DEPARTMENT OF HEALTH AND HUMAN SERVICES MEMORANDUM

Food and Drug Administration
Office of Device Evaluation
9200 Corporate Boulevard
Rockville, MD 20850

Premarket Notification [510(k)] Review
Traditional/Abbreviated
K152519

Date: October 29, 2015
To: The Record
From: Charles N. Durfor, Ph.D.

Office: ODE
Division: DSD/PRSB-2

510(k) Holder: Medical Technology Research Inc.
Device Name: MTR550 Antimicrobial Silver Wound Gel
Contact: Bhalchandra M. Karandikar, Ph.D.
Phone: (503) 902-6279
Fax: (503) 980-7931
Email: bhalchak@mtrmedical.com

I. Purpose and Submission Summary

The 510(k) holder would like to introduce MTR550 Antimicrobial Silver Wound Gel into interstate commerce.

II. Administrative Requirements

	Yes	N	N/A
Indications for Use page (Indicate if: Prescription or OTC)	X		
Truthful and Accuracy Statement	X		
510(k) Summary or 510(k) Statement	X		
Standards Form	p. 5/5		

III. Device Description

	Yes	No	N/A
Is the device life-supporting or life sustaining?		X	
Is the device an implant (implanted longer than 30 days)?		X	
Does the device design use software?		X	
Is the device sterile?		X	
Is the device reusable (not reprocessed single use)? Are "cleaning" instructions included for the end user?		X	

IV. Indications for Use

Prescription Use:

- Stage I-IV pressure ulcers
- Diabetic & foot ulcers
- Partial and full thickness wounds
- Graft and donor sites
- Post-operative surgical wounds
- Trauma wounds (dermal lesions, trauma injuries and incisions)
- 1 & 2nd degree burns
- Abrasions and lacerations

Predicate Device Comparison

- *K062212 SilverShield Antimicrobial Skin & Wound Gel (Product Code MGQ)* - is intended to be used by health care professional in the management of Stage I-IV Pressure Ulcers and Full thickness Wounds, Diabetic Foot and leg Ulcers, grafted and donor sites, abrasions, and lacerations. (Primary Predicate Device)

- *K110458 and K073197 Silver Antimicrobial Wound Gel (Product Code FRO)* - is indicated under the medical supervision of a healthcare professional for the management of dry to moderate exuding partial to full thickness wounds, such as: (Reference Device)

  * Pressure Ulcers
  * Leg Ulcers
  * Diabetic Ulcers
  * Graft and donor sites
  * Post-operative surgical wounds
  * Trauma wounds (dermal lesions, trauma injuries or incisions)
  * First and second degree bums
  * Abrasions and lacerations

Discussion

The proposed Indications for use (i.e., types of wounds for coverage) are substantially equivalent to legally marketed wound dressings.

V. Device Composition

Proposed Device – is a spreadable water-based wound gel with a synthetic clay thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that in turns aids in wound healing. The presence of active silver compounds acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms and may help in reducing infection. The gel is clear to hazy and does not stain the skin tissue. Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures.

The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

Composition (page 4 – Device Description)

Component	Amount (weight %)
(b)(4)	

Properties of MTR550 gel properties (not specification) (page 4 – Device Description)

Property/Characteristic	Value/Description
Appearance	Transparent to slightly hazy
Texture	Smooth
Odor	Odorless
Density (g/mL) @ ~25C	Between 1 and 1.1
Surface tension (mPa.s) @ ~ 25C	N/A
Viscosity (mPa.s) @ ~ 25C	N/A
(b)(4)	
Water absorption (g/g x100) @ ~ 37C	~ 10% @ 24h; ~ 30% @ 72h
Water loss by evaporation (g/g x100) @ ~ 25C	~ < 10% @ 5h
pH	Between 8 and 10
Silver content (ppm)	~ 550 ppm
Compatibility	Incompatible with cationic polymers and reducing agents

The information on page 4 states that the gel properties presented are not specifications. The final product specifications are presented in Attachment 3 (Appendix 1), see below.

Mechanism of Action (page 3 – Device Description)

- The amorphous antimicrobial silver hydrogel maintains moisture in wounds by absorbing or donating moisture depending on wound's condition and
- It provides long lasting antimicrobial protective barrier by inhibiting the growth of common pathogens.
- The moisture donation may assist in the debridement of dry necrotic wounds and by absorbing exudate may reduce skin maceration.

- The antimicrobial effect is provided by ionic silver released from the device upon contact with the wound. (page 3- Device Description) **This is a drug claim. The silver ion can only function within the dressing.**

Device Manufacture - (page 9 – Device Description and Attachment 3) - MTR550 gel is produced by a controlled process in a GMP facility outside the US. Each batch of gel is produced in 100 kilo quantity. The finished gel is packaged tubes (net 43g) employing a semi-automated tube filling machine housed in the same GMP facility.

The gel samples collected from the 100 kilo batch are analyzed and the batch is released after product specifications are met. The manufacturing process (gel production and packaging) along with the product specification and the ingredients specifications are described in an internal technical report (see Attachment 3). An internal technical report (see Attachment 4) summarizes the results of gel properties measurements of samples from the 100 kilo batch

that showed the produced batch was within specifications.

Device Components - page 8 of the Device Description

Properties of Ingredients Used to Produce MTR550 Gel	
	(b)(4)
CAS No.	
Chemical formula	
Formula weight	
Form	
Density@ 25C	
MP	
BP	
pKa	
pH of sat. soln at 25C	
Water solubility at 25C	

Regarding the silver component - The source of silver is a (b)(4) (b)(4) The gel with (b)(4) (b)(4) (b)(4) (b)(4) (b)(4)

(b)(4) Ingredient Information

(b)(4) Ingredient Information

[redacted]

Regarding claims of product performance:

- "The presence of active silver compounds acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms and may help in reducing infection." **Data supporting this claim are discussed below.**

- Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures. **Data supporting this claim are discussed below.**

- The gel is clear to hazy and does not stain the skin tissue. **Data supporting this claim are discussed below.**

The device description (section 6) states:

- The presence of antimicrobial silver in the gel layer provides a protective barrier to entry into the wounds by inhibiting the growth of infectious pathogens that include gram positive and gram negative bacteria, yeast and fungi. **Data for this claim is discussed below.**

- As a result, the viscous gel sustains broad spectrum antimicrobial effectiveness for prolonged period i.e. at least a period of 3 days. **Data for this claim is discussed below.**

(b)(4) Deficiency

[redacted]

Predicate Device Comparison

- K062212 SilverShield Antimicrobial Skin & Wound Gel (Product Code MGQ) - is a clear, amorphous hydrogel wound dressing that helps maintain a moist wound environment that is conducive to healing, by either absorbing the wound exudate or donating the moisture while delivering anti-microbial silver. It is composed of a silver complex an inert viscosity-modifying agent that imparts viscous hydrogel properties. Silver Shield Antimicrobial Skin and Wound Gel will be supplied in collapsible blind ended, heat sealed, co-extruded tubes fitted with a "flip top" dispenser closure (net content 1.5 oz/ 45 grams). The reusable tube will be placed in a clipboard dispenser box with a package insert. The following ingredients are in the device:

Ingredient Percentage	% (w/w)
(b)(4)	

- K073197 and K110458 Silver Antimicrobial Wound Gel (Product Code FRO) - is an opaque, amorphous hydrogel containing a high (>80%) water content and hydrophilic polymer chains. This formulation increases the moisture within the wound through water donation which makes the gel effective in assisting the debridement and desloughing process in dry necrotic wounds, whilst maintaining a moist wound environment for optimal wound healing.

  Silver Antimicrobial Wound Gel contains an antimicrobial silver compound (b)(4) that is an effective barrier to bacterial penetration by inhibiting the growth of broad spectrum of microorganisms which come into contact with the gel.

K110458 was a Special 510(k) in which the (b)(4) content of the product was changed from (b)(4) and all other components and their percentages remain the same as the device cleared under K073197.

Discussion

The composition and design of the proposed and Primary Predicate Device (K062212 SilverShield Antimicrobial Skin & Wound Gel) are similar in the following ways:

- Both devices are water rich formulations (>80%) which provide moisture to the wound, which makes them appropriate for managing dry to moderately exuding wounds.
- As aqueous formulations, the density values at room temperature densities of both products are similar ~ 1.03 gm/mL).
- Both products have similar physical properties, i.e., pH, water loss by evaporation and water absorption over time values (see Attachment 4).

The composition and design of the proposed and Primary Predicate Device (K062212 SilverShield Antimicrobial Skin & Wound Gel) differ in the following ways:

- The composition of the silver compounds in the two products are different (i.e., the proposed device contains (b)(4) compounds and the primary predicate device contains (b)(4) (b)(4) To address this information the sponsor has provided biocompatibility and in vivo wound healing data (see below). In addition, considerable safety information was provided for the (b)(4)

- The amount of gelling agent (b)(4) in the proposed (b)(4) and primary predicate (b)(4) devices are different. However this difference does not appear to be significant.

- The silver concentration in the proposed device 550ppm. The silver concentration in the cited primary predicate device (i.e., K062212 SilverShield Antimicrobial Skin & Wound) Gel is Silver Lactate) is (b)(4) However, the silver content in the cited reference device (i.e., Silver Antimicrobial Wound Gel- K073197 and K110458) is (b)(4) states that the silver content is: (b)(4) (b)(4) (b)(4)

Thus, the chemical compositions of the proposed and predicate devices are not identical, however may be considered substantially equivalent if the supporting data are adequate and the product label is appropriate.

VI. Labeling (Section 5.0)

- *Cardboard box label –*

Side 2 of the label states:

MTR550™ Antimicrobial silver wound gel

- Contains 0.055% (550 ug/g) silver
- Provides lasting antimicrobial barrier as burn and wound dressing (b)(4) (b)(4)
- Helps maintain moist wound environment
- May help lower bio-burden (b)(4) (b)(4)

Side three claims

- Latex free (b)(4) (b)(4)

- *Tube Label*

  Side 2 states: (see comments on the Cardboard box label above)
  - Contains 0.055% (550 ug/g) silver
  - (b)(4) (b)(4)
  - Helps maintain moist wound environment
  - May help lower bio-burden

- *Package Insert*

  - Product Description:

The dressing is formulated to provide an antimicrobial barrier lasting up to 3 days by inhibiting the growth of common pathogens such Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, antibiotic resistant strains MRSA and VRE and yeast and fungi including

Candida albicans, Aspergillus niger and other microorganisms. (b)(4)

(b)(4)

(b)(4) Deficiencies

[redacted]

VII. Sterilization/Shelf Life/Reuse

- *Sterility* – The finished device (in packaged form) is not sterilized. (Page 1 – Device Description)

- *Shelf-life* – The antimicrobial silver in the gel exerts its preservative effect and maintains the device sterile over its 3 year prescribed shelf life. (Page 1 – Device Description) **Data**

- *Packaging* - The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert. (page 2/3 – 510(k) Summary

VIII. Biocompatibility

Proposed Device

(b)(4) Testing [redacted]

Predicate Device

- K062212 SilverShield™ Antimicrobial Skin & Wound Gel (Product Code MGQ)
  - Cytotoxicity
  - Sensitization
  - Irritation
  - Systemic Toxicity

- K110458 Silver Antimicrobial Wound Gel (Product Code FRO)
  - Cytotoxicity – (b)(4) [redacted]
  - Sensitization
  - Irritation
  - (b)(4) [redacted]

(b)(4) [redacted]

(b)(4) [redacted] - FDA review

IX. Software N/A

X. Electromagnetic Compatibility and Electrical, Mechanical and Thermal Safety N/A

XI. Performance Testing – Bench

Proposed Device

(b)(4) Testing

below.

Predicate Device

(b)(4) Testing

XII. Performance Testing – Animal

Proposed Device

(b)(4) Testing

(b)(4) Testing

[redacted]

XIII. Performance Testing – Clinical

Proposed Device - No data submitted.

Predicate Device

- K062212 SilverShield™ Antimicrobial Skin & Wound Gel (Product Code MGQ) - No data submitted.
- K073197 and K110458 Silver Antimicrobial Wound Gel (Product Code FRO) - No data submitted.

Other Information:

- *Attachment 1* – Labeling for Silver-Sept Antimicrobial Skin and Wound Gel (by Anacapa Technologies) – Product label for K062212 SilverShield™ Antimicrobial Skin & Wound Gel (Product Code MGQ)
- *Attachment 2* – Labeling for Normlgel Ag by Molnlycke Health Care AB – this label does not appear to be for either the Primary Predicate Device or the Reference Device.
- *Attachment 3* - Manufacturing Process for (b)(4) [redacted]
  - *Appendix 1 – Product manufacture*

Producing (b)(4)

(b)(4) (b)(4)

Packaging in pre-set amount in tubes

(b)(4)

Appendix 1

Physical Properties	Value/Description
Appearance	Clear to translucent
Texture	Smooth
Odor	Odorless
Density (g/mL) @ ~25C	1.05 g/ml
Form	Amorphous gel
Rheological behavior	(b)(4)
Yield stress (Pa) @ ~ 25C	

Chemical Component	Amount (weight %)
Water	(b)(4)
Others	(b)(4)
Silver	550ppm

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

XIV. Substantial Equivalence Discussion

	Yes	No	
1. Same Indication Statement?			If **YES** = Go To 3
2. Do Differences Alter The Effect Or Raise New Issues of Safety Or Effectiveness?			If **YES** = Stop **NSE**
3. Same Technological Characteristics?			If **YES** = Go To 5
4. Could The New Characteristics Affect Safety Or Effectiveness?			If **YES** = Go To 6
5. Descriptive Characteristics Precise Enough?			If **NO** = Go To 8 If **YES** = Stop **SE**
6. New Types Of Safety Or Effectiveness Questions?			If **YES** = Stop **NSE**
7. Accepted Scientific Methods Exist?			If **NO** = Stop **NSE**
8. Performance Data Available?			If **NO** = Request Data
9. Data Demonstrate Equivalence?			Final Decision:

Note: See http://eroom.fda.gov/eRoomReg/Files/CDRH3/CDRHPremarketNotification510kProgram/0_4148/FLOWCHART%20DECISION%20TREE%20.DOC for Flowchart to assist in decision-making process. Please complete the following table and answer the corresponding questions. "Yes" responses to questions 2, 4, 6, and 9, and every "no" response requires an explanation.

1. Explain how the new indication differs from the predicate device's indication:

2. Explain why there is or is not a new effect or safety or effectiveness issue:

3. Describe the new technological characteristics:

4. Explain how new characteristics could or could not affect safety or effectiveness:

5. Explain how descriptive characteristics are not precise enough:

6. Explain new types of safety or effectiveness question(s) raised or why the question(s) are not new:

7. Explain why existing scientific methods can not be used:

8. Explain what performance data is needed:

9. Explain how the performance data demonstrates that the device is or is not substantially equivalent:

XV. Deficiencies

The sponsor should receive an Additional Information request Letter with the following deficiencies:

(b)(4) Deficiencies

[redacted]

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

[redacted]

XVI. Contact History

XVII. Recommendation

Regulation Number: 21 CFR XXX.XXXX [Only one regulation can be used.]
Regulation Name:
Regulatory Class: Class I, II, III, or Unclassified [Should correspond to regulation.]
Product Code: XYZ [Note: The first code should correspond with the regulation and class thereafter, multiple product codes can be used even if they fall under a different regulation and class.]

Digital Signature Concurrence Table	
Reviewer Sign-Off	Charles N. Durfor -S 2015.10.29 13:08:28 -04'00'
Branch Chief Sign-Off	Jiyoung Dang -S 2015.10.30 19:30:45 -04'00'
Division Sign-Off	

DEPARTMENT OF HEALTH AND HUMAN SERVICES **MEMORANDUM**

Food and Drug Administration
Office of Device Evaluation
9200 Corporate Boulevard
Rockville, MD 20850

Premarket Notification [510(k)] Review
Traditional/Abbreviated
K152519/A001

Date: May 5, 2016
To: The Record
From: Charles N. Durfor, Ph.D.

Office: ODE
Division: DSD/PRSB-2

510(k) Holder: Medical Technology Research Inc.
Device Name: MTR550 Antimicrobial Silver Wound Gel
Contact: Bhalchandra M. Karandikar, Ph.D.
Phone: (503) 902-6279
Fax: (503) 980-7931
Email: bhalchak@mtrmedical.com

Background:

Indications for Use - Prescription Use for Stage I-IV pressure ulcers, Diabetic & foot ulcers, Partial and full thickness wounds, Graft and donor sites, Post-operative surgical wounds, Trauma wounds (dermal lesions, trauma injuries and incisions), 1 & 2nd degree burns and Abrasions and lacerations

Device Composition – a water-based wound gel with a synthetic clay thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that in turns aids in wound healing. The presence of active silver compounds acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms and may help in reducing infection. The gel is clear to hazy and does not stain the skin tissue. Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures. The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

Review: On October 30, 2015 FDA issued an Additional Information request letter that stated:
(b)(4)
[redacted]

Consequently on April 28, 2016 (i.e., the 181th day after issuance of the Additional Information request letter), FDA notified the sponsor that: "On 10/30/2015, we requested additional information from you regarding the submission referenced below. Your response was due on

4/27/2016. Because we have not yet received a complete response, we consider this submission to be withdrawn and have deleted it from our review system."

On the same day FDA received a response from the sponsor and issued an Acknowledgement Letter for K152519/A001. K152519/A001 was logged in as an amendment after the final decision, because the 510(k) had already been deleted from the FDA system.

The 5/2/2016 (12:01 PM) email from Michael Bailey of the 510(k) staff provide insight into how this amendment after the final decision should be handled, i.e.,

"Technically, you do not review the amendment. It was logged in by DCC as we need to keep a record of what was received (even if it came in a day or two after the due date). At this point, as a new 510(k) is needed, I would recommend logging it out as PMNY – needs a 510(k). You could also make a note to this effect in CTS.

Recommendation

This amendment should be logged out as PMNY – needs a 510(k).

Digital Signature Concurrence Table	
Reviewer Sign-Off	Charles N. Durfor -S 2016.05.05 09:50:52 -04'00'
Branch Chief Sign-Off	
Division Sign-Off	

DEPARTMENT OF HEALTH AND HUMAN SERVICES **MEMORANDUM**

Food and Drug Administration
Office of Device Evaluation
9200 Corporate Boulevard
Rockville, MD 20850

Premarket Notification [510(k)] Review
Traditional/Abbreviated
K152519/S001

Date: May 20, 2016
To: The Record
From: Charles N. Durfor, Ph.D.

Office: ODE
Division: DSD/PRSB-2

510(k) Holder: Medical Technology Research Inc.
Device Name: MTR550 Antimicrobial Silver Wound Gel
Contact: Bhalchandra M. Karandikar, Ph.D.
Phone: (503) 902-6279
Fax: (503) 980-7931
Email: bhalchak@mtrmedical.com

Background:

Indications for Use - Prescription Use for Stage I-IV pressure ulcers, Diabetic & foot ulcers, Partial and full thickness wounds, Graft and donor sites, Post-operative surgical wounds, Trauma wounds (dermal lesions, trauma injuries and incisions), 1 & 2nd degree burns and Abrasions and lacerations

Device Composition – a water-based wound gel with a synthetic clay thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that in turns aids in wound healing. The presence of active silver compounds acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms and may help in reducing infection. The gel is clear to hazy and does not stain the skin tissue. Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures. The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

Issues associated with the AINN Letter of October 30, 2015 were also discussed with the sponsor in Q-sub Q15860.

Review:

Supplement 1 provides a response to the FDA AINN letter dated October 30, 2015. The issues cited in that letter are presented below in italics, the sponsor's responses are in plain text and this reviewer's comments are in bold.

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

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(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

[redacted]

This response is acceptable.

Recommendation

The concerns identified during this review were communicated to the sponsor via email on 5/20/16. A response was received on 5/20/16.

Digital Signature Concurrence Table	
Reviewer Sign-Off	Charles N. Durfor -S 2016.05.21 11:01:30 -04'00'
Branch Chief Sign-Off	Jiyoung Dang -S 2016.06.02 07:44:19 -04'00'
Division Sign-Off	

DEPARTMENT OF HEALTH AND HUMAN SERVICES **MEMORANDUM**

Food and Drug Administration
Office of Device Evaluation
9200 Corporate Boulevard
Rockville, MD 20850

Premarket Notification [510(k)] Review
Traditional/Abbreviated
K152519

Date: May 21, 2016
To: The Record
From: Charles N. Durfor, Ph.D.

Office: ODE
Division: DSD/PRSB-2

510(k) Holder: Medical Technology Research Inc.
Device Name: MTR550 Antimicrobial Silver Wound Gel
Contact: Bhalchandra M. Karandikar, Ph.D.
Phone: (503) 902-6279
Fax: (503) 980-7931
Email: bhalchak@mtrmedical.com

I. Purpose and Submission Summary

The 510(k) holder would like to introduce MTR550 Antimicrobial Silver Wound Gel into interstate commerce.

II. Administrative Requirements

	Yes	N	N/A
Indications for Use page (Indicate if: Prescription or OTC)	X		
Truthful and Accuracy Statement	X		
510(k) Summary or 510(k) Statement	X		
Standards Form	p. 5/5		

III. Device Description

	Yes	No	N/A
Is the device life-supporting or life sustaining?		X	
Is the device an implant (implanted longer than 30 days)?		X	
Does the device design use software?		X	
Is the device sterile?		X	
Is the device reusable (not reprocessed single use)? Are "cleaning" instructions included for the end user?		X	

IV. Indications for Use

Prescription Use: - For the management of dry to low/moderate exuding wounds such as:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

Predicate Device Comparison

- *K062212 SilverShield Antimicrobial Skin & Wound Gel (Product Code MGQ)* - is intended to be used by health care professional in the management of Stage I-IV Pressure Ulcers and Full thickness Wounds, Diabetic Foot and leg Ulcers, grafted and donor sites, abrasions, and lacerations. (Primary Predicate Device)

- *K110458 and K073197 Silver Antimicrobial Wound Gel (Product Code FRO)* - is indicated under the medical supervision of a healthcare professional for the management of dry to moderate exuding partial to full thickness wounds, such as: (Reference Device)

  * Pressure Ulcers
  * Leg Ulcers
  * Diabetic Ulcers
  * Graft and donor sites
  * Post-operative surgical wounds
  * Trauma wounds (dermal lesions, trauma injuries or incisions)
  * First and second degree bums
  * Abrasions and lacerations

Discussion

The proposed Indications for use (i.e., types of wounds for coverage) are substantially equivalent to legally marketed wound dressings.

V. Device Composition

Proposed Device – is a spreadable water-based wound gel with a synthetic clay thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that in turns aids in wound healing. The silver compounds in the product act as an effective barrier to microbial penetration by inhibiting the growth of microorganisms within the dressing. The gel is clear to hazy and in testing was found not to stain the skin after 3 days of exposure. The device is packaged primarily in tamper-proof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

Composition (page 4 of original 510(k) – Device Description)

Component	Amount (weight %)
(b)(4)	

(b)(4) Ingredients [redacted]

The final product specifications are presented in Supplement 1 (i.e., Items # 027 - Technical Device Description (page 1/11) and Item # 028 - Final product specifications). These revised specifications (b)(4) [redacted]

- Revised pyrogen Specification - (b)(4) [redacted]

Property/Characteristic	Value/Description
Appearance	Transparent to slightly hazy
Texture	Smooth
Odor	Odorless
Density (g/mL) @ ~25C	Between 1 and 1.1
Surface tension (mPa.s) @ ~ 25C	N/A
Viscosity (mPa.s) @ ~ 25C	N/A
(b)(4) [redacted]	
Water absorption (g/g x100) @ ~ 37C	~ 10% @ 24h; ~ 30% @ 72h
Water loss by evaporation (g/g x100) @ ~ 25C	~ < 10% @ 5h
pH	Between 8 and 10
Silver content (ppm)	~ 550
Compatibility	Incompatible with cationic polymers and reducing agents
Bioburden level	(b)(4) [redacted]
Bacterial endotoxin (pyrogen) level	

Mechanism of Action (page 3 – Device Description)

- The amorphous antimicrobial silver hydrogel maintains moisture in wounds by absorbing or donating moisture depending on wound's condition and
- It provides an antimicrobial protective barrier for up to 3 days by inhibiting the growth of common pathogens within the dressing.
- The moisture donation may assist in the debridement of dry necrotic wounds and by absorbing exudate may reduce skin maceration.

Device Manufacture - (page 9 – Device Description and Attachment 3 – Original 510(k)) - MTR550 gel is produced by a (b)(4) [redacted]

(b)(4) [redacted]

(b)(4) (b)(4) [redacted]

The manufacturing process (gel production and packaging) along with the product specification and the ingredients specifications are described in an internal technical report (see Attachment 3). An internal technical report (see Attachment 4) summarizes the results (b)(4) [redacted]

(b)(4) [redacted]

Device Components - page 8 of the Device Description

Properties of Ingredients Used to Produce MTR550 Gel
(b)(4) [redacted]

(b)(4) Ingredients

(b)(4) Ingredients

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

[redacted]

This response is acceptable.

(b)(4) Deficiencies

[redacted]

- K062212 SilverShield Antimicrobial Skin & Wound Gel (Product Code MGQ) - is a clear, amorphous hydrogel wound dressing that helps maintain a moist wound environment that is conducive to healing, by either absorbing the wound exudate or donating the moisture while delivering anti-microbial silver. It is composed of a silver complex an inert viscosity-modifying agent that imparts viscous hydrogel properties. Silver Shield Antimicrobial Skin and Wound Gel will be supplied in collapsible blind ended, heat sealed, co-extruded tubes fitted with a "flip top" dispenser closure (net content 1.5 oz/ 45 grams). The reusable tube will be placed in a clipboard dispenser box with a package insert. The following ingredients are in the device:

Ingredient	Percentage % (w/w)
(b)(4)	

- K073197 and K110458 Silver Antimicrobial Wound Gel (Product Code FRO) - is an opaque, amorphous hydrogel containing a high (>80%) water content and hydrophilic polymer chains. This formulation increases the moisture within the wound through water donation which makes the gel effective in assisting the debridement and desloughing process in dry necrotic wounds, whilst maintaining a moist wound environment for optimal wound healing.

  Silver Antimicrobial Wound Gel contains an antimicrobial silver compound (Silver carbonate) that is an effective barrier to bacterial penetration by inhibiting the growth of broad spectrum of microorganisms which come into contact with the gel.

K110458 was a Special 510(k) in which the (b)(4))(4) (b)(4) and all other components and their percentages remain the same as the device cleared under K073197.

Discussion

The composition and design of the proposed and Primary Predicate Device (K062212 SilverShield Antimicrobial Skin & Wound Gel) are similar in the following ways:

- Both devices are water rich formulations (>80%) which provide moisture to the wound, which makes them appropriate for managing dry to moderately exuding wounds.
- As aqueous formulations, the density values at room temperature densities of both products are similar ~ 1.03 gm/mL).
- Both products have similar physical properties, i.e., pH, water loss by evaporation and water absorption over time values (see Attachment 4).

The composition and design of the proposed and Primary Predicate Device (K062212 SilverShield Antimicrobial Skin & Wound Gel) differ in the following ways:

- The composition of the (b)(4) (b)(4) (b)(4) To address this information the sponsor has provided biocompatibility and (b)(4) (b)(4) (see below). In addition, considerable safety information was provided for t (b)(4)

- The amount of (b)(4) (b)(4) However this difference does not appear to be significant.
- The silver concentration in the proposed device 550ppm. (b)(4) (b)(4)

Thus, the chemical compositions of the proposed and predicate devices are not identical, but can be considered substantially equivalent based on the biocompatibility and performance testing described below and in Supplement 1.

VI. Labeling (Section 5.0)

- *Cardboard box label –*

Side 2 of the label states:

- Contains 0.055% (550 ug/g) silver (as preservative)
- Provides lasting antimicrobial barrier as burn and wound dressing up to 3 days
- Helps maintain moist wound environment
- As preservative, silver reduces microbial growth within the dressing and exerts no microbial action in or on the wound

These claims are acceptable and supported by data.

Side three claims

- For external use only; not to use in or around the eyes
- Not made with natural rubber latex
- Contraindicated for use on patients with sensitivity to silver and s-triazine compounds

These claims are acceptable and supported by data.

- *Tube Label*
  Side 2 states: (see comments on the Cardboard box label above)

- Contains 0.055% (550 ug/g) silver (as preservative)
- Provides lasting antimicrobial barrier as burn and wound dressing up to 3 days
- Helps maintain moist wound environment
- As preservative, silver reduces microbial growth within the dressing and exerts no microbial action in or on the wound

These claims are acceptable and supported by data.

- *Package Insert*

- Product Description:

MTR550™ gel is spreadable amorphous burn and wound dressing that contains 550 ppm of an antimicrobial silver preservative. The dressing is formulated to provide an antimicrobial barrier lasting up to 3 days by inhibiting the growth of common pathogens such *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli*, antibiotic resistant strains MRSA and VRE and yeast and fungi including *Candida albicans, Aspergillus niger* and other microorganisms within the dressing. As a preservative, the presence of silver in the dressing reduces pathogen counts within the dressing and has no effect on the pathogens in or on the wound. In *in vitro* tests, antimicrobial silver within MTR550™ gel dressing was found to exert preservative action against several common pathogens.

The gel dressing helps maintain moisture in wound environment which may help promote wound healing. The gel is suitable for use on dry to low/moderately exuding wounds. MTR550™ gel will not stain or discolor tissue when used daily over 3 days. However, it is not known if staining will occur if gel use is continued beyond 3 days.

These claims are acceptable and supported by data.

Regarding the 510(k) Summary – Subsequent to FDA review and comment the sponsor has provided an acceptable 510(k) Summary.

VII. Sterilization/Shelf Life/Reuse

- *Sterility* – The finished device (in packaged form) is not sterilized. (Page 1 – Device Description)
- *Shelf-life* – Initially the sponsor proposed an expiration date of 3 years. However, based on (b)(4) [redacted]
- *Packaging* - The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert. (page 2/3 – 510(k) Summary

VIII. Biocompatibility

Proposed Device

- *In-vitro cytotoxicity* (Attachment 5) - (b)(4) [redacted] (b)(4) [redacted]
- *Dermal irritation* (Attachment 6) - (b)(4) [redacted] (b)(4) [redacted]

- *Sensitization* (Attachment 7) - (b)(4) [redacted]
- *Systemic Toxicity* (Attachment 8) - (b)(4) [redacted]

(b)(4) [redacted]

Predicate Device

- K062212 SilverShield™ Antimicrobial Skin & Wound Gel (Product Code MGQ)
  - Cytotoxicity
  - Sensitization
  - Irritation
  - Systemic Toxicity

- K110458 Silver Antimicrobial Wound Gel (Product Code FRO)
  - Cytotoxicity – (b)(4) [redacted]
  - Sensitization
  - Irritation
  - (b)(4) [redacted]

(b)(4) [redacted]

(b)(4) [redacted] - FDA review

IX. Software **N/A**

X. Electromagnetic Compatibility and Electrical, Mechanical and Thermal Safety **N/A**

XI. Performance Testing – Bench

Proposed Device

(b)(4) Testing [redacted]

Predicate Device

(b)(4) Testing

[redacted]

XII. Performance Testing – Animal

Proposed Device

(b)(4) Testing

[redacted]

(b)(4) Testing

[redacted]

(b)(4) Testing

(b)(4) Testing

[redacted]

XIII. Performance Testing – Clinical

Proposed Device - No data submitted.

Predicate Device

(b)(4) Testing

[redacted]

(b)(4) Testing

Other Information:

- *Attachment 1* – Labeling for Silver-Sept Antimicrobial Skin and Wound Gel (by Anacapa Technologies) – Product label for K062212 SilverShield™ Antimicrobial Skin & Wound Gel (Product Code MGQ)

- *Attachment 2* – Labeling for Normlgel Ag by Molnlycke Health Care AB – this label does not appear to be for either the Primary Predicate Device or the Reference Device.

- *Attachment 3* - Manufacturing Process for MTR550 Gel – (b)(4)

(b)(4)

[redacted]

  - *Appendix 1 – Product manufacture*

(b)(4)

[redacted]

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

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(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

[redacted]

XIV. Substantial Equivalence Discussion

	Yes	No	
1. Same Indication Statement?	x		If **YES** = Go To 3
2. Do Differences Alter The Effect Or Raise New Issues of Safety Or Effectiveness?			If **YES** = Stop **NSE**
3. Same Technological Characteristics?	x		If **YES** = Go To 5
4. Could The New Characteristics Affect Safety Or Effectiveness?			If **YES** = Go To 6
5. Descriptive Characteristics Precise Enough?		x	If **NO** = Go To 8 If **YES** = Stop **SE**
6. New Types Of Safety Or Effectiveness Questions?			If **YES** = Stop **NSE**
7. Accepted Scientific Methods Exist?			If **NO** = Stop **NSE**
8. Performance Data Available?	x		If **NO** = Request Data
9. Data Demonstrate Equivalence?	x		Final Decision: SE

Note: See

1. Explain how the new indication differs from the predicate device's indication: **The Indication for use is the same as a legally marketed predicate device.**

2. Explain why there is or is not a new effect or safety or effectiveness issue: **The composition of the device is not identical, but similar to other silver-containing wound dressings. The safety, performance and chemical analyses tests indicate that the new device composition will display substantially equivalent clinical performance to other cleared predicate devices.**

3. Describe the new technological characteristics: **This will be** (b)(4) [redacted] (b)(4) [redacted] **preservative.**

4. Explain how new characteristics could or could not affect safety or effectiveness: **Biocompatibility, performance and chemical analyses indicate that the new characteristics will not affect device safety or effectiveness characteristics.**

5. Explain how descriptive characteristics are not precise enough: **As stated above biocompatibility, performance and chemical analyses were needed to evaluate the product performance.**

6. Explain new types of safety or effectiveness question(s) raised or why the question(s) are not new: **There were no new/different types of safety or effectiveness questions.**

7. Explain why existing scientific methods cannot be used: **N/A**

8. Explain what performance data is needed: **See review.**

9. Explain how the performance data demonstrates that the device is or is not substantially equivalent: **See review.**

XV. Deficiencies N/A

XVI. Contact History

- 10/30/15 – AINN Letter sent to sponsor.
- 5/20/16- Request changes in product Indications for Use, 510(k) Summary and Product Label.

XVII. Recommendation

Regulation Number: None
Regulation Name: Dressing, Wound, Drug
Regulatory Class: Unclassified
Product Code: FRO

Digital Signature Concurrence Table	
Reviewer Sign-Off	Charles N. Durfor -S 2016.05.21 11:07:09 -04'00'
Branch Chief Sign-Off	Jiyoung Dang -S 2016.06.02 09:12:55 -04'00'
Division Sign-Off	

510(k) SUBMISSION

TABLE OF CONTENTS

INDICATIONS FOR USE

K NUMBER: **152519**

DEVICE NAME: **MTR550 ANTIMICROBIAL SILVER WOUND GEL**

INDICATIONS FOR USE

For the management of dry to low/moderate exuding wounds such as:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

TYPE OF USE: **Prescription Use Only (Part 21 CFR 801 Subpart D)**

510(k) SUMMARY

1. SUBMITTER/OWNER

MEDICAL TECHNOLOGY RESEARCH INC.
2650 PROGRESS WAY
WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact Person: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Date Prepared: August 28, 2015

2. DEVICE

Name of Device:	MTR550 (Tentative) Antimicrobial Silver Wound Gel
Common Name:	Antimicrobial Wound Dressing
Classification Name:	Dressing Wound, Drug
Regulatory Class:	Unclassified
Product Code:	FRO

3. PREDICATE DEVICE

MTR550 gel is substantially equivalent to the following legally marketed predicate devices:

(i) SilverShield™ Antimicrobial Skin and Wound Gel (# K062212) from Anacapa Technologies of San Dimas, CA and distributed presently under SilverSept® name

(ii) Silver Antimicrobial Wound Gel from Advanced Medical Solutions of Winsford, UK (#K110458) and marketed by Molynyke under the name Normlgel Ag®

4. DEVICE DESCRIPTION

The product is a spreadable amorphous water based wound gel comprising a synthetic clay as the thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that it turns aids in wound healing. The presence of active silver compounds acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms and may help in reducing infection. The gel is clear to hazy and does not stain the skin tissue. Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures.

The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

5. INDICATIONS FOR USE

The device is indicated for use by prescription only. It is indicated for use for:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

Contraindications: The gel should not be used on patients with known sensitivity to silver and s-triazine compounds.

6. MANUFACTURING

The MTR550 gel is manufactured in accordance with good manufacturing practices at a GMP facility. The batch production of the gel and packaging has been demonstrated to meet product specification. The contract manufacturer has met all requirements for QC/QA to release the product which is safe and effective.

7. BIOCOMPATIBILITY TESTING

The device has been tested for in-vitro cytotoxicity, dermal irritation and sensitization in accordance with ISO 10993 -1 (Biological Evaluation of Medical Devices).

No systemic toxicity was associated with the antimicrobial silver compounds. Animal wound healing study employing rats showed no adverse effects due to the device.

8. PERFORMANCE

The antimicrobial efficacy of the device has been demonstrated by 28 days Antimicrobial preservative challenge test in accordance with USP Chapter 51 guidelines. Employing in vitro assays, the antimicrobial barrier property was demonstrated against 17 different micro-organisms that included bacteria, yeast and fungi. The tested micro-organisms included, MRSA, VRE, Candida A. and Aspergillus niger. Sustenance of antimicrobial barrier activity was demonstrated for 3 days in a serial transfer assay.

Animal wound healing study showed the device exhibited no adverse effect on wound healing compared to a positive control group. The positive control was in the form of (b)(4)

Despite its silver content at 550ppm, the device was shown to be non-staining to dermal tissue and its properties were unaffected by extreme heat or intense light.

9. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH PREDICATE DEVICE

With respect to its physical and chemical properties, the device is substantially equivalent to the predicate device.
The mechanism of antimicrobial action is also similar. Both device exert toxicity towards micro-organisms as a result of silver ions.

The key differences between our device and predicate device are:

(i) Silver content
(ii) Source of antimicrobial silver
(iii) Content of gelling agent

These differences though real raise no safety or effectiveness issue and establish our device is substantially equivalent to the predicate device.

10. CONCLUSIONS

Based on the indications for use, biocompatibility, invivo studies, performance data, the MTR550 gel is substantially similar to SilverShield™ Antimicrobial Skin and Wound Gel (#K062212)

510(k) SUMMARY

1. SUBMITTER/OWNER

MEDICAL TECHNOLOGY RESEARCH INC.
2650 PROGRESS WAY
WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact Person: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Date Prepared: April 25, 2016

2. DEVICE

Name of Device: MTR550 (Tentative) Antimicrobial Silver Wound Gel
Common Name: Antimicrobial Wound Dressing
Classification Name: Dressing Wound, Drug
Regulatory Class: Unclassified
Product Code: FRO

3. PREDICATE DEVICE

MTR550 gel is substantially equivalent to the following legally marketed predicate devices:

(i) SilverShield™ Antimicrobial Skin and Wound Gel (# K062212) from Anacapa Technologies of San Dimas, CA and distributed presently under SilverSept® name

(ii) Silver Antimicrobial Wound Gel from Advanced Medical Solutions of Winsford, UK (#K110458) and marketed by Molynyke under the name Normlgel Ag®

4. DEVICE DESCRIPTION

The product is a spreadable amorphous water based wound gel comprising a synthetic clay as the thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that it turns aids in wound healing. The presence of active silver compounds within the gel provides preservative action and acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms within the dressing. The gel is clear to hazy and will not stain the skin tissue when used over a period of 3 days though its potential to stain skin beyond 3 day use is not known.

Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures.
The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

5. INDICATIONS FOR USE

The device is indicated for use by prescription only. It is indicated for use in the management of dry to low/moderate exuding partial and fullness thickness wounds, stage I-IV pressure ulcers, diabetic and foot ulcers, graft wounds and donor sites, first and second degree wounds, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations.

Contraindications: The gel should not be used on patients with known sensitivity to silver and s-triazine compounds.

6. MANUFACTURING

The MTR550 gel will be manufactured in a production facility in accordance with good manufacturing practices consistent with US FDA guidelines. The batch production of the gel and packaging will be verified to meet product specifications to ensure the product is safe and effective.

7. BIOCOMPATIBILITY TESTING

The device has been tested for in-vitro cytotoxicity, dermal irritation and sensitization in accordance with ISO 10993 -1 (Biological Evaluation of Medical Devices).

No systemic toxicity was associated with the antimicrobial silver compounds. Animal study employing rats showed no adverse effects on animals due to the device.

8. PERFORMANCE

The antimicrobial efficacy of the device has been demonstrated by 28 days Antimicrobial preservative challenge test in accordance with USP Chapter 51 guidelines. Employing in vitro assays, the antimicrobial barrier property was demonstrated against 17 different micro-organisms that included bacteria, yeast and fungi. The tested micro-organisms included, MRSA, VRE, Candida A. and Aspergillus niger. Sustenance of antimicrobial barrier activity was demonstrated for 3 days in a serial transfer assay.

Animal study employing rats showed the device exhibited no adverse effect on animals. In a porcine study examining deep partial thickness burn wound healing, MTR550 device was found to be safe and effective as the predicate device.

Despite its silver content at 550ppm, the device was shown to be non-staining to dermal tissue over 3 days use. The device showed no discoloration despite exposure to intense light or elevated environmental temperatures.

9. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH PREDICATE DEVICE

With respect to its physical and chemical properties, the device is substantially equivalent to the predicate device.

The mechanism of antimicrobial action is also similar. Both device exert toxicity towards micro-organisms as a result of silver ions.

The device is substantially equivalent to legally marketed predicate devices in composition and intended use.

10. CONCLUSIONS

Based on the indications for use, biocompatibility, invivo studies, performance data, the MTR550 gel is substantially similar to SilverShield™ Antimicrobial Skin and Wound Gel (#K062212)

6.0 SPECIFICATION:

6.1 General Device Description

The device is an amorphous aqueous gel containing antimicrobial silver. It is transparent to slightly hazy in appearance. In wounds, it helps maintain moist environment by either donating moisture or by absorbing low to moderate amount of exudate. Moisture management may also help processes that promote wound healing. The presence of antimicrobial silver in the gel layer provides a protective barrier to entry into the wounds by inhibiting the growth of infectious pathogens that include gram positive and gram negative bacteria, yeast and fungi. The device's antimicrobial activity is exerted by ionic silver and similar to other silver containing devices.

The viscous/gel character of amorphous composition serve several purposes. (b)(4)

(b)(4)

The amorphous gel is supplied in a net amount of 43g in heat sealed plastic tubes with a screw-on cap. To prevent tampering, the dispensing end of the tube is sealed with a coated aluminum foil. For the first use, cap is unscrewed and the foil seal is peeled off. The gel also may be packaged in larger tubes or other packaging such as 8 oz. jars. In place of foil seal to prevent tampering, the tube or the jar may be sealed with shrink wrap covering the entire cap and a portion of the tube or jar.

As finished device (in packaged form), the amorphous gel is not sterilized. However, antimicrobial silver in the gel exerts its preservative effect and maintains the device sterile over its 3 year prescribed shelf life.

Breadth of Antimicrobial Activity

In in-vitro assays, its broad spectrum antimicrobial activity was demonstrated against the following 17 microorganisms:

(b)(4)

Indications for Use

As wound dressing, MTR550 antimicrobial gel is intended for use by prescription only. As topical gel, it is indicated for external use only. It is indicated for use under the medical supervision of a licensed healthcare professional for:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

Contraindications and Precautions:

There are several contraindications and precautions associated with the administration of the device. It may not be used on patients with known sensitivity to silver or s-triazine compounds. It is not for ophthalmic use. The antimicrobial gel may be left in the wounds for a period of up to 3 days before a dressing change is required.

How does MTR550 gel works? Rationale for Use of Silver as Antimicrobial Agent

As a wound dressing, the amorphous antimicrobial silver hydrogel provides two functions; it maintains moisture in wounds by absorbing or donating moisture depending on wound's condition and it provides long lasting antimicrobial protective barrier by inhibiting the growth of common pathogens. As an added benefit, the moisture donation may assist in the debridement of dry necrotic wounds and by absorbing exudate may reduce skin maceration.

The antimicrobial effect is provided by ionic silver released from the device upon contact with the wound. Silver's antimicrobial efficacy and safety in wound products has been well established as evidenced by a large number of silver containing devices cleared by FDA under 510(k) submissions over the past decade.

As antimicrobial agent, silver offers several advantages. First, with silver there is practically no risk of microorganisms developing resistance because of the way it exerts its antimicrobial activity. Silver irreversibly binds to surfaces of cells through multiple pathways via functional groups such as carboxyl, amines and thiols making it harder for resistant microorganisms to evolve. Second, silver singularly is effective against multitude of organisms. Thus, the formulators need only one rather than many active components in their formulations or devices simplifying product design. Third, silver is very effective against infectious pathogens at very low levels and yet at these minimum inhibitory concentrations for such organisms, silver is not toxic to humans.

Despite these advantages, silver suffers from a major limitation. Product formulations or devices containing silver often have short shelf lives. The very property that silver gives its potent antimicrobial activity also makes it susceptible to reduction to inactive elemental silver induced by light or heat making it ineffective. The unpredictable nature of silver susceptibility to reduction can cause large batch to batch variability in product shelf lives.

The MTR550 gel incorporates silver as the antimicrobial agent. The source of silver is a pair of silver cyanurate compounds; di-silver cyanurate and silver cyanurate ligand complex. The gel with these two cyanurate compounds retains all the benefits mentioned above but overcomes its major drawback. The two silver cyanurate compounds have unique ability to resist light and heat induced discoloration (the visual indicator of the reduction reaction) even in water rich environment of the amorphous gel without loss of antimicrobial activity. The insensitivity to light and heat of the two silver cyanurate compounds also reduces the risk of premature silver reduction occurring thus ensuring effectiveness over its entire shelf life.

6.2 Technical Device Description

Finished composition and Ingredients

The device is an aqueous amorphous gel composition containing antimicrobial silver in the nominal concentration of 550ppm by weight. The amounts of components by weight in the gel are tabulated below:

Nominal Composition of MTR550 Gel

Component	Amount (weight %)
(b)(4)	

The pertinent physical and chemical properties of MTR550 gel are tabulated below.

Typical MTR550 gel properties (not specification)

Property/Characteristic	Value/Description
Appearance	Transparent to slightly hazy
Texture	Smooth
Odor	Odorless
Density (g/mL) @ ~25C	Between 1 and 1.1
Surface tension (mPa.s) @ ~ 25C	N/A
Viscosity (mPa.s) @ ~ 25C	N/A
Yield stress (Pa) @ ~ 25C	~ 250
Water absorption (g/g x100) @ ~ 37C	~ 10% @ 24h; ~ 30% @ 72h
Water loss by evaporation (g/g x100) @ ~ 25C	~ < 10% @ 5h
pH	Between 8 and 10
Silver content (ppm)	~ 550
Compatibility	Incompatible with cationic polymers and reducing agents

The device is transparent to slightly hazy in appearance though the appearance does not affect its performance. The viscous gel like behavior is imparted by (b)(4)

Glycerol acts as humectant and helps gel retain moisture. Water acts as inert vehicle for other

ingredients including the antimicrobial agents - silver cyanurate compounds. (b)(4) [redacted]. The hazy appearance of the gel in part is due to very finely dispersed particles of insoluble silver cyanurate compounds within the gel.

Inactive Ingredients

(b)(4) [redacted]

(b)(4) Ingredients

(b)(4) Ingredients

(b)(4) Ingredients

6.3 Manufacturing process for MTR550 gel

(b)(4)

6.4 Biocompatibility Testing

The FDA draft guidance document 1811 issued April 23, 2013 titled "Use of International Standard ISO 10993- Biological evaluation of medical devices Part 1: Evaluation and Testing" provides guidelines on biocompatibility testing for industry and FDA staff. Of the information provided, the matrix in Attachment A of that document is most relevant. It suggests for a surface device such as MTR550 gel, initial biocompatibility testing should include cytotoxicity, irritation, sensitization and systemic toxicity.

(b)(4)

Cytotoxicity (see Attachment 5)

(b)(4)

Dermal Irritation (see Attachment 6)

(b)(4)

Dermal Sensitization (see Attachment 7)

(b)(4)

(b)(4) **toxicity** (see Attachment 8)

(b)(4)

Wound healing study (see Attachment 9)

(b)(4)

(b)(4)
[redacted].

6.5 Performance Testing

The FDA draft guidance document issued on July 19, 2007 titled "Pre-market notifications (510(k)) submissions for medical devices that include antimicrobial agents" recommends performance testing to demonstrate antimicrobial effectiveness of the finished device. The testing may include both invitro tests and invivo (animal) tests as required.

Accordingly, the following tests were conducted on MTR550 gel to demonstrate antimicrobial effectiveness.

- Antimicrobial Preservative Challenge Test in accordance with USP Chapter 51 (see Attachment 10)

(b)(4)
[redacted]

5.0 PROPOSED LABELING

The proposed labeling on the thin gauge cardboard box, the tube and the packaging insert is disclosed below. For comparison the product inserts from predicate devices (K062212 & K110458) are attached (see Attachments 1 & 2).

Product cardboard box labeling

Side 1

MTR550™ Gel (Note the MTR550™ name is tentative and may be replaced)

Antimicrobial silver wound gel

Rx Only / Net weight (1.5 oz/43g)

Side 2

MTR550™ Antimicrobial silver wound gel

- Contains 0.055% (550 μg/g) silver (as preservative)
- Provides lasting antimicrobial barrier as burn and wound dressing up to 3 days
- Helps maintain moist wound environment
- As preservative, silver reduces microbial growth within the dressing and exerts no microbial action in or on the wound

Read instructions for use (see insert inside package)
Store at 15C (59F) to 30C (77F)

Side 3

Rx Only
Intended for use for the management of dry to low/moderate exuding wounds such as: partial and full thickness wounds, Stage I-IV pressure ulcers, diabetic and foot ulcers and 1st and 2nd degree burns, graft and donor sites, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations

- For external use only; not to use in or around the eyes
- Not made with natural rubber latex
- Contraindicated for use on patients with sensitivity to silver and s-triazine compounds

Side 4

Manufactured in China for:
Medical Technology Research Inc.
2650 Progress Way, Woodburn, OR 97071

US and corresponding international patents pending
Reorder Number XXXXX/Questions & Comments: 1-800-MTR-MEDI (Tentative)

Tube labeling

Side 1

MTR550™ Antimicrobial Silver Wound Gel*

*US & Int. patents pending

Rx Only / Net wt. 1.5 oz (43g)

Mfd. for:

Medical Technology Research Inc. Woodburn, OR 97071
www.mtrmedical.com

Side 2

MTR550™ Antimicrobial Silver Wound Gel

- Contains 0.055% (550 μg/g) silver (as preservative)
- Provides lasting antimicrobial barrier as burn and wound dressing up to 3 days
- Helps maintain moist wound environment
- As preservative, silver reduces microbial growth within the dressing and exerts no microbial action in or on the wound

Intended for use for the management of dry to low/moderate exuding wounds such as: partial and full thickness wounds, Stage I-IV pressure ulcers, diabetic and foot ulcers and 1st and 2nd degree burns, graft and donor sites, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations

Store at 15C – 30C; Read instructions for use (see package insert)

Package Insert

MTR550™ Antimicrobial Silver Wound Gel*

Reorder Number XXXXX

Product Description:

MTR550™ gel is spreadable amorphous burn and wound dressing that contains 550 ppm of an antimicrobial silver preservative. The dressing is formulated to provide an antimicrobial barrier lasting up to 3 days by inhibiting the growth of common pathogens such *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* antibiotic resistant strains MRSA and VRE and yeast and fungi including *Candida albicans, Aspergillus niger* and other microorganisms within the dressing. As a preservative, the presence of silver in the dressing reduces pathogen counts within the dressing and has no effect on the pathogens in or on the wound. In *in vitro* tests, antimicrobial silver within MTR550™ gel dressing was found to exert preservative action against several common pathogens.

The gel dressing helps maintain moisture in wound environment which may help promote wound healing. The gel is suitable for use on dry to low/moderately exuding wounds. MTR550™ gel will not stain or discolor tissue when used daily over 3 days. However, it is not known if staining will occur if gel use is continued beyond 3 days.

It is intended for sale or for use by prescription only on patients under the medical supervision of licensed healthcare professionals. It is against the Federal law to contravene this restriction.

Indications for use:

It is indicated for use for the management of dry to low/moderate exuding wounds such as:

- Stage I-IV pressure ulcers
- Diabetic & foot ulcers
- Partial and full thickness wounds
- Graft and donor sites
- Post-operative surgical wounds
- Trauma wounds (dermal lesions, trauma injuries and incisions)
- 1 & 2nd degree burns
- Abrasions and lacerations

Contraindications for use:

- Not to be used on patients with sensitivity to silver and s-triazine compounds
- Patients may experience mild skin irritation in and around area of gel application, but if it persists or redness or swelling develops, discontinue using the gel

Directions for use:

- ✓ Cleanse and debride wound as required
- ✓ Dispense MTR550™ gel onto the wound directly and evenly spread it to a 0.1" to 0.2" thick layer. It is recommended the applied gel layer extend 0.2" to 0.25" beyond the edge of the wound for effective barrier function.
- ✓ Cover with a sterile secondary dressing (such as gauze, foam or similar) and secure it in place
- ✓ In between dressing changes, maintain moist environment in the wound bed
- ✓ MTR550™ gel dressing may be maintained for up to 3 days though heavy exuding wounds may require more frequent dressing changes as per clinical protocols

Rx Only
External Use Only. Not to be used in the proximity of eyes.
Not made with natural rubber latex

*US & INTL. PATENTS PENDING

Manufactured in China for:
Medical Technology Research Inc.
2650 Progress Way, Woodburn, OR 97071
www.mtrmedical.com
For questions or comments: 1-800-MTR-MEDI (tentative)

MTR
Medical Technology
Research Inc

STATEMENT OF NO PRIOR SUBMISSIONS

K Number: K152519

This statement is to verify that no prior submission of MTR550™ antimicrobial silver wound gel of any kind (Pre-submission or IDE or NSE or Prior 510(k)) was made to the FDA.

Signature

Bhalchandra M Karandikar
Name of the authorized person

Submission Date: August 28, 2015

Submitter/Owner: MEDICAL TECHNOLOGY RESEARCH INC.

Address: 2650 PROGRESS WAY
WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Email address: bhalchak@mtrmedical.com

Department of Health and Human Services
Food and Drug Administration

STANDARDS DATA REPORT FOR 510(k)s

(To be filled in by applicant)

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TYPE OF 510(K) SUBMISSION

☒ Traditional ☐ Special ☐ Abbreviated

STANDARD TITLE [1]

AAMI ANSI ISO 10993-5 Biological evaluation of medical devices Part 5: Tests for in vitro cytotoxicity (2009)

Please answer the following questions	Yes	No
Is this standard recognized by FDA [2]?	☒	☐
FDA Recognition number [3]	# 2-153	
Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)?	☒	☐
Is a summary report [4] describing the extent of conformance of the standard used included in the 510(k)? If no, complete a summary report table.	☒	☐
Does the test data for this device demonstrate conformity to the requirements of this standard as it pertains to this device?	☐	☒
Does this standard include acceptance criteria? If no, include the results of testing in the 510(k).	☒	☐
Does this standard include more than one option or selection of tests? If yes, report options selected in the summary report table.	☒	☐
Were there any deviations or adaptations made in the use of the standard?	☐	☒
If yes, were deviations in accordance with the FDA supplemental information sheet (SIS) [5]?	☐	☐
Were deviations or adaptations made beyond what is specified in the FDA SIS? If yes, report these deviations or adaptations in the summary report table.	☐	☒
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Is there an FDA guidance [6] that is associated with this standard?	☒	☐
If yes, was the guidance document followed in preparation of this 510k?	☒	☐

Title of guidance: FDA Bluebook Memo G95-1 Use of ISO10993-Biol. Eval. of Med. Dev. Pt 1: Evaluation & Testing

[1] The formatting convention for the title is: [SDO] [numeric identifier] [title of standard] [date of publication]

[2] Authority [21 U.S.C. 360d], http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Standards/default.htm

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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE
AAMI ANSI ISO 10993-5 Biological evaluation of medical devices Part 5: Tests for in vitro cytotoxicity

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
Part 5	Test for in vitro cytotoxicity	☐ Yes ☒ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED ◆
Agarose Overlay Method

DESCRIPTION
The method determines the reactivity of monolayer of L929 mouse fibroblast cells coated with agarose layer through which the active from the test device diffuses and reacts with the cell monolayer.

JUSTIFICATION
The test device, a topical gel is applied as a stagnant layer covering the wound. The active silver diffuses slowly through the gel layer to exerts its antimicrobial effect on the surrounding. This scenario is similar to active silver diffusing from device to agarose layer.

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED ◆

DESCRIPTION

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED ◆

DESCRIPTION

JUSTIFICATION

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STANDARDS DATA REPORT FOR 510(k)s

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TYPE OF 510(K) SUBMISSION

☒ Traditional ☐ Special ☐ Abbreviated

STANDARD TITLE [1]

AAMI ANSI ISO 10993-10 Biological evaluation of medical devices Part 10: Tests for irritation and skin sensitization (2010)

Please answer the following questions	Yes	No
Is this standard recognized by FDA [2]?	☒	☐
FDA Recognition number [3] # 2-173		
Was a third party laboratory responsible for testing conformity of the device to this standard identified in the 510(k)?	☒	☐
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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE
AAMI ANSI ISO 10993-10 Biological evaluation of medical devices Part 10: Tests for irritation and skin sensitization

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
Part 10	Test for skin irritation	☐ Yes ☒ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED ◆
Intracutaneous injection of test article instead of test article extract

DESCRIPTION
The method evaluates local dermal irritation due to the test article (neat) following intracutaneous injection in rabbits and compares reactions at test article sites with reactions observed for control article sites

JUSTIFICATION
Instead of using test article extract, using the test article (gel composition) directly is more realistic as the test article in wounds will be contacting the skin directly.

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED ◆

DESCRIPTION

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED ◆

DESCRIPTION

JUSTIFICATION

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Form Approved: OMB No. 0910-0120; Expiration Date: 1/31/2017

Department of Health and Human Services
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EXTENT OF STANDARD CONFORMANCE SUMMARY REPORT TABLE

STANDARD TITLE
AAMI ANSI ISO 10993-10 Biological evaluation of medical devices Part 10: Tests for irritation and skin sensitization

CONFORMANCE WITH STANDARD SECTIONS*

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
Part 10	Test for skin sensitization	☒ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED ◆
Kligman Guinea Pig Maximization Test

DESCRIPTION
The method evaluates allergenic potential or sensitizing capacity of the test article in guinea pigs.

JUSTIFICATION
The albino guinea pigs have been historically used for sensitization studies. And guinea pigs are believed to be the most sensitive animal model for this type of study.

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED ◆

DESCRIPTION

JUSTIFICATION

SECTION NUMBER	SECTION TITLE	CONFORMANCE?
		☐ Yes ☐ No ☐ N/A

TYPE OF DEVIATION OR OPTION SELECTED ◆

DESCRIPTION

JUSTIFICATION

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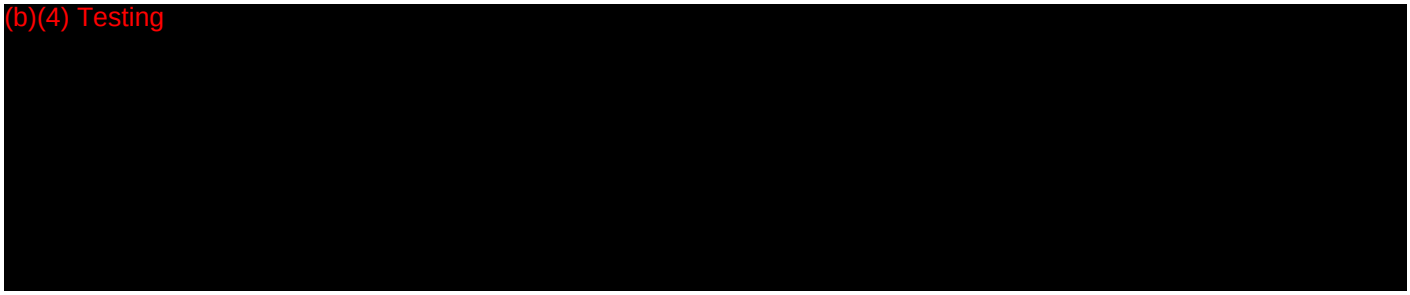
(b)(4) Test Device Performance (b)(4)

(b)(4)

(b)(4) Testing

(b)(4)

(b)(4) Testing



From: Balu Karandikar
To: Durfor, Charles
Subject: In re: K152519
Date: Thursday, September 10, 2015 11:12:54 PM
Attachments: 001 Item 1 (b)(4)
002 Item 2
003 Item 2
004 Item 2
005 Item 3
006 Item 4
007 Item 4
008 Item 4
009 Item 5

Hello Mr. Durfor:
I have attached a set of 9 PDF's as per the eCopy guidance format that constitute our response to the list of 5 items in your email to us on Wednesday Sept 9, 2015.

Please note the Item numbers in PDF file name correspond to the numeral of item in your email. For example, the Item 1 in your email refers to the request for (b)(4) (b)(4) in the PDF file.

I hope your review of the information submitted will conclude that our response has met the requirement threshold for a complete revised 510k submission.

Please let us know immediately should you see any information not consistent with the requirements.

Thank you

Respectfully

Bhalchandra M Karandikar
Chief Technology Officer
Medical Technology Research Inc.

(b)(6) ld appreciate your insight on this issue:

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

Dear Dr. Karandikar:

Subsequent to the filing review of your 510(k), we request the submission of a revised 510(k) that addresses the following concerns:

(b)(4) Deficiencies

[redacted]

(b)(4) Deficiencies

Please provide this information by COB on 9/11/15 or inform FDA that this information will be arriving at a later date. If you have any questions or comments please feel to contact me at 301-796-6970.

Charles Durfor
PRSB-2/DSD/ODE/CDRH/FDA

510(k) SUMMARY

1. SUBMITTER/OWNER

MEDICAL TECHNOLOGY RESEARCH INC.
2650 PROGRESS WAY
WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact Person: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Date Prepared: April 25, 2016

2. DEVICE

Name of Device: MTR550 (Tentative) Antimicrobial Silver Wound Gel
Common Name: Antimicrobial Wound Dressing
Classification Name: Dressing Wound, Drug
Regulatory Class: Unclassified
Product Code: FRO

3. PREDICATE DEVICE

MTR550 gel is substantially equivalent to the following legally marketed predicate devices:

(i) SilverShield™ Antimicrobial Skin and Wound Gel (# K062212) from Anacapa Technologies of San Dimas, CA and distributed presently under SilverSept® name

(ii) Silver Antimicrobial Wound Gel from Advanced Medical Solutions of Winsford, UK (#K110458) and marketed by Molynyke under the name Normlgel Ag®

4. DEVICE DESCRIPTION

The product is a spreadable amorphous water based wound gel comprising a synthetic clay as the thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that in turns aids in wound healing. The presence of active silver compounds within the gel provides preservative action and acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms within the dressing. The gel is clear to hazy and will not stain the skin tissue when used over a period of 3 days though its potential to stain skin beyond 3 day use is not known.

Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures.
The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

5. INDICATIONS FOR USE

The device is indicated for use by prescription only. It is indicated for use in the management of dry to low/moderate exuding partial and fullness thickness wounds, stage I-IV pressure ulcers, diabetic and foot ulcers, graft wounds and donor sites, first and second degree wounds, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations.

Contraindications: The gel should not be used on patients with known sensitivity to silver and s-triazine compounds.

6. MANUFACTURING

The MTR550 gel will be manufactured in a production facility in accordance with good manufacturing practices consistent with US FDA guidelines. The batch production of the gel and packaging will be verified to meet product specifications to ensure the product is safe and effective.

7. BIOCOMPATIBILITY TESTING

The device has been tested for in-vitro cytotoxicity, dermal irritation and sensitization in accordance with ISO 10993 -1 (Biological Evaluation of Medical Devices).

No systemic toxicity was associated with the antimicrobial silver compounds. Animal study employing rats showed no adverse effects on animals due to the device.

8. PERFORMANCE

The antimicrobial efficacy of the device has been demonstrated by 28 days Antimicrobial preservative challenge test in accordance with USP Chapter 51 guidelines. Employing in vitro assays, the antimicrobial barrier property was demonstrated against 17 different micro-organisms that included bacteria, yeast and fungi. The tested micro-organisms included, MRSA, VRE, Candida A. and Aspergillus niger. Sustenance of antimicrobial barrier activity was demonstrated for 3 days in a serial transfer assay.

Animal study employing rats showed the device exhibited no adverse effect on animals. In a porcine study examining deep partial thickness burn wound healing, MTR550 device was found to be safe and effective as the predicate device.

Despite its silver content at 550ppm, the device was shown to be non-staining to dermal tissue over 3 days use. The device showed no discoloration despite exposure to intense light or elevated environmental temperatures.

9. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH PREDICATE DEVICE

With respect to its physical and chemical properties, the device is substantially equivalent to the predicate device.

The mechanism of antimicrobial action is also similar. Both device exert toxicity towards micro-organisms as a result of silver ions.

The device is substantially equivalent to legally marketed predicate devices in composition and intended use.

10. CONCLUSIONS

Based on the indications for use, biocompatibility, invivo studies, performance data, the MTR550 gel is substantially similar to SilverShield™ Antimicrobial Skin and Wound Gel (#K062212)

510(k) SUMMARY

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WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact Person: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Date Prepared: April 25, 2016

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Product Code: FRO

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Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures.
The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

5. INDICATIONS FOR USE

The device is indicated for use by prescription only. It is indicated for use in the management of dry to low/moderate exuding partial and fullness thickness wounds, stage I-IV pressure ulcers, diabetic and foot ulcers, graft wounds and donor sites, first and second degree wounds, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations.

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The mechanism of antimicrobial action is also similar. Both device exert toxicity towards micro-organisms as a result of silver ions.

The device is substantially equivalent to legally marketed predicate devices in composition and intended use.

10. CONCLUSIONS

Based on the indications for use, biocompatibility, invivo studies, performance data, the MTR550 gel is substantially similar to SilverShield™ Antimicrobial Skin and Wound Gel (#K062212)

5.0 PROPOSED LABELING

The proposed labeling on the thin gauge cardboard box, the tube and the packaging insert is disclosed below. For comparison the product inserts from predicate devices (K062212 & K110458) are attached (see Attachments 1 & 2).

Product cardboard box labeling

Side 1

MTR550™ Gel (Note the MTR550™ name is tentative and may be replaced)

Antimicrobial silver wound gel

Rx Only / Net weight (1.5 oz/43g)

Side 2

MTR550™ Antimicrobial silver wound gel

- Contains 0.055% (550 μg/g) silver (as preservative)
- Provides lasting antimicrobial barrier as burn and wound dressing up to 3 days
- Helps maintain moist wound environment
- As preservative, silver reduces microbial growth within the dressing and exerts no microbial action in or on the wound

Read instructions for use (see insert inside package)
Store at 15C (59F) to 30C (77F)

Side 3

Rx Only
Intended for use for the management of dry to low/moderate exuding wounds such as: partial and full thickness wounds, Stage I-IV pressure ulcers, diabetic and foot ulcers and 1st and 2nd degree burns, graft and donor sites, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations

- For external use only; not to use in or around the eyes
- Not made with natural rubber latex
- Contraindicated for use on patients with sensitivity to silver and s-triazine compounds

Side 4

Manufactured in China for:
Medical Technology Research Inc.
2650 Progress Way, Woodburn, OR 97071

US and corresponding international patents pending
Reorder Number XXXXX/Questions & Comments: 1-800-MTR-MEDI (Tentative)

Tube labeling

Side 1

MTR550™ Antimicrobial Silver Wound Gel*

*US & Int. patents pending

Rx Only / Net wt. 1.5 oz (43g)

Mfd. for:

Medical Technology Research Inc. Woodburn, OR 97071
www.mtrmedical.com

Side 2

MTR550™ Antimicrobial Silver Wound Gel

- Contains 0.055% (550 μg/g) silver (as preservative)
- Provides lasting antimicrobial barrier as burn and wound dressing up to 3 days
- Helps maintain moist wound environment
- As preservative, silver reduces microbial growth within the dressing and exerts no microbial action in or on the wound

Intended for use for the management of dry to low/moderate exuding wounds such as: partial and full thickness wounds, Stage I-IV pressure ulcers, diabetic and foot ulcers and 1st and 2nd degree burns, graft and donor sites, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations

Store at 15C – 30C; Read instructions for use (see package insert)

Package Insert

MTR550™ Antimicrobial Silver Wound Gel*

Reorder Number XXXXX

Product Description:

MTR550™ gel is spreadable amorphous burn and wound dressing that contains 550 ppm of an antimicrobial silver preservative. The dressing is formulated to provide an antimicrobial barrier lasting up to 3 days by inhibiting the growth of common pathogens such *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* antibiotic resistant strains MRSA and VRE and yeast and fungi including *Candida albicans, Aspergillus niger* and other microorganisms within the dressing. As a preservative, the presence of silver in the dressing reduces pathogen counts within the dressing and has no effect on the pathogens in or on the wound. In *in vitro* tests, antimicrobial silver within MTR550™ gel dressing was found to exert preservative action against several common pathogens.

The gel dressing helps maintain moisture in wound environment which may help promote wound healing. The gel is suitable for use on dry to low/moderately exuding wounds. MTR550™ gel will not stain or discolor tissue when used daily over 3 days. However, it is not known if staining will occur if gel use is continued beyond 3 days.

It is intended for sale or for use by prescription only on patients under the medical supervision of licensed healthcare professionals. It is against the Federal law to contravene this restriction.

Indications for use:

It is indicated for use for the management of dry to low/moderate exuding wounds such as:

- Stage I-IV pressure ulcers
- Diabetic & foot ulcers
- Partial and full thickness wounds
- Graft and donor sites
- Post-operative surgical wounds
- Trauma wounds (dermal lesions, trauma injuries and incisions)
- 1 & 2nd degree burns
- Abrasions and lacerations

Contraindications for use:

- Not to be used on patients with sensitivity to silver and s-triazine compounds
- Patients may experience mild skin irritation in and around area of gel application, but if it persists or redness or swelling develops, discontinue using the gel

Directions for use:

- ✓ Cleanse and debride wound as required
- ✓ Dispense MTR550™ gel onto the wound directly and evenly spread it to a 0.1" to 0.2" thick layer. It is recommended the applied gel layer extend 0.2" to 0.25" beyond the edge of the wound for effective barrier function.
- ✓ Cover with a sterile secondary dressing (such as gauze, foam or similar) and secure it in place
- ✓ In between dressing changes, maintain moist environment in the wound bed
- ✓ MTR550™ gel dressing may be maintained for up to 3 days though heavy exuding wounds may require more frequent dressing changes as per clinical protocols

Rx Only
External Use Only. Not to be used in the proximity of eyes.
Not made with natural rubber latex

*US & INTL. PATENTS PENDING

Manufactured in China for:
Medical Technology Research Inc.
2650 Progress Way, Woodburn, OR 97071
www.mtrmedical.com
For questions or comments: 1-800-MTR-MEDI (tentative)

INDICATIONS FOR USE

K NUMBER: **152519**

DEVICE NAME: **MTR550 ANTIMICROBIAL SILVER WOUND GEL**

INDICATIONS FOR USE

For the management of dry to low/moderate exuding wounds such as:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

TYPE OF USE: **Prescription Use Only (Part 21 CFR 801 Subpart D)**

INDICATIONS FOR USE

K NUMBER: **152519**

DEVICE NAME: **MTR550 ANTIMICROBIAL SILVER WOUND GEL**

INDICATIONS FOR USE

For the management of dry to low/moderate exuding wounds such as:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

TYPE OF USE: **Prescription Use Only (Part 21 CFR 801 Subpart D)**

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center - WO66-G609
Silver Spring, MD 20993-0002

K152519
Medical Technology Research Inc.
Trade Name: MTR550 Antimicrobial Silver Wound Gel
Contact Name: Bhalchandra M. Karandikar, Ph.D.

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above. We cannot determine if the device is substantially equivalent to a legally marketed predicate device based solely on the information you provided. To complete the review of your submission, we require responses to the following questions and comments:

(b)(4) Deficiencies

[redacted]

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

(b)(4) Deficiencies

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

Close-Out Letter – Deletion of Submission for Lack of Response to Additional Information Request

4/28/2016

Bhalchandra M. Karandikar, Cheif Technology Officer
MEDICAL TECHNOLOGY RESEARCH INC.
2650 PROGRESS WAY
WOODBURN, OR 97071
UNITED STATES

Dear Bhalchandra M. Karandikar:

On 10/30/2015, we requested additional information from you regarding the submission referenced below. Your response was due on 4/27/2016. Because we have not yet received a complete response, we consider this submission to be withdrawn and have deleted it from our review system.

Submission Number: K152519
Received: 9/3/2015
Applicant: MEDICAL TECHNOLOGY RESEARCH INC.
Device: MTR550 (Tentative) Antimicrobial Silver Wound Gel

Pursuant to 21 CFR 20.29, your submission will remain in our records. Confidentiality of your submission will be maintained in accordance with the provision outlined in 21 CFR sections 20.61 and 20.81.

If you decide to send another submission for this product, a new number will be assigned and a new review will begin. Please include a valid eCopy of your new submission. You may incorporate by reference the information contained in the withdrawn submission. Please be advised that any outstanding deficiencies raised by FDA should be addressed in your new submission. New submissions must be sent to:

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center - WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

In addition, your new submission may be subject to a user fee. The Federal Food, Drug, and Cosmetic Act (the Act), as amended by the Medical Device User Fee and Modernization Act of 2002 (MDUFMA) and the FDA Amendments Act of 2007 (Public Law 110-85), authorizes FDA to collect user fees for certain types of submissions. For more information on MDUFMA, please refer to our website at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/MedicalDeviceUserFeeandModernizationActMDUFMA/default.htm.

You may not market this device until you have received a letter from FDA allowing you to do so. If you

market the device without FDA clearance/approval, you will be in violation of the Federal Food, Drug, and Cosmetic Act.

For information about CDRH review regulations and policies, please refer to http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm. If you have specific questions regarding this withdrawal action, please contact the Program Operations Staff at (301) 796-5640 or at CDRHPremarketProgramOperations@fda.hhs.gov.

Sincerely yours,

Center for Devices and Radiological Health

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center WO66 G609
Silver Spring, MD 20993 0002

June 2, 2016

Medical Technology Research, Inc.
Bhalchandra M. Karandikar, Ph.D
Chief Technology Officer
2650 Progress Way
Woodburn, OR 97071

Re: K152519
Trade/Device Name: MTR550 (Tentative) Antimicrobial Silver Wound Gel
Regulatory Class: Unclassified
Product Code: FRO
Dated: April 25, 2016
Received: May 5, 2016

Dear Dr. Karandikar:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you; however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Jennifer R. Stevenson -A

For Binita S. Ashar, M.D., M.B.A., F.A.C.S.
Director
Division of Surgical Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

INDICATIONS FOR USE

K NUMBER: K**152519**

DEVICE NAME: MTR550 ANTIMICROBIAL SILVER WOUND GEL

INDICATIONS FOR USE

For the management of dry to low/moderate exuding wounds such as:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

TYPE OF USE: Prescription Use Only (Part 21 CFR 801 Subpart D)

510(k) SUMMARY

1. SUBMITTER/OWNER

MEDICAL TECHNOLOGY RESEARCH INC.
2650 PROGRESS WAY
WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact Person: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Date Prepared: April 25, 2016

2. DEVICE

Name of Device: MTR550 (Tentative) Antimicrobial Silver Wound Gel
Common Name: Antimicrobial Wound Dressing
Classification Name: Dressing Wound, Drug
Regulatory Class: Unclassified
Product Code: FRO

3. PREDICATE DEVICE

MTR550 gel is substantially equivalent to the following legally marketed predicate devices:

(i) SilverShield™ Antimicrobial Skin and Wound Gel (# K062212) from Anacapa Technologies of San Dimas, CA and distributed presently under SilverSept® name

(ii) Silver Antimicrobial Wound Gel from Advanced Medical Solutions of Winsford, UK (#K110458) and marketed by Molynyke under the name Normlgel Ag®

4. DEVICE DESCRIPTION

The product is a spreadable amorphous water based wound gel comprising a synthetic clay as the thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that in turns aids in wound healing. The presence of active silver compounds within the gel provides preservative action and acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms within the dressing. The gel is clear to hazy and will not stain the skin tissue when used over a period of 3 days though its potential to stain skin beyond 3 day use is not known.

Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures.
The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

5. INDICATIONS FOR USE

The device is indicated for use by prescription only. It is indicated for use in the management of dry to low/moderate exuding partial and fullness thickness wounds, stage I-IV pressure ulcers, diabetic and foot ulcers, graft wounds and donor sites, first and second degree wounds, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations.

Contraindications: The gel should not be used on patients with known sensitivity to silver and s-triazine compounds.

6. MANUFACTURING

The MTR550 gel will be manufactured in a production facility in accordance with good manufacturing practices consistent with US FDA guidelines. The batch production of the gel and packaging will be verified to meet product specifications to ensure the product is safe and effective.

7. BIOCOMPATIBILITY TESTING

The device has been tested for in-vitro cytotoxicity, dermal irritation and sensitization in accordance with ISO 10993 -1 (Biological Evaluation of Medical Devices).

No systemic toxicity was associated with the antimicrobial silver compounds. Animal study employing rats showed no adverse effects on animals due to the device.

8. PERFORMANCE

The antimicrobial efficacy of the device has been demonstrated by 28 days Antimicrobial preservative challenge test in accordance with USP Chapter 51 guidelines. Employing in vitro assays, the antimicrobial barrier property was demonstrated against 17 different micro-organisms that included bacteria, yeast and fungi. The tested micro-organisms included, MRSA, VRE, Candida A. and Aspergillus niger. Sustenance of antimicrobial barrier activity was demonstrated for 3 days in a serial transfer assay.

Animal study employing rats showed the device exhibited no adverse effect on animals. In a porcine study examining deep partial thickness burn wound healing, MTR550 device was found to be safe and effective as the predicate device.

Despite its silver content at 550ppm, the device was shown to be non-staining to dermal tissue over 3 days use. The device showed no discoloration despite exposure to intense light or elevated environmental temperatures.

9. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH PREDICATE DEVICE

With respect to its physical and chemical properties, the device is substantially equivalent to the predicate device.

The mechanism of antimicrobial action is also similar. Both device exert toxicity towards micro-organisms as a result of silver ions.

The device is substantially equivalent to legally marketed predicate devices in composition and intended use.

10. CONCLUSIONS

Based on the indications for use, biocompatibility, invivo studies, performance data, the MTR550 gel is substantially similar to SilverShield™ Antimicrobial Skin and Wound Gel (#K062212)

From: Balu Karandikar
To: Durfor, Charles
Subject: Re: In re: K152519
Date: Friday, September 11, 2015 3:12:37 PM
Attachments: (b)(4) Deficiencies

Dear Mr. Durfor:
Ar per your request outlined in Sept 11, 2015, we have revised the (b)(4) Deficiencies (b)(4) Deficiencies. The revised files are attached in the e Copy guidance format.

Thanks once again for your input.

Respectfully,

Bhalchandra M Karandikar
Medical Technology Research Inc.

On Fri, Sep 11, 2015 at 6:57 AM, Durfor, Charles <Charles.Durfor@fda.hhs.gov> wrote:

> Dear Dr. Karandikar
>
> Please re-submit a revised (b)(4) Deficiencies. The current (b)(4) Deficiencies
>
> (b)(4) Deficiencies

(b)(4) Deficiencies

[redacted]

All three documents should present the same statement.

C. Durfor

From: Balu Karandikar [mailto:bhalchak@mtrmedical.com]
Sent: Thursday, September 10, 2015 11:13 PM
To: Durfor, Charles
Subject: In re: K152519

Hello Mr. Durfor:

I have attached a set of 9 PDF's as per the eCopy guidance format that constitute our response to the list of 5 items in your email to us on Wednesday Sept 9, 2015.

Please note the Item numbers in PDF file name correspond to the numeral of item in your email. For example, the Item 1 in your email refers to the request for (b)(4) Deficiencies in the PDF file.

I hope your review of the information submitted will conclude that our response has met the requirement threshold for a complete revised 510k submission.

Please let us know immediately should you see any information not consistent with the requirements.

Thank you

Respectfully

Bhalchandra M Karandikar

Chief Technology Officer

Medical Technology Research Inc.

From: Bertram, James
To: Durfor, Charles
Cc: Dang, Jiyoung M; Yeatts, Andrew; k152519@docs.fda.gov
Subject: RE: J(b)(4) Question - MTR550 Antimicrobial Silver Wound Gel - K152519 -
Date: Tuesday, September 08, 2015 2:02:13 PM

Hi Charles,
Thanks for the email. So I recommend we proceed with the review for the below reasons. That being said, I am definitely open to conversation if you have concerns or see otherwise.

(b)(4)

Again, would be happy to discuss if you or the branch feel I'm mistaken or am missing anything, but overall, believe the (b)(4), I would be happy to reach out to OCP on this, but ultimately believe we will end up in the same situation we are currently in.

Thanks again,
James

From: Durfor, Charles
Sent: Tuesday, September 08, 2015 12:52 PM
To: Bertram, James
Cc: Dang, Jiyoung M; Yeatts, Andrew; k152519@docs.fda.gov
Subject: (b)(4) Question - MTR550 Antimicrobial Silver Wound Gel - K152519 -

James

I would appreciate your insight on this issue:

(b)(4)

(b)(4) Deficiencies

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

June 2, 2016

Medical Technology Research, Inc.
Bhalchandra M. Karandikar, Ph.D
Chief Technology Officer
2650 Progress Way
Woodburn, OR 97071

Re: K152519
Trade/Device Name: MTR550 (Tentative) Antimicrobial Silver Wound Gel
Regulatory Class: Unclassified
Product Code: FRO
Dated: April 25, 2016
Received: May 5, 2016

Dear Dr. Karandikar:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you; however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Jennifer R. Stevenson -A

For Binita S. Ashar, M.D., M.B.A., F.A.C.S.
Director
Division of Surgical Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

Contains Nonbinding Recommendations

Print Form

Acceptance Checklist for Traditional 510(k)s

(Should be completed within 15 days of DCC receipt)

The following information is not intended to serve as a comprehensive review.

510(k) #: K152519 Date Received by DCC: September 3, 2015

Lead Reviewer: Charles Durfor

Branch: PRSB2 Division: DSD Center/Office: CDRH/ODE

Note: If an element is left blank on the checklist, it does not mean the checklist is incomplete. It means the reviewer did not assess the element during RTA and the element will be assessed during the substantive review.

Preliminary Questions

Answers in the shaded blocks indicate consultations with Center advisor is needed	Yes	No
1) Is the product a device (per section 201(h) of the FD&C Act) or a combination product (per 21 CFR 3.2(e)) with a device constituent part subject to review in a 510(k)? If it appears not to be a device (per section 201(h) of the FD&C Act) or such a combination product, or you are unsure, consult with the CDRH Jurisdictional Officer or the CBER Office Jurisdiction Liaison to determine the appropriate action, and inform division management. *Provide a summary of the Jurisdictional Officer's/Liaison's determination.* If the product does not appear to be a device or such a combination product, mark "No."	X	
Comments?		
2. Is the application with the appropriate Center? If the product is a device or a combination product with a device constituent part, is it subject to review by the Center in which the submission was received? If you believe the application is not with the appropriate Center or you are unsure, consult with the CDRH Jurisdictional Officer or CBER Office Jurisdiction Liaison to determine the appropriate action and inform your division management. *Provide a summary of the Jurisdictional Officer's/Liaison's determination.* If application should not be reviewed by your Center mark "No."	X	
Comments?		
3) If a Request for Designation was submitted for the device or combination product with a device constituent part and assigned to your center, identify the RFD # and confirm the following: a) Is the device or combination product the same (e.g., design, formulation) as that presented in the RFD submission? b) Are the indications for use for the device or combination product identified in the 510(k) the same as those identified in the RFD submission ? If you believe the product or the indications presented in the 510(k) have changed from the RFD, or you are unsure, consult with the CDRH Jurisdictional Officer or appropriate CBER Jurisdiction Liaison to determine the appropriate action and inform your division management. Provide summary of Jurisdictional Officer's/Liaison's determination. If the answer to either question is no, mark "No." If there was no RFD, skip this question.		
Comments?		
4) Is this device type eligible for a 510(k) submission? If a 510(k) does not appear to be appropriate (e.g., Class III type and PMA required, or Class I or II type and 510(k)-exempt), you should consult with the CDRH 510(k) Program Director or appropriate CBER staff during the acceptance review. If 510(k) is not the appropriate regulatory submission, mark "No."	X	
Comments?		

5) Is there a pending PMA for the same device with the same indications for use? If yes, consult division management and the CDRH 510(k) Program Director or appropriate CBER staff to determine the appropriate action.		X
Comments?		
6) If clinical studies have been submitted, is the submitter the subject of an Application Integrity Policy (AIP)? If yes, consult with the CDRH Office of Compliance/Division of Bioresearch Monitoring (OC/DBM - BIMO) or CBER Office of Compliance and Biologics Quality/Division of Inspections and Surveillance/Bioresearch Monitoring Branch (OCBQ/DIS/BMB) to determine the appropriate action. Check on web at http://www.fda.gov/ICECI/EnforcementActions/ApplicationIntegrityPolicy/ucm134453.htm		X
Comments?		

If the answer to 1 or 2 appears to be "No," then stop review of the 510(k) and issue the "Original Jurisdictional Product" letter.
If the answer to 3a or 3b appears to be "No," then stop the review and contact the CDRH Jurisdictional Officer or CBER Office of Jurisdiction Liaison.
If the answer to 4 is "No," the lead reviewer should consult division management and other Center resources to determine the appropriate action.
If the answer to 5 is "Yes," then stop review of the 510(k), contact the CDRH 510(k) Staff and PMA Staff, or appropriate CBER staff.
If the answer to 6 is "Yes," then contact CDRH/OC/DBM-BIMO or CBER/OCBQ/DIS/BMB, provide a summary of the discussion with the BIMO Staff, and indicate BIMO's recommendation/action.

Organizational Elements

Failure to include these items alone generally should not result in an RTA designation.

	Yes	No
1) Submission contains a Table of Contents	X	
2) Each section is labeled (e.g., headings or tabs designating Device Description section, Labeling section, etc.)	X	
3) All pages of the submission are numbered.		X
4) Type of 510(k) is identified (i.e., traditional, abbreviated, or special)	X	
Comments?		

Elements of a Complete Submission (RTA Items)

(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
A. Administrative				
1) All content used to support the submission is written in English (including translations of test reports, literature articles, etc.)	X			
2) Submission identifies the following (such as in CDRH Premarket Review Submission Cover Sheet (Form 3514) or 510(k) cover letter):	X			
a) Device trade name or proprietary name	X			
b) Device common name	X			
c) Device class and panel or Classification regulation or Statement that device has not been classified with rationale for that conclusion	X			
3) Submission contains Indications for Use Statement with Rx and/or OTC designation (see also 21 CFR 801.109).	X			
4) Submission contains 510(k) Summary or 510(k) Statement	X			
a) Summary contains all elements per 21 CFR 807.92 *(See also* 510(k) Summary Checklist*)*	X			
b) Statement contains all elements per 21 CFR 807.93			X	
5) Submission contains Truthful and Accuracy Statement per 21 CFR 807.87(k) *See recommended* format.	X			
6) Submission contains Class III Summary and Certification. *See recommended* content.			X	
7) Submission contains clinical data			X	
8) If submission references use of a national or international standard as part of demonstration of substantial equivalence, submission contains Standards Data Report for 510(k)s (Form 3654) or includes detailed information about how and the extent to which the standard has been followed.	X			
9) The submission identifies prior submissions for the same device for which FDA provided feedback related to the data or information needed to support substantial equivalence (e.g., submission numbers for Pre-Submission, IDE, prior not substantially equivalent (NSE) determination, prior 510(k) that was deleted or withdrawn) or states that there were no prior submissions for the subject device.	X			
a) If there were prior submissions, the submitter has identified where in the current submission any issues related to a determination of substantial equivalence outlined in prior communications are addressed. For additional information regarding the Pre-Submission process, please refer to the Draft Guidance "Medical Devices: The Pre-Submission Program and Meetings with FDA Staff." Once finalized, this guidance will represent the Agency's current thinking on this topic.			X	
B. Device Description				
10)				

Elements of a Complete Submission (RTA Items)
(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
a) If there are requirements regarding the device description, such as special controls, in a device-specific regulation that are applicable to the device, the submission includes device description information to establish that the submitter has followed the device-specific requirement.			X	
b) If there is a device-specific guidance, other than a special controls guidance document, applicable to the device, the submission includes device description information to establish that the submitter has addressed the recommendations or otherwise has met the applicable statutory or regulatory criteria through an alternative approach.			X	
11) Descriptive information is present and consistent within the submission (e.g., the device description section is consistent with the device description in the labeling), including:				
a) A description of the principle of operation and mechanism of action for achieving the intended effect.	X			
b) A description of proposed conditions of use, such as surgical technique for implants; anatomical location of use; user interface; how the device interacts with other devices; and/or how the device interacts with the patient.	X			
c) A list and description of each device for which clearance is requested.	X			
12) Submission contains representative engineering drawing(s), schematics, illustrations and/or figures of the device that are clear, legible, labeled, and include dimensions.			X	
13) If device is intended to be marketed with multiple components, accessories, and/or as part of a system			X	
C. Substantial Equivalence Discussion				
14) Submitter has identified a predicate device.	X			
a) Predicate's 510(k) number, trade name, and model number (if applicable) provided. For predicates that are preamendments devices, information is provided to document preamendments status. *Information regarding documenting preamendment status is available online.*	X			
b) The identified predicate(s) is consistent throughout the submission (i.e., the predicate(s) identified in the Substantial Equivalence section is the same as that listed in the 510(k) Summary (if applicable) and that used in comparative performance testing.	X			
15) Submission includes a comparison of the following for the predicate(s) and subject device				
a) Indications for Use	X			
b) Technology, including features, materials, and principles of operation	X			
16) Submission includes an analysis of why any differences between the subject device and predicate(s) do not render the device NSE (e.g., does not constitute a new intended use; and any differences in technological characteristics are accompanied by information that demonstrates the device is as safe and effective as the predicate and do not raise different questions of safety and effectiveness than the predicate), affect safety or effectiveness, or raise different questions of safety and effectiveness (see section 513(i)(1)(A) of the FD&C Act and 21 CFR 807.87(f))	X			

Elements of a Complete Submission (RTA Items)
(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
D. Proposed Labeling (see also 21 CFR part 801)				
If *in vitro* diagnostic (IVD) device, criteria 17 & 19 may be omitted.				
17) Submission includes proposed package labels and labeling (e.g., instructions for use, package insert, operator's manual) that include a description of the device, its intended use, and the directions for use.	X			
a) Indications for use are stated in labeling and are identical to Indications for Use form and 510(k) Summary (if 510(k) Summary provided).	X			
b) Submission includes directions for use that - include statements of all conditions, purposes or uses for which the device is intended (e.g., hazards, warnings, precautions, contraindications) AND - includes directions for layperson (see 21 CFR 801.5) OR submission states that device qualifies for exemption per 21 CFR 801 Subpart D	X			
18) If indicated for prescription use, labeling includes the prescription use statement (see 21 CFR 801.109(b)(1)) or "Rx only" symbol [See also Alternative to Certain Prescription Device Labeling Requirements]	X			
19) General labeling provisions				
a) Labeling includes name and place of business of the manufacturer, packer, or distributor (21 CFR 801.1).	X			
b) Labeling includes device common or usual name. (21 CFR 801.61)			X	
20)				
a) If there are requirements regarding labeling, such as special controls, in a device-specific regulation that are applicable to the device, the submission includes labeling to establish that the submitter has followed the device-specific requirement.			X	
b) If there is a device-specific guidance, other than a special controls guidance document, applicable to the device, the submission includes labeling to establish that the submitter has addressed the recommendations or otherwise has met the applicable statutory or regulatory criteria through an alternative approach.			X	
c) If there is a special controls document applicable to the device, the submission includes labeling to establish that the submitter has complied with the particular mitigation measures set forth in the special controls document or uses alternative mitigation measures but provides a rationale to demonstrate that those alternative measures identified by the firm will provide at least an equivalent assurance of safety and effectiveness.			X	
21) If the device is an in vitro diagnostic device, provided labeling includes all applicable information required per 21 CFR 809.10.			X	
E. Sterilization				
If IVD device and sterilization is not applicable, select "N/A" and criteria below will be omitted from checklist.				
Submission states that the device and/or accessories are: (one of the below must be checked)				

Elements of a Complete Submission (RTA Items)

(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
provided sterile				
provided non-sterile but sterilized by the end user				
× non-sterile when used				
Information regarding the sterility status of the device is not provided.				
This information will determine whether and what type of additional information may be necessary for a substantial equivalence determination.				
22) Assessment of the need for sterilization information				
a) Identification of device, and/or accessories, and/or components that are provided sterile.				
b) Identification of device, and/or accessories, and/or components that are end user sterilized.			×	
c) Identification of device, and/or accessories, and/or components that are reusable and cleaning /disinfection instructions are provided.			×	
25)				
a) If there are requirements regarding sterility, such as special controls, in a device-specific regulation that are applicable to the device, the submission includes sterility information to establish that the submitter has followed the device-specific requirement.			×	
b) If there is a device-specific guidance, other than a special controls guidance document, applicable to the device, the submission includes sterility information to establish that the submitter has addressed the recommendations or otherwise has met the applicable statutory or regulatory criteria through an alternative approach.			×	
c) If there is a special controls document applicable to the device, the submission includes sterility information to establish that the submitter has complied with the particular mitigation measures set forth in the special controls document or uses alternative mitigation measures but provides a rationale to demonstrate that those alternative measures identified by the firm will provide at least an equivalent assurance of safety and effectiveness.			×	
F. Shelf Life				
26) Proposed shelf life/expiration date stated	×			
27) For sterile device, submission includes summary of methods used to establish that device will remain sterile through the proposed shelf life or a rationale for why testing to establish shelf life is not applicable.			×	
28) Submission includes summary of methods used to establish that device performance is not adversely affected by aging or includes a rationale for why the storage conditions are not expected to affect device safety or effectiveness.	×			
G. Biocompatibility				
If IVD device, select "N/A" and the below criteria will be omitted from checklist.				

Elements of a Complete Submission (RTA Items)

(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
Submission states that there: (one of the below must be checked)				
× are direct or indirect (e.g., through fluid infusion) patient-contacting components.				
are no direct or indirect (e.g., through fluid infusion) patient-contacting components.				
Information regarding the patient contact status of the device is not provided.				
This information will determine whether and what type of additional information may be necessary for a substantial equivalence determination.				
29) Submission includes list of patient-contacting device components and associated materials of construction, including identification of color additives, if present	×			
30) Submission identifies contact classification (e.g., surface-contacting, less then 24 hour duration, etc.)	×			
31) Biocompatibility assessment of patient-contacting components Submission includes: Test protocol (including identification and description of test article), methods, pass/fail criteria, and results provided for each completed test, OR a statement that biocompatibility testing is not needed with a rationale (e.g., materials and manufacturing/processing are identical to the predicate).	×			
H. Software				
Submission states that the device: (one of the below must be checked)				
does contain software/firmware.				
× does not contain software/firmware.				
Information regarding whether the device contains software is not provided.				
This information will determine whether and what type of additional information may be necessary for a substantial equivalence determination.				
I. EMC and Electrical Safety				
Submission states that the device: (one of the below must be checked)				
does require EMC and Electrical Safety evaluation.				
× does not require EMC and Electrical Safety evaluation.				
Information regarding whether the device requires EMC and Electrical Safety evaluation is not provided.				
This information will determine whether and what type of additional information may be necessary for a substantial equivalence determination.				
J. Performance Data - General If IVD device, select "N/A" and the below criteria will be omitted from checklist. Performance data criteria relating to IVD devices will be addressed in Section K.				

Elements of a Complete Submission (RTA Items)

(21 CFR 807.87 unless otherwise indicated)

Submission should be designated RTA if not addressed.

Check "Yes" if item is present, "N/A" if it is not needed and "No" if it is not included but needed.

- Any "No" answer will result in a "Refuse to Accept" decision. - Each element on the checklist should be addressed within the submission. An applicant may provide a rationale for omission for any criteria that are deemed not applicable. If a rationale is provided, the criteria is considered Present (Yes). An assessment of the rationale will be considered during the review of the submission.	Yes	No	N/A	Comment
36) Full test report is provided for each completed test. A full test report includes: objective of the test, description of the test methods and procedures, study endpoint(s), pre-defined pass/fail criteria, results summary, conclusions, and an explanation of how the data generated from the test supports a finding of substantial equivalence.	X			
37)				
a) If there are requirements regarding performance data, such as special controls, in a device-specific regulation that are applicable to the device, the submission includes performance data to establish that the submitter has followed the device-specific requirement.			X	
b) If there is a device-specific guidance, other than a special controls guidance document, applicable to the device, the submission includes performance data to establish that the submitter has addressed the recommendations or otherwise has met the applicable statutory or regulatory criteria through an alternative approach.			X	
c) If there is a special controls document applicable to the device, the submission includes performance data to establish that the submitter has complied with the particular mitigation measures set forth in the special controls document or uses alternative mitigation measures but provides a rationale to demonstrate that those alternative measures identified by the firm will provide at least an equivalent assurance of safety and effectiveness.			X	
38) If literature is referenced in the submission, submission includes:				
a) Legible reprints or a summary of each article.	X			
b) Discussion of how each article is applicable to support the substantial equivalence of the subject device to the predicate.	X			
39) For each completed nonclinical (i.e., animal) study conducted				
a) Submission includes a study protocol which includes all elements as outlined in 21 CFR 58.120.	X			
b) Submission includes final study report which includes all elements outlined in 21 CFR 58.185.	X			
c) Submission contains a statement that the study was conducted in compliance with applicable requirements in the GLP regulation (21 CFR Part 58), or, if the study was not conducted in compliance with the GLP regulation, the submission explains why the noncompliance would not impact the validity of the study data provided to support a substantial equivalence determination.	X			

K. Performance Characteristics - In Vitro Diagnostic Devices Only

(Also see 21 CFR 809.10(b)(12))

Submission states that the device: (one of the below must be checked)	
	is an in vitro diagnostic device.
X	is not an in vitro diagnostic device.

Decision: (•) Accept ( ) Refuse to Accept

If Accept, notify applicant.

If Refuse to Accept, notify applicant in writing and include a copy of this checklist.

Digital Signature Concurrence Table

Reviewer Sign-Off	Charles N. Durfor -S Digitally signed by Charles N. Durfor -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300048677, cn=Charles N. Durfor -S Date: 2015.09.11 15:40:17 -04'00'
Branch Chief Sign-Off (digital signature optional)*	Peter Yang -S 2015.09.14 10:36:31 -04'00'
Division Sign-Off (digital signature optional)*	

* Branch and Division review of checklist and concurrence with decision required. Branch and Division digital signature optional.

From: Balu Karandikar
To: Durfor, Charles
Cc: k152519@docs.fda.gov
Subject: Re: K152519 - MTR550 Antimicrobial Silver Wound Gel
Date: Friday, May 20, 2016 4:21:37 PM
Attachments: 001_Revised IFU 05 20 2016.pdf
002_Revised 510K Summary 05 20 2016.pdf
003_Revised Product Carton Label 05 20 2016.pdf

Dear Dr. Durfor:
As requested in your letter of May 20, 2016, I have made revisions to (b)(4) (b)(4) and have attached herewith the revised documents in PDF. This a complete response to all issues raised in your letter of May 20, 2016.

Please inform us immediately should you have difficulty in downloading PDFs to your system. Thank you.

Respectfully

Bhalchandra M Karandikar, PhD
CTO
Medical Technology Research Inc.
2650 Progress Way
Woodburn, OR 97071
503.902.6279

On Fri, May 20, 2016 at 11:33 AM, Durfor, Charles <Charles.Durfor@fda.hhs.gov> wrote:

> Dear Dr. Karandikar
>
> The revised documents may be submitted in pdf form via email to me. I will then add them to the record.
>
> Many thanks!
>
> C. Durfor
>
> **From:** Balu Karandikar [mailto:bhalchak@mtrmedical.com]
> **Sent:** Friday, May 20, 2016 2:33 PM
> **To:** Durfor, Charles
> **Cc:** k152519@docs.fda.gov
> **Subject:** Re: K152519 - MTR550 Antimicrobial Silver Wound Gel
>
> Dear Dr. Durfor:
>
> Thank you for your comments. I presume you want the suggested revisions to be submitted to FDA through the DCC mechanism. I was wondering since the changes are relatively straightforward and manageable, if I could instead submit revised documents in PDF to you directly. Please advise. Thank you.
>
> Respectfully,
>
> Bhalchandra M Karandikar, PhD
> CTO
> Medical Technology Research Inc.
> 2650 Progress Way
> Woodburn, OR 97071
> 503.902.6279

On Fri, May 20, 2016 at 10:15 AM, Durfor, Charles <Charles.Durfor@fda.hhs.gov> wrote:

Dear Dr. Karandikar

Many thanks for your thoughtful revisions to your 510(k). Upon completing my review of your 510(k), I believe there are a few final corrections that are needed to complete your file. Please let me know if you have any questions.

Date: May 20, 2016

To: The Record
Office: ODE

From: Charles N. Durfor, Ph.D. Division: DSD/PRSB-2

510(k) Holder: Medical Technology Research Inc.

Device Name: MTR550 Antimicrobial Silver Wound Gel

Contact: Bhalchandra M. Karandikar, Ph.D.

Phone: (503) 902-6279

Fax: (503) 980-7931

Email: bhalchak@mtrmedical.com

To complete our review of your 510(k) please provide the following responses by May 25, 2016 or inform FDA that this information will be arriving at a later date.

(b)(4)

(b)(4) Deficiencies

INDICATIONS FOR USE

K NUMBER: **152519**

DEVICE NAME: **MTR550 ANTIMICROBIAL SILVER WOUND GEL**

INDICATIONS FOR USE

For the management of dry to low/moderate exuding wounds such as:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

TYPE OF USE: **Prescription Use Only (Part 21 CFR 801 Subpart D)**

510(k) SUMMARY

1. SUBMITTER/OWNER

MEDICAL TECHNOLOGY RESEARCH INC.
2650 PROGRESS WAY
WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact Person: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Date Prepared: April 25, 2016

2. DEVICE

Name of Device:	MTR550 (Tentative) Antimicrobial Silver Wound Gel
Common Name:	Antimicrobial Wound Dressing
Classification Name:	Dressing Wound, Drug
Regulatory Class:	Unclassified
Product Code:	FRO

3. PREDICATE DEVICE

MTR550 gel is substantially equivalent to the following legally marketed predicate devices:

(i) SilverShield™ Antimicrobial Skin and Wound Gel (# K062212) from Anacapa Technologies of San Dimas, CA and distributed presently under SilverSept® name

(ii) Silver Antimicrobial Wound Gel from Advanced Medical Solutions of Winsford, UK (#K110458) and marketed by Molynyke under the name Normlgel Ag®

4. DEVICE DESCRIPTION

The product is a spreadable amorphous water based wound gel comprising a synthetic clay as the thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that it turns aids in wound healing. The presence of active silver compounds within the gel provides preservative action and acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms within the dressing. The gel is clear to hazy and will not stain the skin tissue when used over a period of 3 days though its potential to stain skin beyond 3 day use is not known.

Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures.
The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

5. INDICATIONS FOR USE

The device is indicated for use by prescription only. It is indicated for use in the management of dry to low/moderate exuding partial and fullness thickness wounds, stage I-IV pressure ulcers, diabetic and foot ulcers, graft wounds and donor sites, first and second degree wounds, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations.

Contraindications: The gel should not be used on patients with known sensitivity to silver and s-triazine compounds.

6. MANUFACTURING

The MTR550 gel will be manufactured in a production facility in accordance with good manufacturing practices consistent with US FDA guidelines. The batch production of the gel and packaging will be verified to meet product specifications to ensure the product is safe and effective.

7. BIOCOMPATIBILITY TESTING

The device has been tested for in-vitro cytotoxicity, dermal irritation and sensitization in accordance with ISO 10993 -1 (Biological Evaluation of Medical Devices).

No systemic toxicity was associated with the antimicrobial silver compounds. Animal study employing rats showed no adverse effects on animals due to the device.

8. PERFORMANCE

The antimicrobial efficacy of the device has been demonstrated by 28 days Antimicrobial preservative challenge test in accordance with USP Chapter 51 guidelines. Employing in vitro assays, the antimicrobial barrier property was demonstrated against 17 different micro-organisms that included bacteria, yeast and fungi. The tested micro-organisms included, MRSA, VRE, Candida A. and Aspergillus niger. Sustenance of antimicrobial barrier activity was demonstrated for 3 days in a serial transfer assay.

Animal study employing rats showed the device exhibited no adverse effect on animals. In a porcine study examining deep partial thickness burn wound healing, MTR550 device was found to be safe and effective as the predicate device.

Despite its silver content at 550ppm, the device was shown to be non-staining to dermal tissue over 3 days use. The device showed no discoloration despite exposure to intense light or elevated environmental temperatures.

9. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH PREDICATE DEVICE

With respect to its physical and chemical properties, the device is substantially equivalent to the predicate device.

The mechanism of antimicrobial action is also similar. Both device exert toxicity towards micro-organisms as a result of silver ions.

The device is substantially equivalent to legally marketed predicate devices in composition and intended use.

10. CONCLUSIONS

Based on the indications for use, biocompatibility, invivo studies, performance data, the MTR550 gel is substantially similar to SilverShield™ Antimicrobial Skin and Wound Gel (#K062212)

5.0 PROPOSED LABELING

The proposed labeling on the thin gauge cardboard box, the tube and the packaging insert is disclosed below. For comparison the product inserts from predicate devices (K062212 & K110458) are attached (see Attachments 1 & 2).

Product cardboard box labeling

Side 1

MTR550™ Gel (Note the MTR550™ name is tentative and may be replaced)

Antimicrobial silver wound gel

Rx Only / Net weight (1.5 oz/43g)

Side 2

MTR550™ Antimicrobial silver wound gel

- Contains 0.055% (550 µg/g) silver (as preservative)
- Provides lasting antimicrobial barrier as burn and wound dressing up to 3 days
- Helps maintain moist wound environment
- As preservative, silver reduces microbial growth within the dressing and exerts no microbial action in or on the wound

Read instructions for use (see insert inside package)
Store at 15C (59F) to 30C (77F)

Side 3

Rx Only
Intended for use for the management of dry to low/moderate exuding wounds such as: partial and full thickness wounds, Stage I-IV pressure ulcers, diabetic and foot ulcers and 1st and 2nd degree burns, graft and donor sites, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations

- For external use only; not to use in or around the eyes
- Not made with natural rubber latex
- Contraindicated for use on patients with sensitivity to silver and s-triazine compounds

Side 4

Manufactured in China for:
Medical Technology Research Inc.
2650 Progress Way, Woodburn, OR 97071

US and corresponding international patents pending
Reorder Number XXXXX/Questions & Comments: 1-800-MTR-MEDI (Tentative)

Tube labeling

Side 1

MTR550™ Antimicrobial Silver Wound Gel*

*US & Int. patents pending

Rx Only / Net wt. 1.5 oz (43g)

Mfd. for:

Medical Technology Research Inc. Woodburn, OR 97071
www.mtrmedical.com

Side 2

MTR550™ Antimicrobial Silver Wound Gel

- Contains 0.055% (550 µg/g) silver (as preservative)
- Provides lasting antimicrobial barrier as burn and wound dressing up to 3 days
- Helps maintain moist wound environment
- As preservative, silver reduces microbial growth within the dressing and exerts no microbial action in or on the wound

Intended for use for the management of dry to low/moderate exuding wounds such as: partial and full thickness wounds, Stage I-IV pressure ulcers, diabetic and foot ulcers and 1st and 2nd degree burns, graft and donor sites, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations

Store at 15C – 30C; Read instructions for use (see package insert)

Package Insert

MTR550™ Antimicrobial Silver Wound Gel*

Reorder Number XXXXX

Product Description:

MTR550™ gel is spreadable amorphous burn and wound dressing that contains 550 ppm of an antimicrobial silver preservative. The dressing is formulated to provide an antimicrobial barrier lasting up to 3 days by inhibiting the growth of common pathogens such *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli*, antibiotic resistant strains MRSA and VRE and yeast and fungi including *Candida albicans, Aspergillus niger* and other microorganisms within the dressing. As a preservative, the presence of silver in the dressing reduces pathogen counts within the dressing and has no effect on the pathogens in or on the wound. In *in vitro* tests, antimicrobial silver within MTR550™ gel dressing was found to exert preservative action against several common pathogens.

The gel dressing helps maintain moisture in wound environment which may help promote wound healing. The gel is suitable for use on dry to low/moderately exuding wounds. MTR550™ gel will not stain or discolor tissue when used daily over 3 days. However, it is not known if staining will occur if gel use is continued beyond 3 days.

It is intended for sale or for use by prescription only on patients under the medical supervision of licensed healthcare professionals. It is against the Federal law to contravene this restriction.

Indications for use:

It is indicated for use for the management of dry to low/moderate exuding wounds such as:

- Stage I-IV pressure ulcers
- Diabetic & foot ulcers
- Partial and full thickness wounds
- Graft and donor sites
- Post-operative surgical wounds
- Trauma wounds (dermal lesions, trauma injuries and incisions)
- 1 & 2nd degree burns
- Abrasions and lacerations

Contraindications for use:

- Not to be used on patients with sensitivity to silver and s-triazine compounds
- Patients may experience mild skin irritation in and around area of gel application, but if it persists or redness or swelling develops, discontinue using the gel

Directions for use:

- ✓ Cleanse and debride wound as required
- ✓ Dispense MTR550™ gel onto the wound directly and evenly spread it to a 0.1" to 0.2" thick layer. It is recommended the applied gel layer extend 0.2" to 0.25" beyond the edge of the wound for effective barrier function.
- ✓ Cover with a sterile secondary dressing (such as gauze, foam or similar) and secure it in place
- ✓ In between dressing changes, maintain moist environment in the wound bed
- ✓ MTR550™ gel dressing may be maintained for up to 3 days though heavy exuding wounds may require more frequent dressing changes as per clinical protocols

Rx Only
External Use Only. Not to be used in the proximity of eyes.
Not made with natural rubber latex

*US & INTL. PATENTS PENDING

Manufactured in China for:
Medical Technology Research Inc.
2650 Progress Way, Woodburn, OR 97071
www.mtrmedical.com
For questions or comments: 1-800-MTR-MEDI (tentative)

1h152519

MTR
Medical Technology
Research Inc

CDRH PREMARKET REVIEW COVER LETTER

510(k) SUBMISSION

TYPE OF 510(k): TRADITIONAL

Submission Date:	August 28, 2015
Submitter/Owner:	MEDICAL TECHNOLOGY RESEARCH INC.
Address:	2650 PROGRESS WAY WOODBURN, OR 97071
Phone: Fax:	503-902-6279 503-980-7931
Contact:	Bhalchandra M. Karandikar, PhD Chief Technology Officer
Email address:	bhalchak@mtrmedical.com
Establishment Registration No.:	Not available
Common Name:	Antimicrobial Wound Dressing
Trade Name:	MTR550 Antimicrobial Silver Wound Gel (Tentative)
Classification Name:	Dressing Wound, Drug
Classification:	Unclassified
Product Code:	FRO
Reason for 510(k):	New Device (Finished Device)
Identified Predicate Devices:	K062212 SilverShield™ Antimicrobial Skin & Wound Gel (Product Code MGQ) K110458 Silver Antimicrobial Wound Gel (Product Code FRO)

Phone: 503-902-6279 | 2650 Progress Way, Woodburn Oregon 97071 USA | bhalchak@mtrmedical.com

MTR
Medical Technology
Research Inc

COMPANY COVER LETTER FOR E-COPY

510(k) SUBMISSION

TYPE OF 510(k): TRADITIONAL

The eCopy is the exact duplicate of the paper copy except the MTR Inc. internal technical reports in the eCopy lack ink signatures. But still they carry the same weight as the signed copies included in the paper copy.

Signature

Bhalchandra M Karandikar
Name of the authorized person

Submission Date: August 28, 2015

Submitter/Owner: MEDICAL TECHNOLOGY RESEARCH INC.

Address: 2650 PROGRESS WAY
WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Email address: bhalchak@mtrmedical.com

Form Approved OMB No. 0910-0511 Expiration Date April 30, 2016. See Instructions for OMB Statement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES FOOD AND DRUG ADMINISTRATION **MEDICAL DEVICE USER FEE COVER SHEET**	PAYMENT IDENTIFICATION NUMBER: (b)(4) Write the Payment Identification number on your check.

A completed cover sheet must accompany each original application or supplement subject to fees. If payment is sent by U.S. mail or courier, please include a copy of this completed form with payment. Payment and mailing instructions can be found at: http://www.fda.gov/oc/mdufma/coversheet.html

1. COMPANY NAME AND ADDRESS (include name, street address, city state, country, and post office code)	2. CONTACT NAME
MEDICAL TECHNOLOGY RESEARCH INC 2650 Progress Way Woodburn Marion OR 97071 US	Bhalchandra Karandikar 2.1 E-MAIL ADDRESS bhalchak@mtrmedical.com 2.2 TELEPHONE NUMBER (include Area code) 503-902-6279 2.3 FACSIMILE (FAX) NUMBER (Include Area code) 503-980-7931
1.1 EMPLOYER IDENTIFICATION NUMBER (EIN) *****9952	

3. TYPE OF PREMARKET APPLICATION (Select one of the following in each column; if you are unsure, please refer to the application descriptions at the following web site: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm345263.htm

Select an application type:	3.1 Select a center
[X] Premarket notification(510(k)); except for third party	[X] CDRH
[ ] 513(g) Request for Information	[ ] CBER
[ ] Biologics License Application (BLA)	3.2 Select one of the types below
[ ] Premarket Approval Application (PMA)	[X] Original Application
[ ] Modular PMA	Supplement Types:
[ ] Product Development Protocol (PDP)	[ ] Efficacy (BLA)
[ ] Premarket Report (PMR)	[ ] Panel Track (PMA, PMR, PDP)
[ ] 30-Day Notice	[ ] Real-Time (PMA, PMR, PDP)
	[ ] 180-day (PMA, PMR, PDP)

4. ARE YOU A SMALL BUSINESS? (See the instructions for more information on determining this status)

[X] YES, I meet the small business criteria and have submitted the required qualifying documents to FDA

NO, I am not a small business

4.1 If Yes, please enter your Small Business Decision Number: SBD155377

5. FDA WILL NOT ACCEPT YOUR SUBMISSION IF YOUR COMPANY HAS NOT PAID AN ESTABLISHMENT REGISTRATION FEE THAT IS DUE TO FDA. HAS YOUR COMPANY PAID ALL ESTABLISHMENT REGISTRATION FEES THAT ARE DUE TO FDA?

[X] YES (All of our establishments have registered and paid the fee, or this is our first device, and we will register and pay the fee within 30 days of FDA's approval/clearance of this device.)

[ ] NO (If "NO," FDA will not accept your submission until you have paid all fees due to FDA. This submission will not be processed; see http://www.fda.gov/cdrh/mdufma for additional information)

6. IS THIS PREMARKET APPLICATION COVERED BY ANY OF THE FOLLOWING USER FEE

EXCEPTIONS? IF SO, CHECK THE APPLICABLE EXCEPTION.

[ ] This application is the first PMA submitted by a qualified small business, including any affiliates

[ ] The sole purpose of the application is to support conditions of use for a pediatric population

[ ] This biologics application is submitted under section 351 of the Public Health Service Act for a product licensed for further manufacturing use only

[ ] The application is submitted by a state or federal government entity for a device that is not to be distributed commercially

7. IS THIS A SUPPLEMENT TO A PREMARKET APPLICATION FOR WHICH FEES WERE WAIVED DUE TO SOLE USE IN A PEDIATRIC POPULATION THAT NOW PROPOSES CONDITION OF USE FOR ANY ADULT POPULATION? (If so, the application is subject to the fee that applies for an original premarket approval application (PMA).

[ ] YES [X] NO

PAPERWORK REDUCTION ACT STATEMENT

Public reporting burden for this collection of information is estimated to average 18 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the address below.

Department of Health and Human Services, Food and Drug Administration, Office of Chief Information Officer, 8455 Colesville Road, COLE-14-14253 Silver Spring, MD 20993-0002
[Please do NOT return this form to the above address, except as it pertains to comments on the burden estimate.]

8. USER FEE PAYMENT AMOUNT SUBMITTED FOR THIS PREMARKET APPLICATION

(b)(4) 25-Aug-2015

Form FDA 3601 (05/13)

"Close Window" Print Cover sheet

Medical Technology Research Inc

CDRH PREMARKET REVIEW COVER LETTER

510(k) SUBMISSION

TYPE OF 510(k): TRADITIONAL

Submission Date:	August 28, 2015
Submitter/Owner:	MEDICAL TECHNOLOGY RESEARCH INC.
Address:	2650 PROGRESS WAY WOODBURN, OR 97071
Phone: Fax:	503-902-6279 503-980-7931
Contact:	Bhalchandra M. Karandikar, PhD Chief Technology Officer
Email address:	bhalchak@mtrmedical.com
Establishment Registration No.:	Not available
Common Name:	Antimicrobial Wound Dressing
Trade Name:	MTR550 Antimicrobial Silver Wound Gel (Tentative)
Classification Name:	Dressing Wound, Drug
Classification:	Unclassified
Product Code:	FRO
Reason for 510(k):	New Device (Finished Device)
Identified Predicate Devices:	K062212 SilverShield™ Antimicrobial Skin & Wound Gel (Product Code MGQ) K110458 Silver Antimicrobial Wound Gel (Product Code FRO)

MTR
Medical Technology
Research Inc

COMPANY COVER LETTER FOR E-COPY

510(k) SUBMISSION

TYPE OF 510(k): TRADITIONAL

The eCopy is the exact duplicate of the paper copy except the MTR Inc. internal technical reports in the eCopy lack ink signatures. But still they carry the same weight as the signed copies included in the paper copy.

Signature

Bhalchandra M Karandikar
Name of the authorized person

Submission Date: August 28, 2015

Submitter/Owner: MEDICAL TECHNOLOGY RESEARCH INC.

Address: 2650 PROGRESS WAY
WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Email address: bhalchak@mtrmedical.com

INDICATIONS FOR USE

K NUMBER: _____

DEVICE NAME: MTR550 ANTIMICROBIAL SILVER WOUND GEL

INDICATIONS FOR USE

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

TYPE OF USE: Prescription Use Only (Part 21 CFR 801 Subpart D)

510(k) SUMMARY

1. SUBMITTER/OWNER

MEDICAL TECHNOLOGY RESEARCH INC.
2650 PROGRESS WAY
WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact Person: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Date Prepared: August 28, 2015

2. DEVICE

Name of Device:	MTR550 (Tentative) Antimicrobial Silver Wound Gel
Common Name:	Antimicrobial Wound Dressing
Classification Name:	Dressing Wound, Drug
Regulatory Class:	Unclassified
Product Code:	FRO

3. PREDICATE DEVICE

MTR550 gel is substantially equivalent to the following legally marketed predicate devices:

(i) SilverShield™ Antimicrobial Skin and Wound Gel (# K062212) from Anacapa Technologies of San Dimas, CA and distributed presently under SilverSept® name

(ii) Silver Antimicrobial Wound Gel from Advanced Medical Solutions of Winsford, UK (#K110458) and marketed by Molynyke under the name Normlgel Ag®

4. DEVICE DESCRIPTION

The product is a spreadable amorphous water based wound gel comprising a synthetic clay as the thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that it turns aids in wound healing. The presence of active silver compounds acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms and may help in reducing infection. The gel is clear to hazy and does not stain the skin tissue. Even with silver content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures.

The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

5. INDICATIONS FOR USE

The device is indicated for use by prescription only. It is indicated for use in the management of low to moderate exuding wounds, partial and fullness thickness wounds, pressure and leg ulcers, diabetic foot ulcers, graft wounds and donor sites, first and second degree wounds, surgical wounds.

Contraindications: The gel should not be used on patients with known sensitivity to silver and s-triazine compounds.

6. MANUFACTURING

The MTR550 gel is manufactured in accordance with good manufacturing practices at a GMP facility. The batch production of the gel and packaging has been demonstrated to meet product specification. The contract manufacturer has met all requirements for QC/QA to release the product which is safe and effective.

7. BIOCOMPATIBILITY TESTING

The device has been tested for in-vitro cytotoxicity, dermal irritation and sensitization in accordance with ISO 10993 -1 (Biological Evaluation of Medical Devices).

No systemic toxicity was associated with the antimicrobial silver compounds. Animal wound healing study employing rats showed no adverse effects due to the device.

8. PERFORMANCE

The antimicrobial efficacy of the device has been demonstrated by 28 days Antimicrobial preservative challenge test in accordance with USP Chapter 51 guidelines. Employing in vitro assays, the antimicrobial barrier property was demonstrated against 17 different micro-organisms that included bacteria, yeast and fungi. The tested micro-organisms included, MRSA, VRE, Candida A. and Aspergillus niger. Sustenance of antimicrobial barrier activity was demonstrated for 3 days in a serial transfer assay.

Animal wound healing study showed the device exhibited no adverse effect on wound healing compared to a positive control group. The positive control was in the form of a cream containing silver sulfadiazine and zinc sulfadiazine.

Despite its silver content at 550ppm, the device was shown to be non-staining to dermal tissue and its properties were unaffected by extreme heat or intense light.

9. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH PREDICATE DEVICE

With respect to its physical and chemical properties, the device is substantially equivalent to the predicate device.
The mechanism of antimicrobial action is also similar. Both device exert toxicity towards micro-organisms as a result of silver ions.

The key differences between our device and predicate device are:

(i) Silver content
(ii) Source of antimicrobial silver
(iii) Content of gelling agent

These differences though real raise no safety or effectiveness issue and establish our device is substantially equivalent to the predicate device.

10. CONCLUSIONS

Based on the indications for use, biocompatibility, invivo studies, performance data, the MTR550 gel is substantially similar to SilverShield™ Antimicrobial Skin and Wound Gel (#K062212)

PREMARKET NOTIFICATION TRUTH AND ACCURATE STATEMENT

(As required by 21CFR 807.87(k))

I certify that, in my capacity as the Chief Technology Officer of Medical Technology Research Inc., I believe to the best of my knowledge, that all data and information submitted in the premarket notification are truthful and accurate and that no material fact has been omitted.

B M Karandikar

Signature

BHALCHANDRA. M. KARANDIKAR

Name

AUG 28, 2015

Date

Premarket Notification 510(k) Number

5.0 PROPOSED LABELING

The proposed labeling on the thin gauge cardboard box, the tube and the packaging insert is disclosed below. For comparison the product inserts from predicate devices (K062212 & K110458) are attached (see Attachments 1 & 2).

Product cardboard box labeling

Side 1

MTR550™ Gel (Note the MTR550™ name is tentative and may be replaced)

Antimicrobial silver wound gel

Rx Only / Net weight (1.5 oz/43g)

Side 2

MTR550™ Antimicrobial silver wound gel

- Contains 0.055% (550 μg/g) silver
- Provides lasting antimicrobial barrier as burn and wound dressing
- Helps maintain moist wound environment
- May help lower bio-burden

Read instructions for use (see insert inside package)
Store at 15C (59F) to 30C (77F)

Side 3

Rx Only

- Intended for use for management of partial and full thickness wounds including pressure ulcers, diabetic foot ulcers and 1st and 2nd degree burns
- For external use only; not to use in or around the eyes
- Latex free
- Contraindicated for use on patients with sensitivity to silver and s-triazine compounds

Side 4

Manufactured in China for:
Medical Technology Research Inc.
2650 Progress Way, Woodburn, OR 97071
US and corresponding international patents pending
Reorder Number XXXXX/Questions & Comments: 1-800-MTR-MEDI (Tentative)

Tube labeling

Side 1

MTR550™ Antimicrobial Silver Wound Gel*

*US & Int. patents pending

Rx Only / Net wt. 1.5 oz (43g)

Mfd. for:

Medical Technology Research Inc. Woodburn, OR 97071
www.mtrmedical.com

Side 2

MTR550™ Antimicrobial Silver Wound Gel

- Contains 0.055% (550 μg/g) silver
- Provides lasting antimicrobial barrier as burn and wound dressing
- Helps maintain moist wound environment
- May help lower bio-burden

Intended for use in the management of partial & full thickness wounds (pressure ulcers, diabetic & foot ulcers, 1st and 2nd degree burns)

Store at 15C – 30C; Read instructions for use (see package insert)

Package Insert

MTR550™ Antimicrobial Silver Wound Gel*

Reorder Number XXXXX

Product Description:

MTR550™ gel is spreadable amorphous burn and wound dressing that contains 550 ppm antimicrobial silver. The dressing is formulated to provide an antimicrobial barrier lasting up to 3 days by inhibiting the growth of common pathogens such Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, antibiotic resistant strains MRSA and VRE and yeast and fungi including Candida albicans, Aspergillus niger and other microorganisms. In invitro tests, MTR550™ gel was found to lower bio-burden but its implication in clinical setting is not known.

The gel dressing helps maintain moisture in wound environment which may help promote wound healing. MTR550™ gel will not stain or discolor tissue.

It is intended for sale or for use by prescription only on patients under the medical supervision of licensed healthcare professionals. It is against the Federal law to contravene this restriction.

Indications for use:

It is indicated for use in the management of dry to low/moderate exuding partial and full thickness wounds including:

- Stage I-IV pressure ulcers
- Diabetic & foot ulcers
- Partial and full thickness wounds
- Graft and donor sites
- Post-operative surgical wounds
- Trauma wounds (dermal lesions, trauma injuries and incisions)
- 1 & 2nd degree burns
- Abrasions and lacerations

Contraindications for use:

- Not to be used on patients with sensitivity to silver and s-triazine compounds
- Patients may experience mild skin irritation in and around applied area, but if it persists, discontinue its use

Directions for use:

- ✓ Cleanse and debride wound as required
- ✓ Dispense MTR550™ gel onto the wound directly and evenly spread it to a 0.1” to 0.2” thick layer.

- ✓ Cover with a sterile secondary dressing (such as gauze, foam or similar) and secure it in place
- ✓ In between dressing changes, maintain moist environment in the wound bed
- ✓ MTR550™ gel dressing may be maintained for up to 3 days though heavy exuding wounds may require more frequent dressing changes as per clinical protocols

Rx Only
External Use Only. Not to be used in the proximity of eyes.
Latex free

*US & INTL. PATENTS PENDING

Manufactured in China for:
Medical Technology Research Inc.
2650 Progress Way, Woodburn, OR 97071
www.mtrmedical.com
For questions or comments: 1-800-MTR-MEDI (tentative)

6.0 SPECIFICATION:

6.1 General Device Description

The device is an amorphous aqueous gel containing antimicrobial silver. It is transparent to slightly hazy in appearance. In wounds, it helps maintain moist environment by either donating moisture or by absorbing low to moderate amount of exudate. Moisture management may also help processes that promote wound healing. The presence of antimicrobial silver in the gel layer provides a protective barrier to entry into the wounds by inhibiting the growth of infectious pathogens that include gram positive and gram negative bacteria, yeast and fungi. The device's antimicrobial activity is exerted by ionic silver and similar to other silver containing devices.

The viscous/gel character of amorphous composition serve several purposes. (b)(4)

(b)(4)

The amorphous gel is supplied in a net amount of 43g in heat sealed plastic tubes with a screw-on cap. To prevent tampering, the dispensing end of the tube is sealed with a coated aluminum foil. For the first use, cap is unscrewed and the foil seal is peeled off. The gel also may be packaged in larger tubes or other packaging such as 8 oz. jars. In place of foil seal to prevent tampering, the tube or the jar may be sealed with shrink wrap covering the entire cap and a portion of the tube or jar.

As finished device (in packaged form), the amorphous gel is not sterilized. However, antimicrobial silver in the gel exerts its preservative effect and maintains the device sterile over its 3 year prescribed shelf life.

Breadth of Antimicrobial Activity

In in-vitro assays, its broad spectrum antimicrobial activity was demonstrated against the following 17 microorganisms:

(b)(4)

Indications for Use

As wound dressing, MTR550 antimicrobial gel is intended for use by prescription only. As topical gel, it is indicated for external use only. It is indicated for use under the medical supervision of a licensed healthcare professional for the management of dry to low/moderate exuding wounds such as:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

Contraindications and Precautions:

There are several contraindications and precautions associated with the administration of the device. It may not be used on patients with known sensitivity to silver or s-triazine compounds. It is not for ophthalmic use. The antimicrobial gel may be left in the wounds for a period of up to 3 days before a dressing change is required.

How does MTR550 gel works? Rationale for Use of Silver as Antimicrobial Agent

As a wound dressing, the amorphous antimicrobial silver hydrogel provides two functions; it maintains moisture in wounds by absorbing or donating moisture depending on wound's condition and it provides long lasting antimicrobial protective barrier by inhibiting the growth of common pathogens. As an added benefit, the moisture donation may assist in the debridement of dry necrotic wounds and by absorbing exudate may reduce skin maceration.

The antimicrobial effect is provided by ionic silver released from the device upon contact with the wound. Silver's antimicrobial efficacy and safety in wound products has been well established as evidenced by a large number of silver containing devices cleared by FDA under 510(k) submissions over the past decade.

As antimicrobial agent, silver offers several advantages. First, with silver there is practically no risk of microorganisms developing resistance because of the way it exerts its antimicrobial activity. Silver irreversibly binds to surfaces of cells through multiple pathways via functional groups such as carboxyl, amines and thiols making it harder for resistant microorganisms to evolve. Second, silver singularly is effective against multitude of organisms. Thus, the formulators need only one rather than many active components in their formulations or devices simplifying product design. Third, silver is very effective against infectious pathogens at very low levels and yet at these minimum inhibitory concentrations for such organisms, silver is not toxic to humans.

Despite these advantages, silver suffers from a major limitation. Product formulations or devices containing silver often have short shelf lives. The very property that silver gives its potent antimicrobial activity also makes it susceptible to reduction to inactive elemental silver induced by light or heat making it ineffective. The unpredictable nature of silver susceptibility to reduction can cause large batch to batch variability in product shelf lives.

The MTR550 gel incorporates silver as the antimicrobial agent. The source of (b)(4)

(b)(4)

[redacted]

6.2 Technical Device Description

Finished composition and Ingredients

The device is an aqueous amorphous gel composition containing antimicrobial silver in the nominal concentration of 550ppm by weight. The amounts of components by weight in the gel are tabulated below:

Nominal Composition of MTR550 Gel

Component	Amount (weight %)
(b)(4) [redacted]	

The pertinent physical and chemical properties of MTR550 gel are tabulated below.

Typical MTR550 gel properties (not specification)

Property/Characteristic	Value/Description
Appearance	Transparent to slightly hazy
Texture	Smooth
Odor	Odorless
Density (g/mL) @ ~25C	Between 1 and 1.1
Surface tension (mPa.s) @ ~ 25C	N/A
Viscosity (mPa.s) @ ~ 25C	N/A
Yield stress (Pa) @ ~ 25C	~ 250
Water absorption (g/g x100) @ ~ 37C	~ 10% @ 24h; ~ 30% @ 72h
Water loss by evaporation (g/g x100) @ ~ 25C	~ < 10% @ 5h
pH	Between 8 and 10
Silver content (ppm)	~ 550
Compatibility	Incompatible with cationic polymers and reducing agents

The device is transparent to slightly hazy in appearance though the appearance does not affect its performance. The viscous gel like behavior is imparted by (b)(4) [redacted]

(b)(4) [redacted]

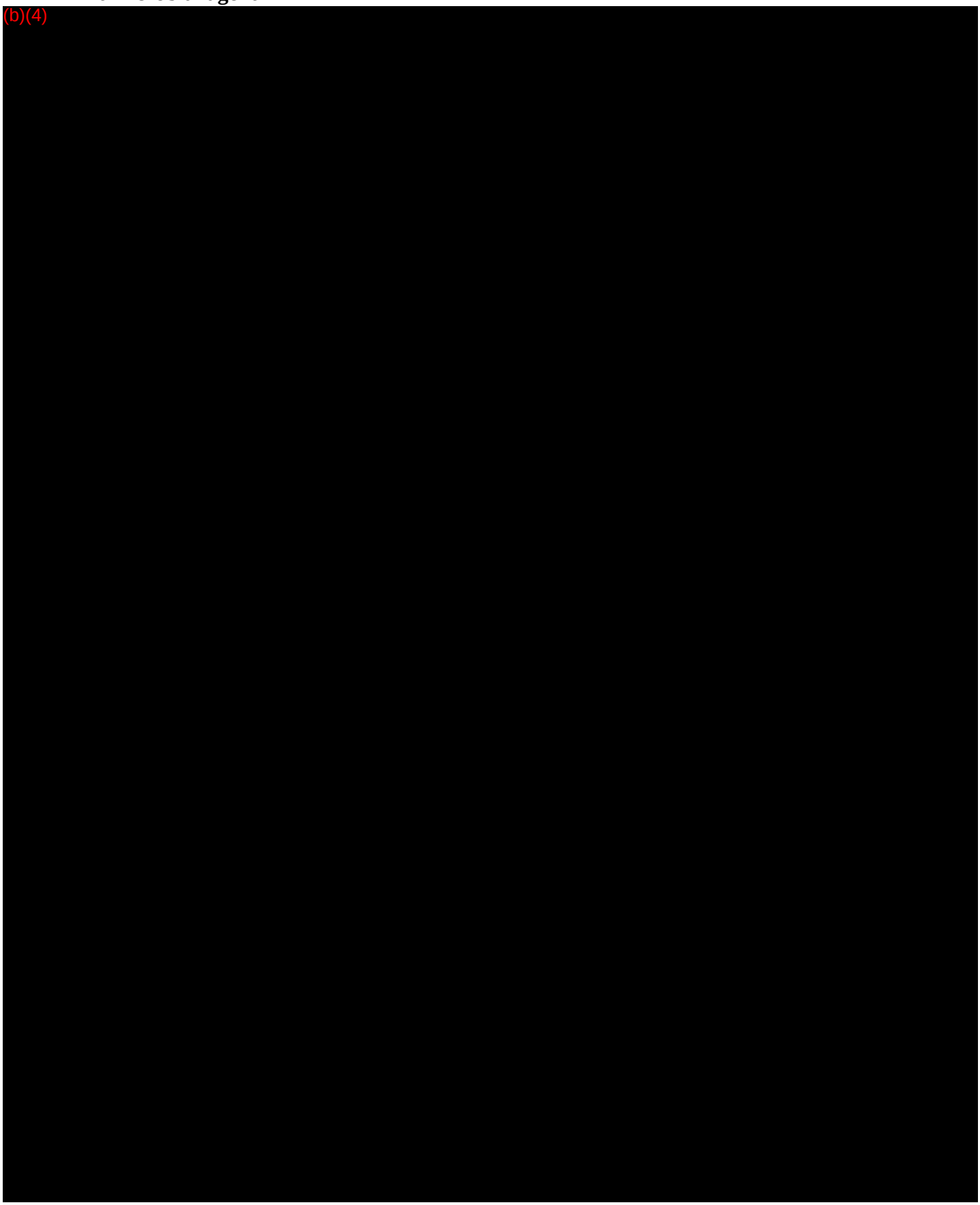
(b)(4)

Inactive Ingredients

(b)(4)

Antimicrobial agent

(b)(4)



(b)(4) Ingredients

(b)(4) Ingredients

[redacted]

The table below lists the properties of ingredients used to produce MTR550 gel.

Properties of Ingredients Used to Produce MTR550 Gel

(b)(4) Ingredients

[redacted]

6.3 Manufacturing process for MTR550 gel

(b)(4)

Device Sterility

As finished gel, the device is not sterile. However, it contains sufficient level of silver to exert its preservative and antimicrobial activity and remains microorganism free. Sterilization by traditional methods of gamma irradiation or e-beam is not recommended as the gel may discolor and degrade.

6.4 Biocompatibility Testing

The FDA draft guidance document 1811 issued April 23, 2013 titled "Use of International Standard ISO 10993- Biological evaluation of medical devices Part 1: Evaluation and Testing" provides guidelines on biocompatibility testing for industry and FDA staff. Of the information provided, the matrix in Attachment A of that document is most relevant. It suggests for a surface device such as MTR550 gel, initial biocompatibility testing should include cytotoxicity, irritation, sensitization and systemic toxicity.

(b)(4)

Cytotoxicity (see Attachment 5)

(b)(4)

Dermal Irritation (see Attachment 6)

(b)(4)

Dermal Sensitization (see Attachment 7)

(b)(4)

Systemic toxicity (see Attachment 8)

(b)(4)

Wound healing study (see Attachment 9)

(b)(4)

(b)(4) Testing

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6.5 Performance Testing

The FDA draft guidance document issued on July 19, 2007 titled "Pre-market notifications (510(k)) submissions for medical devices that include antimicrobial agents" recommends performance testing to demonstrate antimicrobial effectiveness of the finished device. The testing may include both invitro tests and invivo (animal) tests as required.

Accordingly, the following tests were conducted on MTR550 gel to demonstrate antimicrobial effectiveness.

- Antimicrobial Preservative Challenge Test in accordance with USP Chapter 51 (see Attachment 10)

(b)(4) Testing

7.0 SUBSTANTIAL EQUIVALENCE COMPARISON

The table below compares the characteristics and attributes of our device with its predicate devices. A discussion on similarities and differences between our device and the primary predicate device follows. Despite the differences, we show there are no issues of safety and effectiveness and that our device is substantially equivalent to its predicate devices.

Attribute/Characteristic	MTR550 gel (Present Device)	Primary Predicate Device (K062212)	Secondary Predicate Device (K110458)
Intended use	Wound dressing Prescription only	Wound dressing OTC and Prescription	Wound dressing Prescription only
Indications for use	Stage I-IV pressure ulcers Diabetic foot and leg ulcers Partial and full thickness wounds Graft and donor sites Post-operative surgical wounds Trauma wounds (dermal lesions, trauma injuries and incisions) 1 & 2nd degree burns Abrasions and lacerations	Stage I-IV pressure ulcers Diabetic foot & leg ulcers Partial and full thickness wounds Graft and donor sites 1 & 2nd degree burns Abrasions and lacerations	Pressure ulcers Diabetic leg ulcers Partial and full thickness wounds Graft and donor sites Post-operative surgical wounds Trauma wounds (dermal lesions, trauma injuries and incisions) 1 & 2nd degree burns Abrasions and lacerations
Duration of use	3 days	3 days	3 days
Wound condition	Dry to moderate exuding partial and full thickness wounds	Low exuding wounds	Dry to moderate exuding partial and full thickness wounds
Target population	Not limited to any age group		
Anatomical site	External use only Not for ophthalmic use	External use only Not for ophthalmic use	External use only

Technological characteristic			
Form	Amorphous viscous gel (thixotropic)	Amorphous viscous gel (thixotropic)	Amorphous viscous gel
Antimicrobial active agent	Silver ([redacted])	Silver ([redacted])	Silver ([redacted])
Silver content (ppm)	[redacted]	[redacted]	[redacted]
Water content (% weight)	[redacted]		
Yield Stress (Pa)	[redacted]		
Performance (Antimicrobial invitro testing)	[redacted]		

Biocompatibility - Invitro testing	(b)(4)
Biocompatibility - Invivo testing	

Skin staining	Does not stain skin	Does not stain skin	Possibility of skin staining on prolonged use
Ingredients safety – Viscosity modifying agent	(b)(4) [redacted]		
Ingredient safety – Antimicrobial active agent			
Ingredient safety – (b)(4) [redacted]			

Discussion on Substantial Equivalence Comparison

The MTR550 gel is substantially equivalent to the Primary Predicate Device (K062212), referenced hereon as PPD with respect to the intended use and indications for use except MTR550 gel proposed use is limited to by prescription only. The duration for use of MTR550 gel and the PPD are substantially equivalent. Both can be used for 3 days. A serial transfer assay showed MTR550 gel sustained antimicrobial effectiveness for that period (See Attachment 12). And the product label (see Attachment 1) of the PPD indicates it can be used for 3 days.

Technologically, both MTR550 gel and PPD are water rich formulations (>80%) and can donate moisture to the wound. But MTR550 gel can absorb nearly 30% its weight (see Attachment 4) over 72h which actually is better than ~ 10% absorption by PPD that we measured over the same period. Thus, MTR550 gel is suitable for managing dry to moderately exuding wounds and in this respect at least equivalent to the PPD or better.

As MTR550 gel and the PPD are both aqueous formulations, density values at room temperature are comparable (MTR550 gel density at ~ 25C is ~ 1.03 gm/mL). As viscous gels, both are thixotropic and exhibit yield stress, though their yield stress values are different. The lower value of yield stress of PPD indicates it is less firm of a gel compared to MTR550 gel but this difference has no impact on the ability of the gels to be spread.

MTR550 gel and the PPD also exhibit comparable pH values, water loss by evaporation and water absorption over time (see Attachment 4) suggest they are substantially equivalent in terms of these physical properties.

The key differences between MTR550 gel and PPD are the amount of silver (b)(4) the source of silver ((b)(4)) and the amount of gelling agent (b)(4) We established the amount of (b)(4) (see the internal technical report as Attachment 15).

(b)(4) Ingredient Information

(b)(4) Ingredient Information

(b)(4) Ingredient Information

[redacted]

Antimicrobial performance

(b)(4)

[redacted]

Other performance testing

MTR550 and the PPD with respect to their ability to not stain or discolor tissue are substantially similar (see Attachment 13). MTR550 gel has been demonstrated to withstand environmental stresses of intense light and heat better than the PPD.

Biocompatibility

(b)(4)

Animal testing

(b)(4)

Toxicity testing (b)(4)

(b)(4)

Animal wound healing study

(b)(4)

(b)(4) Testing

8.0 CONCLUSION

MTR550 gel and PPD have several similarities. Some differences are minor and raise no safety issues. Others that are significant have been discussed at length and explained to show they raise no safety or effectiveness concerns. Thus, MTR550 gel and PPD can be considered substantially equivalent.

NDC 67180-3015

SILVER-SEPT

Silver Antimicrobial Skin & Wound Gel

PRODUCT DESCRIPTION:

Silver-Sept® Silver Antimicrobial Skin & Wound Gel, containing 200µg/gram total silver, is a clear, amorphous hydrogel wound dressing that helps to maintain a moist wound environment that is conducive to healing. Silver-Sept® will not stain or discolor tissue.

Silver-Sept® Gel functions as a long-lasting antimicrobial barrier by inhibiting the growth of common bacteria such as: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli Proteus mirabilis, Serratia marcescens,* including the antibiotic resistant strains: *MRSA & VRE,* as wellas fungi such as, *Candida albicans* and *Aspergillus niger.*

INDICATIONS:

Silver-Sept® Gel is intended for OTC use for Abrasions & Lacerations and under the supervision of a healthcare professional in the management of:

- Stage I-IV Pressure Ulcers
- Partial and Full Thickness Wounds
- 1st and 2nd degree burns
- Diabetic Foot and Leg Ulcers
- Grafted and Donor Sites

DIRECTIONS FOR USE:

1) Cleanse or debride wound as necessary
2) Apply a generous amount of Silver-Sept® Gel directly onto wound bed (1/8" to 3/16"Thick)
3) Cover with a sterile gauze or other appropriate secondary dressing and secure in place.
4) Maintain a moist wound environment between dressing changes.

NOTE: Silver-Sept® Silver Antimicrobial Skin & Wound Gel may remain in the wound bed for up to 3 days. More frequent dressing changes may be required dependent upon the amount of wound exudate present and the condition of the secondary dressing.

EXTERNAL USE ONLY. NOT FOR OPTHALMIC USE

Reorder Information: Catalog no. 3015 S

Manufactured by:
Anacapa® Technologies, Inc., San Dimas, CA 91773

(800)-489-2591
www.anacapa-tech.net

Made in the USA
Rev02

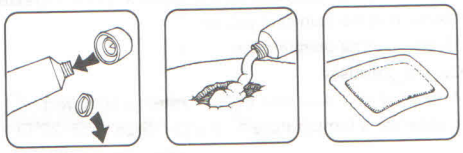
ANACAPA TECHNOLOGIES

Normlgel® Ag

1.5oz/45g

en Silver Antimicrobial Wound Gel

fr Gel antimicrobien à l'argent pour le traitement des plaies



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Normlgel® Ag

en

Silver Antimicrobial Wound Gel

℞ only

Caution: Federal (US) Law restricts this device to sale by or on the order of a physician (or properly licensed practitioner).

Product Description

Normlgel Ag is an opaque, amorphous hydrogel containing a high (>80%) water content and water soluble polymer chains. This formulation increases the moisture within the wound through water donation which makes the gel effective in assisting the debridement and desloughing process in dry necrotic wounds, whilst maintaining a moist wound environment for optimal wound healing.

Normlgel Ag contains an antimicrobial silver compound (silver carbonate) that is an effective barrier to bacterial penetration by inhibiting the growth of broad spectrum of microorganisms which come into contact with the gel.

The nominal silver content is 0.11%.(1100 µg/gram)

In-vitro testing

In-vitro testing has shown that Normlgel Ag is effective against the following broad spectrum of microorganisms; *Staphylococcus aureus*, including MRSA, *Staphylococcus epidermidis*, including MRSE, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* and fungi such as *Candida albicans* and *Aspergillus brasiliensis* when they come into contact with the gel. The clinical implications of the in-vitro findings are unknown.

Indications

Normlgel Ag is indicated under the medical supervision of a healthcare professional for the management of dry to moderate exuding partial and full thickness wounds, such as:

- diabetic ulcers
- pressure ulcers
- leg ulcers
- graft and donor sites
- post-operative surgical wounds
- trauma wounds (dermal lesions, trauma injuries or incisions)
- 1st and 2nd degree burns
- Abrasions and lacerations

Contraindication

Do not use on patients with a known sensitivity to Silver or Propylene glycol.

Directions for Use

1. Cleanse or gently flush the wound with normal saline solution or an appropriate wound cleanser.
2. Apply Normlgel Ag onto the wound bed (approximately 0.2 inches thick).
3. Ensure that Normlgel Ag does not cover healthy skin.
4. Cover the gel with an appropriate moisture retentive secondary dressing (such as Mepilex Border or Mepilex Border Lite). The choice depends on the exudate level.

Changing the dressing

1. Normlgel Ag may be left in place for up to 3 days. Dressing changes may be required more frequently dependent upon wound conditions and the levels of exudate.
2. Carefully remove the secondary dressing. Cleanse or gently flush the wound with normal saline solution to remove necrotic debris.

Cautions:

- Frequent or prolonged use of this preparation may result in permanent discoloration of skin and mucous membranes.
- Discard the tube and any remaining gel 28 days after opening.
- For single patient use only.
- For external use only.
- Do not sterilise.
- Do not use if individual tube is damaged or opened.
- Store at room temperature (15-25°C/59-77°F).

Normlgel® Ag

fr

Gel antimicrobien à l'argent pour le traitement des plaies

Description du produit

Normlgel Ag est un hydrogel opaque, amorphe, contenant une teneur élevée en eau (> 80 %) et des chaînes polymères hydrosolubles. Cette préparation augmente l'humidité dans la plaie grâce à l'apport en eau, ce qui favorise le processus de débridement et de décollement des plaies nécrotiques sèches, tout en maintenant un milieu humide pour une cicatrisation optimale de la plaie.

Normlgel Ag contient un composé antimicrobien à base d'argent (carbonate d'argent) qui constitue une barrière efficace à la pénétration bactérienne en inhibant la croissance d'un large spectre de micro-organismes entrant en contact avec le gel.

La teneur nominale en argent est de 0.11 % (1100 µg/g).

Tests in vitro

Les tests in vitro ont montré que Normlgel Ag pour le traitement des plaies est efficace contre le spectre de micro-organismes suivant: *Staphylococcus aureus*, y compris les SARM, *Staphylococcus epidermidis*, y compris les SERM, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* et les champignons tels que *Candida albicans* et *Aspergillus brasiliensis* lorsqu'ils entrent en contact avec le gel. Les implications cliniques des résultats in vitro sont méconnues.

Indications

Normlgel Ag est indiqué, sous le contrôle d'un professionnel de santé, dans le traitement des plaies partielles et profondes sèches à modérément exsudatives telles que:

- les ulcères diabétiques;
- les escarres;
- les ulcères de jambe;
- les sites donneurs et de greffons;
- les plaies chirurgicales post-opératoires;
- les plaies traumatiques (lésions du derme, blessures traumatiques ou incisions);
- les brûlures du premier et du deuxième degré;
- les abrasions et lacérations.

Contre-indications

Ne pas utiliser chez les patients présentant une allergie connue à l'argent ou au propylène glycol.

Mode d'emploi

1. Nettoyer ou rincer délicatement la plaie avec une solution saline normale ou un nettoyant pour plaie approprié.
2. Appliquer Normlgel Ag dans le lit de la plaie (environ 0,5 cm d'épaisseur).
3. Veiller à ce que Normlgel Ag ne couvre pas la peau saine.
4. Recouvrir le gel d'un pansement secondaire adapté au niveau d'exsudat qui va aussi maintenir l'équilibre hydrique de la plaie (tel que Mepilex Border ou Mepilex Border Lite).

Renouvellement du pansement

1. Normlgel Ag peut rester en place jusqu'à trois jours. Le renouvellement du pansement peut être nécessaire plus souvent selon l'état de la plaie et la quantité d'exsudat.
2. Retirer délicatement le pansement secondaire. Nettoyer ou rincer délicatement la plaie avec une solution saline normale pour retirer les débris nécrotiques.

Attention:

- Une utilisation fréquente ou prolongée de cette préparation peut conduire à une décoloration permanente de la peau et des muqueuses.
- Jeter le tube et tout reste de gel 28 jours après l'ouverture.
- Usage réservé à un seul patient.
- Usage externe uniquement.
- Ne pas stériliser.
- Ne pas utiliser si le tube est endommagé ou ouvert.
- Conserver à température ambiante (15-25°C/59-77°F).

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Assessment of using Laponite® cross-linked poly(ethylene oxide) for controlled cell adhesion and mineralization

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ABSTRACT

The in vitro cytocompatibility of silicate (Laponite® clay) cross linked poly(ethylene oxide) (PEO) nano composite films using MC3T3 E1 mouse preosteoblast cells was investigated while cell adhesion, spread ing, proliferation and mineralization were assessed as a function of film composition. By combining the advantageous characteristics of PEO polymer (hydrophilic, prevents protein and cell adhesion) with those of a synthetic and layered silicate (charged, degradable and potentially bioactive) some of the physical and chemical properties of the resulting polymer nanocomposites could be controlled. Hydration, disso lution and mechanical properties were examined and related to cell adhesion. Overall, this feasibility study demonstrates the ability of using model Laponite cross linked PEO nanocomposites to create bio active scaffolds.

1. Introduction

In order for polymer silicate nanocomposites to be employed in biomedical technology they need to have designed mechanical properties as well as favorable interactions with biological inter faces [1,2]. Silicate nanoparticles from clay have been extensively used to improve the modulus, strength and toughness of polymers, thus offering strategies to tailor the mechanical properties of nano composites [3 7]. Research interest in these materials arises from the possibility of synergistically combining the properties of the individual components [8 12]. However, much of the literature in this area has focused on reinforcing polymers with silicate (clay) nanoparticles and little has been reported on the biological con straints that are biomedically relevant [1,2]. Only a few reports have attempted to merge knowledge from disciplines such as cell biology and materials science to better understand how nanocomposites composed of a polymer and layered silicates interface with cells. One such study described the synthesis of porous poly(lactic acid) montmorillonite (MMT clay) nanocomposites using microcom pounding and polymer/particle leaching techniques [13]. Addition of MMT nanoparticles was found to improve the compression prop erties of the polymer, to a level close to that of cancellous bone. The hydrophilicity of the polymer nanocomposite surface was found to directly affect cell adhesion and spreading [13]. In another study, intercalated gelatin chitosan clay nanocomposites were found to have lower degradation rates when compared with a gelatin chitosan control. As expected, polymer degradation was dependent on the clay concentration [14]. Enhanced cell adhesion and proliferation of rat stromal cells on the surfaces of these MMT gelatin chitosan nanocomposite films was observed.

Other studies of polymer nanocomposites that covered mechanical properties and cell growth include work on materials made of poly[ethylene co (vinyl acetate)] and Cloisite® clay [15]. Clay dependent mechanical properties as well as clay dependent cell adhesion were observed. The adhesion and growth of human dermal fibroblasts on the materials surfaces turned out to be max imal at ~10% clay. Cells cultured on substrates with a higher clay content had only poor growth curves. On adsorption of iron onto the clay the resulting material became magnetic and MC3T3 osteo blast cell proliferation could be maximized when cultured in a con stant magnetic field [15].

A silicate often used as either a physical or covalent cross linker in polymer hydrogels is Laponite clay, $Na^{+}_{0.7}[(Mg_{5.5}Li_{0.3})Si_8O_{20}(OH)_4]_{0.7}$, which is comprised of synthetic and charged silicate nano particles with a negative charge of 0.7 per unit cell (http://www.laponite.com). Poly(ethylene oxide)s (PEO)s have frequently been used to synthesize physically cross linked PEO Laponite hydrogels and poly(N isopropyl acrylamide) (PNIPAAm) based polymers have been used to synthesize mostly covalently cross linked polymer Laponite hydrogels [16 18]. Although the exact molecular interac tions between the polymer and the Laponite are still not clear, covalently cross linking PNIPAAm to Laponite requires monomer polymerization to be initiated at the silicate nanoparticle surface [19,20]. The first PNIPAAm Laponite hydrogels were developed by Haraguchi et al., who later used the stimuli responsiveness of

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PNIPAAm for cell cultivation on and cell sheet detachment from the hydrogel surfaces [19,20]. This group studied cell adhesion and proliferation of human hepatoma cells, dermal fibroblasts and umbilical vein endothelial cells on PNIPAAm Laponite hydrogels, which were found to be strongly dependent on the silicate concen tration [20].

Successful formulations of physically cross linked hydrogel net works made from PEO and Laponite have strong specific interac tions, such as hydrogen bonding and ionic interactions [21 23]. Since the Laponite nanoplatelets are synthetic polyions they act as multifunctional cross linkers to the PEO polymer chains. The cross linking of these hydrogels is reversible because, under defor mation, the PEO chains may attach and detach from the nanopar ticle surfaces [24 27]. According to published research the shear deforms large Laponite PEO aggregates within the hydrogels and exposes fresh Laponite surface area for the formation of new poly mer bridges, which rapidly form a network that spans the entire solution and forms a gel [22]. More quantitative studies by Nelson et al. found that PEO adsorbed onto Laponite particles forms a com pact layer of mostly trains and loops on the face of the nanoparticle surface, and large loops around the edges [28].

The biomedical relevance of physically cross linked PEO Lapo nite hydrogels has been suggested only recently, when preliminary cell growth studies showed that murine fibroblast cells cultured on the surfaces of PEO Laponite gels attach and proliferate easily [3,29,30]. Cell adhesion on PEO Laponite nanocomposites could be tuned by formulating the composition and structure of the polymer nanocomposites [30]. Addition of chitosan to the PEO Laponite hydrogels improved cell adhesion and spreading, while maintaining mechanical strength, injectability and self healing properties without hampering the mechanical strength of the injectable hydrogels [29]. In the presence of phosphate buffer solu tion (PBS) the PEO Laponite network can be made stable via the hydrogel formulation. The charges on the Laponite surfaces are negative and the edge charges are pH dependent, with negative charged edges favored at high pH (pH $\sim$ 10) and positive charged edges present at pH = 7 [23,31]. Thus the elstrostatic interactions of the PEO macromolecules (oxygen atoms) with ions, e.g. from PBS, may stabilize the hydrogels and prevent flocculation.

Controlling and guiding cell adhesion on biomaterials is impor tant to a variety of applications in tissue engineering and biotech nology. Biocompatibility can be improved by limiting non specific adsorption of proteins and promoting specific cell matrix interac tions. Specific interactions between cells and the matrix are impor tant because they regulate cell function, tissue homeostasis and matrix remodeling [32,33] and because cell shape influences dif ferentiation and viability [34,35].

Synthetic silicate nanoparticles (Laponite) share some similarity to bioactive materials, such as bioactive glasses, in terms of chem ical composition and dissolution products. The silicate nanoparti cles have been shown to degrade into non toxic products (Na^+, $Si(OH)_4$, Mg^{2+}, Li^+), some of which are similar to the degradation products of bioactive glasses (Na^+, $Si(OH)_4$, Ca^{2+}, PO_4^{3} [31,36,37]. Several reports have suggested that silica may act as a cross linking agent in connective tissue [38] and that ionic dissolution products of bioactive glasses enhance cell proliferation [39,40]. Moreover, divalent cations, such as magnesium ions (dissolution products of some silicates), play significant roles in cellular adhe sion to biomaterial surfaces that are mediated mainly by adhesion proteins belonging to the integrin family [41,42]. Other studies have shown that silicate degradation products from bioactive glasses can be effectively cleared from the body [43]. Thus silicate nanoparticles such as Laponite may have the potential to be used as next generation bioactive materials not only due to their unique biological properties but also due to advantages such as high pur ity, low cost and easy processing.

Here we present in vitro cytocompatibility data for Laponite cross linked PEO hydrogel films using MC3T3 E1 mouse preosteo blast cells. First we investigate the material properties, such as hydration, dissolution/degradation and mechanical strength, all of which influence cell adhesion, growth and mineralization. Then we present cell adhesion, spreading, proliferation and mineraliza tion data as a function of film composition. With this feasibility study we assess the potential of using Laponite cross linked PEO nanocomposites to create bioactive scaffold materials with con trolled cell adhesion and mineralization properties.

2. Methods and materials

2.1. Preparation of silicate cross linked PEO nanocomposites

PEO with a molecular weight M_w of 10^6 g mol^{-1} and a molecular mass distribution of 1.5 was purchased from Polysciences Inc. Lapo nite (LRD), from Southern Clay Products Inc., is a synthetic hectorite type silicate composed of polyions $Na^+_{0.7}[(Mg_{5.5}Li_{0.3})Si_8O_{20}(OH)_4]_{0.7}$. The Laponite nanoparticles have an average diameter of 25 30 nm and a thickness of approximately 1 nm. Laponite cross linked PEO films were prepared via gel/solution exfoliation while optimal solu tions were obtained for a particular polymer silicate ratio, pH and ionic strength [3,44,45]. Hydrogels composed of X wt.% PEO, 5 X wt.% LRD and 95 wt.% water were prepared at ambient tem perature. The solution pH and ionic strength of the hydrogels was controlled by adding 10^{-4} M NaOH and 10^{-3} M NaCl, respectively. Hydrogels were spread onto glass slides and dried at 25 °C in desic cators and subsequently under vacuum [3]. The composition of the films after solvent evaporation (Fig. 1a) was calculated from the ini tial weight of polymer and silicate (by mass fraction). After drying, all films were 70 100 μm thick.

2.2. Hydration kinetics

The hydration behavior of nanocomposite films was assessed in PBS at physiological temperature (37 °C) for 24 h (n = 3). The swol len films were removed from PBS and were weighed after blotting off excess water from the sample surface with a filter paper. The hydration degree was defined as the weight ratio of the net liquid uptake to the dried nanocomposite film.

$$\text{Hydration Degree} \quad \frac{M_{wet} \quad M_{init}}{M_{init}} \times 100\%$$

M_{init} is the initial weight and M_{wet} is the wet weight of the nano composite films.

2.3. In vitro dissolution properties

The effect of silicate on the dissolution of nanocomposites was determined by soaking the nanocomposite films (cut into 1.5 cm diameter discs, n = 3) in PBS at 37 °C. The medium was changed every other day. At predetermined time points films were removed and lyophilized to determine their dried weight. The dissolution degree was calculated by measuring the mass loss of the sample.

2.4. Protein release kinetics

To determine the effect of silicate on protein release 0.05 wt.% albumin was entrapped within the nanocomposite hydrogels using solvent mixing. Films were fabricated from the well mixed hydro gel using solvent evaporation. Release kinetics of the entrapped protein were determined by soaking the nanocomposite films (1.5 cm diameter discs, n = 6) in 1 ml of PBS at 37 °C in 24 well plates. At predetermined time points 1 ml was withdrawn from

(a)

Nanocomposites	PEO (%)	LRD (%)
40% Silicate	60	40
50% Silicate	50	50
60% Silicate	40	60
70% Silicate	30	70

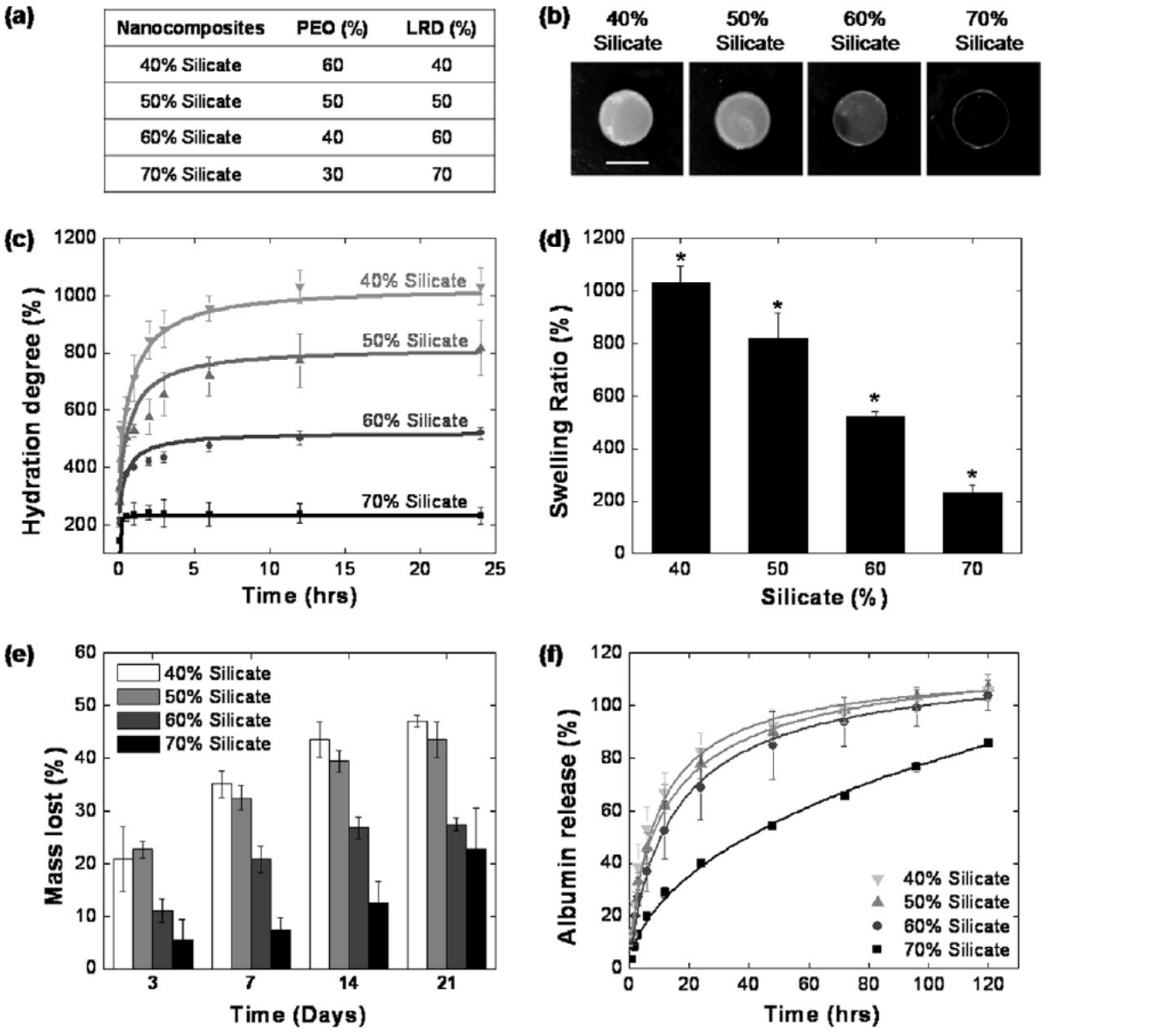

Fig. 1. Silicate enhances the structural stability of nanocomposites and retards release of entrapped protein. (a) Composition of dried PEO–Laponite nanocomposite films. Laponite (LRD) is a synthetic and layered silicate clay. (b) Nanocomposite films after submersion in PBS (24 h). Films containing low amounts of silicate readily swell and become opaque. Addition of silicate improves optical transmission. Scale bar represents 1.5 cm. (c) and (d) Time and concentration dependent swelling of PEO–Laponite nanocomposite films measured at 37 °C. A pure PEO film (not shown here) readily dissolves in PBS within minutes. Addition of silicate reduces hydration and improves the structural stability of films when submersed in PBS. The saturated hydration degrees of the nanocomposite films are significantly different from each other and are indicated by an asterisk (Student's t-test, $P < 0.05$, $n = 3$). (e) Time transient mass loss of nanocomposite films in PBS solution. The solutions were changed every other day. A trend can be seen showing that the addition of silicate retards the dissolution of nanocomposite films. The mass lost for all the samples are significantly different for days 3, 7 and 14 (except for the 70% sample). (f) Cross-linking of the PEO network by silicate nanoparticles retards the release of entrapped albumin. Samples having low silicate concentrations suggest a rapid release of albumin. Samples having high silicate concentrations prolonged the protein release.

each well and fresh PBS was added. The albumin concentration released into solution was determined using the Commassie blue assay. The absorbance at 595 nm was recorded with a SpectraMax M5 microplate reader. All release studies were carried out in triplicate. The accumulated albumin in solution was calculated and plotted versus time to determine the protein release kinetics. The results were presented in terms of cumulative release as a function of time:

$$\text{Cumulative amount released} \quad \left(\sum_{t\ 0}^{t\ t} M_t \Big/ M_0\right) \times 100\%$$

where $\sum_{t\ 0}^{t\ t} M_t$ is the cumulative amount of albumin released from the nanocomposite at time t and M_0 is the initial amount of albumin loaded.

2.5. Mechanical properties of nanocomposites

The mechanical properties of fully hydrated nanocomposite films (in PBS at 37 °C) were measured using an AR2000 stress controlled rheometer (TA Instruments Ltd) ($n = 6$). Stress dependent oscillatory shear experiments (frequency ~1 Hz) were performed at 37 °C. The storage modulus G' and loss modulus G'' of the swollen nanocomposite films were measured using a 20 mm parallel plate geometry and a gap of 150 μm. A solvent trap was used to minimize drying of the swollen hydrogel film. Unconfined compressive tests were performed on fully hydrated nanocomposite films using an ARES rheometer (TA Instruments Ltd.) equipped with a 2000 gf transducer ($n = 6$). A cross head speed of

0.01 mm s^{-1} was used and all the samples were pre stressed. The cross section of samples was 15 mm in diameter. The compression limit was 70% strain to protect the load cell.

2.6. Cell adhesion, spreading, proliferation and viability

The biological properties of the nanocomposites were deter mined by seeding MC3T3 E1 subclone 4 mouse preosteoblast cells (American Type Culture Collection) on nanocomposite films. Cells were grown in minimal essential medium alpha (MEM α) (Gibco) supplemented with 10% fetal bovine serum, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Films were cut into 1.5 cm diame ter discs, briefly submersed in 70% ethyl alcohol and allowed to dry under sterile conditions for all experiments. Before seeding the cells on the nanocomposites the films were allowed to hydrate in the medium for 24 h. Tissue culture polystyrene (TCPS) plates were used as a control. Cell adhesion and spreading were deter mined by seeding the nanocomposite wells at 15,000 cells cm^{-2} ($n = 3$). Three hours post seeding the cells were fixed using 3.7% formaldehyde solution and the cytoskeleton of the cells was la beled with Alexa Fluor® 488 phalloidin fluorescent dye (Invitro gen). Fluorescent images were taken with an Olympus FV1000 confocal microscope with an excitation wavelength of 488 nm. Representative images are shown. Cell adhesion and spreading was quantified using ImageJ software (National Institutes of Health, Bethesda, MD) ($n = 6$). To evaluate cell spreading as a func tion of film composition raw confocal images were converted into black and white images using a threshold. The cumulative area fraction was obtained from the processed image and normalized to cell number. Cell proliferation and viability were determined by seeding the films and control wells with the preosteoblast cells (10,000 cells cm^{-2}) in ultra low attachment 24 well plates (Corn ing) and the medium was changed every other day ($n = 3$). Cell number was quantified by CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega) following the manufacturer's proto col. Cell viability was determined using a Multitox Fluor Multiplex Cytotoxicity Assay Kit (Promega). The cytotoxicity of silicate was also tested by preparing Laponite solutions in distilled water. After seeding cells for 3 h the cells were exposed to different concentra tions of silicate and incubated for 24 h ($n = 9$). After this time per iod viability was measured.

2.7. Differentiation of preosteoblast cells

The effect of silicate on cell differentiation was determined by seeding preosteoblast cells on nanocomposites films for 7 and 28 days in osteogenic medium, consisting of MEM α, 10% fetal bo vine serum, 0.284 μM ascorbic acid phosphate, 10 mM β glycero phosphate, 100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin. Alkaline phosphatase (ALP) activity was used as an early marker for osteoblast differentiation. After culture for a predetermined period ($n = 3$) the cell grown nanocomposite films were washed twice with PBS, followed by addition of a cell lysis buffer contain ing 0.2% Triton X 100 for 45 min. Samples were collected and stored at 20 °C. ALP activity was measured using a Quanti Chrom™ Alkaline Phosphatase Assay Kit according to the manufac turer's instructions (BioAssay Systems). ALP activities were normalized to the total protein present in the samples. Total intra cellular protein was quantified with the RC DC Protein Assay fol lowing the manfacturer's protocol (Bio Rad). Calcium phosphate present in mineralized extracellular matrix (ECM) was detected using standard von Kossa staining. Nanocomposite films seeded with preosteoblast cells in osteogenic medium for 28 days were fixed with 3.7% formaldehyde solution ($n = 3$). Samples were then rinsed five times with deionized water. Afterwards samples were incubated with 1 ml of 5% silver nitrate solution and were exposed to UV radiation for 1 h.

2.8. Statistical analysis

Data are presented as mean ± standard error of the mean values. Statistical analysis was performed using Minitab (version 16, Minitab Inc., USA) to determine statistical differences. Statistical comparisons were performed with Student's t test or one way analysis of variance (ANOVA) for an average of 3 5 replicates. After ANOVA was performed on the data set, Tukey's method was used to test all pairwise mean comparisons. Statistical significance for all tests was set at $P < 0.05$.

3. Results and discussions

One of the important factors in developing new biomaterials for biomedical applications is to control interfacial interactions between cells and materials while simultaneously optimizing materials performance. In the following we discuss the material properties and their influence on cell adhesion, growth and mineralization.

3.1. Silicate enhances the structural integrity of nanocomposites

The surface properties of polymeric biomaterials are signifi cantly affected by water at the interfaces, as water molecules directly influence protein and cell adhesion. PEO is a highly hydro philic polymer that has shown significant changes in properties when dissolved in water [46]. As a first step in the development of silicate cross linked PEOs we investigated the effect of silicate on the hydration kinetics of the polymer nanocomposite system under physiological conditions (in PBS and at 37 °C). The hydration degree is defined as the ratio of solvent absorbed by the material to its dried weight. Since both the PEO and the silicate are hygro scopic in nature, the PEO silicate network dissolves slowly when subjected to distilled water. Surprisingly, when the same network is subjected to PBS or other physiological solutions, structural integrity is observed and maintained and the network does not dis solve readily (Fig. 1b). An explanation for this behavior could be the electrostatic interactions of the PEO macromolecules (electro negative oxygen atoms in PEO) with ions, e.g. from PBS, which lead to stabilization of the hydrogels. This effect suggests that the phys ical cross linking between Laponite and PEO is dominated by ionic interactions and these interactions increase when more ions (e.g. from PBS) are present.

Fig. 1c and d shows that the hydration degree is directly propor tional to the silicate concentration. The degrees of "saturated hydration" for all the nanocomposite films are significantly differ ent from each other (Student's t test, $P < 0.05$, $n = 3$). Nanocompos ite films containing lower amounts of silicate have higher degrees of hydration and vice versa. Thus the presence of silicate and its ionic interactions with the polymer and surrounding water molecules are responsible for the structural stability of the nano composite network when submersed in PBS. The degree of satura tion hydration of the nanocomposites also suggests different porosities of the networks. For example, nanocomposites contain ing low amounts of silicate cross linker (high hydration degrees) should have larger pores compared with nanocomposites contain ing high silicate concentrations (low hydration degrees).

The effect of silicate on the short term stability of nanocompos ites under physiological conditions was determined by monitoring transient weight loss over time of the nanocomposite films in PBS and at 37 °C. From the literature we know that Laponite disinte grates very slowly at pH < 9 [31,36], whereas PEO is hydrolytically

stable under physiological conditions [31,46]. Time dependent rheological measurements over a period of approximately 2 months (data not shown) suggested that the very slow dissolution of Laponite did not influence the mechanical testing data or hydrogel stability. The effect of silicate composition on the dissolution properties of the nanocomposite is shown in Fig. 1e. All sample compositions show weight loss, and a trend is visible. Statistical analysis of these data reveals a significant difference between the samples (except between the 40% and 50% samples) when measured on days 3, 7 and 14. Films having low silicate compositions show higher dissolution degrees compared with films having high silicate compositions. This effect indicates that the addition of silicate retards the dissolution of the nanocomposite films by cross linking more PEO chains within the hydrogel. PEO that is not cross linked may diffuse slowly out of the hydrogel and lead to weight loss. Thus short term dissolution of the physically cross linked hydrogel is dominated by dissolution of PEO chains that are not or not sufficiently cross linked to the Laponite. Long term dissolution of the hydrogel due to Laponite dissolution over a period of months will be evaluated in more detail in the future. Overall the data suggest that further hydrogel formulation will allow the dissolution properties to be tailored to fit specific applications in bone repair.

3.2. Polymer nanocomposites retard the release of entrapped protein

PEO Laponite based hydrogel cell constructs as well as cell free hydrogels containing growth factors can be envisioned for use as scaffolds. To test if macromolecular model proteins interact with the Laponite or can be released easily from the hydrogel we have measured the effect of silicate concentration on the release kinetics of albumin (Fig. 1f). All the nanocomposite films show an initial burst release, with the release becoming more sustained as the silicate concentration increased. Pure PEO films dissolve in PBS and release entrapped protein immediately, thus the release was too fast to be measured.

The burst release of entrapped albumin can be influenced by parameters such as the processing conditions, surface characteristics, surface adsorption, film morphology and porosity [47]. Fig. 1f shows that the release of entrapped protein appears to follow a similar trend to the hydration kinetics (Fig. 1c) and the dissolution properties (Fig. 1d). The initial burst release might be attributed to the network structure and degree of cross linking. A dense network, containing higher amounts of silicate cross linkers, results in a slower release of albumin. Higher amounts of silicate nanoparticles also provide a torturous path for macromolecular protein diffusion, which slows down the release of albumin even more. The charged and PEO covered Laponite nanoparticles may interact with the albumin molecules but do not prevent their release. Thus if one wishes to use Laponite as a drug depot within the hydrogel then a drug could be attached to the charged nanoparticles before these are covered with PEO. Overall, our data can be compared with those of Zhang et al., who demonstrated that the use of inorganic cross linkers in polymer nanocomposites overcame the burst release of entrapped drugs [48].

3.3. Silicate improves mechanical strength and network stability

A consideration of mechanical properties is essential to the design of materials with controlled cell adhesion properties. For a polymer nanocomposite that is to be used for cellular adhesion there is a need to formulate the surface chemistry to be compatible with what cells require for adhesion. In a comparable manner, nanocomposites inhibiting cell adhesion must have an optimized surface chemistry to reduce protein interaction [49,50]. Many of the surface properties depend on structural changes, such as the deformation and orientation of the nanocomposite network, which can be greatly affected by the polymer to silicate ratio. Since appropriate mechanical properties are essential for structural stability, it is imperative to understand those polymer nanoparticle interactions that are influenced by mechanical deformation.

The mechanical properties of nanocomposites films in the fully hydrated state (hydrogel like) were evaluated here by unconfined compression testing and oscillatory stress sweep experiments. Fig. 2a and b summarizes the unconfined compression testing data for fully hydrated nanocomposite films at 37 °C. The results show that all the nanocomposites have biphasic compressive properties. A biphasic model assumes that the overall mechanical properties of a material depend on the matrix deformation as well as on the movement of the entrapped fluid through the pores during the deformation process. The initial deformation (toe region) is caused by the outflow of entrapped fluid within the nanocomposites network, whereas the secondary deformation (knee and linear region) is resistance generated by the nanocomposite network. The biphasic compressive properties of the nanocomposite network are due to the nonlinear permeability response, especially at high pressures and strains. With an increase in compressive strain and hydraulic pressure, permeability decreases. The decrease in permeability is due to the decrease in pore size of the matrix, which is subjected to compressive forces.

Compressive moduli of the hydrated nanocomposite films were taken from both the knee and linear regions of the stress curves. An increase in silicate concentration results in an increase in compressive modulus (Fig. 2b). Statistical analysis of these data shows that the moduli determined in the knee region are statistically different from each other, except for the samples containing 40% and 50% silicate. The compressive moduli from the linear region increase linearly with increasing silicate concentration; they are significantly different from each other. The increase in modulus is mainly attributed to the increase in cross linking density between silicate nanoparticles and polymer.

The oscillatory shear experiments were used to determine the viscoelastic properties of the nanocomposite networks and how these relate to their compositions (Fig. 2c and d). Significant differences (Student's t test, $P < 0.05$, $n = 3$) between the moduli of samples containing different amounts of silicate were observed. Nanocomposites containing low amounts of silicate exhibit shorter linear viscoelastic regions, indicating a network structure that can be disrupted at high stresses. At low stresses the elastic modulus (G') was always larger than the viscous modulus (G'') for samples with low silicate concentrations. For samples having 40% and 50% silicate the cross over stresses and cross over moduli were dependent on the silicate concentration. Nanocomposites containing higher amounts of silicate have broader linear viscoelastic regions (Fig. 2c) and no cross over stress or cross over modulus was observed within the range measured. From these data it is evident that addition of silicate improved the mechanical stability of the nanocomposite network.

3.4. In vitro cytocompatibility of silicate nanoparticles

Laponite nanoparticles are layered magnesium silicates with a well defined structural and chemical composition. They can be reproducibly prepared in large quantities and at high purity. Despite properties such as positive and negative charges on the Laponite as well as dissolution properties, very few studies have proposed using Laponite based polymer nanocomposites for biomedical applications [1,2,20]. To our knowledge there have been no reports on the acute toxicity of Laponite nanoparticles. To evaluate the potential toxicity of Laponite nanoparticles we first subjected preosteoblast cells to different concentrations of silicate nanoparticles (0.0035 35 mg ml^{-1}), the results of which are

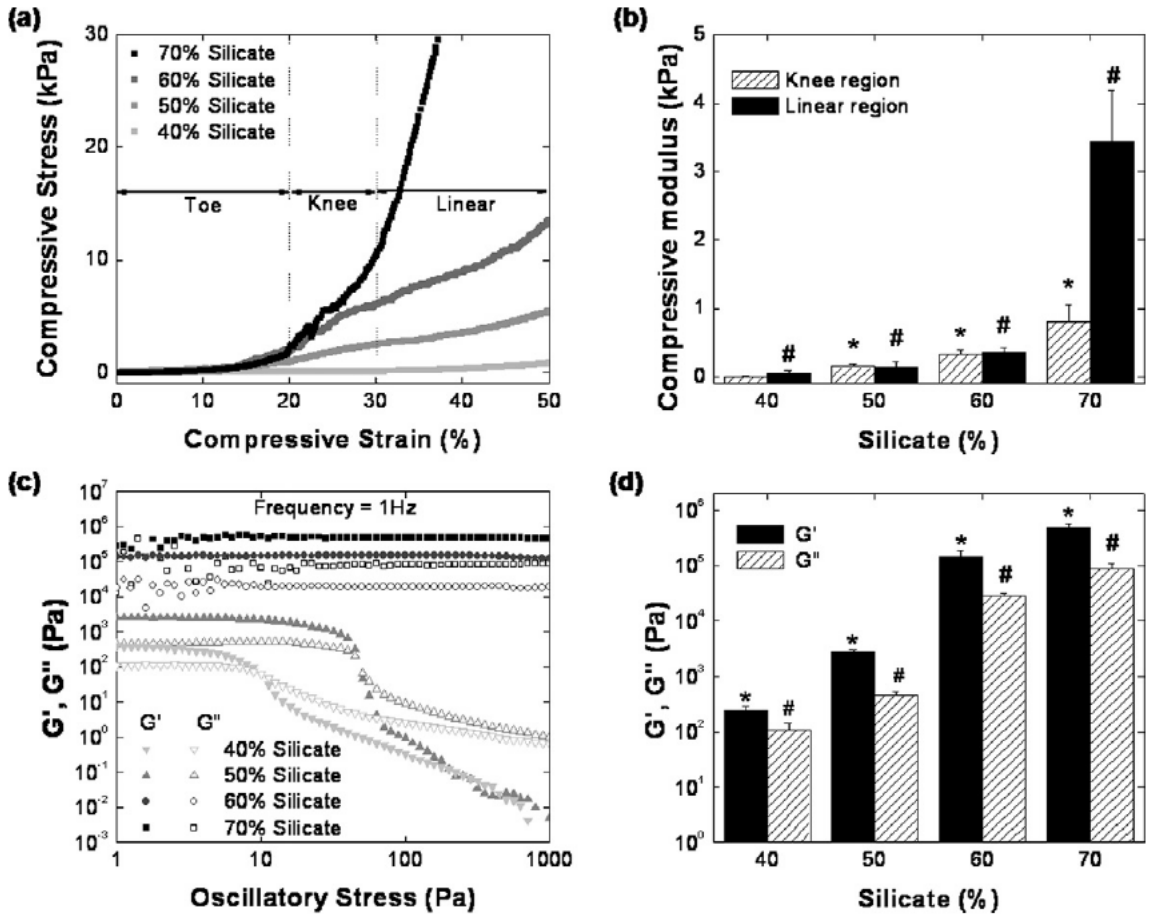

Fig. 2. Silicate improves the mechanical strength and network stability of nanocomposite films. (a) Unconfined compression tests of fully hydrated nanocomposite films suggest biphasic compressive properties. The initial deformation (toe region) is caused by outflow of entrapped fluid within the nanocomposite network, whereas the secondary deformation (knee and linear region) is resistance generated by the nanocomposite network. (b) Addition of silicate significantly enhances compressive modulus. The modulus of the nanocomposites network was determined from the knee and the linear region. The moduli determined from the knee region are significantly different for all the samples (indicated by an asterisk) except between 40% and 50% samples. Whereas, moduli determined from the linear region are significantly different from each other (indicated by hatching). (c) and (d) Viscoelastic properties of swollen nanocomposite films are strongly affected by the silicate concentration. Films containing low amounts of silicate show a crossover point between G' and G'' which indicates yielding of the nanocomposite network structure under deformation. Overall, an increase in silicate concentration improves the network stability and mechanical properties. (d) With an increase in silicate concentration both the elastic (G') and viscous moduli (G'') increase (data taken from (c) at 1 Hz and 5 Pa). Both G' (indicated by an asterisk) and G'' (indicated by hatching) are significantly different (Student's t-test, $P < 0.05$, $n = 3$) for different composition.

Table 1
Cell viability of preosteoblast cells remains unchanged when dosed with different concentrations of Laponite/H_2O solution (average viability ± standard deviation, $n = 9$).

Silicate concentration (Laponite/H_2O solution)	Viability (%)
0 mg ml ^{1}	88 ± 2
0.0035 mg ml ^{1}	84 ± 3
0.35 mg ml ^{1}	92 ± 2
35 mg ml ^{1}	88 ± 4

displayed in Table 1. Overall the data indicate that the Laponite nanoparticles did not show acute toxicity to preosteoblast cells, as the overall viability remained unchanged after administering up to 35 mg ml^{-1} Laponite to cells. It was impossible to add higher silicate concentrations (without further formulation) due to sili cate precipitation. This preliminary study suggests that silicate nanoparticles are non toxic to cells at low concentrations and can potentially be used for various biomedical applications. As a next step we investigated the effect of silicate nanoparticles within polymer nanocomposites on adhesion, proliferation and differenti ation of MC3T3 E1 subclone 4 mouse preosteoblast cells.

3.5. Silicate enhances cell adhesion, spreading and proliferation

Cell adhesion is one of the important parameters that control cell biomaterial interactions. Previous attempts to induce and tune cell adhesion on polymer surfaces include the incorporation of cell recognition motifs, such as the RGD peptide sequence [51,52]. Other methods used are surface patterning to control cell adhesion, e.g. surface modification with hydrophilic or hydropho bic polymers, electrically charged polymers, stimuli responsive polymers or growth factors [53]. Compared with these methods, our addition of Laponite to PEO to control cell adhesion is a simple, highly cost effective and reproducible procedure.

Here, preosteoblast cells were cultured on the nanocomposite surfaces containing different amounts of silicate. Fig. 3a c shows that the addition of silicate results in enhanced cell adhesion and spreading, and a trend is visible. Almost a 4 fold increase in cell adhesion was observed when the silicate concentration was in creased from 40% to 70% (Fig. 3b). The nanocomposite films contain ing 40% and 50% silicate show significantly lower (ANOVA, $P < 0.05$, $n = 5$) cell spreading when compared with the 60% and 70% silicate samples and positive control (TCPS). Thus the presence of silicate particles seems to play a major role in influencing cell adhesion.

At low silicate concentrations preosteoblast cells display a spherical morphology, which is commonly found in "non adhesive" environments, whereas at higher silicate concentrations cells display a flat and well spread morphology that is usually found in two dimensions. Almost a 3 fold increase in cell spreading was observed with an increase in silicate concentration from 40% to 70% (Fig. 3c) (statistically different, ANOVA, $P < 0.05$, $n = 5$). At the higher silicate concentrations cells developed protruding cellular arms and formed connections between adjacent cells.

A considerable difference in cytoskeleton organization was observed when the silicate concentration was increased from 40% to 70% (Fig. 3a). The F actin in cells seeded on 40% silicate nanocomposites appeared disrupted and disorganized, whereas at higher silicate concentrations actin stress fibers were clearly visible, with

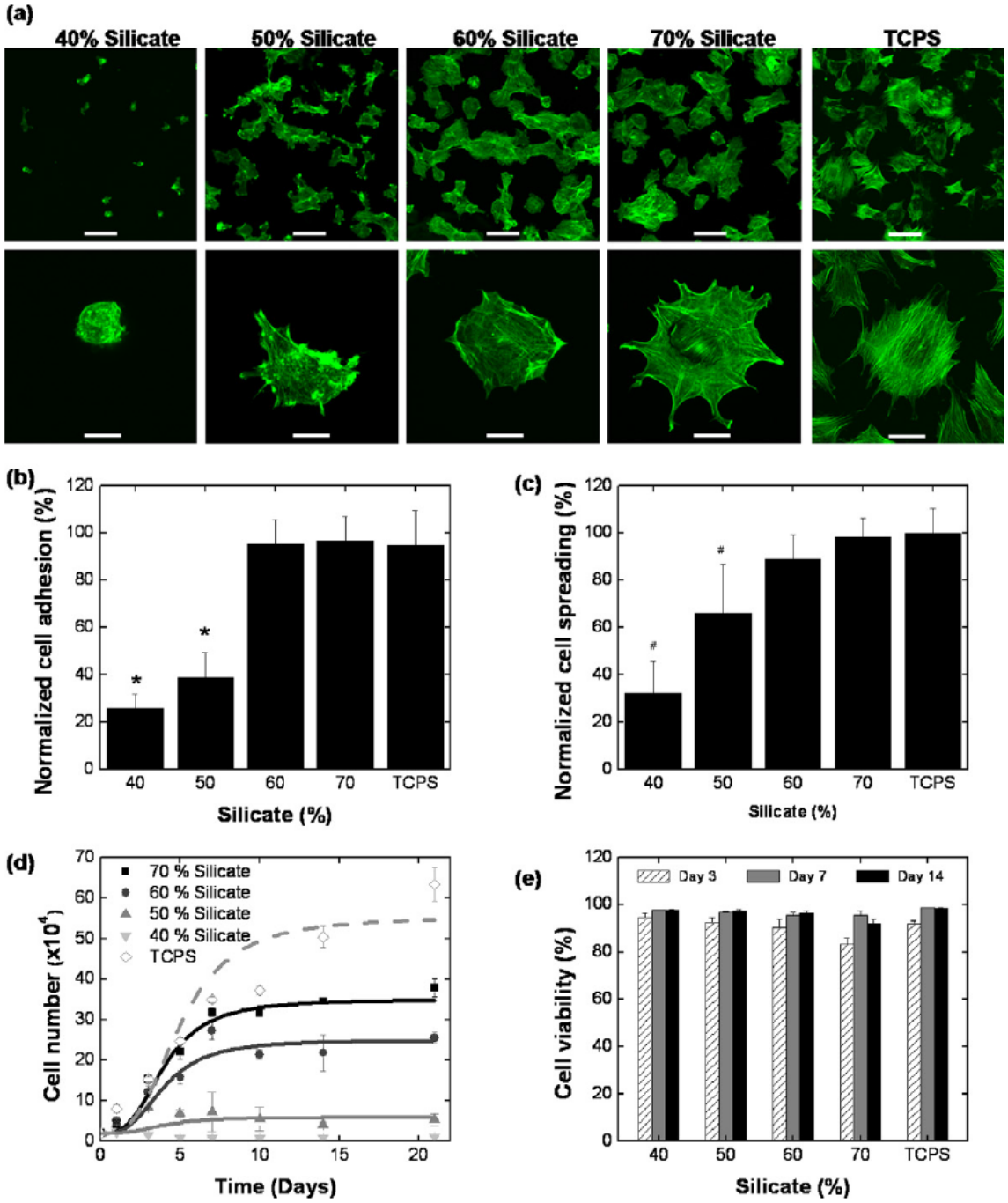

Fig. 3. Silicate enhances cell adhesion, spreading and proliferation on PEO–Laponite surfaces. (a) Confocal images of F-actin stained preosteoblast cells seeded on nanocomposite surfaces and fixed after 3 h. Pure PEO (not shown here) does not support cell adhesion. With an increase in silicate concentration, cells adhere and spread on the nanocomposite surfaces. A significant difference in cytoskeleton organization is observed when the silicate concentration is increased. Lower silicate concentrations show that F-actin in the seeded cells appears somewhat disorganized while cells assume a more or less spherical morphology. High silicate concentrations show that cells have well-organized F-actin bundles. Scale bar represents 100 (top row) and 40 μm (bottom row). Cell adhesion (b) and spreading (c) were quantified using ImageJ (NIH) software. When silicate concentration is increased from 40% to 70% a 4-fold increase in cell adhesion and almost 3-fold increase in cell spreading was found. Nanocomposites containing 40% and 50% silicate have significantly lower cell adhesion (indicated by an asterisk) and spreading (indicated by hatching) compared with 70% and TCPS. (d) Cell growth curves including a TCPS control. Initial seeding density was 10,000 cells cm^{-2}. (e) Cell viability of preosteoblast cells on nanocomposite films with different compositions (no significant difference). Initial seeding viability 97 ± 1% (average viability ± standard deviation, $n = 3$).

F actin very well organized in thick bundles that were stretched in side the cells. A recent study suggests that a complex interplay be tween the cell cytoskeleton, mechanical forces and biochemical signaling networks are responsible for cell cycle progression and cell fate (including switching between growth, differentiation and apoptosis) [54,55].

Cell proliferation on silicate cross linked nanocomposites is summarized in Fig. 3d. Nanocomposites containing 40% silicate do not support cell growth, while cells readily proliferate on higher silicate concentrations. Samples having higher silicate concentra tions exhibit sigmoidal shaped growth curves where the number of cells in the plateau region is proportional to the amount of sili cate. Since pure PEO does not support protein attachment and thus cell proliferation, the presence of silicate must be responsible for the enhanced cell growth. Although cells exhibit a silicate concen tration dependent growth profile, the viability of cells remains high irrespective of the composition (Fig. 3e).

Fig. 3d shows that the number of cells on TCPS at the plateau phase of the cell growth curve is significantly higher compared with all the other nanocomposites compositions. Similar results were observed using fibroblast cell lines where cells growing on nanocomposite surfaces did not reach confluency in the plateau phase [30]. Experiments showed that the cell numbers reached in the plateau phase of the cell growth curves were not a result of contact inhibition between cells. Instead, growth inhibition was found to be dependent on the number and distribution of "cell repellant" PEO and "cell adhesive" silicate regions on the nanocom posite films [30].

3.6. Silicate augments alkaline phosphatase activity and in vitro mineralization

After determining if and how preosteoblast cells adhere to the silicate cross linked PEO surfaces, the activity of osteoblast cells was investigated in more detail. MC3T3 E1 subclone 4 preosteo blast cells can be differentiated into osteoblast cells by adding ascorbic acid and β glycerophosphate to the culture medium [56,57]. Alkaline phosphatase (ALP), an early stage marker of osteoblast phenotype and calcified matrix production [58], has been found to increase with an increase in silicon/silicate concen tration [59]. Fig. 4a shows this normalized to the total protein con centration to account for differences in cell number. These data show that an increased silicate nanoparticle content increases ALP production in a dose dependent manner after 7 and 28 days. When the silicate concentration was increased from 40% to 70% a 6 fold increase in ALP activity was observed on day 7 and a 10 fold increase on day 28. Thus the presence of Laponite enhanced ALP production significantly, suggesting a similarity to bioactive glasses. In addition, changes in cell morphology that result due to the presence of the silicate nanoparticles (which induced changes in hydrogel stiffness) may also influence ALP production because cell morphology plays a vital role in governing cell dynam ics, such as actin polymerization, signaling cascades, protein syn thesis and many other cell functions. Statistical analysis of the data (ANOVA, $P < 0.05$, $n = 3$) indicated that the ALP activity of cells seeded on nanocomposites containing 40%, 50% and 60% silicate were lower compared with the positive control (TCPS).

In addition to investigating ALP activity, von Kossa staining was used to visualize the extent of phosphate in the mineralized matrix produced by osteoblast cells. Fig. 4b and c qualitatively and quanti tatively show mineralized phosphate production. A silicate concen tration dependent response in the amount of mineralized phosphate was observed. When the silicate concentration increased from 40% to 70%, a more than 10 fold increase in mineralized ECM was measured. All the nanocomposites show significantly higher amounts of mineralized matrix compared with the negative control. Moreover, nanocomposites containing 70% silicate showed almost twice the mineralized matrix when compared with the positive control (TCPS). This indicates that the silicate must be responsible for the increase in mineralized ECM.

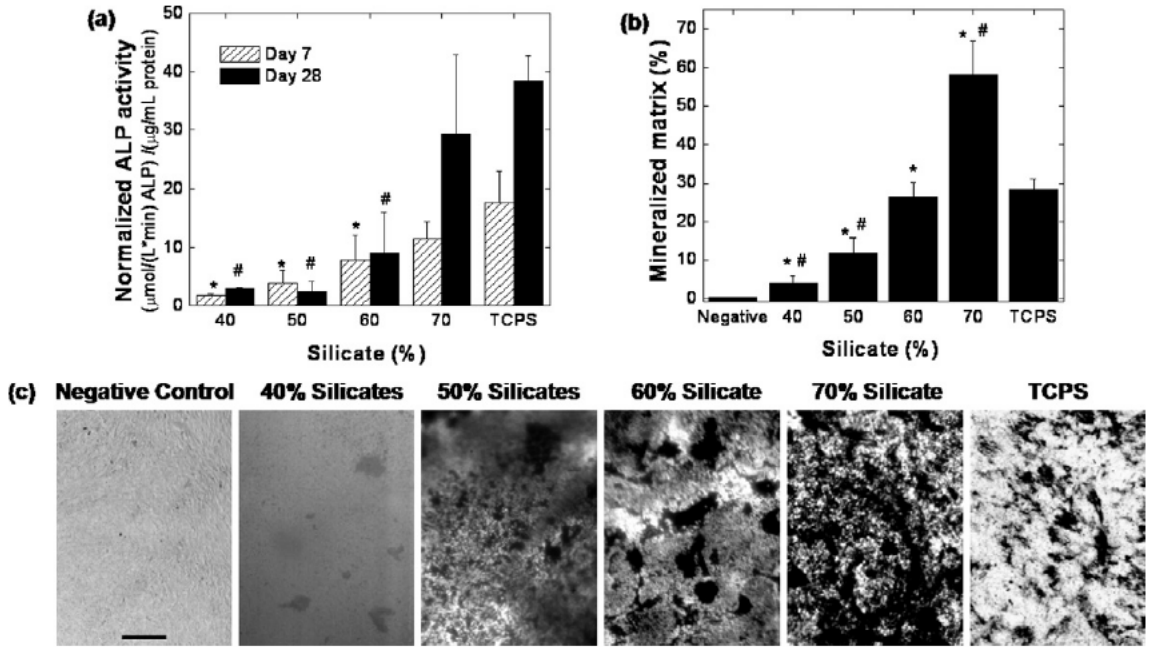

Fig. 4. The presence of silicate influences ALP activity and in vitro mineralization. (a) ALP activity increases with an increase in silicate concentration. When the silicate concentration increases from 40% to 70% (see Fig. 1) a 6-fold increase in ALP activity was observed on day 7 and 10-fold increase on day 28. The APL activity of 40%, 50% and 60% silicate samples are significantly different (Student's *t*-test, $P < 0.05$, $n = 3$) than TCPS on day 7 (indicated by an asterisk) and day 28 (indicated by hatching). (b) and (c) Qualitative and quantitative evaluation of mineralization data from nanocomposites having different silicate compositions. Data were measured after 28 days. All the nanocomposites show significantly higher mineralization compared with the negative control (indicated by an asterisk). When the silicate concentration increased from 40% to 70% a 30-fold increase in mineralized ECM was observed. Nanocomposites containing 70% silicate show significantly enhanced mineralization when compared with the positive control (indicated by hatching), indicating that silicate influences mineralization. Scale bar represents 50 μm for all images.

For better data interpretation our results can be compared with those obtained by Sun et al., who observed very similar effects using the bioactive ceramic akermanite (calcium magnesium sili cate) [60]. This group observed that akermanite enhanced osteo blast differentiation of human bone marrow stromal cells in vitro by up regulating osteogenic gene expression. Several other reports have suggested that divalent cations such as Ca^{2+} (and Mg^{2+}, e.g. from Laponite used in our study) play a significant role in cellular adhesion to biomaterial surfaces, mediated mainly by proteins of the integrin type [41,42]. Moreover, the silicate can activate com plex gene transduction pathways, leading to enhanced cell differ entiation and osteogenesis [40,55]. Therefore, the enhanced differentiation of preosteoblast cells observed in our study may be explained by the presence of silicate nanoparticles (Laponite) that play an important role in stimulatory processes.

4. Conclusions and future directions

Based on the cell culture experiments presented here, we con clude that cellular adhesion, spreading and proliferation can be modified by changing the silicate concentration within the Lapo nite PEO system. Material properties, such as hydration, dissolu tion and mechanical properties, influence cell adhesion and allow the induction of specific functionalities, such as bioactivity, while simultaneously optimizing material performance. The increase in mineralized phosphate produced on the nanocomposite surfaces indicated that the silicate nanoparticles influence the differentia tion of preosteoblast cells.

Overall, this feasibility study demonstrates the ability of using Laponite cross linked PEO nanocomposites to create bioactive materials. So far the new materials presented here represent only model systems, as further optimization of their chemical and phys ical properties is necessary before translation of this research into clinical applications. Future work will investigate the long term dissolution properties of Laponite in order to better control bioac tivity. While we have here used high molecular weight PEO for model PEO Laponite hydrogels with tunable mechanical proper ties and viscoelasticity, PEO by itself is not degradable in vivo and high molecular weight PEO cannot be cleared by the body. Thus, developing the chemistry to include degradable peptide sequences into the PEO chains may allow the development of PEO Laponite based hydrogel networks that can be degraded or dissolved and cleared by the body.

As for potential future applications, PEO Laponite based hydro gel cell constructs or cell free hydrogels containing growth factors can be envisioned for use in orthopedic tissue repair. The charged Laponite nanoparticles used as cross linkers can potentially be used to attach biomolecules to the hydrogels, thus creating sus tained release drug delivery matrices. Other potential applications in the broader biotechnology area include platforms for biosensors that require interactions with cells and injectable hydrogel materi als with self healing properties.

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Appendix

Figures with essential colour discrimination. Certain figures in this article, particularly Fig. 3, is difficult to interpret in black and white. The full colour images can be found in the on line version, at doi: 10.1016/j.actbio.2010.09.015.

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REVIEW ARTICLE

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Development of Soft Nanocomposite Materials and Their Applications in Cell Culture and Tissue Engineering

Kazutoshi Haraguchi

Novel soft nanocomposite materials with unique organic/inorganic network structures have been developed by extending the strategy of “organic/inorganic nanocomposites” to the field of soft materials. The structures described here were synthesized by *in-situ* free-radical polymerization of various monomers in the presence of exfoliated clay (hectorite) in aqueous media. The nanocomposite hydrogels (NC gels) and soft nanocomposites (M-NCs) obtained were flexible and transparent soft materials, regardless of the clay content, that could be prepared in various shapes and surface forms, each consisting of individually different polymer/clay network structures. Owing to these unique network structures, both NC gels and M-NCs showed extraordinary mechanical properties such as ultrahigh elongation at break and widely controlled modulus and strength, which could overcome the problems (e.g., mechanical fragility, optical turbidity, poor processing ability) associated with conventional chemically crosslinked materials. In addition, the NC gels and M-NCs exhibited a number of new characteristics related to optical anisotropy, morphology, biocompatibility, stimulus sensitivity and cell culture. In the present review, we outline the novel features of these soft nanocomposites, and demonstrate their potential as soft culture substrates useful for tissue engineering as well as soft, transparent, absorbing, and mechanically tough biomaterials for many bio-applications.

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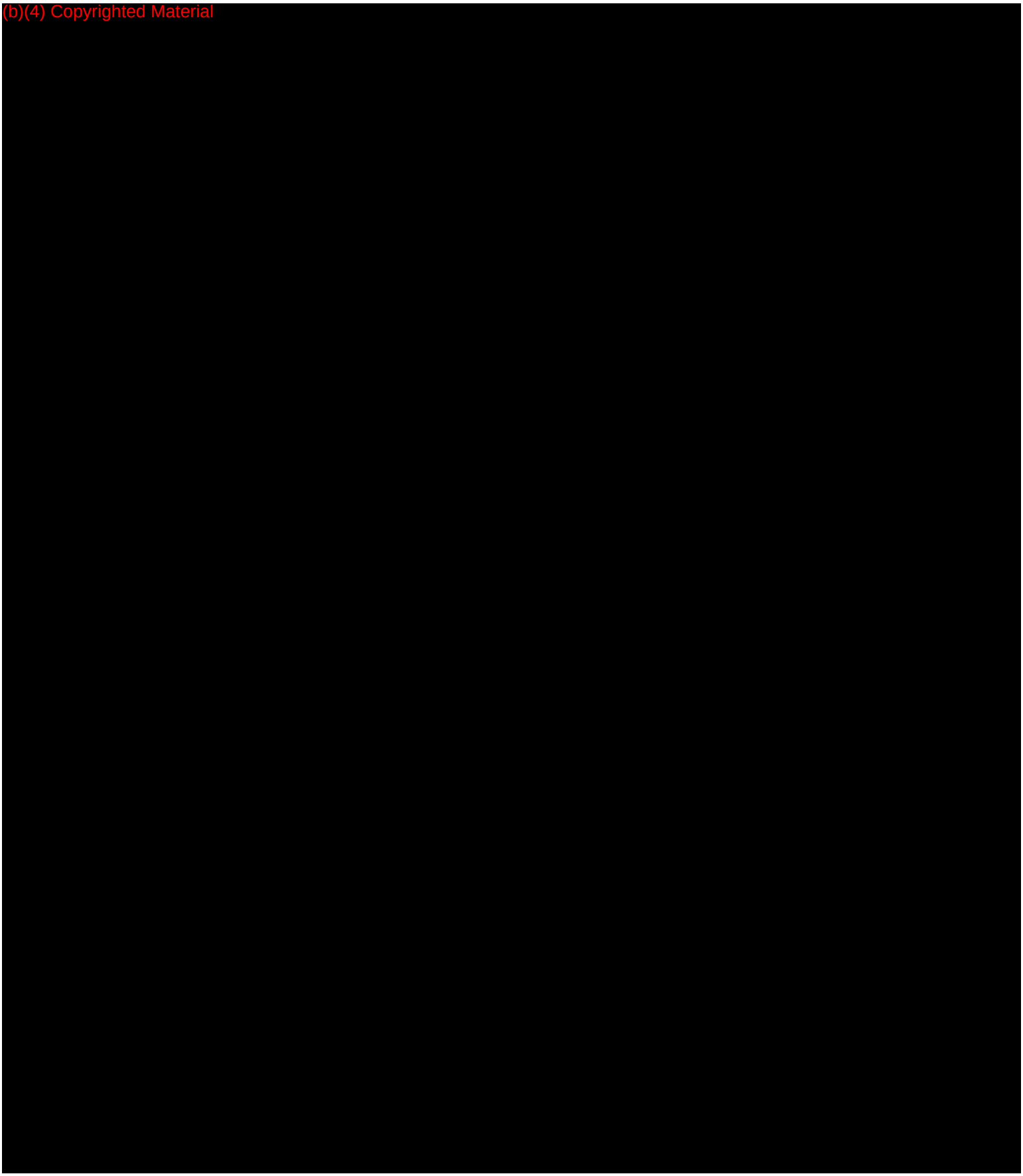

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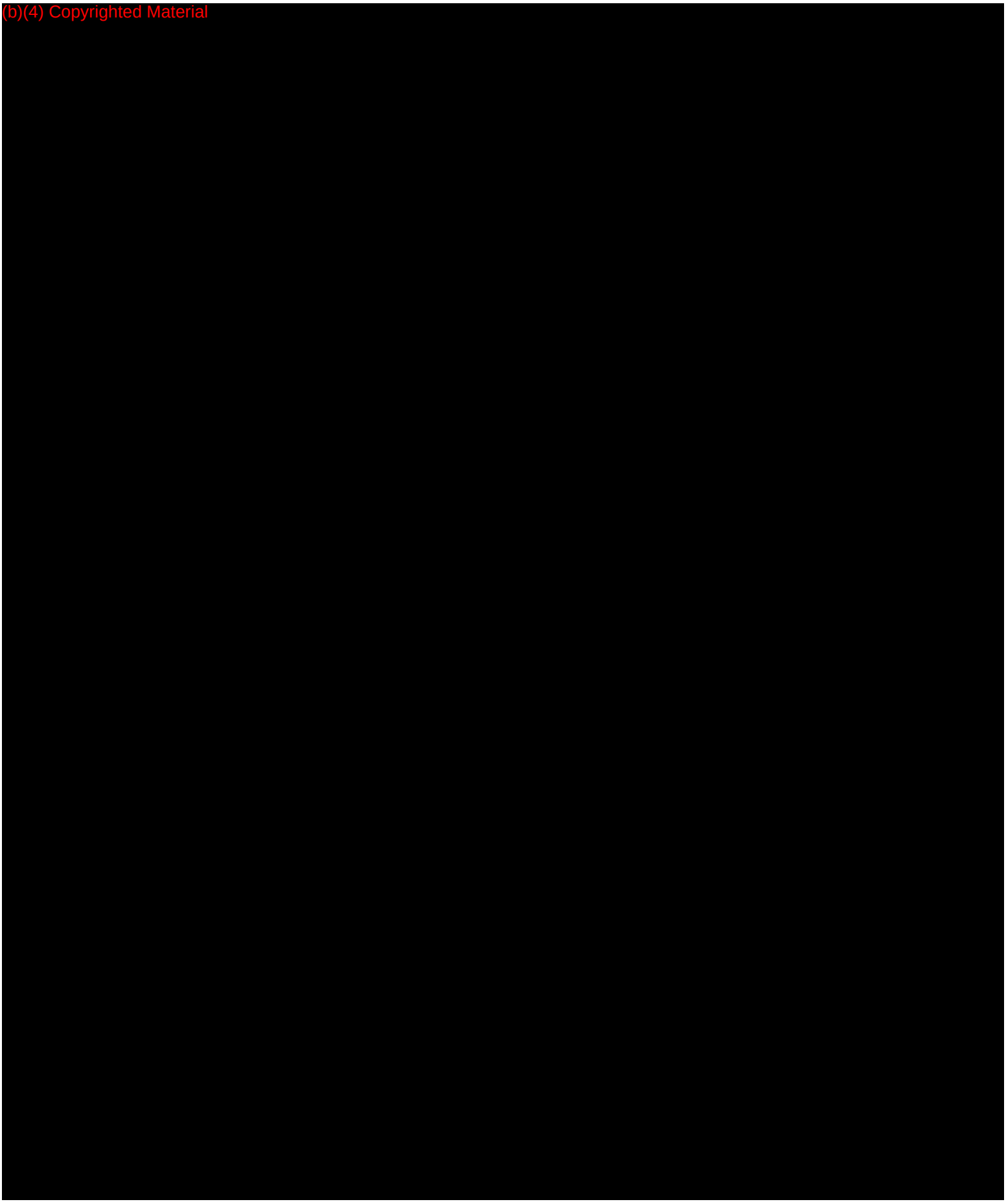

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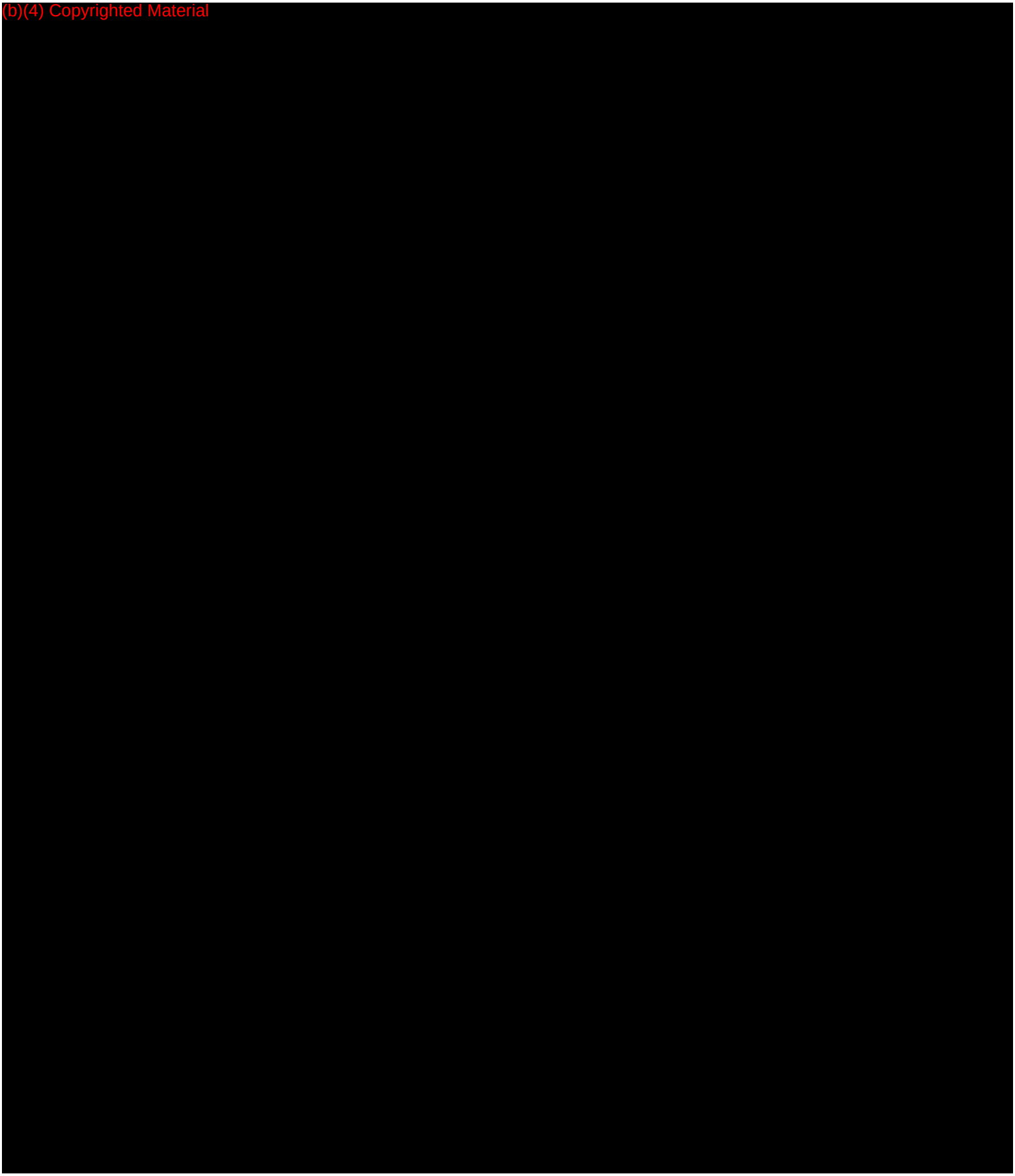

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Research paper

Physico-chemical, mechanical and cytotoxicity characterizations of Laponite®/alginate nanocomposite

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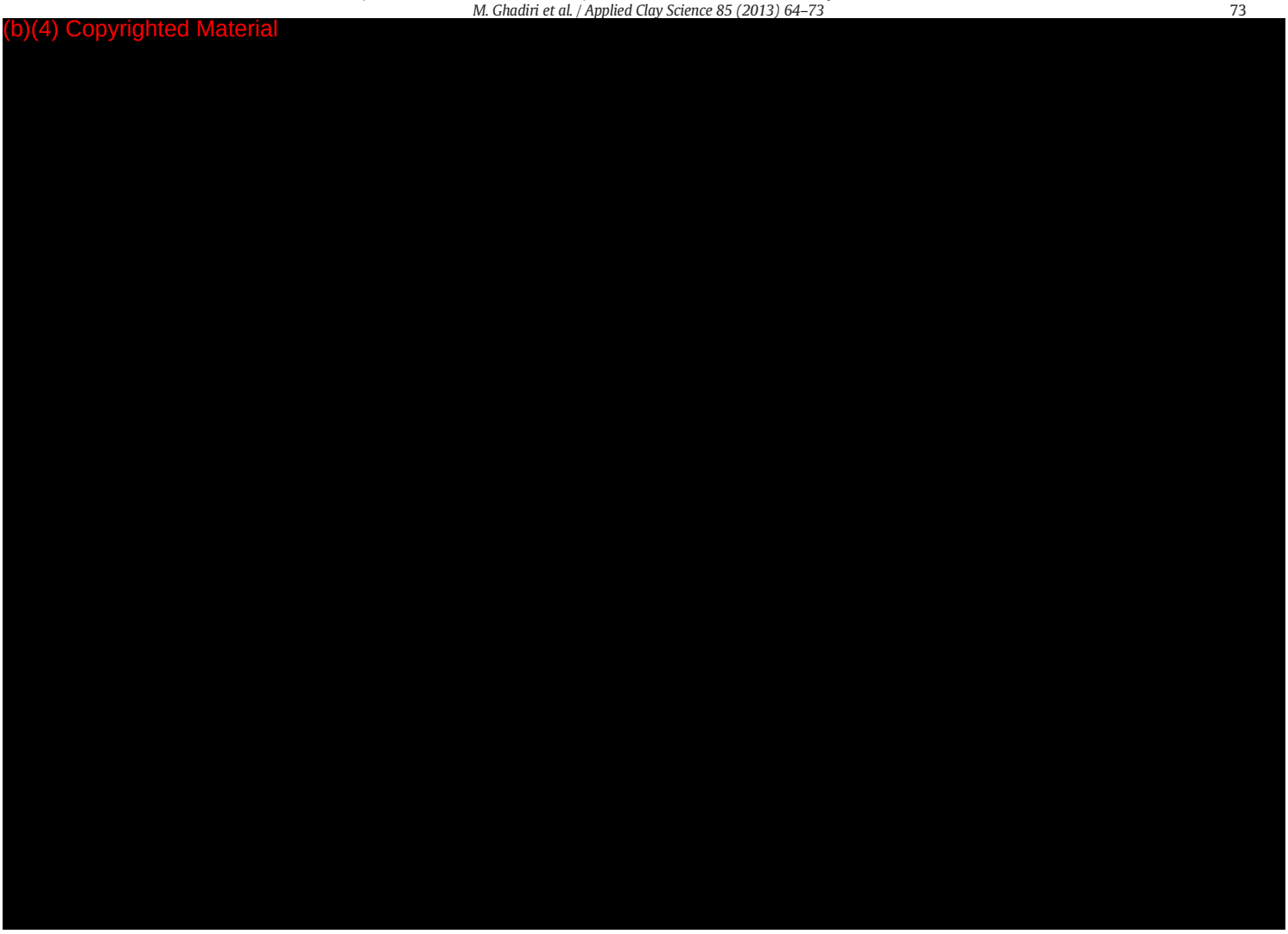

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PLOS ONE

Inactivation of the Antibacterial and Cytotoxic Properties of Silver Ions by Biologically Relevant Compounds

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Abstract

There has been a recent surge in the use of silver as an antimicrobial agent in a wide range of domestic and clinical products, intended to prevent or treat bacterial infections and reduce bacterial colonization of surfaces. It has been reported that the antibacterial and cytotoxic properties of silver are affected by the assay conditions, particularly the type of growth media used *in vitro*. The toxicity of Ag^+ to bacterial cells is comparable to that of human cells. We demonstrate that biologically relevant compounds such as glutathione, cysteine and human blood components significantly reduce the toxicity of silver ions to clinically relevant pathogenic bacteria and primary human dermal fibroblasts (skin cells). Bacteria are able to grow normally in the presence of silver nitrate at >20-fold the minimum inhibitory concentration (MIC) if Ag^+ and thiols are added in a 1:1 ratio because the reaction of Ag^+ with extracellular thiols prevents silver ions from interacting with cells. Extracellular thiols and human serum also significantly reduce the antimicrobial activity of silver wound dressings Aquacel-Ag (Convatec) and Acticoat (Smith & Nephew) to *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli in vitro*. These results have important implications for the deployment of silver as an antimicrobial agent in environments exposed to biological tissue or secretions. Significant amounts of money and effort have been directed at the development of silver-coated medical devices (e.g. dressings, catheters, implants). We believe our findings are essential for the effective design and testing of antimicrobial silver coatings.

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Introduction

In recent years, the emergence and persistence of bacterial strains with resistance to multiple classes of antibiotics has led to renewed interest in the antimicrobial properties of silver. There has been a surge in the number of products on the market, both domestic and clinical, that contain antimicrobial silver compounds or nanoparticles. These include anti odor fabric coatings, deodor ants, washing machine filters, laptop coatings, topical burn creams, wound dressings and medical devices [1 3]. The development of improved antimicrobial silver coatings and silver nanoparticles continues to receive significant research funding worldwide [4 6]. A key aim of this research is to ensure that silver ions are released at a sufficient rate and concentration to be effective as an antimicrobial at levels that are safe for use. This is particularly important for the development of medical devices, such as wound dressings, catheters, bone implants and cardiovascular stents, which are typically tested first *in vitro* (antimicrobial assays and human cell culture) and later *in vivo* (animal models and clinical trials). Topical silver solutions (0.5% silver nitrate) and creams (1% silver sulfadiazine) have been used in the prevention and treatment of wound infections for several decades, but these preparations need to be reapplied frequently in order to penetrate wound tissues due to rapid complexation of silver with wound exudates [7]. Modern advances in silver delivery methods have seen the introduction of sustained release dressings such as the nanocrystal line wound dressing Acticoat (Smith & Nephew) and the hydrogel dressing Aquacel Ag (Convatec). These dressings should release sufficient Ag^+ to prevent or reduce bacterial colonization of the wound bed and support efficient healing. Silver coatings on indwelling medical devices have also been developed, such as the Bardex IC Foley catheter (Bard Medical). These coatings should release sufficient silver to reduce or prevent bacterial attachment and formation of biofilms whilst inducing minimal damage to surrounding human cells and tissue [8]. However, differences in experimental conditions and procedures can make comparisons of antimicrobial efficacy and human toxicity from *in vitro* and *in vivo* experiments difficult [9 11]. A recent study by Greulich *et al.* used identical growth conditions for bacteria and human cells and this revealed that the antibacterial and cytotoxic properties of both silver ions (silver acetate) and silver nanoparticles are within the same range [10].

Several studies have shown the antibacterial and cytotoxic properties of silver are affected by the assay conditions, including the type of growth media and growth supplements such as fetal calf serum [12]. Only a few studies have explored the chemistry behind these differences. Liau *et al.* showed that compounds containing thiol groups reduce the toxicity of silver to *Pseudomonas aeruginosa* [13]. Similarly, equimolar concentrations of the thiol containing amino acid cysteine reduce the toxicity of silver to *Staphylococcus*

epidermidis [14]. The major blood protein serum albumin reduces both the antimicrobial and cytotoxic properties of silver nanopar ticles embedded in hydrogels, although the mechanism of inactivation is not known [15].

Whilst the majority of the thiol groups in the proteins of human cells are in the oxidized state (forming disulphide bridges between cysteine residues in proteins), the thiol groups of bacterial cytoplasmic proteins are mostly in the reduced state due to the redox conditions in the prokaryotic cytoplasm [16]. Animals and bacteria have a thiol based antioxidant system that protects cellular components against oxidative damage from reactive oxygen species (ROS) and free radicals. In humans and many Gram negative bacteria, such as *Escherichia coli* and *P. aeruginosa*, the system utilizes the tripeptide glutathione as the predominant antioxidant. Glutathione is synthesized by specific enzymes from the amino acids glutamate, glycine and cysteine [17,18]. Following oxidation by ROS, the oxidized glutathione (GSSG) is recycled back to the reduced form (GSH) by the enzyme glutathione reductase using NADPH as an electron donor. In other bacteria such as *S. aureus* and *Bacillus* spp. that cannot synthesize glutathione, the predominant cellular antioxidant is typically a low molecular weight compound synthesized from cysteine [19,20].

In this study we present the first detailed analysis of the extent to which biologically relevant compounds such as glutathione, cysteine and human blood components affect toxicity of silver ions to clinically relevant pathogenic bacteria in comparison to human dermal fibroblasts (skin cells). We used the notorious nosocomial opportunistic pathogens *S. aureus* and *P. aeruginosa* in these studies as they are frequently exposed to silver coated dressings and catheters in clinical settings. Our findings have important implications for the future deployment of silver as an antimicrobial agent in environments exposed to biological tissue or secretions.

Materials and Methods

Chemicals and Reagents

Silver nitrate, sodium nitrate, GSH, GSSG, amino acids, human serum albumin and human serum were purchased from Sigma Aldrich and stock solutions were prepared fresh for each assay in sterile Milli Q water, filter sterilized at 0.22 μm (Millex GS, Millipore). Propidium iodide and NucBlue (a cell permeable form of Hoechst 33347) were diluted to the recommended working concentration in Dulbecco's phosphate buffered saline (DPBS + calcium, magnesium, glucose and pyruvate), all purchased from Life Technologies.

Bacterial growth and microbiological assays

Escherichia coli K12, *P. aeruginosa* PA01 [21], *S. aureus* MSSA476 and MRSA252 [22] were recovered from frozen (80°C) glycerol (15% *v/v*) stocks on Luria Bertani (LB) agar plates at 37°C for 24 hr. Single colonies were grown in 10 mL LB broth, 250 rpm, at 37°C for 16 18 hr. Bacteria were then sub cultured (1:100) in 10 mL LB broth, 250 rpm, at 37°C for 2 5 hr to exponential phase (OD_{600} 0.4 0.6). Cultures were adjusted to OD_{600} 0.3 and diluted in LB (1:50) prior to use in microbiological assays unless otherwise stated.

Stock solutions of chemicals were diluted in sterile Milli Q water at 50× the concentration desired in the assay. These were then diluted 1:25 in LB broth, human serum albumin 100 μg/mL dissolved in LB, or 100% human serum where stated. 100 μL of this 2× solution was aliquoted into the appropriate wells of a 96 well flat bottom transparent plate (Greiner) with 100 μL of bacterial culture prepared as described above (equivalent to $\sim 5\times10^5$ bacteria/well) in technical duplicates, with three biolog ical replicates for each strain. Microplates were incubated in a Fluostar Omega plate reader (BMG) for 24 hr, with continuous orbital shaking at 300 rpm, and absorbance measurements taken at 600 nm every 6 min (20 flashes/well/cycle). The optical density of each individual culture at 16 hr or 24 hr was plotted in OriginPro8 (OriginLab) and Sigmoidal curves fitted using the Boltzman function. Fitted values for each individual curve were used to calculate the mean minimum inhibitory concentration (MIC).

To test the effect of R SH on the antimicrobial activity of wound dressings, 20 mL molten LB agar (42°C) was inoculated with approximately 1×10^5 bacterial cells and 200 uL of the appropriate concentration of GSH, mixed well and poured into a standard 90 mm Petri dish. For human serum tests, 2 mL molten LB agar (42°C) was mixed with 2 mL human serum and approximately 2×10^4 bacterial cells and poured into wells in a 6 well tissue culture dish (Corning). Squares (1.25 cm×1.25 cm) of Aquacel (Convatec), Aquacel Ag (Convatec) and Acticoat (Smith & Nephew) dressings were applied to the surface of the solidified agar. Plates were incubated for 24 hr at 37°C and the zones of inhibition surrounding the dressings were measured (n 3). Statistical significance was calculated using Student's *t* test.

Human cell culture and cytotoxicity assays

Primary adult human dermal fibroblasts were purchased from the American Type Culture Collection (PCS 201 012). All incubations were at 37°C, 5% CO_2/95% air in a humidified incubator. Cells were cultured in 75 cm^2 tissue culture flasks in Medium 106 supplemented with low serum growth supplement (Life Technologies) to a confluence of ~80% for up to 8 passages. Cells were detached from tissue culture flasks using trypsin EDTA and trypsin neutralizer solution as per the manufacturer's protocol (Life Technologies).

For cytotoxicity tests, cells were seeded at 5×10^3 cells/cm^2 in 24 well dishes with 500 μL media per well and grown to a confluence of ~80% with media replaced every 24 hr for 2 3 days. Stock solutions of silver nitrate and GSH were diluted 1:50 in Medium 106 supplemented with low serum growth supplement (NB. the pH of the culture medium was not affected). Plates were incubated for 4 or 24 hr and media was replaced with 500 μL propidium iodide solution and incubated for 20 min. This solution was then replaced with 500 μL NucBlue solution and incubated for 20 min. Micrograph images were captured using an EVOS fl digital inverted microscope (Advanced Microscopy Group) with the light microscope, DAPI light cube (excitation at 357 nm, emission at 447 nm, to detect NucBlue stain) and RFP light cube (excitation at 531 nm, emission at 593 nm, to detect propidium iodide) at ×20 magnification. Stained nuclei were counted in captured images using ImageJ [23] with means and standard errors of the mean calculated from two technical replicate images per well and four biological replicates per condition. The percentages of viable cells (ratio of cells stained with propidium iodide vs. NucBlue) were plotted in OriginPro8 (OriginLab) and Sigmoidal curves fitted using the Boltzman function. Fitted values representing a 50% reduction in viability for each individual curve were used to calculate the mean cytotoxic concentration (CC_{50}).

Quantification of silver

Overnight cultures of *S. aureus* were sub cultured in 50 mL LB broth in sterile 250 mL Erlenmeyer flasks and grown for 2 3 hr at

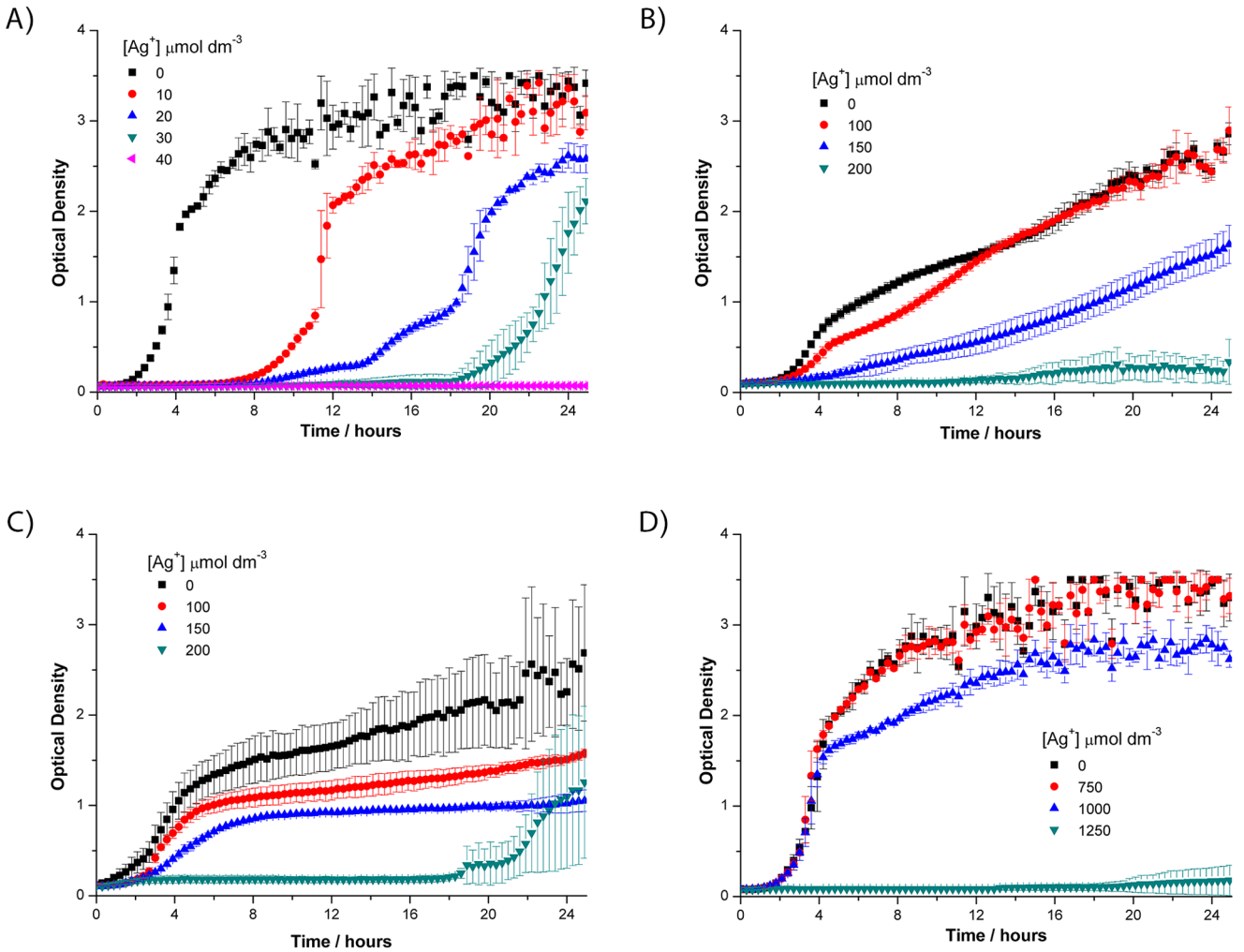

Figure 1. The effect of silver nitrate on the growth of *Staphylococcus aureus* MSSA476 in different media. $AgNO_3$ was added to growth media at the indicated concentrations (μmol dm ^{3}) in (A) LB; (B) LB + 50 mg/mL HSA; (C) LB + 50% human serum (*v/v*); (D) LB + 1 mmol dm ^{3} GSH. GSH, reduced glutathione; HSA, human serum albumin (the major blood protein). Error bars = SD, n = 3.
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37°C with aeration (250 rpm shaking), to OD_{600} 0.5 0.8. 10 mL aliquots of culture were diluted 1:2 into LB with or without $AgNO_3$ and with or without GSH to a final concentration of 1 mmol dm ^{3}. Cultures were incubated for 1 hr at 37°C, 250 rpm and cells harvested by centrifugation at 4°C. The supernatant was discarded, cell pellets were washed 3× in 1 mL PBS and re suspended in 200 μL 70% ethanol. Samples were boiled at 90°C for 1 hr to lyse the cells and dry the pellets. Pellets were weighed, re suspended in 3 mL nH_20 and transferred to digestion tubes. 10.5 mL concentrated hydrochloric acid and 3.5 mL concentrated nitric acid was added to each sample to cold digest overnight. Samples were heated to 140°C for 2.5 hr, allowed to cool, and filtered through Cu impregnated filter papers (prepared by soaking Whatman no. 540 filter paper in 0.1 M copper nitrate and rinsing 3× in nH_2O). Samples were made to volume in 100 mL volumetric flasks with 0.5 M nitric acid and diluted 1:2 with nH_20 prior to analysis by ICP OES. Three blank samples were prepared without cell pellets as negative controls to set the detection limit.

Results

Antibacterial activity of silver nitrate in different conditions

The pathogenic clinical isolates *S. aureus* MSSA476 and *P. aeruginosa* PA01 were grown overnight in LB broth with a range of concentrations of silver nitrate, which readily dissolves in culture media to Ag^+ and NO_3 . Each increase in the concentration of silver nitrate below the minimum inhibitory concentration (MIC) resulted in a prolonged lag phase (i.e. the time between the inoculation of bacteria and the onset of exponential growth) for both strains, but once growth had initiated the growth rate was then comparable to that in LB (Fig. 1A and 2A). The MIC of silver nitrate in LB broth was 33 μmol dm ^{3} to *S. aureus* MSSA476, 13 μmol dm ^{3} to *P. aeruginosa* PA01 and 37 μmol dm ^{3} to *E. coli* K12, at 16 hr (Table 1). A methicillin resistant *S. aureus* strain, MRSA252, was also tested and the MIC was equivalent to that of MSSA476. We found that the MIC was not affected by the number of bacteria in the starting inoculum as 10 fold dilutions of bacteria from 1×10^{6} to 1×10^{2} bacteria per well resulted in comparable MIC values.

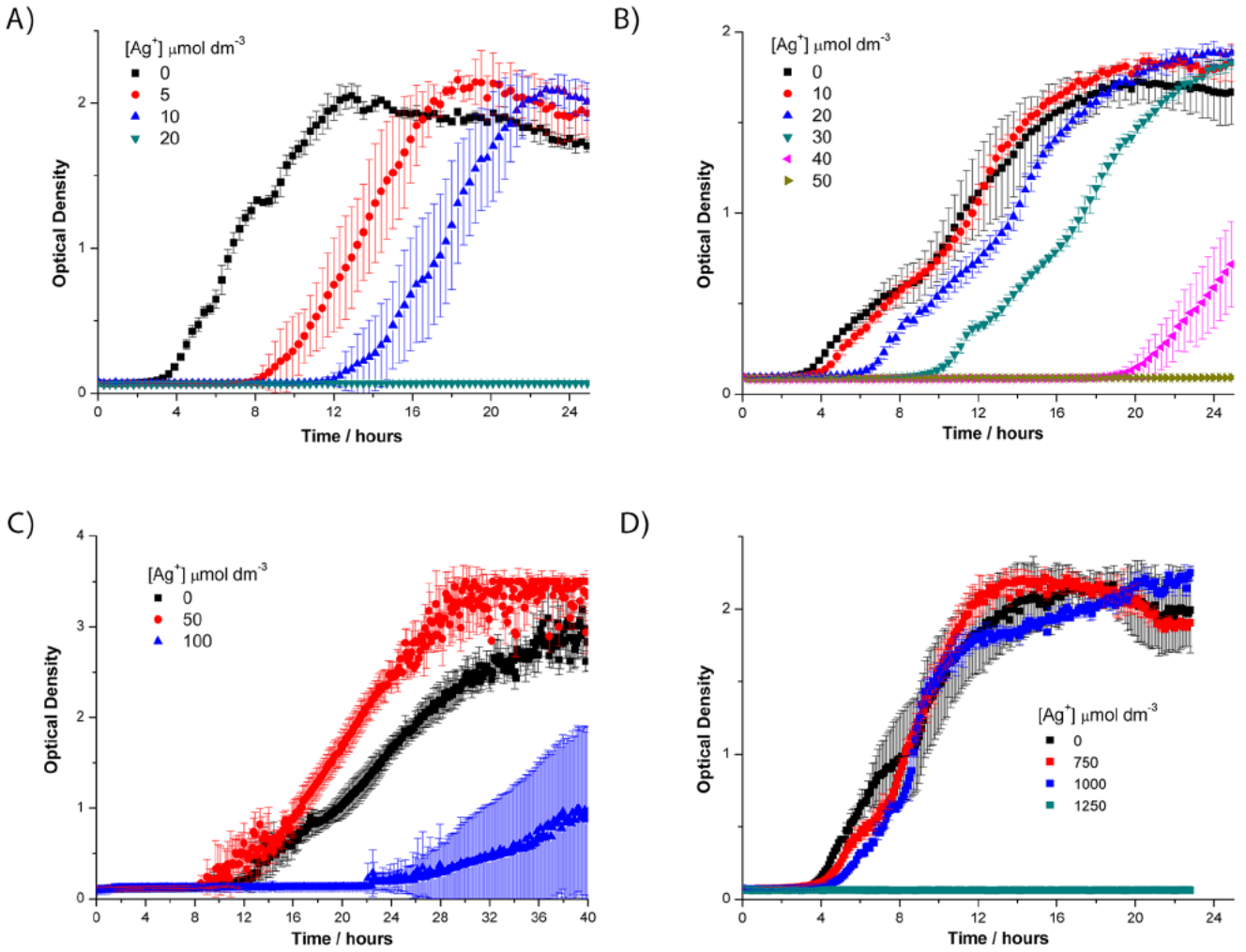

Figure 2. The effect of silver nitrate on the growth of *Pseudomonas aeruginosa* PA01 in different media. $AgNO_3$ was added to growth media at the indicated concentrations (µmol dm $^{-3}$) in (A) LB; (B) LB + 50 mg/mL HSA; (C) LB + 50% human serum (*v/v*); (D) LB + 1 mmol dm $^{-3}$ GSH. GSH, reduced glutathione; HSA, human serum albumin (the major blood protein). Error bars = SD, n = 3.
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Coatings on medical devices such as bandages and catheters contact human blood and tissue. To assess whether the components of blood affect the antimicrobial efficacy of silver ions, the MIC of silver was determined in LB supplemented with human serum (blood depleted of cells and clotting factors) and human serum albumin (HSA, the major blood protein present in serum). Both of these blood components increased the MIC of $AgNO_3$ to *S. aureus* (Figure 1B and 1C) and *P. aeruginosa* (Figure 2B and 2C), with serum being more potent than HSA alone indicating the presence of additional components within serum that inactivate Ag^+ toxicity. Note that only 50% serum was used in this experiment so the protective effect of whole blood is

Table 1. The effect of biologically relevant compounds on the minimum inhibitory concentration (MIC) and cytotoxic concentration (CC_{50}) of silver nitrate to bacteria and human cells.

Growth conditions	*P. aeruginosa* µmol dm^{-3}	*S. aureus* µmol dm^{-3}	*E. coli* µmol dm^{-3}	Human Fibroblasts µmol dm^{-3}
Media only	13±2	33±3	37±5	23±1
Media + HSA 50 mg/ml	44±2	158±10	50±10	ND
Media + human serum 50% *v/v*	81±7	174±17	ND	ND
Media + 1 mmol dm^{-3} GSH	1126±9	1121±80	1020±65	982±72

The MIC of $AgNO_3$ to *P. aeruginosa*, *S. aureus* and *E. coli* was determined in Luria Bertani broth and the CC_{50} of $AgNO_3$ to primary human dermal fibroblasts in Medium 106 supplemented with low serum growth supplement (± SD, n≥3). GSH, reduced glutathione; HSA, human serum albumin; ND, Not determined (conditions do not support growth).
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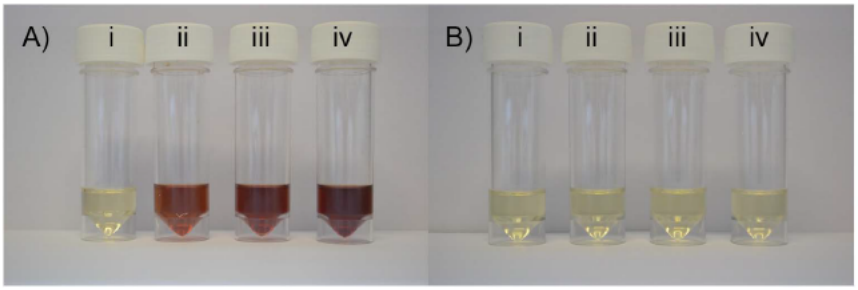

Figure 3. Photochemical reduction of Ag^+ in LB medium. $AgNO_3$ was added to (A) LB and (B) LB + 1 mmol dm^3 GSH at (i) 0 mmol dm^3, (ii) 0.75 mmol dm^3, (iii) 1.0 mmol dm^3, (iv) 1.25 mmol dm^3. GSH, reduced glutathione.
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potentially greater *in vivo*. The inclusion of 1 mmol dm^{-3} GSH in LB enabled *S. aureus*, *P. aeruginosa* and *E. coli* to grow in the presence of up to, but not in excess of, 1 mmol dm^{-3} $AgNO_3$ (Table 1). The lag phase and growth rate in LB with 1 mmol dm^{-3} $AgNO_3$ + 1 mmol dm^{-3} GSH was remarkably similar to that in LB with 1 mmol dm^{-3} GSH alone (Figure 1 and 2) indicating GSH causes complete loss of silver ion toxicity in a 1:1 molar ratio. The addition of 1 mmol dm^{-3} cysteine to LB showed the same protective effect as GSH enabling normal growth up to, but not in excess of, 1 mmol dm^{-3} $AgNO_3$ (data not shown). This suggests that silver ions bind to glutathione and cysteine (which both contain one thiol group) in a 1:1 ratio and that these complexes are not toxic to bacteria. In contrast, the addition of glutamate, glycine, methionine, histidine or cystine (cysteine disulphide) at 1 mmol dm^{-3} did not rescue growth of either *P. aeruginosa* or *S. aureus* at 200 µmol dm^{-3} $AgNO_3$ in LB. The addition of 1 mmol dm^{-3} GSSG was toxic (data not shown).

We speculate that the addition of excess GSSG would lead to depletion of the cellular pool of reductant as the bacteria attempt to convert it back to GSH. The addition of 1 mmol dm^{-3} sodium nitrate to LB did not affect the growth of the bacterial strains compared to LB alone, indicating NO_3 does not influence the toxicity of $AgNO_3$.

After performing these assays, the surplus media was left on the lab bench and we noted that the LB + $AgNO_3$ solutions became increasingly dark brown over time, but this was prevented by the addition of 1 mmol dm^{-3} GSH (Figure 3) or cysteine and these solutions remained clear for over 3 months.

Antibacterial activity of silver-coated dressings in different conditions

Silver coated wound dressings come into contact with biological secretions within the wound bed. The antibacterial properties of

Table 2. The effect of biologically relevant compounds on the antimicrobial efficacy of Aquacel-Ag (Convatec) wound dressings.

Growth conditions	*P. aeruginosa* mm	*S. aureus* mm	*E. coli* mm
LB agar only	8.7±0.6	3.0±0.0	4.3±0.6
LB agar + 0.1 mmol dm^{-3} GSH	6.3±0.6 *	2.0±0.0	2.5±0.5
LB agar + 0.5 mmol dm^{-3} GSH	5.2±0.3 *	0.2±0.3 *	0.3±0.3 *
LB agar + 1 mmol dm^{-3} GSH	1.7±0.6 *	0.0±0.0 *	0.0±0.0 *
LB agar + human serum 50% *v/v*	2.3±0.6 *	0.8±0.3 *	ND

The average zone of inhibition (mm) surrounding 1.25×1.25 cm dressing samples applied to bacterial lawns. ± SD, n = 3, * denotes a significant difference from LB agar only control (Student's *t* test $P<0.01$). GSH, reduced glutathione; LB, Luria Bertani; ND, Not determined (conditions do not support growth).
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Table 3. The effect of biologically relevant compounds on the antimicrobial efficacy of Acticoat (Smith & Nephew) wound dressings.

Growth conditions	*P. aeruginosa* mm	*S. aureus* mm	*E. coli* mm
LB agar only	9.0±1.0	3.0±0.0	4.3±0.6
LB agar + 0.1 mmol dm^{-3} GSH	7.3±0.6	2.2±0.3 *	2.5±0.5
LB agar + 0.5 mmol dm^{-3} GSH	5.8±0.3 *	0.3±0.3 *	0.8±0.3 *
LB agar + 1 mmol dm^{-3} GSH	4.3±0.6 *	0.0±0.0 *	0.0±0.0 *
LB agar + human serum 50% *v/v*	2.3±0.6 *	1.7±0.3 *	ND

The average zone of inhibition (mm) surrounding 1.25×1.25 cm dressing samples applied to bacterial lawns. ± SD, n = 3, * denotes a significant difference from LB agar only control (Student's *t* test $P<0.01$). GSH, reduced glutathione; LB, Luria Bertani; ND, Not determined (conditions do not support growth).
doi:10.1371/journal.pone.0094409.t003

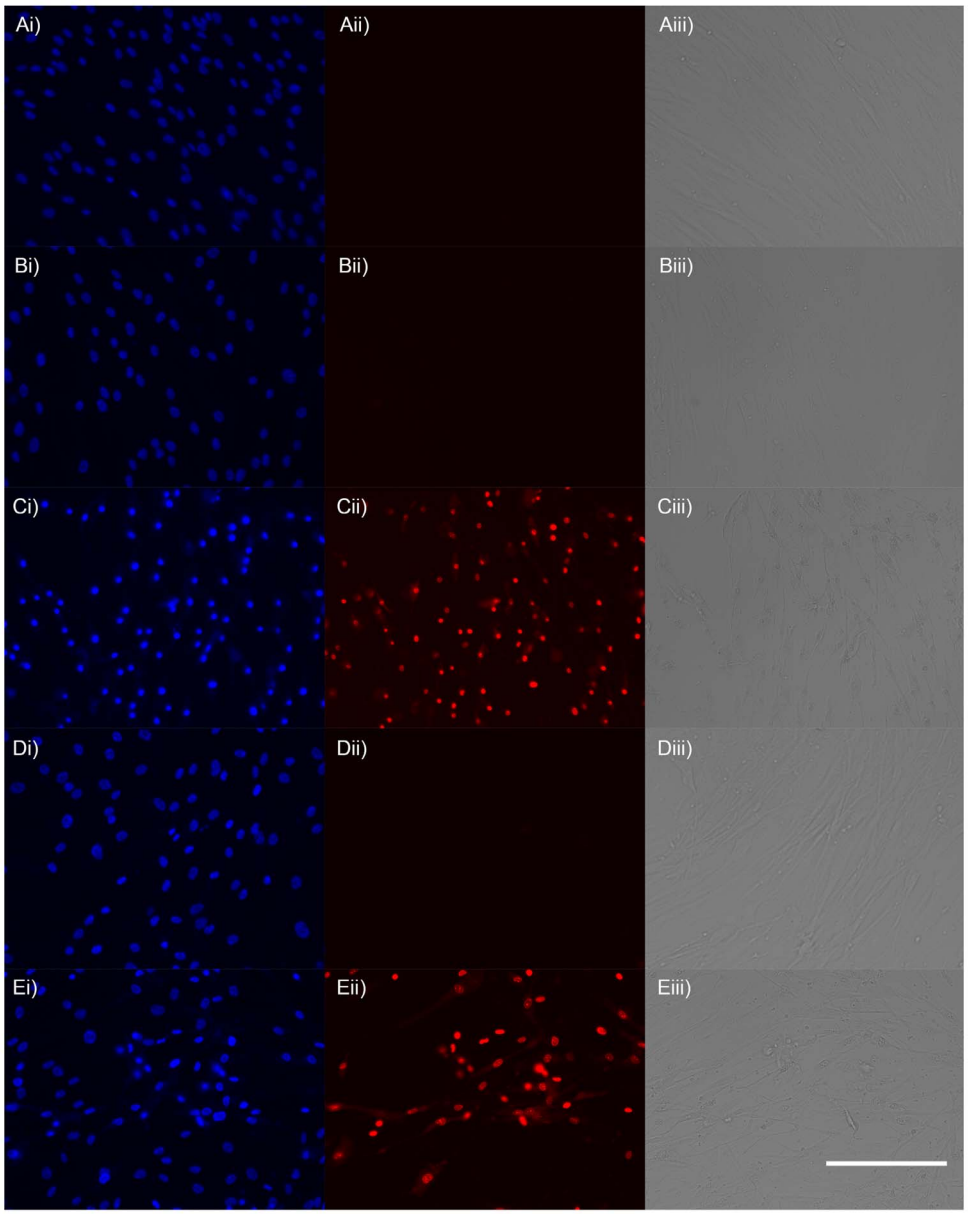

Figure 4. Micrographs of primary adult human dermal fibroblasts exposed to silver nitrate. Cells were exposed to $AgNO_3$ at the indicated concentration for 24 hr: (A) 0 μmol dm ^{3} $AgNO_3$; (B) 10 μmol dm ^{3} $AgNO_3$; (C) 25 μmol dm ^{3} $AgNO_3$; (D) 750 μmol dm ^{3} $AgNO_3$ + 1 mmol dm ^{3} GSH; (E) 1 mmol dm ^{3} $AgNO_3$ + 1 mmol dm ^{3} GSH. Images were captured for the same cells stained with i) NucBlue (Hoechst 33347), which stains all cell nuclei and ii) Propidium iodide, which stains nuclei of dead cells; (iii) Light microscope images show changes in cell morphology. GSH, reduced glutathione. Scale bar = 200 μm
doi:10.1371/journal.pone.0094409.g004

wound dressings can be tested *in vitro* by measuring the zone of inhibition surrounding a test sample. Silver ions released from dressings diffuse through the agar and prevent bacterial growth where the concentration exceeds the MIC. Aquacel Ag (Table 2) and Acticoat (Table 3) dressings showed similar efficacies against the test bacteria in LB agar, with *P. aeruginosa* displaying the largest zone of inhibition as expected based on the greater sensitivity of this species to silver ion toxicity (Table 1). No zones of inhibition were observed for non silver Aquacel dressings confirming Ag^{+} release is solely responsible for the inhibition of growth caused by Aquacel Ag and Acticoat. Increasing the concentration of GSH in the LB agar caused a corresponding reduction in the size of the zone of inhibition caused by both Aquacel Ag and Acticoat. The inclusion of human serum (50% *v/v*) in the agar significantly reduced the size of the zone of inhibition to *P. aeruginosa* and *S. aureus* (Student's *t* test $P<0.001$). Our results confirm that the antimicrobial effectiveness of these dressings is significantly reduced by the presence of extracellular R SH and human serum (Table 2 and 3).

Cytotoxicity of silver towards primary human fibroblasts

Fibroblasts within the dermal layer of the skin are one of the most important cell types involved in wound healing. This is therefore the cell line of choice for assessing cytotoxicity of silver in wound dressings and medical devices. Primary cells are directly acquired from donor tissue and have a limited lifespan in cell

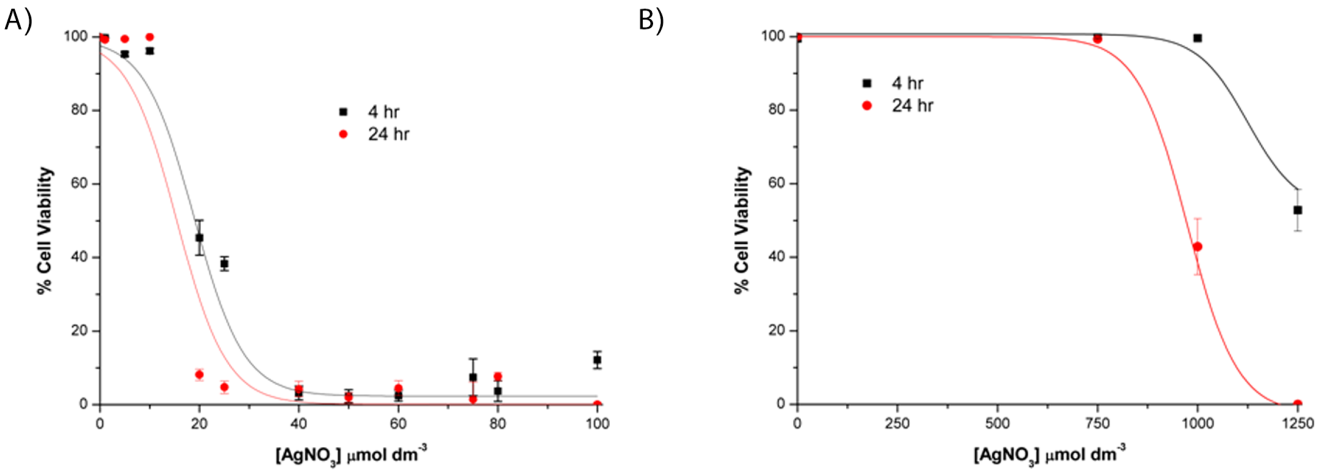

Figure 5. The cytotoxicity of silver nitrate to human skin cells. Viability of primary adult human dermal fibroblasts exposed to $AgNO_3$ for 4 h or 24 h in (A) Medium 106; (B) Medium 106+1 mmol dm ^{3} GSH. Sigmoidal curves were fitted using the Boltzman function in OriginPro8 (OriginLab). Error bars = SEM, n = 4. GSH, reduced glutathione.
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culture. These cells are therefore preferred for cytotoxicity studies as they more closely reflect host responses *in vitro* than immortalized cell lines that may have changed significantly during routine culture in the laboratory.

The cytotoxic concentration (CC_{50}) of $AgNO_3$ to primary human dermal fibroblasts was 23 µmol dm ^{3}, which is in the same range as the MIC to the bacteria tested in this study (Table 1). Exposure of cells to 10 µmol dm ^{3} $AgNO_3$ for 24 hours had no visible effect on cell morphology (cells remained elongated) or viability (cell nuclei stained with NucBlue, but not propidium iodide) and cells maintained a confluent, adherent monolayer (Figure 4B). In contrast, cells exposed to 25 µmol dm ^{3} $AgNO_3$ were rounded (as opposed to elongate) and had begun to detach from the culture plate. The nuclei of the majority of these cells stained with propidium iodide indicating compromised cell membrane integrity (Fig 4Cii) equating to a 60% and 95% reduction in viability at 4 hr and 24 hr respectively (Figure 5A). Furthermore, the nuclei of the cells stained with propidium iodide showed signs of nuclear condensation, which is indicative of apoptosis or "programmed cell death" (Figure 6). The addition of 1 mmol dm ^{3} GSH to the cell culture medium increased the CC_{50} of $AgNO_3$ to 982 µmol dm ^{3} after 24 hr (Table 1, Figure 5B). The addition of 1 mmol dm ^{3} sodium nitrate to the cell culture medium had no effect on cell morphology or viability relative to controls after 24 hr, indicating nitrate does not influence the cytotoxicity of $AgNO_3$ (data not shown).

Mechanism of thiol protection

To determine how extracellular R SH reduce the toxicity of Ag^{+} we used ICP OES to analyse the silver content of *S. aureus* exposed to 1 mmol dm ^{3} $AgNO_3$ with and without the inclusion of an equimolar concentration of GSH in LB broth. Ag was detected in cells exposed to $AgNO_3$ only at a concentration of 326±62 fg Ag/cell and 62.8±5.5 µg Ag/mg cell dry weight (± SEM, n 4). In contrast, Ag was not detectable in cells exposed to LB only and $AgNO_3$ + GSH. We therefore conclude that extracellular R SH prevent Ag^{+} from binding to cells and this inactivates Ag^{+} toxicity.

Discussion

Our findings prove that reduced thiol groups (R SH) in the extracellular environment markedly reduce the antimicrobial efficacy and cytotoxicity of silver ions. When Ag^{+} and R SH are added in a 1:1 ratio the reaction of Ag^{+} with R SH prevents Ag^{+} from interacting with cells thereby inactivating silver toxicity. GSH is the predominant low molecular weight thiol in humans, present in all cell types at a concentration of between 1 and 10 mmol

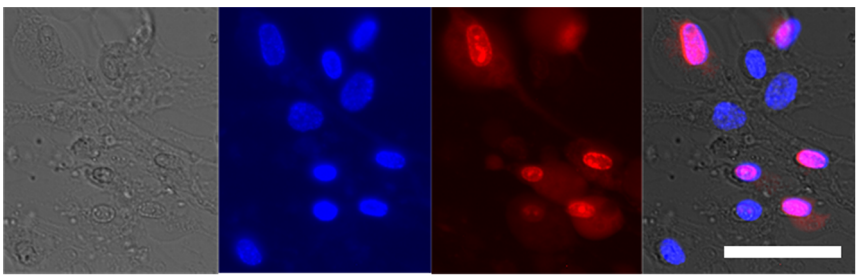

Figure 6. Nuclear condensation in human skin cells exposed to the minimum cytotoxic concentration of silver nitrate. Primary adult human dermal fibroblasts were exposed to 20 µmol dm ^{3} $AgNO_3$ for 4 hr. Images were captured for the same cells: (A) Light microscope image shows cellular morphology; (B) stained with NucBlue (Hoechst 33347), which stains all cell nuclei; (C) stained with propidium iodide, which stains nuclei of dead cells (NB. areas of nuclear condensation are indicative of apoptosis); (D) Composite image of A, B and C. GSH, reduced glutathione. Scale bar = 50 µm.
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dm ^{3} and in blood at approximately 1 mmol dm ^{3} [24,25]. Our results show the addition of 1 mmol dm ^{3} GSH results in complete loss of antibacterial activity of Aquacel Ag (Convatec) and Acticoat (Smith & Nephew) dressings to both *S. aureus* and *E. coli in vitro*. Given recent evidence that the toxicity of silver nanoparticles is dependent on the rate of dissolution of free Ag^+ [6,26,27], extracellular R SH will similarly reduce their antibac terial efficacy. The negative effect of complex formation between biological R SH groups and Ag^+ should be considered in the future development of all novel silver coatings and nanoparticles. *In vitro* testing of silver coated dressings and medical devices should be performed in biologically relevant media as the concentration of R SH in standard bacterial culture media is typically much lower than in human blood and tissue. This is particularly relevant to the testing of sustained release devices as the presence of R SH in biological tissues could significantly affect the rate of dissolution of Ag^+ and the duration of antimicrobial efficacy. Another consideration is that *in vitro* tests are typically performed in closed systems, which could exaggerate the longevity of antimicrobial action due to saturation of R SH with Ag^+. It seems likely that the constant replenishment of biological fluids containing R SH would continue to limit the antimicrobial efficacy of Ag^+ released from dressings/devices *in vivo*. Furthermore, the concentration and rate of Ag^+ dissolution from antibacterial coatings on medical devices and wound dressings should be carefully controlled to minimize cytotoxicity towards dermal fibroblasts and other human cell types because this could reduce the rate of wound healing, as suggested elsewhere [28,29]. Indeed, a recent Cochrane systematic review of the use of topical silver including silver sulphadiazine in the treatment of burns suggested that there is insufficient clinical evidence to support the hypothesis that such dressings do indeed promote healing or prevent infection [30].

Whilst it was not possible to use identical culture conditions for the bacterial and human cell assays in this study, we found that the toxicity of $AgNO_3$ to both bacteria and human cells was within the same range, which is in agreement with the results of Greulich *et al.* [10]. The toxicity of silver is attributed to multiple factors including cell membrane damage, inhibition of respiratory enzymes, perturbation of metal ion homeostasis and generation of ROS that damage cellular components such as DNA and lipids. Several studies have demonstrated that the major target site(s) of Ag^+ in Gram negative bacteria are intracellular. Firstly, low level silver resistance by adaptation of *E. coli* to increasing concentra tions of $AgNO_3$ was achieved by both decreased outer membrane permeability (due to a decrease in porin proteins that form membrane channels in the outer membrane) and active efflux of Ag^+ from the cell [31]. Secondly, all known high level silver resistance mechanisms in bacteria involve efflux pumps [32,33]. Only one silver efflux system has been characterised at the molecular level to date and is encoded by the *sil* genes (*silRSE silCBA silP*) on the pMG101 plasmid of *Salmonella*. This system utilises a periplasmic Ag^+ binding protein (SilE), which surprisingly lacks cysteine residues and instead coordinates 10 silver ions per polypeptide via 10 histidine residues [34]. The binding of silver ions to exposed thiol groups within a cell would have two complementary negative effects. Firstly, it might impair the functionality of any biomolecules to which it became bound and secondly it would reduce the cell's ability to neutralize natural ROS by depleting the effector molecules of the homeostatic antioxidant system such as GSH and cysteine. This would explain why silver ions often induce a measurable increase in intracellular ROS in both bacterial [35] and human cells [36 38], but do not directly generate ROS via Fenton type reactions [35]. It should be noted that bacterial cells are much smaller than human cells and therefore contain less total GSH (or alternative low molecular weight thiols) per cell. Human cells also produce several forms of the cysteine rich protein metallothionein (MT) that protect against oxidative damage and metal ion toxicity [39,40]. MT gene expression is induced by treatment with sub inhibitory concen trations of silver, suggesting a role in cytoprotection against this specific stress [41,42]. Furthermore, the majority of cytoplasmic proteins in a bacterium are maintained in the reduced state [16] and should therefore be more susceptible to Ag^+ binding. With this in mind, it is surprising that bacterial cells are not much more sensitive to Ag^+ than human cells. One possible explanation is that the most sensitive "targets" in bacteria and human cells lay in common essential biological processes or pathways. Xu *et al.* recently showed that silver specifically inhibits the activity of several dehydratases in *E. coli*, leading to destruction of the exposed 4Fe 4S clusters and the release of iron ions [43], which would generate intracellular ROS via Fenton type reactions. In eukaryotic cells this would cause mitochondrial damage and trigger apoptosis, as observed in response to silver treatment [37,42,44,45]. Whilst the exact mechanisms of silver toxicity are still unclear, this study has shown that extracellular thiols inactivate Ag^+ toxicity to both prokaryotic and eukaryotic cells. By understanding the mechanisms of silver toxicity and the inactivation of this by thiols, it may be possible to design silver based antibacterial coatings with improved efficacy and reduced cytotoxicity *in vivo*.

Conclusions

In conclusion, we have demonstrated that biologically relevant compounds that contain reduced thiol groups such as GSH and cysteine, and other human blood components, significantly reduce the toxicity of silver ions to clinically relevant bacteria and human dermal fibroblasts (skin cells). These findings have important implications for the development and testing of novel antimicro bial coatings, particularly those intended for use in environments exposed to biological tissues or secretions such as wound dressings and indwelling medical devices.

Author Contributions

Conceived and designed the experiments: GM ATAJ NRW. Performed the experiments: GM. Analyzed the data: GM ATAJ NRW. Contributed reagents/materials/analysis tools: GM ATAJ NRW. Wrote the paper: GM ATAJ NRW.

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INDICATIONS FOR USE

K NUMBER: **152519**

DEVICE NAME: **MTR550 ANTIMICROBIAL SILVER WOUND GEL**

INDICATIONS FOR USE

The gel dressing provides antimicrobial barrier function for the management of dry to low/moderate exuding wounds such as:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

TYPE OF USE: **Prescription Use Only (Part 21 CFR 801 Subpart D)**

510(k) SUMMARY

1. SUBMITTER/OWNER

MEDICAL TECHNOLOGY RESEARCH INC.
2650 PROGRESS WAY
WOODBURN, OR 97071

Phone: 503-902-6279
Fax: 503-980-7931

Contact Person: Bhalchandra M. Karandikar, PhD
Chief Technology Officer

Date Prepared: April 25, 2016

2. DEVICE

Name of Device:	MTR550 (Tentative) Antimicrobial Silver Wound Gel
Common Name:	Antimicrobial Wound Dressing
Classification Name:	Dressing Wound, Drug
Regulatory Class:	Unclassified
Product Code:	FRO

3. PREDICATE DEVICE

MTR550 gel is substantially equivalent to the following legally marketed predicate devices:

(i) SilverShield™ Antimicrobial Skin and Wound Gel (# K062212) from Anacapa Technologies of San Dimas, CA and distributed presently under SilverSept® name

(ii) Silver Antimicrobial Wound Gel from Advanced Medical Solutions of Winsford, UK (#K110458) and marketed by Molynyke under the name Normlgel Ag®

4. DEVICE DESCRIPTION

The product is a spreadable amorphous water based wound gel comprising a synthetic clay as the thickening agent. The gel provides for optimal moisture management of the wound bed by donating/absorbing water that it turns aids in wound healing. The presence of active silver compounds within the gel provides preservative action and acts as an effective barrier to microbial penetration by inhibiting the growth of microorganisms. The gel is clear to hazy and will not stain the skin tissue when used over a period of 3 days though its potential to stain skin beyond 3 day use is not known. Even with silver

content at 550ppm, the gel is not discolored by incidental exposure to intense light such sunlight or to elevated environment temperatures.
The device is packaged primarily in tamperproof tubes (43g) with screw caps though it may be available in other sizes and containers. The tube is contained in a cardboard box with product insert.

5. INDICATIONS FOR USE

The device is indicated for use by prescription only. It is indicated for use in the management of dry to low/moderate exuding partial and fullness thickness wounds, stage I-IV pressure ulcers, diabetic and foot ulcers, graft wounds and donor sites, first and second degree wounds, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations.

Contraindications: The gel should not be used on patients with known sensitivity to silver and s-triazine compounds.

6. MANUFACTURING

The MTR550 gel will be manufactured in a production facility in accordance with good manufacturing practices consistent with US FDA guidelines. The batch production of the gel and packaging will be verified to meet product specifications to ensure the product is safe and effective.

7. BIOCOMPATIBILITY TESTING

The device has been tested for in-vitro cytotoxicity, dermal irritation and sensitization in accordance with ISO 10993 -1 (Biological Evaluation of Medical Devices).

No systemic toxicity was associated with the antimicrobial silver compounds. Animal study employing rats showed no adverse effects on animals due to the device.

8. PERFORMANCE

The antimicrobial efficacy of the device has been demonstrated by 28 days Antimicrobial preservative challenge test in accordance with USP Chapter 51 guidelines. Employing in vitro assays, the antimicrobial barrier property was demonstrated against 17 different micro-organisms that included bacteria, yeast and fungi. The tested micro-organisms included, MRSA, VRE, Candida A. and Aspergillus niger. Sustenance of antimicrobial barrier activity was demonstrated for 3 days in a serial transfer assay.

Animal study employing rats showed the device exhibited no adverse effect on animals. In a porcine study examining deep partial thickness burn wound healing, MTR550 device was found to be safe and effective as the predicate device.

Despite its silver content at 550ppm, the device was shown to be non-staining to dermal tissue over 3 days use but its potential to stain skin after more than 3 day use is not known. The device showed no discoloration despite exposure to intense light or elevated environmental temperatures.

9. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH PREDICATE DEVICE

With respect to its physical and chemical properties, the device is substantially equivalent to the predicate device.
The mechanism of antimicrobial action is also similar. Both device exert toxicity towards micro-organisms as a result of silver ions.

The key differences between our device and predicate device are:

(i) Silver content
(ii) Source of antimicrobial silver
(iii) Content of gelling agent

These differences though real raise no safety or effectiveness issue and establish our device is substantially equivalent to the predicate device.

10. CONCLUSIONS

Based on the indications for use, biocompatibility, invivo studies, performance data, the MTR550 gel is substantially similar to SilverShield™ Antimicrobial Skin and Wound Gel (#K062212)

5.0 PROPOSED LABELING

The proposed labeling on the thin gauge cardboard box, the tube and the packaging insert is disclosed below. For comparison the product inserts from predicate devices (K062212 & K110458) are attached (see Attachments 1 & 2).

Product cardboard box labeling

Side 1

MTR550™ Gel (Note the MTR550™ name is tentative and may be replaced)

Antimicrobial silver wound gel

Rx Only / Net weight (1.5 oz/43g)

Side 2

MTR550™ Antimicrobial silver wound gel

- Contains 0.055% (550 μg/g) silver
- Provides lasting antimicrobial barrier as burn and wound dressing up to 3 days
- Helps maintain moist wound environment

Read instructions for use (see insert inside package)
Store at 15C (59F) to 30C (77F)

Side 3

Rx Only
Intended for use for the management of dry to low/moderate exuding wounds such as: partial and full thickness wounds, Stage I-IV pressure ulcers, diabetic and foot ulcers and 1st and 2nd degree burns, graft and donor sites, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations

- For external use only; not to use in or around the eyes
- Not made with natural rubber latex
- Contraindicated for use on patients with sensitivity to silver and s-triazine compounds

Side 4

Manufactured for:
Medical Technology Research Inc.
2650 Progress Way, Woodburn, OR 97071
US and corresponding international patents pending
Reorder Number XXXXX/Questions & Comments: 1-800-MTR-MEDI (Tentative)

Tube labeling

Side 1

MTR550™ Antimicrobial Silver Wound Gel*

*US & Int. patents pending

Rx Only / Net wt. 1.5 oz (43g)

Manufactured for:

Medical Technology Research Inc. Woodburn, OR 97071
www.mtrmedical.com

Side 2

MTR550™ Antimicrobial Silver Wound Gel

- Contains 0.055% (550 μg/g) silver
- Provides lasting antimicrobial barrier as burn and wound dressing up to 3 days
- Helps maintain moist wound environment

Intended for use for the management of dry to low/moderate exuding wounds such as: partial and full thickness wounds, Stage I-IV pressure ulcers, diabetic and foot ulcers and 1st and 2nd degree burns, graft and donor sites, post-operative surgical wounds, trauma wounds (dermal lesions, trauma injuries and incisions), abrasions and lacerations

Store at 15C – 30C; Read instructions for use (see package insert)

Package Insert

MTR550™ Antimicrobial Silver Wound Gel*

Reorder Number XXXXX

Product Description:

MTR550™ gel is spreadable amorphous burn and wound dressing that contains 550 ppm antimicrobial silver. The dressing is formulated to provide an antimicrobial barrier lasting up to 3 days by inhibiting the growth of common pathogens such *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, antibiotic resistant strains MRSA and VRE and yeast and fungi including *Candida albicans, Aspergillus niger* and other microorganisms. In *in vitro* tests, antimicrobial silver within MTR550™ gel dressing was found to exert preservative action against several common pathogens.

The gel dressing helps maintain moisture in wound environment which may help promote wound healing. The gel is suitable for use on dry to low/moderately exuding wounds. MTR550™ gel will not stain or discolor tissue when used daily over 3 days. However, it is not known if staining will occur if gel use is continued beyond 3 days.

It is intended for sale or for use by prescription only on patients under the medical supervision of licensed healthcare professionals. It is against the Federal law to contravene this restriction.

Indications for use:

It is indicated for use for the management of dry to low/moderate exuding wounds such as:

- Stage I-IV pressure ulcers
- Diabetic & foot ulcers
- Partial and full thickness wounds
- Graft and donor sites
- Post-operative surgical wounds
- Trauma wounds (dermal lesions, trauma injuries and incisions)
- 1 & 2nd degree burns
- Abrasions and lacerations

Contraindications for use:

- Not to be used on patients with sensitivity to silver and s-triazine compounds
- Patients may experience mild skin irritation in and around area of gel application, but if it persists or redness or swelling develops, discontinue using the gel

Directions for use:

- ✓ Cleanse and debride wound as required

- ✓ Dispense MTR550™ gel onto the wound directly and evenly spread it to a 0.1" to 0.2" thick layer. It is recommended the applied gel layer extend 0.2" to 0.25" beyond the edge of the wound for effective barrier function.
- ✓ Cover with a sterile secondary dressing (such as gauze, foam or similar) and secure it in place
- ✓ In between dressing changes, maintain moist environment in the wound bed
- ✓ MTR550™ gel dressing may be maintained for up to 3 days though heavy exuding wounds may require more frequent dressing changes as per clinical protocols

Rx Only
External Use Only. Not to be used in the proximity of eyes.
Not made with natural rubber latex

*US & INTL. PATENTS PENDING

Manufactured for:
Medical Technology Research Inc.
2650 Progress Way, Woodburn, OR 97071
www.mtrmedical.com
For questions or comments: 1-800-MTR-MEDI (tentative)

6.0 SPECIFICATION:

6.1 General Device Description

The device is an amorphous aqueous gel containing antimicrobial silver. It is transparent to slightly hazy in appearance. In wounds, it helps maintain moist environment by either donating moisture or by absorbing low to moderate amount of exudate. Moisture management may also help processes that promote wound healing. The presence of antimicrobial silver in the gel layer provides a protective barrier to entry into the wounds by inhibiting the growth of infectious pathogens that include gram positive and gram negative bacteria, yeast and fungi. The device's antimicrobial activity is exerted by ionic silver and similar to other silver containing devices.

The viscous/gel character of amorphous composition serve several purposes. As gel it can be spread easily and stay in place over the wound. Its viscous character provides flow resistance and anti-settling property that helps maintain uniform distribution of the micro-particles of the antimicrobial silver compounds. As diffusion is inversely proportional to viscosity, mass transport of the active by diffusion is controlled. That in turn limits its bolus thus preventing the gel matrix from depleting quickly and losing effectiveness. As a result, the viscous gel sustains broad spectrum antimicrobial effectiveness for prolonged period i.e. at least a period of 3 days. Unlike the viscosity build up caused by high molecular weight cellulose polymers and other natural polymers, the viscosity build up in the present gel is due to an inorganic clay mineral. The interaction of the hydrated particles of the clay mineral with other solutes in the gel form unique ordered structure on a molecular level that yields a thixotropic gel. Such gel until disturbed by shear force remains stiff (solid like) and non-flowing. However, beyond a specific force value, it rapidly loses viscous property, begins to flow and thus can be spread with minimum effort.

The amorphous gel is supplied in a net amount of 43g in heat sealed plastic tubes with a screw-on cap. To prevent tampering, the dispensing end of the tube is sealed with a coated aluminum foil. For the first use, cap is unscrewed and the foil seal is peeled off. The gel also may be packaged in larger tubes or other packaging such as 8 oz. jars. In place of foil seal to prevent tampering, the tube or the jar may be sealed with shrink wrap covering the entire cap and a portion of the tube or jar.

As finished device (in packaged form), the amorphous gel is not sterilized. However, antimicrobial silver in the gel exerts its preservative effect and maintains low to negligible bioburden of bacteria, yeast or molds in the finished device ($\leq$ 11 cfu/g).

Breadth of Antimicrobial Activity

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Indications for Use

As wound dressing, MTR550 antimicrobial gel is intended for use by prescription only. As topical gel, it is indicated for external use only. It is indicated for use under the medical supervision of a licensed healthcare professional for the management of dry to low/moderate exuding wounds such as:

Stage I-IV pressure ulcers
Diabetic & foot ulcers
Partial and full thickness wounds
Graft and donor sites
Post-operative surgical wounds
Trauma wounds (dermal lesions, trauma injuries and incisions)
1 & 2nd degree burns
Abrasions and lacerations

Contraindications and Precautions:

There are several contraindications and precautions associated with the administration of the device. It may not be used on patients with known sensitivity to silver or s-triazine compounds. It is not for ophthalmic use. The antimicrobial gel may be left in the wounds for a period of up to 3 days before a dressing change is required.

How does MTR550 gel works? Rationale for Use of Silver as Antimicrobial Agent

As a wound dressing, the amorphous antimicrobial silver hydrogel provides two functions; it maintains moisture in wounds by absorbing or donating moisture depending on wound's condition and it provides long lasting antimicrobial protective barrier by inhibiting the growth of common pathogens. As an added benefit, the moisture donation may assist in the debridement of dry necrotic wounds and by absorbing exudate may reduce skin maceration.

The antimicrobial effect is provided by ionic silver present in the device (wound dressing) which is lethal to bacteria or other organisms. Silver's antimicrobial efficacy and safety in wound products has been well established as evidenced by a large number of silver containing devices cleared by FDA under 510(k) submissions over the past decade.

As antimicrobial agent, silver offers several advantages. First, with silver there is practically no risk of microorganisms developing resistance because of the way it exerts its antimicrobial activity. Silver irreversibly binds to surfaces of cells through multiple pathways via functional groups such as carboxyl, amines and thiols making it harder for resistant microorganisms to evolve. Second, silver singularly is effective against multitude of organisms. Thus, the formulators need only one rather than many active components in their formulations or devices simplifying product design. Third, silver is very effective against infectious pathogens at very low levels and yet at these minimum inhibitory concentrations for such organisms, silver is not toxic to humans.

Despite these advantages, silver suffers from a major limitation. Product formulations or devices containing silver often have short shelf lives. The very property that silver gives its potent antimicrobial activity also makes it susceptible to reduction to inactive elemental silver induced by light or heat making it ineffective. The unpredictable nature of silver susceptibility to reduction can cause large batch to batch variability in product shelf lives.

The MTR550 gel incorporates silver as the antimicrobial agent. The source of silver is a pair of silver cyanurate compounds; di-silver cyanurate and silver cyanurate ligand complex. The gel with these two cyanurate compounds retains all the benefits mentioned above but overcomes its major drawback. The two silver cyanurate compounds have unique ability to resist light and heat induced discoloration (the visual indicator of the reduction reaction) even in water rich environment of the amorphous gel without loss of antimicrobial activity. The insensitivity to light and heat of the two silver cyanurate compounds also reduces the risk of premature silver reduction occurring thus ensuring effectiveness over its entire shelf life.

6.2 Technical Device Description

Finished composition and Ingredients

The device is an aqueous amorphous gel composition containing antimicrobial silver in the nominal concentration of 550ppm by weight. The amounts of components by weight in the gel are tabulated below:

Nominal Composition of MTR550 Gel

Component	Amount (weight %)
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The pertinent physical and chemical properties of MTR550 gel are tabulated below.

Typical MTR550 gel properties (not specification)

Property/Characteristic	Value/Description
Appearance	Transparent to slightly hazy
Texture	Smooth
Odor	Odorless
Density (g/mL) @ ~25C	Between 1 and 1.1
Surface tension (mPa.s) @ ~ 25C	N/A
Viscosity (mPa.s) @ ~ 25C	N/A
Yield stress (Pa) @ ~ 25C	~ 250
Water absorption (g/g x100) @ ~ 37C	~ 10% @ 24h; ~ 30% @ 72h
Water loss by evaporation (g/g x100) @ ~ 25C	~ < 10% @ 5h
pH	Between 8 and 10
Silver content (ppm)	~ 550
Compatibility	Incompatible with cationic polymers and reducing agents
Bioburden level	(b)(4)
Bacterial endotoxin (pyrogen) level	

(b)(4) Ingredients

[redacted]

Inactive Ingredients

(b)(4) Ingredients

[redacted]

(b)(4) Ingredients

(b)(4) Ingredients

(b)(4) Ingredients

[redacted]

The table below lists the properties of ingredients used to produce MTR550 gel.

Properties of Ingredients Used to Produce MTR550 Gel

(b)(4) Ingredients

[redacted]

6.3 Manufacturing process for MTR550 gel

(b)(4)

Device Sterility

As finished gel, the device is not sterile. However, it contains sufficient level of silver to exert its antimicrobial activity and maintains very low to negligible bioburden ($\leq$11 cfu/g). Sterilization by traditional methods of gamma irradiation or e-beam is not recommended as the gel may discolor and degrade.

6.4 Biocompatibility Testing

The FDA draft guidance document 1811 issued April 23, 2013 titled "Use of International Standard ISO 10993- Biological evaluation of medical devices Part 1: Evaluation and Testing" provides guidelines on biocompatibility testing for industry and FDA staff. Of the information provided, the matrix in Attachment A of that document is most relevant. It suggests for a surface device such as MTR550 gel, initial biocompatibility testing should include cytotoxicity, irritation, sensitization and systemic toxicity.

(b)(4)

Cytotoxicity (see Attachment 5)

(b)(4)

Dermal Irritation (see Attachment 6)

(b)(4)

Dermal Sensitization (see Attachment 7)

(b)(4)

Systemic toxicity (see Attachment 8)

(b)(4)

Wound healing study (see Attachment 9)

(b)(4)

(b)(4) Testing

[redacted]

6.5 Performance Testing

The FDA draft guidance document issued on July 19, 2007 titled "Pre-market notifications (510(k)) submissions for medical devices that include antimicrobial agents" recommends performance testing to demonstrate antimicrobial effectiveness of the finished device. The testing may include both invitro tests and invivo (animal) tests as required.

Accordingly, the following tests were conducted on MTR550 gel to demonstrate antimicrobial effectiveness.

- Antimicrobial Preservative Challenge Test in accordance with USP Chapter 51 (see Attachment 10)

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[redacted]

Date initiated: March 10, 2016	MEDICAL TECHNOLOGY RESEARCH INC. TECHNICAL REPORT	Report No. (b)(4) [redacted]
Date completed: April 22, 2016	Title: Manufacturing Process for MTR550 Gel	1st author initials: _____
Date released:	Author(s): Bhalchandra M Karandikar, PhD	Reviewer initials: _____

(b)(4) Manufacturing Information [redacted]

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WHO/SDE/WSH/07.01/16/Rev/1
English only

Nitrate and nitrite in drinking-water

Background document for *development* of
WHO *Guidelines for Drinking-water Quality*

Rev/1: Revisions indicated with a vertical line in the left margin.

Nitrate and Nitrite in Drinking-water
Background document for development of WHO *Guidelines for Drinking-water Quality*

Preface

One of the primary goals of the World Health Organization (WHO) and its Member States is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water". A major WHO function to achieve such goals is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002. The third edition of the GDWQ was published in 2004, the first addendum to the third edition was published in 2006 and the second addendum to the third edition was published in 2008. The fourth edition will be published in 2011.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Japan, the United Kingdom and the United States of America (USA) prepared the documents for the fourth edition.

Under the oversight of a group of coordinators, each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meeting on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

Acknowledgements

The original draft of Nitrate and nitrite in drinking-water, Background document for development of WHO *Guidelines for Drinking-water Quality*, was prepared by G.J.A. Speijers. It has been updated and revised by Mr J.K. Fawell of the United Kingdom.

The work of the following working group coordinators was crucial in the development of this document and others contributing to the fourth edition:

Dr J. Cotruvo, J. Cotruvo & Associates, USA (*Materials and chemicals*)
Mr J.K. Fawell, United Kingdom (*Naturally occurring and industrial contaminants* and *Pesticides*)
Ms M. Giddings, Health Canada (*Disinfectants and disinfection by-products*)
Mr P. Jackson, WRc-NSF, United Kingdom (*Chemicals – practical aspects*)
Professor Y. Magara, Hokkaido University, Japan (*Analytical achievability*)
Dr A.V. Festo Ngowi, Muhimbili University of Health and Allied Sciences, United Republic of Tanzania (*Pesticides*)
Dr E. Ohanian, Environmental Protection Agency, USA (*Disinfectants and disinfection by-products*)

The draft text was discussed at the Expert Consultation for the fourth edition of the GDWQ, held in December 2011. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants at the meeting is gratefully acknowledged.

The WHO coordinators were Mr R. Bos and Mr B. Gordon, WHO Headquarters. Ms C. Vickers provided a liaison with the International Programme on Chemical Safety, WHO Headquarters. Mr M. Zaim, Public Health and the Environment Programme, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

Ms P. Ward provided invaluable administrative support throughout the review and publication process. Ms M. Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comments are greatly appreciated.

Acronyms and abbreviations used in the text

DNA	deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
GDWQ	*Guidelines for Drinking-water Quality*
Hb	haemoglobin
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
metHb	methaemoglobin
NADH	reduced nicotinamide adenine dinucleotide
NOEL	no-observed-effect level
USA	United States of America
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

Table of contents

1. GENERAL DESCRIPTION

1.1 Identity

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle. The nitrate ion (NO_3^-) is the stable form of combined nitrogen for oxygenated systems. Although chemically unreactive, it can be reduced by microbial action. The nitrite ion (NO_2^-) contains nitrogen in a relatively unstable oxidation state. Chemical and biological processes can further reduce nitrite to various compounds or oxidize it to nitrate (ICAIR Life Systems, Inc., 1987).

1.2 Physicochemical properties (ICAIR Life Systems, Inc., 1987)[1]

Property	*Nitrate*	*Nitrite*
Acid	Conjugate base of strong acid HNO_3; $pK_a = -1.3$	Conjugate base of weak acid HNO_2; $pK_a = 3.4$
Salts	Very soluble in water	Very soluble in water
Reactivity	Unreactive	Reactive; oxidizes antioxidants, Fe^{2+} of haemoglobin to Fe^{3+}, and primary amines; nitrosates several amines and amides

1.3 Major uses and sources in drinking-water

Nitrate is used mainly in inorganic fertilizers. It is also used as an oxidizing agent and in the production of explosives, and purified potassium nitrate is used for glass making. Sodium nitrite is used as a food preservative, especially in cured meats. Nitrate is sometimes also added to food to serve as a reservoir for nitrite. Nitrates occur naturally in plants, for which it is a key nutrient. Nitrate and nitrite are also formed endogenously in mammals, including humans. Nitrate is secreted in saliva and then converted to nitrite by oral microflora.

Nitrate can reach both surface water and groundwater as a consequence of agricultural activity (including excess application of inorganic nitrogenous fertilizers and manures), from wastewater treatment and from oxidation of nitrogenous waste products in human and animal excreta, including septic tanks. Nitrite can also be formed chemically in distribution pipes by *Nitrosomonas* bacteria during stagnation of nitrate-containing and oxygen-poor drinking-water in galvanized steel pipes or if chloramination is used to provide a residual disinfectant and the process is not sufficiently well controlled.

1.4 Environmental fate

In soil, fertilizers containing inorganic nitrogen and wastes containing organic nitrogen are first decomposed to give ammonia, which is then oxidized to nitrite and nitrate. The nitrate is taken up by plants during their growth and used in the synthesis of organic nitrogenous compounds. Surplus nitrate readily moves with the groundwater (USEPA, 1987; van Duijvenboden & Matthijsen, 1989).

[1] Conversion to nitrogen: 1 mg/l as nitrate = 0.226 mg/l as nitrate-nitrogen; 1 mg/l as nitrite = 0.304 mg/l as nitrite-nitrogen.

Under aerobic conditions, nitrate can percolate in relatively large quantities into the aquifer when there is no growing plant material to take up the nitrate and when the net movement of soil water is downward to the aquifer. Degradation or denitrification occurs only to a small extent in the soil and in the rocks forming the aquifer. Under anaerobic conditions, nitrate may be denitrified or degraded almost completely to nitrogen. The presence of high or low water tables, the amount of rainwater, the presence of other organic material and other physicochemical properties are also important in determining the fate of nitrate in soil (van Duijvenboden & Loch, 1983; Mesinga, Speijers & Meulenbelt, 2003; Fewtrell, 2004; Dubrovsky & Hamilton, 2010). In surface water, nitrification and denitrification may also occur, depending on the temperature and the pH. The uptake of nitrate by plants, however, is responsible for most of the nitrate reduction in surface water.

Nitrogen compounds are formed in the air by lightning or discharged into it from industrial processes, motor vehicles and intensive agriculture. Nitrate is present in air primarily as nitric acid and inorganic aerosols, as well as nitrate radicals and organic gases or aerosols. These are removed by wet and dry deposition.

2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

2.1 Air

Atmospheric nitrate concentrations ranging from 0.1 to 0.4 µg/m^3 have been reported, the lowest concentrations being found in the South Pacific (Prospero & Savoie, 1989). Higher concentrations ranging from 1 to 40 µg/m^3 have also been reported, with annual means of 1–8 µg/m^3. Mean monthly nitrate concentrations in air in the Netherlands range from 1 to 14 µg/m^3 (Janssen, Visser & Roemer, 1989). Indoor nitrate aerosol concentrations of 1.1–5.6 µg/m^3 were found to be related to outdoor concentrations (Yocom, 1982).

2.2 Water

Concentrations of nitrate in rainwater of up to 5 mg/l have been observed in industrial areas (van Duijvenboden & Matthijsen, 1989). In rural areas, concentrations are somewhat lower.

The nitrate concentration in surface water is normally low (0–18 mg/l) but can reach high levels as a result of agricultural runoff, refuse dump runoff or contamination with human or animal wastes. The concentration often fluctuates with the season and may increase when the river is fed by nitrate-rich aquifers. Nitrate concentrations have gradually increased in many European countries in the last few decades and have sometimes doubled over the past 20 years. In the United Kingdom, for example, an average annual increase of 0.7 mg/l has been observed in some rivers (Young & Morgan-Jones, 1980).

The natural nitrate concentration in groundwater under aerobic conditions is a few milligrams per litre and depends strongly on soil type and on the geological situation. In the United States of America (USA), naturally occurring levels do not exceed 4–9 mg/l for nitrate and 0.3 mg/l for nitrite (USEPA, 1987). As a result of agricultural activities, the nitrate concentration can easily reach several hundred milligrams per litre (WHO, 1985b). For example, concentrations of up to 1500 mg/l were found in groundwater in an agricultural area of India (Jacks & Sharma, 1983).

In the USA, nitrates are present in most surface water and groundwater supplies at levels below 4 mg/l, with levels exceeding 20 mg/l in about 3% of surface waters and 6% of groundwaters. In 1986, a nitrate concentration of 44 mg/l (10 mg of nitrate-nitrogen per litre) was exceeded in 40 surface water and 568 groundwater supplies. Nitrite levels were not surveyed but are expected to be much lower than 3.3 mg/l (USEPA, 1987).

The increasing use of artificial fertilizers, the disposal of wastes (particularly from animal farming) and changes in land use are the main factors responsible for the progressive increase in nitrate levels in groundwater supplies over the last 20 years. In Denmark and the Netherlands, for example, nitrate concentrations are increasing by 0.2–1.3 mg/l per year in some areas (WHO, 1985b). Because of the delay in the response of groundwater to changes in soil, some endangered aquifers have not yet shown the increase expected from the increased use of nitrogen fertilizer or manure. Once the nitrate reaches these aquifers, the aquifers will remain contaminated for decades, even if there is a substantial reduction in the nitrate loading of the surface.

In most countries, nitrate levels in drinking-water derived from surface water do not exceed 10 mg/l. In some areas, however, concentrations are higher as a result of runoff and the discharge of sewage effluent and certain industrial wastes. In 15 European countries, the percentage of the population exposed to nitrate levels in drinking-water above 50 mg/l ranged from 0.5% to 10% (WHO, 1985b; ECETOC, 1988); this corresponds to nearly 10 million people. Individual wells in agricultural areas throughout the world especially contribute to nitrate-related toxicity problems, and nitrate levels in the well water often exceed 50 mg/l.

Nitrite levels in drinking-water are usually below 0.1 mg/l. In 1993, a maximum value of 0.21 mg/l was detected in the Netherlands (RIVM, 1993).

Chloramination may give rise to the formation of nitrite within the distribution system, and the concentration of nitrite may increase as the water moves towards the extremities of the system. Nitrification in distribution systems can increase nitrite levels, usually by 0.2–1.5 mg of nitrite per litre, but potentially by more than 3 mg of nitrite per litre (AWWARF, 1995).

2.3 Food

Vegetables and cured meat are in general the main sources of nitrate and nitrite in the diet, but small amounts may be present in fish and dairy products. Meat products may contain <2.7–945 mg of nitrate per kilogram and <0.2–6.4 mg of nitrite per kilogram; dairy products may contain <3–27 mg of nitrate per kilogram and <0.2–1.7 mg of nitrite per kilogram (ECETOC, 1988). Several vegetables and fruits contain 200–2500 mg of nitrate per kilogram (van Duijvenboden & Matthijsen, 1989). The nitrate content of vegetables can be affected by processing of the food, the use of fertilizers and growing conditions, especially the soil temperature and (day)light intensity (Gangolli et al., 1994; FAO/WHO, 1995). Vegetables such as beetroot, lettuce, radish and spinach often contain nitrate concentrations above 2500 mg/kg, especially when they are cultivated in greenhouses. Nitrite levels in food are very low (generally well below 10 mg/kg) and rarely exceed 100 mg/kg. Exceptions to this are vegetables that have been damaged, poorly stored or stored for extended periods, as well as pickled or fermented vegetables. In such circumstances, nitrite levels of up to 400 mg/kg have been found (FAO/WHO, 1995).

2.4 Estimated total exposure and relative contribution of drinking-water

Air pollution appears to be a minor source of nitrate exposure. In general, vegetables will be the main source of nitrate intake when nitrate levels in drinking-water are below 10 mg/l (Chilvers, Inskip & Caygill, 1984; USEPA, 1987; ECETOC, 1988).

When nitrate levels in drinking-water exceed 50 mg/l, drinking-water will be the major source of total nitrate intake, especially for bottle-fed infants. In the Netherlands, the average population exposure is approximately 140 mg of nitrate per day (including the nitrate in drinking-water). The contribution of drinking-water to nitrate intake is usually less than 14%. For bottle-fed infants, daily intake from formula made with water containing 50 mg of nitrate per litre would average about 8.3–8.5 mg of nitrate per kilogram of body weight per day.

The mean dietary intakes determined by the duplicate portion technique (WHO, 1985a) range from 43 to 131 mg of nitrate per day and from 1.2 to 3 mg of nitrite per day. Estimates of the total nitrate intake based on the proportion of nitrate excreted in the urine (Bartholomew et al., 1979) range from 39 to 268 mg/day, the higher values applying to vegetarian and nitrate-rich diets (ECETOC, 1988). The estimated total daily intake of nitrate ranged in the United Kingdom from 50 to 81 mg per person (Bonnell, 1995; Schuddeboom, 1995), in Denmark from 70 to 172 mg per person (Bonnell, 1995) and in Germany from 70 to 110 mg per person (Bonnell, 1995). According to the United States Environmental Protection Agency (USEPA), the average nitrate intake from food is approximately 40–100 mg/day for males. The daily nitrite intake ranges from 0.3 to 2.6 mg/day, primarily from cured meat (NAS, 1981). Nitrite present in cured meat has been reported to account for up to 70% of total dietary intake of this substance, depending on the intake of such meat and the origin and type of cured meat consumed. Mean dietary nitrite intake from all food

sources has been reported to range from <0.1 to 8.7 mg of nitrite per person per day for European diets (FAO/WHO, 1995). EFSA (2008) indicated that average adult consumption of nitrate from all dietary sources, including water for the United Kingdom and France, was 91 and 141 mg per person, respectively, indicating that average intakes have remained relatively stable. For some individuals and communities where vegetables with particularly high nitrate levels are consumed or where well water contains elevated concentrations of nitrate, concumption may be significantly higher.

3. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

3.1 Absorption, distribution and elimination

Ingested nitrate is readily and completely absorbed from the upper small intestine. Nitrite may be absorbed directly from both the stomach and the upper small intestine. Part of the ingested nitrite reacts with gastric contents prior to absorption. At least 25% of the ingested nitrate is transported into the saliva, where the concentration is approximately 10 times greater than that in plasma as a result of bioconcentration. About 20% of the nitrate in saliva is converted to nitrite by commensal bacteria on the surface of the tongue. Individuals with gastroenteritis have a higher conversion rate (EFSA, 2008). There is evidence that the use of antibacterial mouthwashes may reduce this conversion (van Maanen et al., 1996; Govoni et al., 2008).

Nitrate is rapidly distributed throughout the tissues. Approximately 25% of ingested nitrate is actively secreted into saliva, where it is partly (20%) reduced to nitrite by the oral microflora; nitrate and nitrite are then swallowed and re-enter the stomach. Bacterial reduction of nitrate may also take place in other parts of the human gastrointestinal tract, but not normally in the stomach; exceptions are reported in humans with low gastric acidity, such as artificially fed infants, certain patients in whom hydrochloric acid secretion is slower than normal or patients using antacids (Colbers et al., 1995). In rats, active secretion and reduction of nitrate in saliva are virtually absent (Walker, 1995). Total nitrate reduction is probably less in rats than in humans.

Absorbed nitrite is rapidly oxidized to nitrate in the blood. Nitrite in the bloodstream is involved in the oxidation of haemoglobin (Hb) to methaemoglobin (metHb): the Fe^{2+} present in the haem group is oxidized to its Fe^{3+} form, and the remaining nitrite binds firmly to this oxidized haem. The Fe^{3+} form does not allow oxygen transport, owing to the strong binding of oxygen (Jaffé, 1981; United States National Research Council, 1995). Therefore, methaemoglobinaemia can lead to cyanosis.

Nitrite has been shown to cross the placenta and cause the formation of fetal methaemoglobinaemia in rats. It may react in the stomach with nitrosatable compounds (e.g. secondary and tertiary amines or amides in food) to form *N*-nitroso compounds. Such endogenous nitrosation has been shown to occur in human as well

as animal gastric juice both in vivo and in vitro, mostly at higher pH values, when both nitrite and nitrosatable compounds were present simultaneously (Shephard, 1995; FAO/WHO, 1996).

The major part of the ingested nitrate is eventually excreted in urine as nitrate, ammonia or urea, faecal excretion being negligible. Little nitrite is excreted (WHO, 1985b; ICAIR Life Systems, Inc., 1987; Speijers et al., 1989).

3.2 Endogenous synthesis of nitrate and nitrite

The excess nitrate excretion that has often been observed after low nitrate and nitrite intake originates from endogenous synthesis, which amounts, in normal healthy humans, to 1 mmol/day on average, corresponding to 62 mg of nitrate per day or 14 mg of nitrate-nitrogen per day. Gastrointestinal infections greatly increase nitrate excretion, as a result, at least in part, of increased endogenous (non-bacterial) nitrate synthesis, probably induced by activation of the mammalian reticuloendothelial system (WHO, 1985b; Speijers et al., 1989; Wishnok et al., 1995; FAO/WHO, 1996). This endogenous synthesis of nitrate complicates the risk assessment of nitrate.

Increased endogenous synthesis of nitrate, as reported in animals with induced infections and inflammatory reactions, was also observed in humans. Infections and non-specific diarrhoea played a role in the increased endogenous synthesis of nitrate (Tannenbaum et al., 1978; Green et al., 1981; Hegesh & Shiloah, 1982; Bartholomew & Hill, 1984; Lee et al., 1986; Gangolli et al., 1994). These observations are all consistent with the induction of one or more nitric oxide synthases by inflammatory agents, analogous to the experiments described in animals and macrophages. This induction in humans has been difficult to demonstrate directly, but administration of [^{15}N]arginine to two volunteers resulted in the incorporation of ^{15}N into urinary nitrate in both individuals, confirming the arginine–nitric oxide pathway in humans (Leaf, Wishnok & Tannenbaum, 1989).

Nitrate excretion in excess of nitrate intake by humans was reported in 1916, but this result remained obscure until the end of the 1970s, when it was re-examined because of the potential involvement of nitrate in endogenous nitrosation. A relatively constant daily production of about 1 mmol of nitrate was confirmed. A major pathway for endogenous nitrate production is conversion of arginine by macrophages to nitric oxide and citrulline, followed by oxidation of the nitric oxide to nitrous anhydride and then reaction of nitrous anhydride with water to yield nitrite. Nitrite is rapidly oxidized to nitrate through reaction with Hb. In addition to macrophages, many cell types can form nitric oxide, generally from arginine. Under some conditions, bacteria can form nitric oxide by reduction of nitrite. These processes can lead to nitrosation of amines at neutral pH, presumably by reaction with nitrous anhydride. The question of whether the arginine–nitrate pathway can be associated with increased cancer risk via exposure to *N*-nitroso compounds remains open. Nitric oxide is mutagenic towards bacteria and human cells in culture; it causes deoxyribonucleic acid (DNA) strand breaks, deamination (probably via nitrous anhydride) and oxidative damage; and it

can activate cellular defence mechanisms. In virtually all of these cases, the biological response is paralleled by the final nitrate levels. Thus, whereas endogenously formed nitrate may itself be of relatively minor toxicological significance, the levels of this substance may serve as indicators for those potentially important nitric oxide–related processes that gave rise to it (Wishnok et al., 1995).

As mentioned above, both in vitro and in vivo studies showed that nitrate can be reduced to nitrite by bacterial and mammalian metabolic pathways, via the widespread nitrate reductase (Gangolli et al., 1994). In humans, saliva is the major site for the formation of nitrite. About 5% of dietary nitrate is converted to nitrite (Spiegelhalder, Eisenbrand & Preussmann, 1976; Eisenbrand et al., 1980; Walters & Smith, 1981; Gangolli et al., 1994). A direct correlation between gastric pH, bacterial colonization and gastric nitrite concentration has been observed in healthy people with a range of pH values from 1 to 7 (Mueller et al., 1983, 1986). In individuals with gastrointestinal disorders and achlorhydria, high levels of nitrite can be reached (6 mg/l) (Rudell et al., 1976, 1978; Dolby et al., 1984). The situation in neonates is not clear. It is commonly accepted that infants younger than 3 months may be highly susceptible to gastric bacterial nitrate reduction, as the pH is generally higher than in adults (Speijers et al., 1989). However, the presence of acid-producing lactobacilli in the stomach may be important, as these organisms do not reduce nitrate and may maintain a pH low enough to inhibit colonization by nitrate-reducing bacteria (Bartholomew et al., 1980). As mentioned above, nitrite may also be produced via the arginine–nitric oxide pathway but would be undetectable because of the rapid oxidation to nitrate. One possible example of nitrite production by this route, however, is the methaemoglobinaemia observed in infants suffering from diarrhoea (Gangolli et al., 1994).

In addition to the endogenous production of nitrate and its role in the nitric oxide pathway, there is increasing evidence for the beneficial role of this pathway in human health. There is evidence for its importance in protecting against oral and gastrointestinal diseases (Duncan et al., 1997) and also for its role in vascular fitness and exerting antihypertensive effects (Bryan & Loscalzo, 2011; Carlstrom et al., 2011; Lansley et al., 2011; Montenegro et al., 2011; Tang et al., 2011; Zhu et al., 2011).

4. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO SYSTEMS

4.1 Acute exposure

The acute oral toxicity of nitrate to laboratory animals is low to moderate. Median lethal doses (LD_{50} values) of 1600–9000 mg of sodium nitrate per kilogram of body weight have been reported in mice, rats and rabbits. Ruminants are more sensitive to the effects of nitrate as a result of high nitrate reduction in the rumen; the LD_{50} for cows was 450 mg of sodium nitrate per kilogram of body weight. Nitrite is more toxic than nitrate: LD_{50} values of 85–220 mg of sodium nitrite per kilogram of body weight have been reported for mice and rats (Speijers et al., 1989; FAO/WHO, 1996).

4.2 Short-term exposure

In a 13-week study in which nitrite was given to rats in drinking-water, a dose-related hypertrophy of the adrenal zona glomerulosa was observed at all dose levels (100, 300, 1000 or 3000 mg of potassium nitrite per litre). Increased metHb levels were seen only in the highest dose group (Til et al., 1988). FAO/WHO (1995) concluded that the no-observed-effect level (NOEL) in this study was 100 mg of potassium nitrite per litre (equivalent to 5.4 mg/kg of body weight per day expressed as nitrite ion), because the hypertrophy seen at this dose was not significantly different from the controls.

An additional 13-week study in which nitrite was also given in drinking-water, including lower doses of potassium nitrite and two doses of sodium nitrite (equimolar to the low and high doses of potassium nitrite), confirmed the finding of the adrenal hypertrophy of the zona glomerulosa for potassium nitrite and also revealed hypertrophy in the animals given sodium nitrite. The NOEL for the adrenal hypertrophy of the zona glomerulosa was 50 mg of potassium nitrite per litre (equivalent to 5 mg of potassium nitrite per kilogram of body weight per day) (Kuper & Til, 1995). Since then, studies designed to clarify the etiology of this hypertrophy and to establish its significance for human health have been partly performed and are currently in progress. The studies already performed confirmed the adrenal hypertrophy in another rat strain. However, the effects were seen only at higher dose levels. It was also seen that the hypertrophy was still present after a 30-day recovery period but had disappeared after a 60-day recovery period. At present, the mechanism of hypertrophy induced by nitrite is not clear (Boink, Dormans & Speijers, 1995).

A variety of experimental and field studies in different mammals identified inorganic nitrate as a goitrogenic agent. It could be shown in rats by oral and parenteral application of potassium nitrate (Wyngaarden, Stanbury & Rabb, 1953; Bloomfield et al., 1961; Alexander & Wolff, 1966; Wolff, 1994), of nitrate in hay (Lee, Weiss & Horvath, 1970) and of sodium nitrate (Höring et al., 1985; Seffner & Höring, 1987a,b). Antithyroid effects of nitrate were also found in sheep (Bloomfield et al., 1961) and in pigs by application of potassium nitrate (Jahreis et al., 1986, 1987). Furthermore, nitrate was goitrogenic to livestock: pigs (Körber, Groppel & Leirer, 1983), cattle (Körber, Groppel & Leirer, 1983; Körber, Rossow & Otta, 1985), sheep (Körber, Groppel & Leirer, 1983) and goats (Prassad, 1983).

4.3 Long-term exposure

The only observed effect of nitrate in rats after 2 years of oral administration was growth inhibition; this was seen at dietary concentrations of 5% sodium nitrate and higher. The NOEL in this study was 1%, which corresponds to 370 mg of nitrate per kilogram of body weight per day (Speijers et al., 1989; FAO/WHO, 1996). A more recent long-term study was solely a carcinogenicity study, in which the highest dose levels of 1820 mg of nitrate per kilogram of body weight per day did not show

carcinogenic effects. However, this level could not be considered as a NOEL, because complete histopathological examinations were not performed (FAO/WHO, 1996).

One of the long-term effects of nitrite reported in a variety of animal species is vitamin A deficiency; this is probably caused by the direct reaction of nitrite with the vitamin. The most important effects reported in long-term animal studies were an increase in metHb level and histopathological changes in the lungs and heart in rats receiving nitrite in drinking-water for 2 years. The lowest-observed-adverse-effect level (LOAEL), which gave a metHb level of 5%, was 1000 mg of sodium nitrite per litre; the NOEL was 100 mg of sodium nitrite per litre, equivalent to 10 mg of sodium nitrite per kilogram of body weight per day (or 6.7 mg/kg of body weight per day expressed as nitrite ion) (Speijers et al., 1989).

4.4 Reproductive and developmental toxicity

The reproductive behaviour of guinea-pigs was impaired only at very high nitrate concentrations (30 000 mg of potassium nitrate per litre); the NOEL was 10 000 mg/l (Speijers et al., 1989; FAO/WHO, 1996). In rabbits, dose levels of 250 or 500 mg of nitrate per litre administered during 22 weeks revealed no detrimental effects on reproductive performance after successive gestations. In sheep and cattle, no abortions were observed at dose levels causing severe methaemoglobinaemia (Speijers et al., 1989; FAO/WHO, 1996).

Nitrite appeared to cause fetotoxicity in rats at drinking-water concentrations equivalent to 200 and 300 mg of sodium nitrite per kilogram of body weight per day, causing increased maternal metHb levels. However, after similar doses in feed in other studies, no embryotoxic effects were observed in rats. In a reproductive toxicity study in guinea-pigs at dose levels of 0, 50 or 60 mg of sodium nitrite per kilogram of body weight per day given by subcutaneous injection, fetal death followed by abortion occurred at the highest dose level. Teratogenic effects were not observed in reported studies in mice and rats (Speijers et al., 1989; FAO/WHO, 1996).

4.5 Mutagenicity and related end-points

Nitrate is not mutagenic in bacteria and mammalian cells in vitro. Chromosomal aberrations were observed in the bone marrow of rats after oral nitrite uptake, but this could have been due to exogenous *N*-nitroso compound formation. Nitrite is mutagenic. It causes morphological transformations in in vitro systems; mutagenic activity was also found in a combined in vivo–in vitro experiment with Syrian hamsters. The results of in vivo experiments were controversial (Speijers et al., 1989; FAO/WHO, 1996).

4.6 Carcinogenicity

Nitrate is not carcinogenic in laboratory animals. Some studies in which nitrite was given to mice or rats in the diet showed slightly increased tumour incidence; however,

the possibility of exogenous *N*-nitroso compound formation in these studies could not be excluded. In studies in which high levels of nitrite and simultaneously high levels of nitrosatable precursors were administered, increased tumour incidence was seen (Speijers et al., 1989; FAO/WHO, 1996, 2003a). These types of tumours could be characteristic of the presumed corresponding *N*-nitroso compound endogenously formed. However, this increase in tumour incidence was seen only at extremely high nitrite levels, in the order of 1000 mg/l of drinking-water. At lower nitrite levels, tumour incidence resembled those of control groups treated with the nitrosatable compound only. On the basis of adequately performed and reported studies, it may be concluded that nitrite itself is not carcinogenic to animals (Speijers et al., 1989; FAO/WHO, 1996, 2003a).

5. EFFECTS ON HUMANS

5.1 Methaemoglobinaemia

The toxicity of nitrate to humans is mainly attributable to its reduction to nitrite. The major biological effect of nitrite in humans is its involvement in the oxidation of normal Hb to metHb, which is unable to transport oxygen to the tissues. The reduced oxygen transport becomes clinically manifest when metHb concentrations reach 10% of normal Hb concentrations and above; the condition, called methaemoglobinaemia, causes cyanosis and, at higher concentrations, asphyxia. The normal metHb level in humans is less than 2%; in infants under 3 months of age, it is less than 3%.

The Hb of young infants is more susceptible to metHb formation than that of older children and adults. This higher susceptibility was believed to be the result of the large proportion of fetal Hb still present in the blood of these infants, which was more easily oxidized to metHb, but this has been shown to not be the case (Avery, 1999). However, reduced nicotinamide adenine dinucleotide (NADH)–cytochrome b5–metHb reductase does not reach reference levels until after 4 months of age, with a consequent reduction in the ability to reduce metHb back to Hb. The net result is that a dose of nitrite causes a higher metHb formation in these infants than in adults. With respect to exposure to nitrate, these young infants are also more at risk because of a relatively high intake of nitrate in relation to body weight and, under certain conditions, a higher reduction of nitrate to nitrite by gastric bacteria as a result of the low production of gastric acid (FAO/WHO, 1996). The higher reduction of nitrate to nitrite in the young infants is not quantified very well, and it appears that gastrointestinal infections are important in significantly increasing the risk of higher yield of nitrite and thus higher metHb formation (ECETOC, 1988; Speijers et al., 1989; Möller, 1995; Schuddeboom, 1995; FAO/WHO, 1996). However, there is also evidence that gastrointestinal infections may cause metHb formation through the nitric oxide pathway (Avery, 1999). Other studies have shown that high nitrate concentration, above 100 mg/l, is an important cause of metHb formation and that breastfeeding is protective in exposed populations. However, gastrointestinal infection is a very important contributor. Thus, not only is the microbiological quality of

drinking-water important, but also proper hygiene is essential to prevent such infections (Pollack & Pollack, 1994; Hanukoglu & Danon, 1996; Zeman et al., 2002).

Other groups potentially susceptible to metHb formation include pregnant women and people deficient in glucose-6-phosphate dehydrogenase or metHb reductase (Speijers et al., 1989).

5.2 Adults and children above the age of 3 months

Cases of methaemoglobinaemia have been reported in adults consuming high doses of nitrate by accident or as a medical treatment. Fatalities were reported after single intakes of 4–50 g of nitrate (equivalent to 67–833 mg of nitrate per kilogram of body weight) (Speijers et al., 1989; FAO/WHO, 1996), many of which occurred among special risk groups in whose members gastric acidity was reduced. Toxic doses—with metHb formation as a criterion for toxicity—ranged from 2 to 9 g (equivalent to 33–150 mg of nitrate per kilogram of body weight) (FAO/WHO, 1996). In a controlled study, an oral dose of 7–10.5 g of ammonium nitrate and an intravenous dose of 9.5 g of sodium nitrate did not cause increased metHb levels in adults, although vomiting and diarrhoea occurred (Speijers et al., 1989; FAO/WHO, 1996).

Accidental human intoxications have been reported as a result of the presence of nitrite in food. The oral lethal dose for humans was estimated to range from 33 to 250 mg of nitrite per kilogram of body weight, the lower doses applying to children and elderly people. Toxic doses giving rise to methaemoglobinaemia ranged from 0.4 to 200 mg/kg of body weight (FAO/WHO, 1996).

Another source of information with respect to nitrite toxicity in humans is the use of sodium nitrite as medication for vasodilatation or as an antidote in cyanide poisoning. Doses of 30–300 mg per person (equivalent to 0.5–5 mg/kg of body weight) were reported not to cause toxic effects (FAO/WHO, 1996).

Few cases of methaemoglobinaemia have been reported in older children. A correlation study among children aged 1–8 years in the USA showed that there was no difference in metHb levels between 64 children consuming high-nitrate well water (22–111 mg of nitrate-nitrogen per litre) and 38 children consuming low-nitrate water (<10 mg of nitrate-nitrogen per litre). These concentrations correspond to 100–500 and <44 mg of nitrate per litre, respectively. All the metHb levels were within the normal range, suggesting that older children are relatively insensitive to the effects of nitrate (Craun, Greathouse & Gunderson, 1981).

5.3 Infants under 3 months of age

Cases of methaemoglobinaemia related to lower intakes of nitrate appear to be restricted to infants. In infants under the age of 3 months, the conversion of nitrate to nitrite and metHb formation are high, as discussed above. Gastrointestinal disturbances play a crucial role, also as discussed above. Toxic effects can therefore

be induced at a much lower dose of nitrate than in adults. According to Corré & Breimer (1979), assuming an 80% reduction of nitrate to nitrite in these young infants, the toxic dose ranged from 1.5 to 2.7 mg of nitrate per kilogram of body weight, using 10% formation of metHb as a toxicity criterion. However, in reported cases of methaemoglobinaemia, the amounts of nitrate ingested were higher: 37.1–108.6 mg/kg of body weight, with an average of 56.7 mg of nitrate per kilogram of body weight (FAO/WHO, 1996). In studies in which a possible association between clinical cases of infantile methaemoglobinaemia or subclinically increased metHb levels and nitrate concentrations in drinking-water was investigated, a significant relationship was usually found, most clinical cases (97.7%) occurring at nitrate levels of 44.3–88.6 mg/l or higher (Walton, 1951; FAO/WHO, 1996), and almost exclusively in infants under 3 months of age (Walton, 1951). However, subsequent studies have identified methaemoglobinaemia only at nitrate concentrations in water that are higher than this, mostly in excess of 100 mg/l, and often in the presence of gastrointestinal infections. Some cases of infant methaemoglobinaemia have been described in which increased endogenous nitrate (nitrite) synthesis as a result of gastrointestinal infection appeared to be the only causative factor (FAO/WHO, 1996). As most cases of infantile methaemoglobinaemia reported in the literature have been associated with the consumption of private and often bacterially contaminated well water, the involvement of infections is highly probable. Most of these studies may be therefore less suitable from the point of view of the quantitative assessment of the risk of nitrate intake for healthy infants.

5.4 Carcinogenicity

Nitrite was shown to react with nitrosatable compounds in the human stomach to form *N*-nitroso compounds. Many of these *N*-nitroso compounds have been found to be carcinogenic in all the animal species tested, although some of the most readily formed compounds, such as *N*-nitrosoproline, are not carcinogenic in humans. The *N*-nitroso compounds carcinogenic in animal species are probably also carcinogenic in humans. However, the data from a number of epidemiological studies are at most only suggestive. The endogenous formation of *N*-nitroso compounds is also observed in several animal species, if relatively high doses of both nitrite and nitrosatable compounds are administered simultaneously. Thus, a link between cancer risk and endogenous nitrosation as a result of high intake of nitrate and/or nitrite and nitrosatable compounds is possible (Speijers et al., 1989; FAO/WHO, 1996, 2003a,b).

Several reviews of epidemiological studies have been published; most of these studies are geographical correlation studies relating estimated nitrate intake to gastric cancer risk. The United States National Research Council found some suggestion of an association between high nitrate intake and gastric and/or oesophageal cancer (NAS, 1981). However, individual exposure data were lacking, and several other plausible causes of gastric cancer were present. In a later review by the World Health Organization (WHO, 1985b), some of the earlier associations appeared to be weakened following the introduction of individual exposure data or after adjustment for socioeconomic factors. No convincing evidence was found of an association

between gastric cancer and the consumption of drinking-water in which nitrate concentrations of up to 45 mg/l were present. No firm evidence was found at higher levels either, but an association could not be excluded because of the inadequacy of the data available. More recent geographical correlation and occupational exposure studies also failed to demonstrate a clear relationship between nitrate intake and gastric cancer risk, although these studies were well designed. A case–control study in Canada, in which dietary exposure to nitrate and nitrite was estimated in detail, showed that exogenous nitrite intake, largely from preserved meat, was significantly associated with the risk of developing gastric cancer (ECETOC, 1988). On the other hand, case–control studies based on food frequency questionnaires tend to show a protective effect of the estimated nitrate intake on gastric cancer risk. Most likely this is due to the known strong protective effect of vegetables and fruits on the risk of gastric cancer (Möller, 1995; FAO/WHO, 1996). Studies that have assessed the effect of nitrate from sources other than vegetables, such as the concentration in drinking-water or occupational exposure to nitrate dusts, have not shown a protective effect against gastric cancer risk. For other types of cancer, there are no adequate data with which to establish any association with nitrite or nitrate intake (Gangolli et al., 1994; Möller, 1995; FAO/WHO, 1996).

It has been established that the intake of certain dietary components present in vegetables, such as vitamins C and E, decreases the risk of gastric cancer. This is generally assumed to be at least partly due to the resulting decrease in the conversion of nitrate to nitrite and in the formation of *N*-nitroso compounds. It is possible that any effect of a high nitrate intake per se is masked in correlation studies by the antagonizing effects of simultaneously consumed dietary protective components. However, the absence of any link with cancer in occupational exposure studies is not in agreement with this theory.

5.5 Other effects

Congenital malformations have been related to high nitrate levels in drinking-water in Australia; however, these observations were not confirmed. Other studies also failed to demonstrate a relationship between congenital malformations and nitrate intake (WHO, 1985b; ECETOC, 1988; Manassaram et al., 2007).

Studies relating cardiovascular effects to nitrate levels in drinking-water gave inconsistent results (WHO, 1985b).

Possible relationships between nitrate intake and effects on the thyroid have also been studied. It is known that nitrate can competitively inhibit iodine uptake, as with similar anions. However, what is known to occur in the laboratory may not result in adverse effects in human populations under normal circumstances of exposure. In addition to effects of nitrate on the thyroid observed in experimental animal studies and in livestock, epidemiological studies revealed indications for an antithyroid effect of nitrate in humans. If dietary iodine is available at an adequate range (corresponding to a daily iodine excretion of 150–300 µg/day), the effect of nitrate is likely to be

weak, with a tendency to zero. The nitrate effect on thyroid function may be strong if a nutritional iodine deficiency exists simultaneously (Höring, Nagel & Haerting, 1991; Höring, 1992).

Hettche (1956a,b) described an association between high nitrate concentrations in drinking-water and goitre incidence. As well, Höring & Schiller (1987), Sauerbrey & Andree (1988), Höring, Nagel & Haerting (1991), Höring (1992) and van Maanen et al. (1994) found that inorganic nitrate in drinking-water is associated with endemic goitre. A dose–response relationship could be demonstrated by Höring, Nagel & Haerting (1991) (nitrate in drinking-water versus incidence of goitre) as well as by van Maanen et al. (1994) (nitrate in drinking-water versus thyroid volume). Both the experimental and epidemiological studies give the impression that nitrate in drinking-water has a stronger effect on thyroid function than does nitrate in food. The differences in nitrate kinetics after ingestion through drinking-water and through food could be the cause of the difference in thyroid effects. However, no adequate studies regarding this question exist at present. Furthermore, some of the above-mentioned studies demonstrate that dietary iodine deficiency is much more effective than nitrate exposure in causing goitre.

A number of subsequent studies in Slovakia, Bulgaria, Germany and the USA have reported a correlation between various measures of nitrate intake and effects on thyroid function, but all suffer from methodological and data problems that preclude definitive conclusions being drawn (Gatseva & Dimitrov, 1997; Gatseva et al., 1998; Hampel et al., 2003; Tajtakova et al., 2006; Gatseva & Argirova, 2008a,b; Radikova et al., 2008; Ward et al., 2010).

Other studies, including a clinical study in the Netherlands, did not find any relationship between nitrate intake and thyroid structure or function (Hunault et al., 2007; Blount et al., 2009).

Because there are a number of factors that may complicate the findings of epidemiological studies, including low iodine intake and thiocyanates in the diet, it is important that studies are sufficiently comprehensive and take such factors into account. Where small communities that use a range of wells with varying nitrate concentrations are studied, better characterization of intake is important, rather than relying purely on nitrate concentrations.

In addition to the effect of nitrite on the adrenal zona glomerulosa in rats, a study in humans indicated that sodium nitrite (0.5 mg of sodium nitrite per kilogram of body weight per day, for 9 days) caused a decreased production of adrenal steroids, as reflected by the decreased concentration of 17-hydroxysteroid and 17-ketosteroids in urine (Til et al., 1988; Kuper & Til, 1995). Similar results were also found in rabbits (Violante, Cianetti & Ordine, 1973).

6. PRACTICAL ASPECTS

6.1 Analytical methods and achievability

Spectrometric techniques are used for the determination of nitrate in water. Detection limits range from 0.01 to 1 mg/l (ISO, 1986, 1988). A molecular absorption spectrometric method is available for the determination of nitrite in potable water, raw water and wastewater. The limit of detection lies within the range of 0.005–0.01 mg/l (ISO, 1984). A continuous-flow spectrometric method for the determination of nitrite, nitrate or the sum of both in various types of water is suitable at concentrations ranging from 0.05 to 5 mg/l for nitrite and from 1 to 100 mg/l for nitrite/nitrate, both in the undiluted sample (ISO, 1996).

Nitrate and nitrite can also be determined in water by liquid chromatography, down to a level of 0.1 mg/l for nitrate and 0.05 mg/l for nitrite (ISO, 1992).

6.2 Treatment and control methods and technical performance

The most appropriate means of controlling nitrate concentrations, particularly in groundwater, is the prevention of contamination (Schmoll et al., 2006). This may take the form of appropriate management of agricultural practices, the careful siting of pit latrines and septic tanks, sewer leakage control, as well as management of fertilizer and manure application and storage of animal manures. It may also take the form of denitrification of wastewater effluents.

Methaemoglobinaemia has most frequently been associated with private wells. It is particularly important to ensure that septic tanks and pit latrines are not sited near a well or where a well is to be dug and to ensure that animal manure is kept at a sufficient distance to ensure that runoff cannot enter the well or the ground near the well. It is also particularly important that the household use of manures and fertilizers on small plots near wells should be managed with care to avoid potential contamination. The well should be sufficiently protected to prevent runoff from entering the well. Where there are elevated concentrations of nitrate or where inspection of the well indicated that there are sources of nitrate close by that could be causing contamination, particularly where there are indications that microbiological quality might also be poor, a number of actions can be taken. Water should be boiled or disinfected by an appropriate means before consumption. Where alternative supplies are available for bottle-fed infants, these can be used, taking care to ensure that they are microbiologically safe. Steps should then be taken to protect the well and ensure that sources of both nitrate and microbiological contamination are removed from the vicinity of the well.

In areas where household wells are common, health authorities may wish to take a number of steps to ensure that nitrate contamination is not or does not become a problem. Such steps could include targeting mothers, particularly expectant mothers, with appropriate information about water safety, assisting with visual inspection of

wells to determine whether a problem may exist, providing testing facilities where a problem is suspected, providing guidance on disinfecting water or where nitrate levels are particularly high, providing bottled water from safe sources or providing advice as to where such water can be obtained.

With regard to piped supplies, where nitrate is present, the first potential approach to treatment of drinking-water supplies, if source substitution is not feasible, is to dilute the contaminated water with a low-nitrate source. Where blending is not feasible, a number of treatment techniques are available for drinking-water. The first is disinfection, which may serve to oxidize nitrite to the less toxic nitrate as well as minimize the pathogenic and non-pathogenic reducing bacterial population in the water. Nitrate removal methods include ion exchange (normally for groundwaters) and biological denitrification (normally for surface waters). However, there are disadvantages associated with both approaches, including the need for regeneration and disposal of spent regenerant with ion exchange and the complexities of operation and the potential for microbial and carbon feed contamination of the final water with biological denitrification.

Care should be taken with the use of chloramination for providing a residual disinfectant in the distribution system. It is important to manage this to minimize nitrite formation, either in the main distribution system or in the distribution systems of buildings where chloramines are used to control *Legionella*.

7. GUIDELINE VALUES

The guideline value for nitrate of 50 mg/l as nitrate is based on epidemiological evidence for methaemoglobinaemia in infants, which results from short-term exposure and is protective for bottle-fed infants and, consequently, other population groups. This outcome is complicated by the presence of microbial contamination and subsequent gastrointestinal infection, which can increase the risk for this group significantly. Authorities should therefore be all the more vigilant that water to be used for bottle-fed infants is microbiologically safe when nitrate is present at concentrations near the guideline value. It is recommended that water should not be used for bottle-fed infants when nitrate levels are above 100 mg/l, but that it may be used if medical authorities are vigilant for signs of methaemoglobinaemia when the nitrate concentration is between 50 and 100 mg/l, particularly where a high rate of gastrointestinal infection is present in infants and children in the population The latter is a minor modification of previous guidance to place greater emphasis on the role of microbiological quality.

The guideline for nitrite of 3 mg/l as nitrite is based on human data showing that doses of nitrite that cause methaemoglobinaemia in infants range from 0.4 to more than 200 mg/kg of body weight. By applying the lowest level of the range (0.4 mg/kg of body weight), a body weight of 5 kg for an infant and a drinking-water consumption of 0.75 litre, a guideline value of 3 mg/l (rounded figure) can be derived.

Because of the possibility of the simultaneous occurrence of nitrate and nitrite in drinking-water, the sum of the ratios of the concentration (C) of each to its guideline value (GV) should not exceed 1, i.e.

$$\frac{C_{nitrate}}{GV_{nitrate}} + \frac{C_{nitrite}}{GV_{nitrite}} \leq 1$$

At this time, no other values are proposed for chronic effects, in view of uncertainties regarding differences in the way in which nitrate and nitrite are handled by laboratory animals and significant uncertainties in epidemiological data, particularly for effects on the thyroid.

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UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI INGEGNERIA INDUSTRIALE

CORSO DI LAUREA MAGISTRALE IN INGEGNERIA CHIMICA E DEI PROCESSI INDUSTRIALI

Tesi di Laurea Magistrale in

Ingegneria Chimica e dei Processi Industriali

NOVEL POLYMER BASED HYDROGEL FOR BIOMEDICAL APPLICATIONS. SYNTHESIS, CLASSIFICATION AND RHEOLOGICAL INVESTIGATION OF THE HYDROGELS THIXOTROPIC BEHAVIOR.

IDROGELI POLIMERICI PER LE APPLICAZIONI BIOMEDICALI. SINTESI, CLASSIFICAZIONI E INVESTIGAZIONI REOLOGICHE DELLE PROPRIETÀ TIXOTROPICHE DEGLI IDROGELI.

Relatore: Prof. Michele Modesti

Correlatore: Prof. Dr. Robert Luxenhofer (University of Würzburg)

Laureando: Samson Olufemi Abiodun Junior Akanbi

ANNO ACCADEMICO 2014-2015

Abstract

This study was carried out in the period between 2013 and 2014, at the faculty of Chemistry and Pharmacy, under the supervision of Prof. Dr. Robert Luxenhofer, in Julius-Maximilians Universität, Würzburg, Germany and Prof. Michele Modesti, Industrial Eng. Dept., University of Padova, Italy.

The project is based on the synthesis and characterization of a novel polymer based hydrogel, for biomedical applications. The synthesis of the hydrogel was carried out starting from the mixture of a silicate nanoparticle (Laponite) with both synthetic and natural polymer: poly (ethylene oxide) and collagen I, respectively, in different mediums.

Then, the hydrogels were subjected to both rheological and swelling degree test, in order to be able to understand the mechanical or viscoelastic properties of these gels when deformed under a certain stress or strain, and also, to have a proper idea of the importance of the hydrogels swelling capacity in the area of solute or drug transportation in biomedical applications.

Factors, such as hydrogel inhomogeneity, ageing effect, temperatures, pH and ionic concentration of the medium used in the synthesis of the gels, that could affect the hydrogels rheology, swelling capacity and data reproducibility were also considered. All these factors are to be taken into account when the hydrogel system is needed for a certain application, for instance in drug delivery sector, wound dressing, tissue engineering field, injectable polymeric system and as well for technical products.

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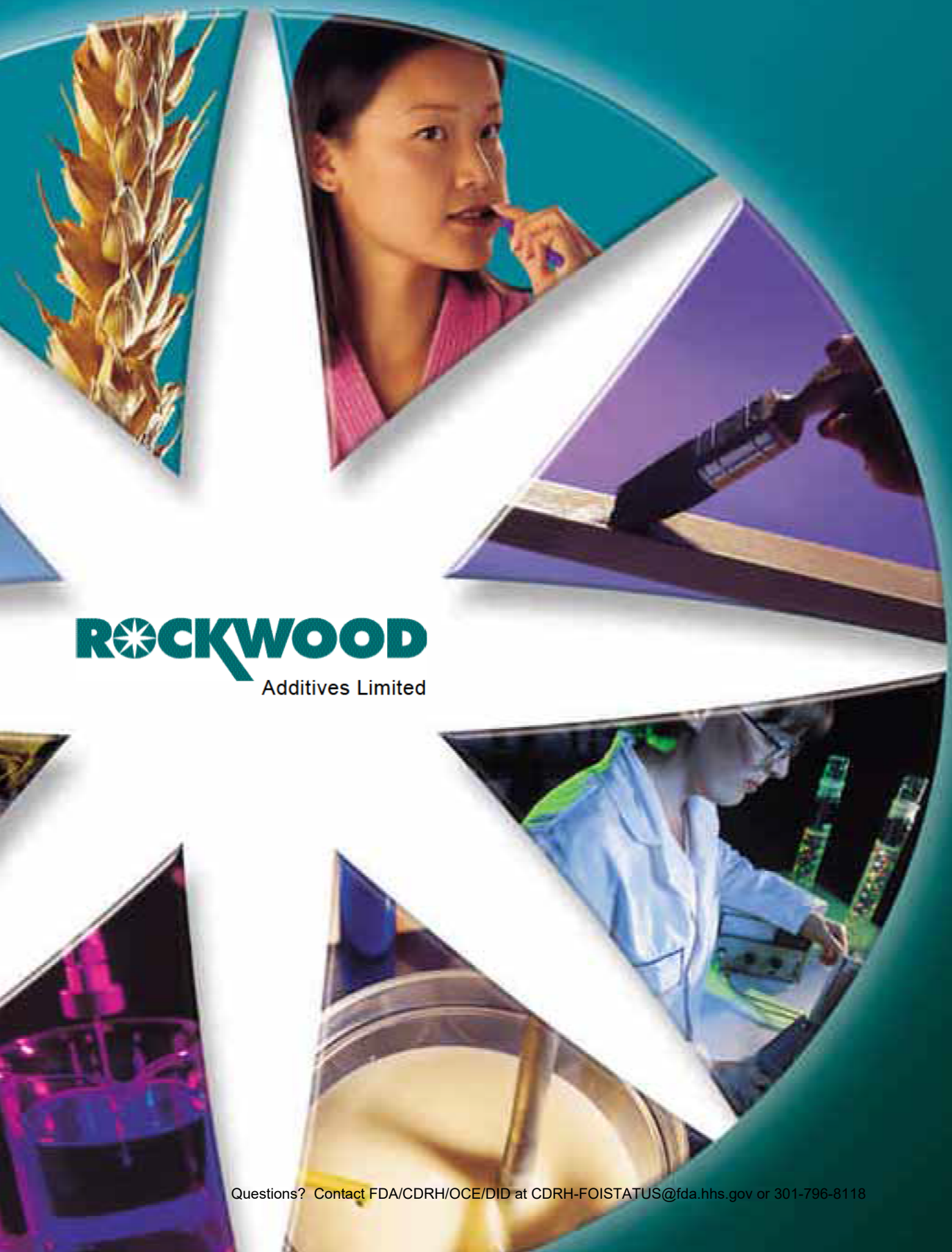
laponite

the performance enhancer



ROCKWOOD
Additives Limited

Laponite: The Performance Enhancer

Laponite, a synthetic layered silicate is a unique speciality additive that improves the performance of a wide range of industrial and consumer products making them more valuable to their users.

There are two key areas of functional use for Laponite.

- **As a rheology modifier** - Laponite may be added to the formulation of many waterborne products such as surface coatings, household cleaners and personal care products. It will impart shear sensitive viscosity and improve stability and syneresis control.

- **As a film former** - Laponite is a film forming agent and is used to produce electrically conductive, antistatic and barrier coatings.

At Rockwood we are continuing to develop Laponite to enhance its performance, consistency and breadth of application.

Guideline formulations showing the extensive application range and versatility of Laponite are available from Rockwood.

In partnership with our customers we are dedicated to ensuring that formulations containing Laponite will consistently offer tangible advantages to their users. Advantages that can enhance the performance and increase the value of your products.

Contents

Product range

Laponite is a colloidal synthetic layered silicate, two basic types are available:

- **gel forming grades**
- **sol forming grades**

Some definitions as they apply to Laponite:

Colloid - a very small particle, often a macromolecle, typically having dimensions <500nm

Gel - a high viscosity colloidal dispersion

Sol - a low viscosity colloidal dispersion

Gel forming grades disperse readily in water, under agitation, to form clear, colourless dispersions. The viscosity of such dispersions depends upon the solids content and the electrolyte content of the water used. At 2% in ***tap water*** highly thixotropic gels are formed; at the same concentration in ***deionised water***, low viscosity sols will be produced. Both forms of dispersion are suitable to use in, or add to formulations at this point. Laponite develops viscosity by interaction with the soluble components in a formulation – see pages 18-22 for more details.

Sol forming grades follow the same dispersion characteristics, but incorporate an inorganic polyphosphate dispersing agent which delays the formation of a thixotropic gel structure. At concentrations of up to 10%, low viscosity sols are formed.

When the sol premix is added to a water based system containing other solids or electrolytes, the effect of the dispersing agent is overcome and viscosity begins to rise. The rate at which the desired level of structure is achieved will depend on the exact composition of the system, but in many formulated products this can often be within minutes of adding the Laponite sol premix – see pages 18-22 for more details.

Sol forming grades of Laponite provide unique flexibility by allowing the formation of structure to be delayed until a predetermined point during manufacture. They are also especially useful in hard water areas.

- the polyphosphate is an effective sequestrant for Ca^{2+} and Mg^{2+} ions.

Specially developed grades of Laponite capable of producing stable sols at over 20% solids are used to make electrically conductive, antistatic and barrier coatings.

Talk to Rockwood if you need special grades to suit your particular requirements.

Summary of Laponite grades and characteristics:

Gel forming grades	Sol forming grades	Function
RD	**RDS**	Rapid dispersion in water for general and industrial use
XLG	**XLS**	Rapid dispersion in water, high purity, low heavy metal content for personal care/cosmetic applications
D, DF	**DS**	Rapid dispersion in sorbitol solution for toothpaste applications
	S, JS	High sol stability grades for electrically conductive, antistatic and barrier films

Quality by action, quality by performance

by our actions....

Laponite is synthesised under carefully controlled conditions to ensure excellent consistency from batch to batch. These are some of the steps we have taken to ensure high quality

- Conformance to international quality standards with BS5750 (ISO 9002) registration since 1989 - Reg No. FM1857
- Computerised information transfer between Production, Quality assurance, Customer Services and Logistics Departments
- Use of statistical quality improvement techniques, such as Statistical process Control (SPC) and Statistical Quality Control (SQC)
- A team of process technologists, dedicated to the Laponite production plant with specific responsibilities for quality and process improvements
- Highly focused and experienced technical support to develop new applications in partnership with our customers.

....and by measuring performance

Chemical composition, moisture content and product particle size are all measured during manufacture to control the consistency of every batch.

However, as a speciality chemical with unique properties and a very wide application base, it is also vitally important to control the performance of the Laponite. Tests have been developed to "fingerprint" and maintain optimum Laponite performance:

- Gel strength
  The low shear rate viscosity of a gel of Laponite in water is measured using a Brookfield viscometer with T-bar and helipath attachment.
- Gel time
  We measure the time taken by a dispersion of Laponite in water to reach a fixed gel strength.
- Dispersion rate
  A dilute, stirred, dispersion of Laponite is pumped through a flow cell fitted in a visible light spectrophotometer. The time taken to achieve the chosen level of clarity (optical density) is measured.
- Clarity
  Laponite will produce exceptionally clear dispersions. We check every batch to assure maximum clarity.

Formulating with Laponite

How to use Laponite

In common with most speciality additives it is crucial that Laponite is introduced into formulations in the correct way. This will ensure that optimum performance and efficiency is achieved. All Laponite types, both gel forming and sol forming, must be added to water and allowed to disperse and hydrate fully before any other components are added. The presence of components such as surfactants, dispersing agents etc., already in solution will interfere with the dispersion of Laponite and in some cases may halt it completely.

Recommended dispersion procedure for Laponite

Add the free flowing Laponite powder to deionised or tap water with rapid agitation at room temperature. Mixer speed should be sufficiently high to produce a vortex which will cause all the powder to fully wet out without the formation of clumps.

Suitable laboratory mixing equipment could be a mechanical stirrer fitted with a propeller blade revolving at 200rpm or a saw tooth (Cowles) blade revolving at 500rpm. Mixing should be continued for at least 20 minutes.

If required, dispersion time may be reduced by increasing the temperature of the mixture up to 40-50˚C after the Laponite powder is fully wetted out or by use of a high shear mixer such as a Silverson.

The viscosity of the Laponite dispersion at this time depends upon the concentration of the premix and the Laponite grade in use.

The typical use level of Laponite in a formulation may range from 0.05% up to 1%, or higher in some cases.


1


2


3

For gel forming grades RD, XLG, D, DF

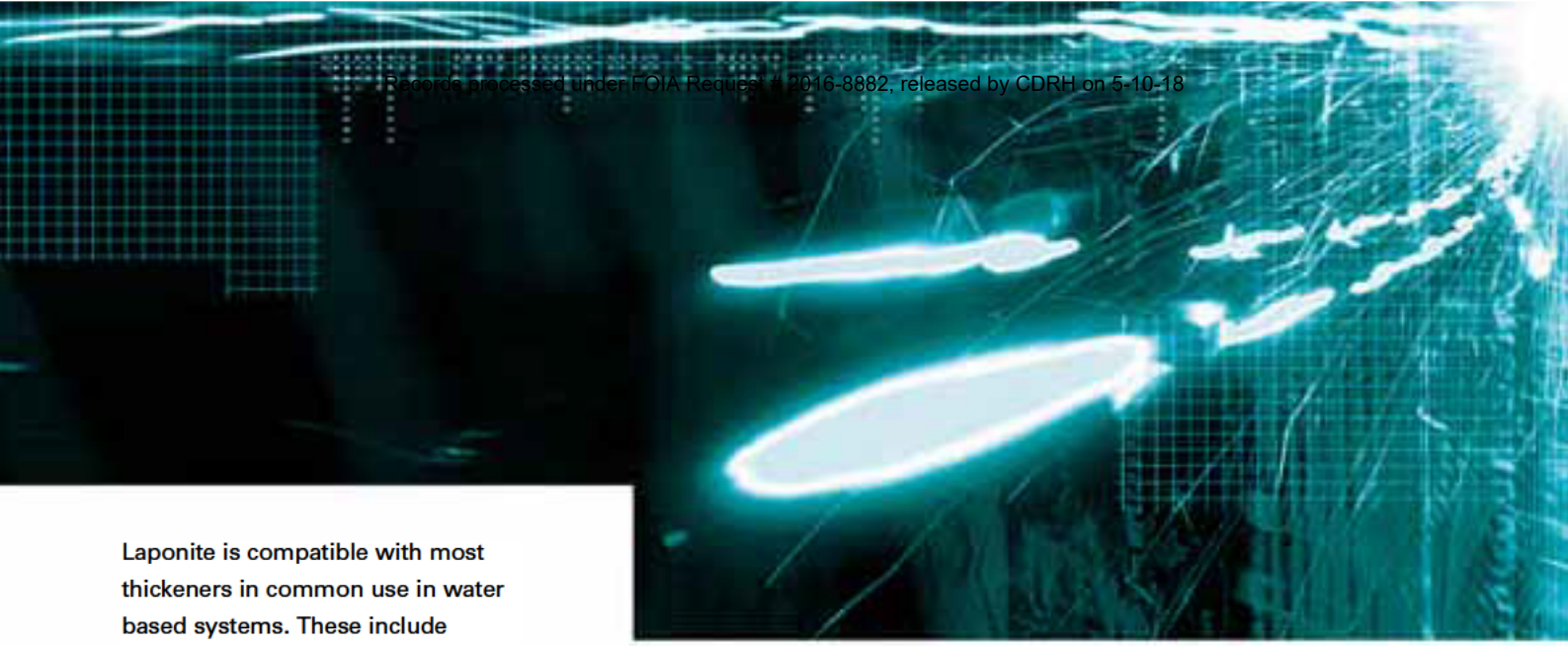
When dispersion is complete these grades produce a clear, colourless colloidal dispersion.

Concentrations above 3% of gel forming grades can build structure very quickly in the Laponite/water premix and will form a highly viscous pregel which can make it difficult to incorporate with other raw materials in a formulation. If insufficient free water is available to allow the preparation of a premix with concentration below 3% then Laponite may be "de-gelled" by the addition of compounds such as tetrasodium pyrophosphate or low molecular weight polyethylene glycols. This degelling effect is overcome on addition of the premix to a formulation.

For sol forming grades RDS, XLS, DS, S, JS

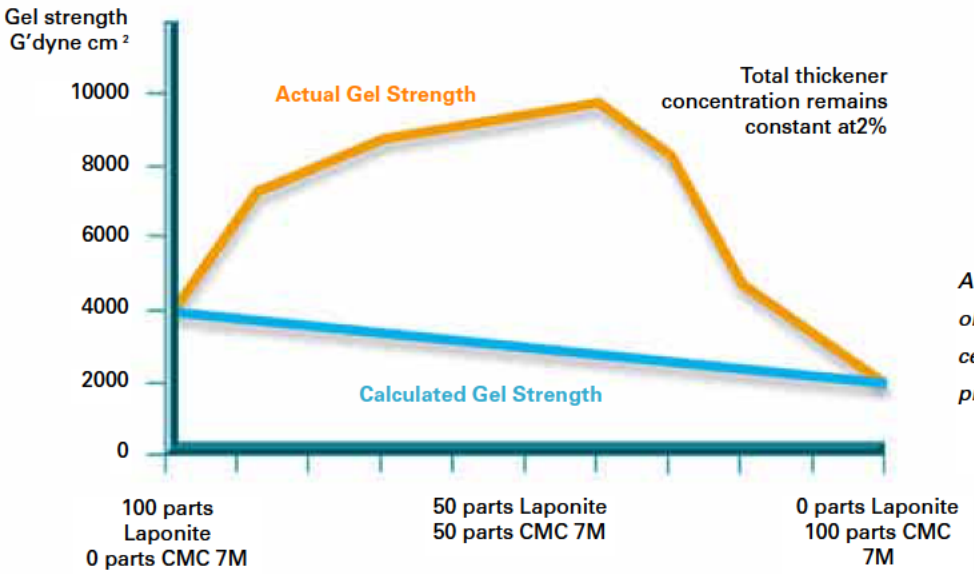
Colourless, translucent and colloidal, low viscosity dispersions known as sols are formed. This liquid premix may be stored and used in successive batches of a formulation. High solids concentrations of sol grades should be aged for up to one hour to allow the hydration process to complete. The length of time for which a sol will remain stable as a liquid is discussed on page 22.

For scale up to pilot trials or production with Laponite you are invited to contact Rockwood to discuss your requirements

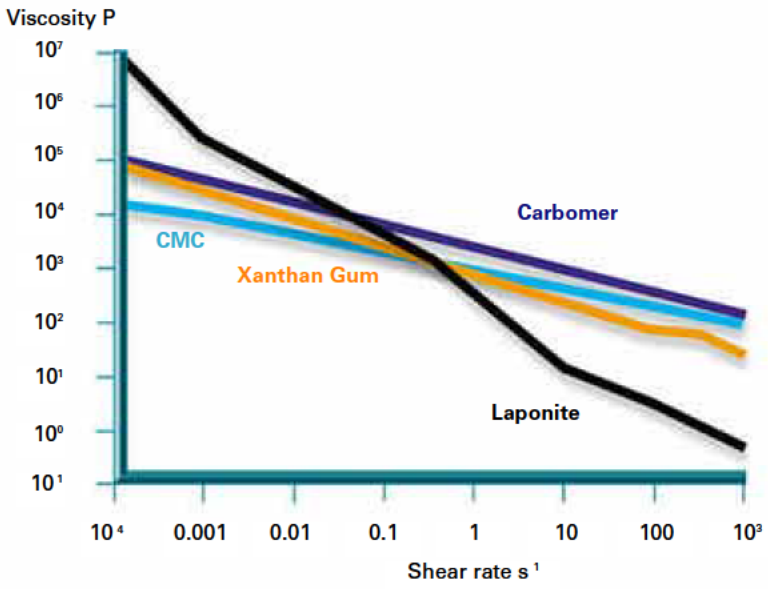


Laponite is compatible with most thickeners in common use in water based systems. These include cellulose ethers, natural gums, polyurethanes, polyacrylic acid polymers and natural clays.

Using Laponite with other thickeners



A synergistic increase in efficiency can be obtained by combining Laponite with certain polymeric thickeners, this can provide significant cost savings.



Laponite is the most highly shear thinning of commonly used rheological additives. At shear rates close to those caused by gravity ($10^{-4}.s^{-1}$), eg when a product is in storage, a gel of Laponite in water at 2% concentration has a viscosity of over 10^{8} cP. Under shear rate conditions comparable with smoothing a cosmetic cream on the skin ($10^{3}.s^{-1}$) the viscosity falls to less than 30 cP – similar to the viscosity of milk.

Formulating with Laponite *Trouble shooting checklist*

How to achieve optimum performance with Laponite

- **Order of addition**
  Adding Laponite powder directly to a finished product, latex or electrolyte solution will result in flocculation or low viscosity build. Laponite products should always be premixed in water before use.

- **Preparation of Laponite premix**
  Laponite powder should be added to water at room temperature with rapid agitation. Slow agitation and short mixing times will produce partially hydrated Laponite particles which may sink to the bottom of the mixing vessel and produce a viscous gel coating which is difficult to redisperse.

- **The rate of hydration of Laponite is temperature dependent.**
- If water temperature is low, 10°C or less, then hydration time will be increased.
- If Laponite powder is added to hot water, 35°C or greater, then the rate of hydration is so rapid that gel coated clumps of powder can form.
- After the powder has wetted out, the temperature of the premix may be raised to increase the rate of hydration.

- **Water quality**
  Calcium and magnesium ions present in very hard water can reduce the rate of hydration in gel forming grades of Laponite, leading to reduced efficiency in viscosity build. This can readily be overcome by the addition of a suitable sequestering agent such as EDTA or a sodium polyphosphate salt – or by using the appropriate sol forming grade.

- **Formulation pH**
  Laponite is most useful in the range pH6 to pH13. However, when correctly stabilised, Laponite can provide effective antisettling and thixotropic properties in highly acidic systems.

- Recommended agents for pH adjustment include:

To lower formulation pH:
- sodiumcitrate/citric acid buffer

To increase formulation pH:
- ammonia solution, sodium hydroxide, potassium hydroxide, sodium silicate, AMP95, DMAMP80.

In certain cases tertiary amines such as TEA or DMEA may be used to neutralise acidic resins, however, neutralisation should be completed before addition of a Laponite premix into the formulation.

- **Compatibility with other components**
  Laponite has one of the most extensive application ranges of all water based rheological additives – clear evidence of its compatibility across a wide spectrum of formulation additives.

Laponite products are anionic, and their use in formulations containing cationic compounds is not recommended.

Properties in formulations

Every time Laponite is evaluated in a new product, the formulator's attention is invariably drawn to the unique and novel rheological properties of this speciality additive.

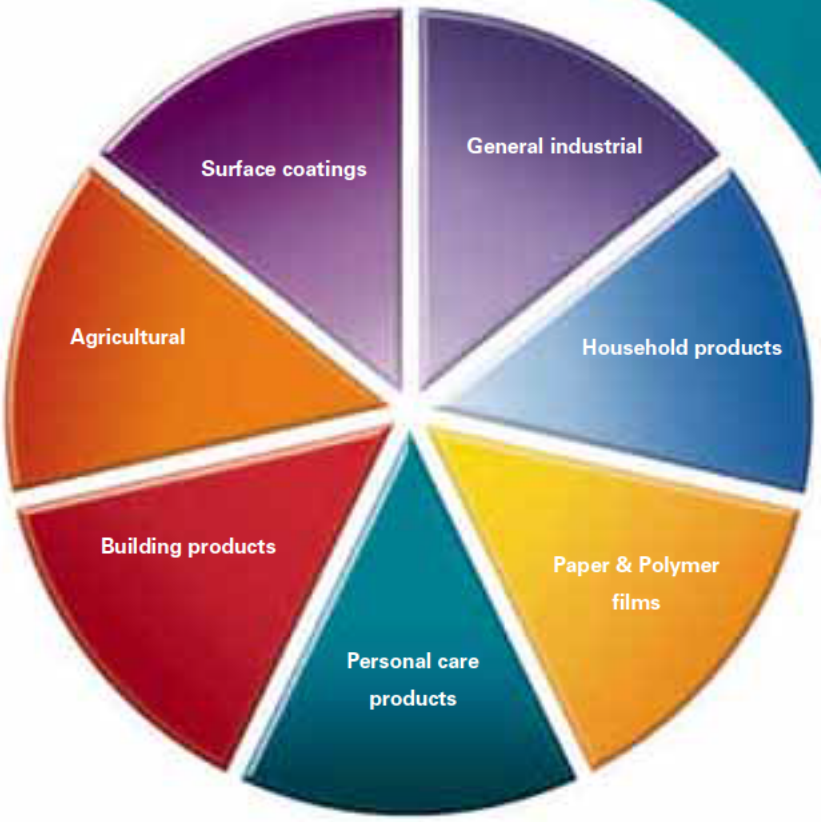
These include:

- ***high viscosity at low shear rates which produces very effective anti-settling properties***
- ***low viscosity at high shear rates***
- ***an unequalled degree of shear thinning***
- ***progressive and controllable thixotropic restructuring after shear***

It is the combinations of these key properties which result in Laponite being one of the most versatile thickeners across the widest range of water based formulated products.

Property	Benefits
• Synthetic layered silicate	• high purity • colourless dispersion • excellent consistency • free from abrasives
• Colloidal sized primary crystal	• produces clear gels or sols in water to give ultra-clear products • disperses rapidly in water without the need for high shear
• Inorganic material	• cannot support microbial growth • not affected by high temperature • non-yellowing • non-toxic • non-flammable

Laponite applications



Surface coatings
- decorative & architectural finishes
- textured coatings
- water-in-water multicolour paint
- automotive OEM & refinish
- clearcoats & varnishes
- industrial & protective coatings
- rust conversion coatings
- water reducible alkyds
- wood stains
- wood varnishes
- printing inks
- artist's & children's paints

An extensive range of guideline starter formulations showing examples of many of these products is available from Rockwood.

General industrial
- pigment suspensions
- mould release suspensions
- childrens toys
- processing aids
- grinding pastes
- oil drilling fluids
- ceramics
- ceramic glazes
- foundry coatings
- rubber latex
- electrorheological fluids

Paper & Polymer films
- static dissipative (antistatic) coatings
- electrographic paper & film
- inert barrier films
- antiblocking coatings
- paper coating slurries
- ink jet coatings
- paper sizing
- industrial speciality papers

Agricultural
- seed germination gels
- plant rooting gels
- agrochemical flowables - herbicides, pesticides
- essential element suspensions

Building products
- plasters & fillers
- setting retardants
- wood treatment suspensions
- wood adhesives
- tile adhesives

Household products
- liquid automatic dishwashing detergents
- oven cleaners
- gelled bleach cleaners
- carpet shampoos
- acidic and alkali toilet cleaners
- hard surface cleaners
- air fresheners
- antistatic products
- antiredeposition agent

Personal care products
- toothpaste
- cosmetics
- gelled skin cleansers
- depilatory creams
- exfoliant cleansers
- astringent cleansers
- nail lacquers
- antiperspirants
- topical pharmaceuticals
- shampoos

Household products

Laponite is used to stabilise many household cleaning products, particularly those which are thickened or which contain suspended solids. It is possible to formulate gelled products for spray applications which will cling to vertical surfaces.

When Laponite is correctly stabilised it is also possible to produce thixotropic cleaners with pH <1.

Laponite can also improve emulsion stability with silicone or mineral oils by increasing the low shear rate viscosity of the aqueous phase.

Laponite is compatible with:

- sodium and potassium hydroxides
- sodium silicate
- condensed phosphate detergent builders
- zeolites
- glycol ethers and alcohols

As a result of its high chemical purity and inorganic nature, Laponite demonstrates a compatibility with sodium hypochlorite bleach which is unsurpassed by other thickeners.

Surfactants

- compatible with non-ionic, anionic and some amphoteric surfactants. However, Laponite itself is a highly anionic material and it is not recommended for use with cationic compounds.

As in many other application areas, Laponite may be used in combination with other thickeners such as alkali swellable polyacrylate, xanthan gum or cellulose ethers where synergistic interactions can produce greatly improved efficiency and performance. Recommended grades for household products are Laponite RD and Laponite RDS.

Personal care products

Because of their high purity, Laponite grades XLG and XLS are the clear choice of rheological additives for many cosmetic and personal care products.

Laponite itself is colourless and does not affect the colour of products into which it is formulated; its unique rheology can improve the performance and appearance of formulations in many ways.

Property	Benefit
• synthesised under carefully controlled conditions from selected inorganic chemicals	• product does not contain crystalline silica • very low heavy metal content • stable to uv • not susceptible to microbial attack • suitable for sterilisation by gamma irradiation or ethylene oxide
• unequalled degree of shear thinning	• gives a light, clean texture to creams and lotions • reduces oily feel of emulsions • gels and pastes readily dispensed
• high gel strength	• improves stability of emulsions, suspended abrasives and solid actives • suitable for making non tacky gels with high yield values
• thixotropic viscosity	• controllable rate of restructure after shear.

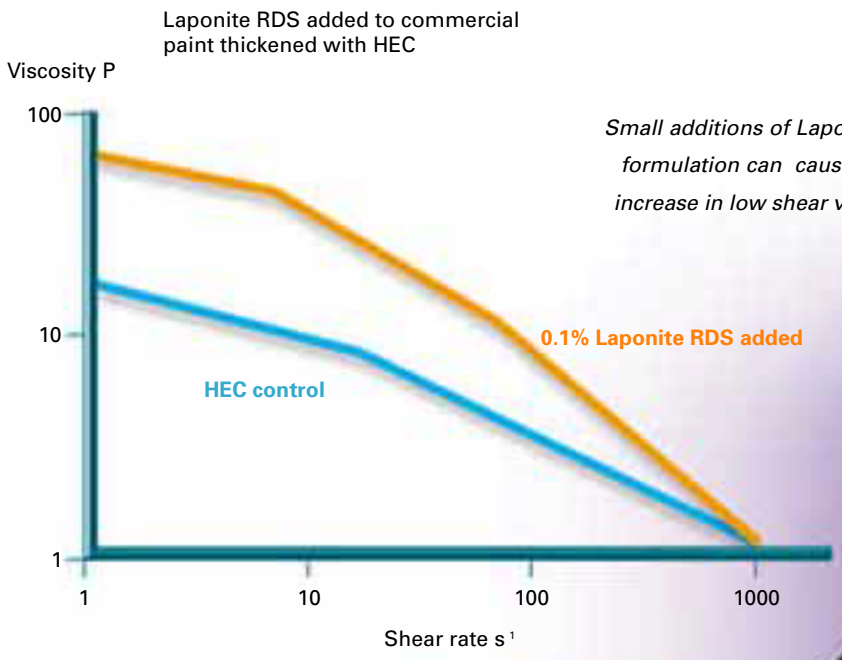
Surface coatings

In aqueous coating systems, thickeners are used to give control over flow as well as to provide adequate stability in storage and suitable rheology for application.

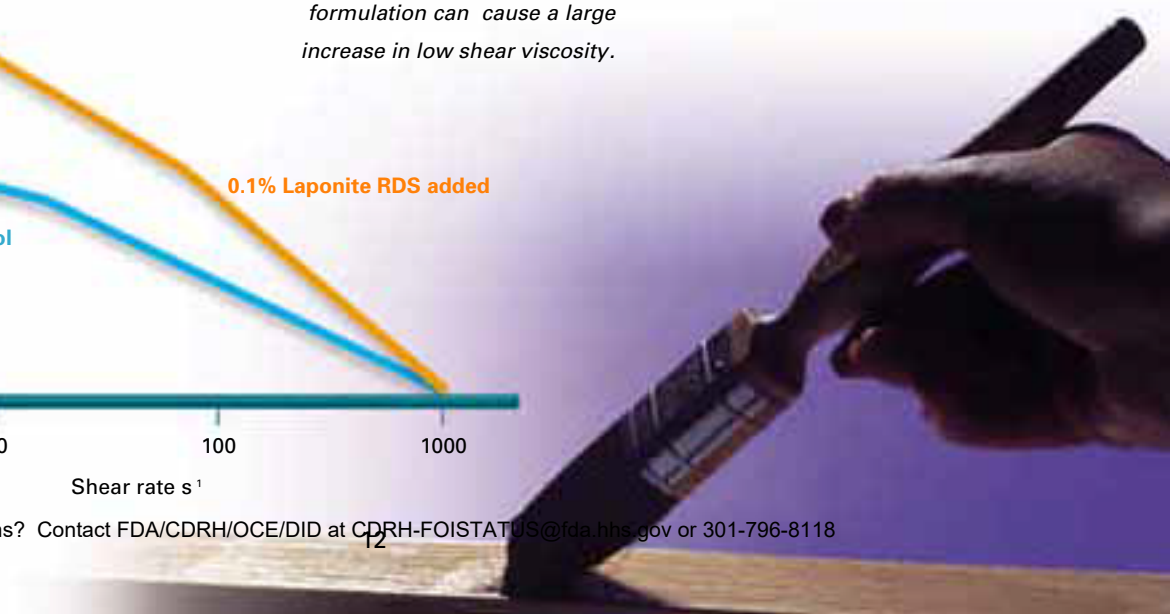
The correct choice of thickeners in a formulation should provide a sufficiently high viscosity at low shear rate to prevent sedimentation of pigment and maintain good in-can appearance and storage stability. The formulation should then shear thin during application and progressively restructure on the surface being coated. This gives the desired combination of easy application with sufficient film build and levelling, but without allowing dripping or sag. Laponite, on its own, or in combination with other thickeners, is used to improve the properties and performance of a wide range of coatings.

Property	*Benefit*
• High viscosity at low shear	• Excellent pigment suspension to give good in-can appearance and syneresis control
• Highly shear thinning	• Readily formulated for brush, roller or spray application
• Progressive restructuring after shear	• Allows good flow and levelling • Prevents sag • Excellent flip-flop in metallic and pearlescent spray coatings
• Interaction with polymeric thickeners	• Allows tailor-made rheological profiles • Unique synergistic increases in viscosity when combined many other types of thickener

Effect on paint viscosity



Small additions of Laponite to a formulation can cause a large increase in low shear viscosity.



Laponite

The recommended grades for use in paint and coatings are the rapidly dispersing gel forming grade, *Laponite RD*, and the delayed gelling, sol forming grade *Laponite RDS*.

Laponite RD should always be pre-dispersed and fully hydrated in water as the first stage in preparation of the mill base.

If the concentration of the Laponite RD premix in water is greater than 2%, then it is recommended that a degelling agent is added. Examples of suitable agents include condensed phosphates, eg tetrasodium pyrophosphate (typical addition level 1%-2% of the weight of Laponite RD in the premix) and some water soluble organic solvents, eg low molecular weight polyethylene glycols (typical addition level 1 part PEG : 1 part Laponite RD).

If free water available to hydrate the Laponite RD is limited, resulting in a premix concentration of >4% Laponite RD, then use of the sol forming grade Laponite RDS is recommended.

Laponite RDS may be prepared as low viscosity, concentrated sol premix at up to 10% solids content in water. This can be added to the coating at any stage during manufacture, although it is frequently found that best results are obtained when it is added as the final component.

Compatibility

Laponite products have been widely used in the coatings industry for over thirty years and show excellent compatibility with commonly used latex systems, pigments and extenders. Laponite does not react with coalescing solvents, biocides and defoamers at normal levels of use.

Formulation pH

Laponite has been successfully formulated into coatings across a wide pH range, for example

- pH 3 – organic acid based rust conversion coating
- pH 13 – water glass based primer coating.

Laponite does not require pH adjustment.

Some speciality applications

1. **Laponite in automotive coatings**

Laponite gives:

- Excellent appearance
- Improved flip-flop
- Improved moisture sensitivity compared with other thickeners

2. **Laponite in water based multicoloured paint**

Multicoloured particles of paint in a single pack are prevented from mixing together by a barrier coating of Laponite gel using a process developed by and freely available from Rockwood.

3. **Laponite in wood coatings**

For both industrial and DIY use, Laponite gives

- Excellent clarity, gloss and smoothness in varnishes
- Suspension of pigment in wood stains
- Improved hold-out properties suited for brush or spray application.

4. **Laponite in pigment suspensions**

- Stability without viscosity. In certain formulations Laponite can be used at very low levels to provide stability of suspended pigment without producing thixotropic viscosity. Applications include liquid printing inks, automotive paints, dip coatings and wood stains.

Toothpaste

Here, as in many other application areas it is the unique and novel properties of Laponite which make it the binder of choice in toothpaste.

In addition to its use in conventional toothpastes, Laponite is highly recommended for speciality products such as:

- ultra-clear gels
- combination toothpaste and mouthwash
- striped pastes

Laponite is compatible with all commonly used toothpaste ingredients

Laponite features and benefits include:	
high gel strength	• stability in the tube ideal for striped pastes
unequalled degree of shear thinning	• gel-like pastes can be readily extruded from tube • ease of filling • improved flavour release as paste flows more readily in mouth
thixotropic restructure after shear	• re-sets after extrusion to give a firm toothpaste ribbon • improves appearance of paste
short, non-elastic texture	• non stringy pastes break cleanly
inorganic	• does not hold flavour in paste by H-bonding

Agricultural and horticultural applications

Laponite is classified as an inert ingredient in formulations applied to growing crops or to raw agricultural crops after harvest.

Applications for Laponite include:

- Anti-settling agent for agrochemical flowables and suspension concentrates
- A medium for seed germination and fluid seed drilling
- A gel medium for rooting of plant cuttings
- Non-toxic antistatic and barrier coating for seeds

Ceramics and enamels

Laponite can be used as a partial or complete replacement for conventional organic polymer or clay based set up agents to increase stability and improve sprayability of ceramic glazes and enamel frits. It is not degraded by high temperature, or high shear dispersion processes.

Laponite has:

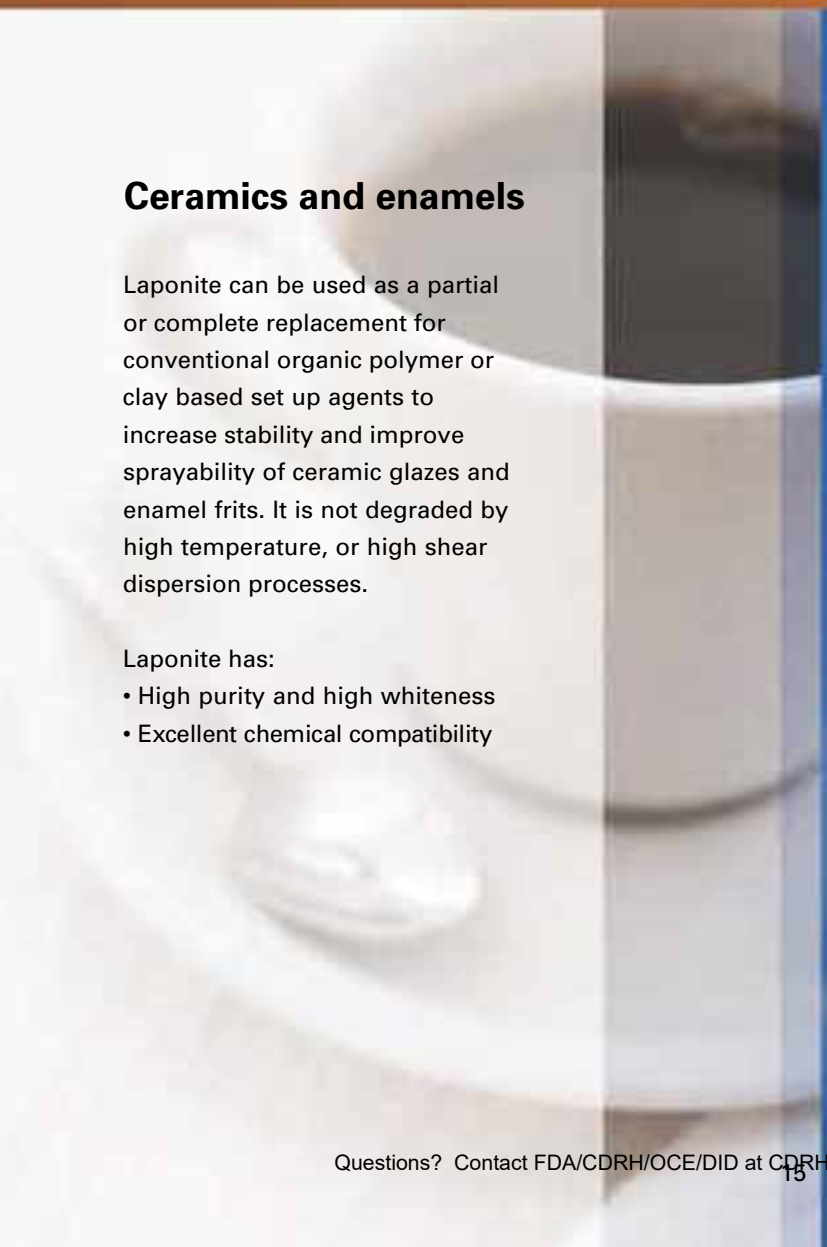
- High purity and high whiteness
- Excellent chemical compatibility

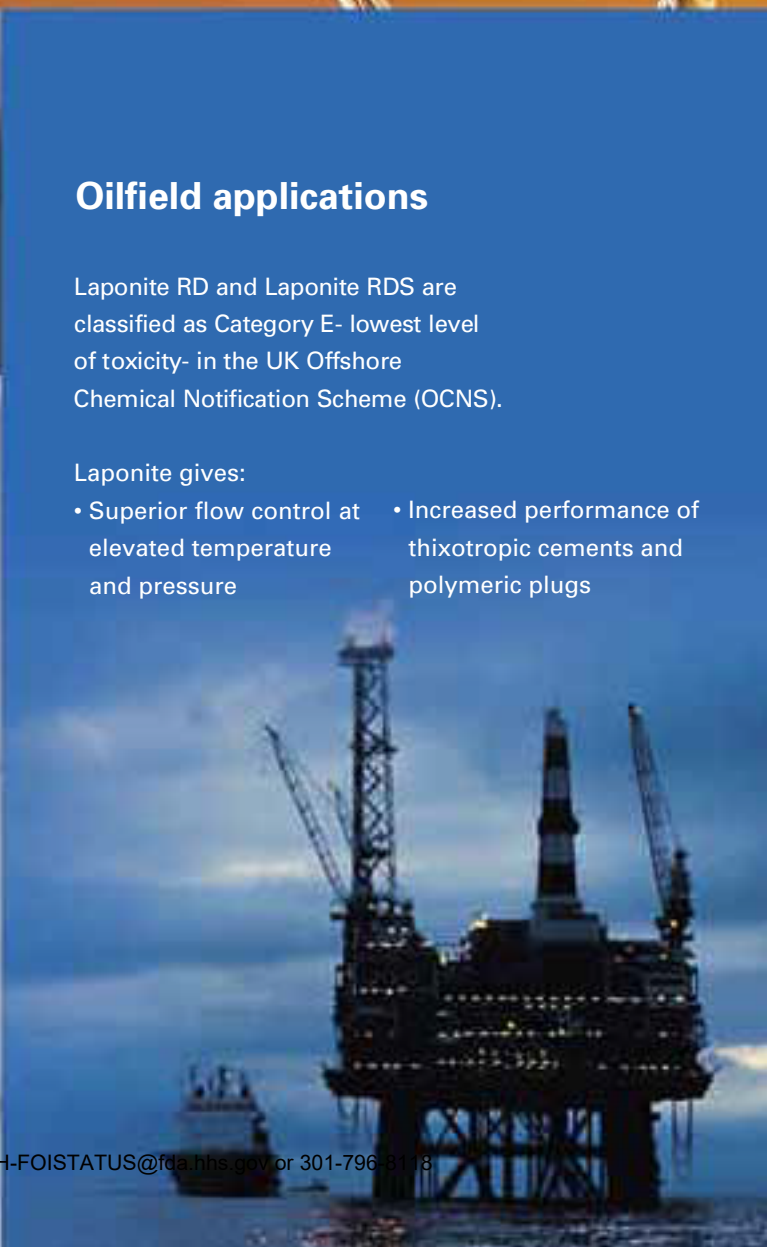
Oilfield applications

Laponite RD and Laponite RDS are classified as Category E- lowest level of toxicity- in the UK Offshore Chemical Notification Scheme (OCNS).

Laponite gives:

- Superior flow control at elevated temperature and pressure
- Increased performance of thixotropic cements and polymeric plugs





Laponite as a film forming agent

Like many other colloidal materials, Laponite is a natural film former. However, the unusual shape of the Laponite crystal, combined with its anionic nature, enables Laponite to produce films which have:

- conductive/antistatic
- barrier
- antiblocking properties

A film of Laponite can be cast onto paper simply from a dispersion of Laponite sol grade in water. Standard coating techniques such as metering bar, dip coating or flexogrphic printing are all suitable. Addition of an emulsion resin binder - for example, polyurethane, acrylic, vinyl acetate or many other types will enable the preparation of coating mixes for a wide range of substrates, including;

- polymeric films, extrudates and mouldings:
  - polypropylene
  - polyethylene
  - polycarbonate
  - polyester
  - acrylic
  - ABS
  - PVC
- glass
- paper

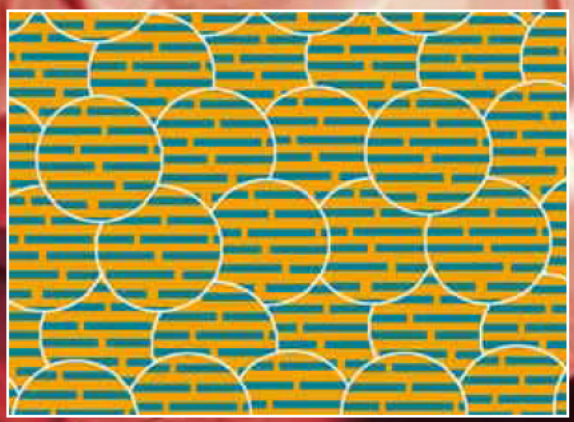
By selecting an appropriate binder and wetting agent system it is possible to produce coatings of Laponite which are clear, highly flexible and moisture resistant.

These unique properties are used to advantage in many applications:

- conductive layer in electrographic and speciality antistatic paper grades
- absorbent ink receiving coatings for ink jet printing
- inert barriers in x-ray and photographic film
- barrier sizing of speciality papers
- paper and polymer antistatic packaging for food and electrical components.

For scale up to pilot trials or machine trials, you are invited to contact Rockwood for technical support.

Figure 1. ***Plan of Laponite coating***



How Laponite works as an antistatic agent

When coated onto a substrate, Laponite conducts electricity using two mechanisms.

1. Electronic

The Laponite coating forms a continuous interlinked and overlapping film of electrically charged particles. This mechanism is not affected by changes in relative humidity (RH). (Figure 1)

2. Ionic

Free moisture - a Laponite film will typically absorb up to 15% free moisture at 50% RH. This is associated as water of hydration of the ions within the Laponite crystal structure. Some of this water is lost at very low humidity.

Structural water - Laponite contains approximately 8% by mass of water which is chemically absorbed into the crystal structure and may only be released at temperatures above 150°C.

Electrical charge may be conducted through this concentrated ionic solution.

Depending upon coat weight applied and the substrate, Laponite can be used to produce coatings with surface resistivity in the range 10^6 - 10^{12} ohms/square.

Benefits of Laponite compared with polymeric resins:

- The conductivity of Laponite coatings is affected less by changes in relative humidity.
- Laponite coatings are not easily redissolved and are suitable for overcoating with aqueous or solvent based coatings.
- Laponite coatings are dry and non tacky to the touch and are suitable surfaces for writing, printing or for use with water or solvent based adhesives.
- Because Laponite is inorganic, its films do not discolour with ageing or heating.
- Polymeric resins operate as antistatic agents by dissipating electrical charge via physically absorbed water molecules. This water is rapidly removed as relative humidity falls, resulting in a significant loss in conductivity.

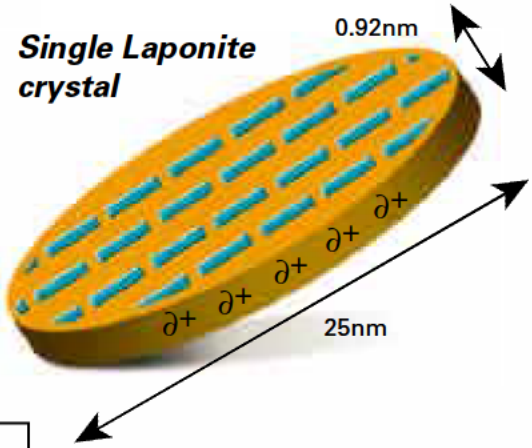
Barrier properties

It has been estimated that Laponite has a physical surface area of over 900$m^2.g^1$. The unusual combination of particle size and shape of the Laponite crystal allows it to be used as a barrier forming substance, both in a film and in dispersion within a medium.

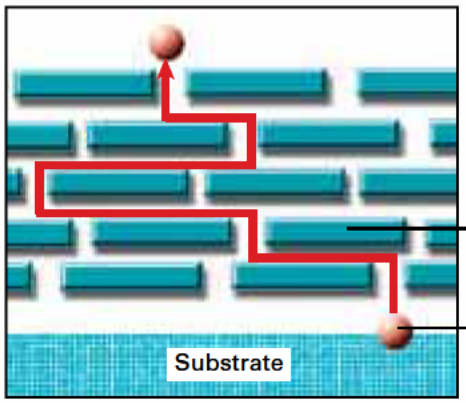
In dispersion in a liquid or gel medium, Laponite particles can provide a structured system which can prevent or reduce the rate of the movement of species between different phases. This effect can be used to develop greatly improved stability in many 2-in-1 type products such as:

- striped toothpaste
- water based multicolour paint
- multilayer films

A film of Laponite can prevent migration of molecular particles between two layers by providing a *tortuous* path for the species to travel through.



Barrier properties



Tortuous Path for a particle to migrate through a film of Laponite crystals

Laponite film

Particle or macromolecule

Manufacture, structure and chemistry

Laponite is an entirely synthetic product. The synthesis process shown in Figure 1 involves combining salts of sodium magnesium and lithium with sodium silicate at carefully controlled rates and temperatures. This produces an amorphous precipitate which is then partially crystalised by a high temperature treatment. The resulting product is filtered, washed, dried and milled to give a fine white powder.

Laponite has a layer structure which, in dispersion in water, is in the form of disc-shaped crystals. It can be seen as a two-dimensional inorganic polymer where the empirical formula forms a unit cell in the crystal as shown in Figure 2.

This shows six octahedral magnesium ions sandwiched between two layers of four tetrahedral silicon atoms. These groups are balanced by twenty oxygen atoms and four hydroxyl groups. The height of the unit cell represents the thickness of the Laponite crystal. The unit cell is repeated many times in two directions, resulting in the disc-shaped appearance of the crystal shown below in Figure 3. It has been estimated that a typical Laponite crystal contains some 30000-40000 unit cells. Macromolecules of this particle size are known as colloids.

Natural clay mineral thickeners such as bentonite and hectorite have a similar disc shaped crystal structure but are an order of magnitude larger in size. The primary particle size of Laponite is compared with those of natural hectorite and bentonite in Figure 4.

The idealised structure shown in Figure 2 would have a neutral charge with six magnesium ions in the octahedral layer, giving a positive charge of twelve. In practice, however, some magnesium ions are substituted by lithium ions and some spaces are empty to give typically a composition which has the empirical formula:

$Na^{+}_{0.7}[(Si_8 Mg_{5.5} Li_{0.3}) O_{20}(OH)_4]^{-0.7}$

This has a charge deficiency of 0.7 per unit cell.

The negative charge becomes neutralised during drying as sodium ions are absorbed onto the surfaces of the crystals. The crystals become arranged into stacks which are held together electrostatically by sharing of sodium ions in the interlayer region between adjacent crystals.

The processes occurring during dispersion of Laponite into water are shown schematically in Figure 5.

At 25°C in tap water and with rapid agitation, this process is substantially complete after 10 minutes. High shear mixing, elevated temperature or chemical dispersants are not required.

Figure 1. ***Production flow diagram***

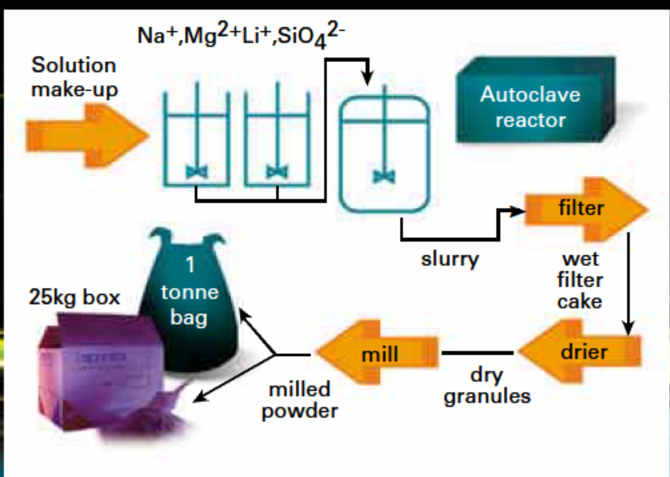


Figure 2. ***Idealised structural formula***

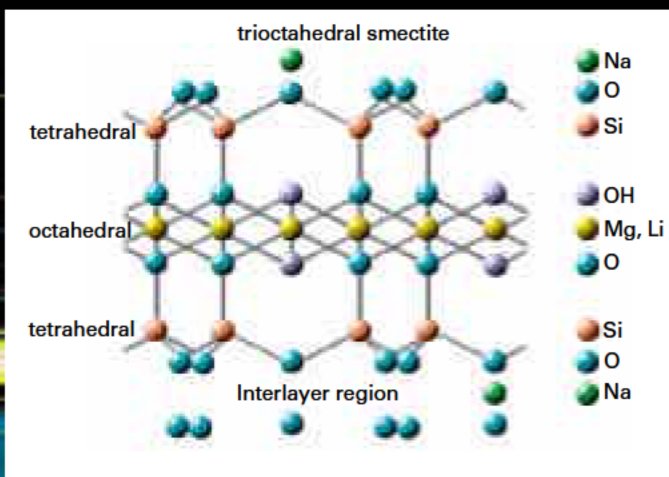


Figure 3. **Single Laponite crystal**

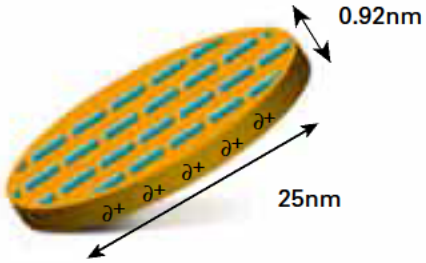


Figure 4. **Comparison of primary particles**

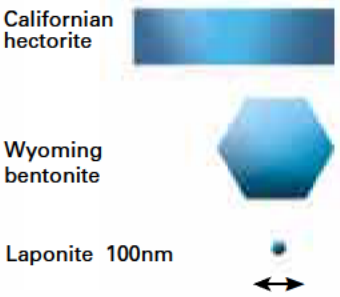
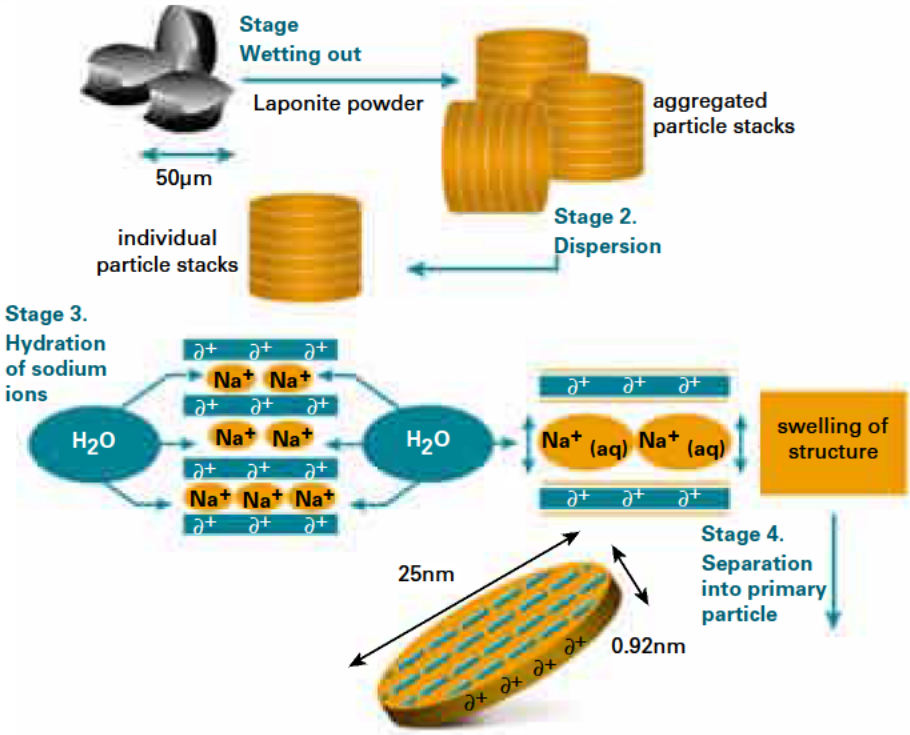


Figure 5. **Addition of Laponite to water** *(schematic)*



Gel formation

A dilute dispersion of Laponite in deionised water may remain a low viscosity dispersion of non-interacting crystals for long periods of time.

The crystal surface has a negative charge of 50-55 m.mol.100g^{-1}. the edges of the crystal have small localised positive charges generated by absorption of hydroxyl groups where the crystal structure terminates. This positive charge is typically 4-5 m.mol.100g^{-1}.

Electrostatic attractions draw the sodium ions in solution towards the crystal surface and osmotic pressure from the bulk of water pulls them away. An equilibrium becomes established where the sodium ions are held in a diffuse region on both sides of the dispersed Laponite crystal as shown in Figure 6. These are known as electrical double layers. When two particles approach their mutual positive charges repel each other and the dispersion exhibits low viscosity and Newtonian type rheology.

The addition of polar compounds in solution (eg simple salts, surfactants, coalescing solvents, soluble impurities and additives in pigments, fillers or binders etc.) to the dispersion of Laponite will reduce the osmotic pressure holding the sodium ions away from the particle surface. This causes the electrical double layer to thin and allows the weaker positive charge on the edge of the crystals to interact with the negative surfaces of adjacent crystals.

The process may continue to give a "house of cards" structure which, in a simple system of Laponite, water and salt, is seen as a highly thixotropic gel. See Figure 7. This gel consists of a single flocculated particle held together by weak electrostatic forces.

continued...

Figure 6. **Dispersed primary particle**

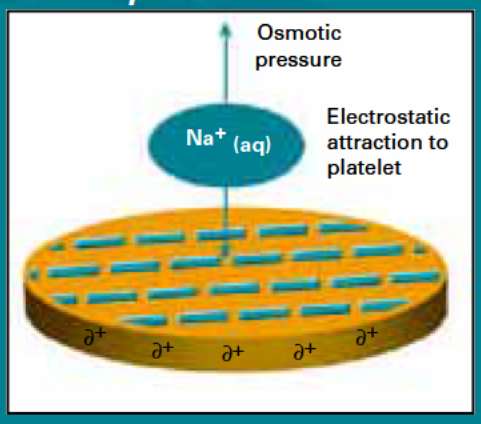


Figure 7. **Gel formation-House of cards**

Gel formation *(...Continued)*

A number of features of the rheology support this type of mechanism for gel formation.

- Solid particles are held within the 3D gel structure and are not stabilised by viscosity alone - this gives excellent suspension properties for materials of all densities.
- as the bonds are ionic viscosity is not affected by temperature.
- The gel structure is readily broken down on application of shear stress. Laponite shows a greater degree of shear thinning than other commonly used thickeners.
- When held under high shear, Laponite dispersions show very little resistance to flow and have low viscosity.
- The gel structure takes time to reform when shear stress is removed as the particles must re-orientate themselves into the house of cards structure.

Formation of sols

Firstly, two general textbook definitions:

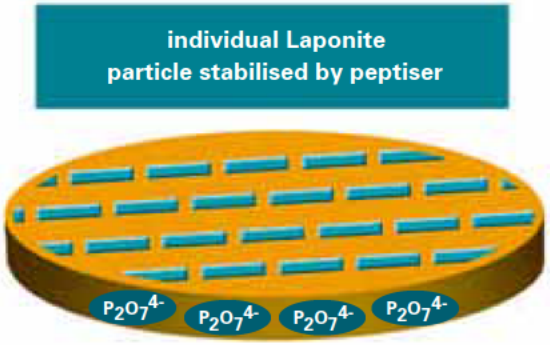
- A high viscosity colloidal dispersion is termed a gel
- A low viscosity colloidal dispersion is termed a sol

It is possible to modify Laponite from a gel forming type to a sol forming type by addition of certain compounds, for example, condensed phosphates, polyethylene glycols, polypropylene glycols and certain non-ionic surfactants. Optimised sol forming grades of Laponite have been developed by combining Laponite with a small proportion of tetrasodium pyrophosphate (TSPP).

When a sol forming grade is added to water the Laponite will disperse as described earlier. As the blended TSPP dissolves, the pyrophosphate $(P_2O_7)^{4-}$ anions become associated with the positively charged edges of the Laponite crystal, as shown in Figure 8, making the whole particle negatively charged. This is subsequently surrounded completely by a loosely held layer of hydrated sodium ions, whose positive charges cause mutual repulsions between the dispersed Laponite crystals. TSPP is said to have a peptising or dispersing effect on Laponite. Sol stability is discussed in detail on the following page.

When a sol dispersion of Laponite is added into a formulated product such as paint or toothpaste, the dispersing effect of the TSPP is rapidly overcome as the pyrophosphate anions are absorbed by the matrix of e.g., fillers, pigments, binders, surfactants, wetting agents, etc. that comprise the formulation. As this happens adjacent Laponite crystals will begin to interact with each other and the house of cards type structure can form, resulting in viscosity increase *in-situ*. This unique feature of Laponite gives the opportunity to develop a viscosity increase at a selected time during formulation and allows it to be used as a post additive or corrective thickener and in water-lean formulations.

Figure 8. ***Sol grades - (schematic)***



Sol stability

Under manufacturing conditions when formulating with Laponite, a sol dispersion should be regarded as a temporary intermediate. The length of time for which a sol can be stored is known as its *sol stability*.

The pyrophosphate ions which stabilise the Laponite sol dispersion are themselves unstable in solution and slowly hydrolyse to produce simple phosphate.

The charge density on the phosphate ion is much higher than on the pyrophosphate ion and does not produce the sol stabilising effect. The phosphate ions return into solution.

$(P_2O_7)^{4-} + 2OH^- \rightarrow 2(PO_4)^{3-} + H_2O$

As the edges of the crystals are once more free then particle-particle interactions can occur between positively charged edges and negatively charged faces. This reduces the mobility of the particles within the dispersion resulting in an increase in viscosity - when the process is complete a highly thixotropic gel is formed.

The length of time for which a sol remains stable, at low viscosity, depends upon a number of factors:

Concentration

- as concentration increases then Laponite crystals are forced into closer contact with each other and viscosity increase will occur earlier

Storage temperature

- at elevated temperatures the rate of hydrolysation of pyrophosphate ion is accelerated and sol stability can be significantly reduced

Electrolyte level/water hardness

- concentrated sols show maximum sol stability when deionised or soft water is used, – in harder water, sol stability may be reduced

- if water soluble compounds (surfactants, polyols, simple electrolytes) or latexes are added, then in some cases sol stability may be reduced. The addition of larger quantities of electrolyte such as would be caused by addition of the sol to a fully formulated toothpaste or paint will cause almost instantaneous viscosity increase.

Typical sol stability (25°C)

Duration of sol stability (days)	Max concentration to achieve sol stability		
	RDS/XLSDS	S	JS
90	6	8	15
28	7.5	9.5	18
3	10	13	19
0.5	11	14	20

Laponite sol stability is defined as being the time in days for which the sol continues to have a viscosity of less than 100cP (Brookfield LV, 60rpm, 25°C)

Product safety, storage and handling

- Laponite products do not contain respirable crystalline silica.

Product safety data and handling information for Laponite can be found on the relevant safety data sheets. These are available on request from the addresses shown on the back cover of this handbook. It is recommended that the safety data sheets are examined before using Laponite.

Laponite grades are not classified as Dangerous Substances or Dangerous Preparations under Directives 67/548/EEC and 88/379/EEC. Laponite is registered under ECOIN (Europe), TSCA (USA), DSL (Canada), ACOIN (Australia) and MITI (Japan).

Proposition 65 - California safe drinking water and Toxic enforcement act - these rules do not apply.

Packaging:

- 25kg polyethylene lined cardboard cartons.
- up to 1000kg in polyethylene lined woven polypropylene intermediate bulk containers - "big bags".

Storage:

Store under dry conditions in original packaging. Seal container after use.

Environmental information:

Laponite is manufactured from abundant inorganic mineral sources and has a chemical composition analogous to that of naturally occurring smectite clay minerals. It is widely viewed as environmentally inert.

Regulatory information:

Laponite Grade	RD, XLG, D, DF	RDS, XLS, DS	S, JS
CAS No	53320-86-8	53320-86-8	64060-48-6
EINECS No	258-476-2	258-476-2	285-349-9
CTFA and INCI name	sodium magnesium silicate	sodium magnesium silicate (and) tetrasodium pyrophosphate	sodium magnesium fluorosilicate (and) tetrasodium pyrophosphate

Who is Rockwood?

Rockwood Specialties Inc. is a leading speciality chemical company headquartered in Princeton, New Jersey, USA.
The Rockwood Group comprises three major Divisions, which have developed superior global market positions.

- **Electronics** - we have leading market positions in photomask and PCB chemicals and are the world leader of chemicals produced for wafer reclaim
- **Speciality Compounds** - we are the global leader in high performance wire and cabling sheathing
- **Pigments and Performance Additives** - we are a world leader in synthetic iron oxide pigments, wood treatment products, pool and spa additives and in clay-based additives. The clay-based additives business is comprised of Rockwood Additives Limited (Widnes, U.K.), Southern Clay Products, (Gonzales, Texas, USA) and Rockwood Specialties (Singapore) Pte, Ltd.

laponite

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Laponite is manufactured at the Rockwood site in Widnes, UK and is marketed worldwide through distributors who hold stocks of product in most major industrial countries. Application development support is available from experienced technical service teams based in Widnes, UK and Gonzales TX, USA.

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Date completed: April 22, 2016	Title: (b)(4) Manufacturing Information	1st author initials: _____
Date released:	Author(s): Bhalchandra M Karandikar, PhD	Reviewer initials: _____

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MTR550 GEL ANTIMICROBIAL (b)(4) TEST REPORT

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