

510(k) "SUBSTANTIAL EQUIVALENCE"
DECISION-MAKING PROCESS

(b)(5) FDA Reviewer Notes

K051647 page 1/2

NOV - 8 2005

510(K) SUMMARY

Submitted by:

Organogenesis Inc.
150 Dan Road
Canton, Massachusetts 02021

Contact

Patrick R. Bilbo
Telephone: (781) 401-1155
Facsimile: (781) 575-1570

Date: October 21, 2005

Device:

Trade Name:	FortaDerm™ Antimicrobial PHMB Wound Dressing coated with 0.1% Polyhexamethylene Biguanide Hydrochloride (PHMB)
Common/Usual Name:	Topical Wound Dressing, Wound Management Biomaterial
Classification Name:	Dressing, Wound (KMF)
Classification:	Unclassified

Predicate Devices:

The relevant predicate devices are FortaDerm™ (PuraPly™) Wound Dressing (K011026) manufactured by Organogenesis, Inc. and Xcell Antimicrobial Cellulose Wound Dressing (K024054) manufactured by Xylos® Corporation.

Statement of Substantial Equivalence:

The FortaDerm™ Antimicrobial PHMB Wound Dressing is similar with respect to intended use, technological characteristics, materials and physical construction to the predicate devices in terms of section 510(k) equivalency.

Intended Use:

The FortaDerm™ Antimicrobial PHMB Wound Dressing is intended for the management of wounds and as an effective barrier to resist microbial colonization within the dressing and reduce microbes penetrating through the dressing. FortaDerm™ Antimicrobial PHMB Wound Dressing may be used for the management of: partial and full thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grfts, post-Moh's surgery, post-laser surgery, wound

K051647 page 2/2

dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, and skin tears) and draining wounds.

The device is intended for one-time use.

Device Description:

FortaDerm™ Antimicrobial PHMB Wound Dressing consists of two layers, crosslinked sheet of fenestrated sheet of porcine intestinal collagen coated with 0.1 % Polyhexamethylene Biguanide hydrochloride (PHMB). FortaDerm Wound Dressing is supplied dry in sheet form in sizes ranging from 4 x 4 cm to 12 x 36 cm. The device is packaged in sterile, sealed single pouches.

Performance Data:

FortaDerm™ Antimicrobial PHMB Wound Dressing was subjected to a number of tests to assess biocompatibility and performance. The device passed the requirements of all tests.



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

**Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850**

NOV - 8 2005

Patrick R. Bilbo, Vice President
Regulatory and Clinical Affairs
Organogenesis, Inc.
150 Dan Road
Canton, Massachusetts 02021

Re: K051647

Trade/Device Name: FortaDerm™ Antimicrobial PHMB Wound Dressing
Regulatory Class: Unclassified
Product Code: FRO
Dated: October 21, 2005
Received: October 25, 2005

Dear Mr. Bilbo:

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.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such 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.

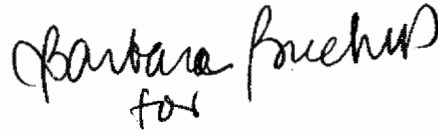
Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); 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.

Page 2- Patrick R. Bilbo, Vice President

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of Compliance at (240) 276-0115. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its Internet address <http://www.fda.gov/cdrh/industry/support/index.html>

Sincerely yours,

Handwritten signature of Barbara Buckner in cursive script, with the word "for" written below it.

Mark N. Melkerson
Acting Director
Division of General, Restorative
and Neurological Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): K051647

Device Name: FortaDerm™ Antimicrobial PHMB Wound Dressing

Indications for Use:

The FortaDerm™ Antimicrobial PHMB Wound Dressing is intended for the management of wounds and as an effective barrier to resist microbial colonization within the dressing and reduce microbes penetrating through the dressing. FortaDerm™ Antimicrobial PHMB Wound Dressing may be used for the management of: partial and full thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grafts, post-Moh's surgery, post-laser surgery, wound dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, and skin tears) and draining wounds.

The device is intended for one-time use.

Prescription Use ✓
(Part 21 CFR 801 Subpart D)

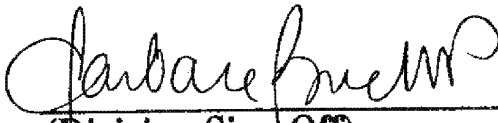
AND/OR

Over-The-Counter Use _____
(21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE
OF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

(Posted November 13, 2003)


(Division Sign-Off)
Division of General, Restorative,
and Neurological Devices

Page ___ of ___

510(k) Number K051647

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

June 22, 2005

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

ORGANOGENESIS, INC.
150 DAN RD.
CANTON, MA 02021
ATTN: PATRICK BILBO

510(k) Number: K051647
Received: 21-JUN-2005
Product: FORTADERM
ANTIMICROBIAL WOUND
DRESSING

The Food and Drug Administration (FDA), Center for Devices and Radiological Health (CDRH), has received the Premarket Notification you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act(Act) for the above referenced product. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in any future correspondence that relates to this submission. We will notify you when the processing of your premarket notification has been completed or if any additional information is required. YOU MAY NOT PLACE THIS DEVICE INTO COMMERCIAL DISTRIBUTION UNTIL YOU RECEIVE A LETTER FROM FDA ALLOWING YOU TO DO SO.

On May 21, 2004, FDA issued a Guidance for Industry and FDA Staff entitled, "FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Performance Assessment". The purpose of this document is to assist agency staff and the device industry in understanding how various FDA and industry actions that may be taken on 510(k)s should affect the review clock for purposes of meeting the Medical Device User Fee and Modernization Act. Please review this document at <http://www.fda.gov/cdrh/mdufma/guidance/1219.html>.

Please remember that all correspondence concerning your submission MUST be sent to the Document Mail Center (DMC)(HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review". Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html.

You should be familiar with the regulatory requirements for medical device available at Device Advice <http://www.fda.gov/cdrh/devadvice/>. If you have other procedural or policy questions, or want information on how to check on the status of your submission, please contact DSMICA at (301) 443-6597 or its toll-free number (800) 638-2041, or at their Internet address <http://www.fda.gov/cdrh/dsmamain.html> or me at (301)594-1190.

Sincerely yours,

Marjorie Shulman
Supervisory Consumer Safety Officer
Office of Device Evaluation
Center for Devices and Radiological Health

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

June 21, 2005

ORGANOGENESIS, INC.
150 DAN RD.
CANTON, MA 02021
ATTN: PATRICK BILBO

510(k) Number: K051647
Received: 21-JUN-2005
Product: FORTADERM
User Fee ID Number: (b)(4) WOUND
DRESSING

The Food and Drug Administration (FDA) Center for Devices and Radiological Health (CDRH), has received the Premarket Notification you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act (Act) for the above referenced product. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in any future correspondence that relates to this submission. YOU MAY NOT PLACE THIS DEVICE INTO COMMERCIAL DISTRIBUTION UNTIL YOU RECEIVE A LETTER FROM FDA ALLOWING YOU TO DO SO.

The Act, as amended by the Medical Device User Fee and Modernization Act of 2002 (MDUFMA) (Public Law 107-250), specifies that a submission shall be considered incomplete and shall not be accepted for filing until fees have been paid (Section 738(f)). Our records indicate that you have not submitted the user fee payment information and therefore your 510(k) cannot be filed and has been placed on hold. Please send a check to one of the addresses listed below:

By Regular Mail

Food and Drug Administration
P.O. Box 956733
St. Louis, MO 63195-6733.

By Private Courier (e.g., Fed Ex, UPS, etc.)

U.S. Bank
956733
1005 Convention Plaza
St. Louis, MO 63101
(314) 418-4983

The check should be made out to the Food and Drug Administration referencing the payment identification number, and a copy of the User Fee Cover sheet should be included with the check. A copy of the Medical Device User Fee Cover Sheet should be faxed to CDRH at (301) 594-2977 referencing the 510(k) number if you have not already sent it in with your 510(k) submission. After the FDA has been notified of the receipt of your user fee payment, your 510(k) will be filed and the review will begin. If payment has not been received within 30 days, your 510(k) will be deleted from the system. Additional information on user fees and how to submit your user fee payment may be found at <http://www.fda.gov/oc/mdufma>.

Please note that since your 510(k) has not been reviewed, additional information may be required during the review process and the file may be placed on hold once again. If you are unsure as to whether or not you need to file an application with FDA or what type of application to file, you should first telephone the Division of Small Manufacturers, International and Consumer Assistance (DSMICA), for guidance at (301)443-6597 or its toll-free number (800)638-2041, or contact them at their Internet address <http://www.fda.gov/cdrh/dsmamain.html>, or you may submit a 513(g) request to the Document Mail Center at the address above. If you have any questions concerning the contents of this letter, you may contact me at (301) 594-1190.

Sincerely yours,

Marjorie Shulman
Consumer Safety Officer
Office of Device Evaluation
Center for Devices and
Radiological Health

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APPENDIX A

PROPOSED LABELING

PROPOSED FORTADERM™ ANTIMICROBIAL PHMB WOUND DRESSING INSTRUCTIONS FOR USE AND PACKAGE INSERT

(b)(4) Draft Labeling

(b)(4) Draft Labeling

(b)(4) Draft Labeling

APPENDIX B

PREDICATE DEVICE LABELING

B.1 Predicate Device Labeling

APPENDIX B.1

FORTADERM™ (PURAPLY™) LABELING



PURAPLY™
Bioengineered Wound Management

INSTRUCTIONS FOR USE AND PACKAGE INSERT

DESCRIPTION

PuraPly consists of a single-layer fenestrated sheet of collagen intended for the management of wounds. PuraPly is supplied dry in sheet form. The device is packaged in sterile, sealed single pouches.

INDICATIONS

PuraPly is supplied sterile in peel-open packages. Intended for one-time use. PuraPly is indicated for the management of wounds including:

- Partial and full-thickness wounds
- Venous ulcers
- Diabetic ulcers
- Drainage wounds
- Pressure ulcers
- Chronic vascular ulcers
- Trauma wounds (abrasions, lacerations, second-degree burns, skin tears)
- Surgical wounds (donor sites/grafts, post-Mohs' surgery, post-laser surgery, wound dehiscence)

CONTRAINDICATIONS

- This device is derived from a porcine source and should not be used in patients with known sensitivity to porcine material.
- The device is not indicated for use in third degree burns.

PRECAUTIONS

- **Do not resterilize.** Discard all open and unused portions of PuraPly.
- Device is sterile if the package is dry, unopened and undamaged. Do not use if the package seal is broken.
- The device must be used prior to the expiration date.
- Discard device if mishandling has caused possible damage or contamination.
- PuraPly should not be applied until excessive exudate, bleeding, acute swelling and infection are controlled.

POTENTIAL COMPLICATIONS

The following complications are possible with the use of wound dressings. If any of these conditions occur, the device should be removed.

- Infection
- Chronic inflammation (Initial application of wound dressings may be associated with transient, mild, localized inflammation.)
- Allergic reaction
- Excessive redness, pain, swelling or blistering

3712-01 Rev. A



PURAPLY™
Bioengineered Wound Management

INSTRUCTIONS FOR USE

Note: These recommendations are designed to serve only as a general guideline. They are not intended to supercede institutional protocols or professional clinical judgment concerning patient care.

CAUTION: Federal (U.S.A.) law restricts this device to sale by or on the order of a physician or properly licensed practitioner.

ALWAYS HANDLE PURAPLY USING ASEPTIC TECHNIQUE.

1. Prepare wound area using standard methods to ensure wound is free of debris and necrotic tissue. If necessary, surgically debride the wound to ensure the wound edges contain viable tissue.
2. To apply, cut the dry sheet to the outline of the wound area. If the wound is larger than a single sheet, then multiple sheets may be used. Overlap adjoining sheets to provide coverage of the entire wound. For ease of handling, apply PuraPly by placing in a dry state over the wound and if necessary, rehydrate the sheet using sterile saline or other isotonic solution.
3. Place the edge of the sheet in contact with the intact tissue. Smooth PuraPly into place to ensure the sheet is in contact with the underlying wound bed.

NOTE: If excess exudate collects under the sheet, small openings can be cut in the sheet to allow the exudate to drain.

IMPORTANT: After application, use an appropriate, non-adherent, secondary dressing to maintain a moist wound environment. The optimum secondary dressing is determined by wound location, size, depth and user preference. Change the secondary dressing as needed to maintain a moist, clean wound area. Frequency of secondary dressing change will be dependent upon volume of exudate produced and type of dressing used. As healing occurs, sections of PuraPly may gradually peel and may be removed during dressing changes. Do not forcibly remove sections of PuraPly that may adhere to the wound. Alternatively, PuraPly may form into a caramel-colored gel, which can be rinsed away with gentle irrigation. On inspection, if PuraPly is no longer covering the wound and the wound has not healed, place an additional piece of PuraPly over the wound.

STORAGE: This device should be stored in a clean, dry location at room temperature.

STERILIZATION: This device has been sterilized with gamma irradiation.

Distributed By
Royce Medical Co.
742 Pancho Road
Camarillo, CA 93012, USA
800-521-0601

Manufactured for:
Organogenesis Inc.
150 Dan Road
Canton, MA 02021, USA

3712-01 Rev. A

APPENDIX B.2

XYLOS[®] XCELL[®] ANTIMICROBIAL CELLULOSE WOUND DRESSING LABELING

XCell[®] Antimicrobial Cellulose Wound Dressing**Instructions for Use****Sterile by Irradiation****Non-pyrogenic****Single Use****Product Description:**

The XCell[®] Antimicrobial Wound Dressing is a sterile product composed of cellulose, water, and 0.3% polyhexamethylene biguanide (PHMB). XCell[®] Antimicrobial Wound Dressing has been shown to be effective in reducing the risk of infection by preventing the migration of bacteria and fungi including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Escherichia coli* and other clinically significant microbes. The dressings are presented in a single-use peelable pouch for ease of removing the dressing.

Indications for Use:

The XCell[®] Antimicrobial Wound Dressing is intended for use on partial and full-thickness wounds as an effective barrier to resist microbial colonization within the dressing and reduce microbial penetration through the dressing. The XCell[®] Antimicrobial Wound Dressing is intended to cover a wound or burn on a patient's skin to absorb areas of wound exudates and to provide a moist wound environment that supports the autolytic debridement of areas of the wound that are necrotic. The dressing may be used on moderately exuding, non-exuding and dry wounds as stated in the Instructions-for-use. The wound dressing also protects against abrasion, desiccation, and external contamination. The moist environment has a cooling effect that may reduce pain.

Under the supervision of a health care professional, the XCell[®] Antimicrobial Wound Dressing is intended for the local management of exuding wounds, including infected and non-infected pressure ulcers, venous ulcers, diabetic ulcers, arterial ulcers, donor sites and other bleeding surface wounds, dermal lesions, trauma injuries or incisions, superficial cuts, minor scalds and burns, minor skin irritations, lacerations and abrasions.

Contraindications:

XCell[®] Dressings may not be used to treat third degree burns.
Do not use on individuals with a known sensitivity to the dressing.
Do not use if frozen

Precautions:

Inspect sealed, sterile pouch before opening. Do not use if pouch is opened or damaged.
Do not reuse or resterilize. Discard all open or unused portion of the device.
Store product in a dry place at 5 – 30°C (41 – 86°F), do not freeze.
Consult a health care professional if any of the following occur: (1) signs of clinical infection, (2) change in wound color and/or odor (3) the wound does not show signs of healing and (4) any other unexpected symptoms. The XCell[®] Dressing may be continued at the discretion of the health care professional.

Instructions for Use:

XCell[®] Dressings should be used under health care professional direction for the following indications: partial to full thickness wounds that are moderately exuding, non-exuding and dry (arterial, venous, pressure and diabetic ulcers), first and second degree burns (severe sunburn, etc.), minor burns, post-operative surgical wounds, skin grafts, donor sites and dermal lesions (cuts, abrasions, etc.)

To apply the primary wound dressing:

1. Cleanse the wound of excess exudate (fluid)
2. The skin surrounding the wound should be clean and dry for secure application
3. Select a XCell[®] Dressing size larger than the wound
4. Peel open the package and remove the wound dressing. XCell[®] Dressings are sterile and should be handled appropriately
5. The dressing may be cut to size with sterile scissors if desirable
 - a. Discard any unused portion of the product after dressing the wound
6. Either side of the dressing may be applied to the wound site. Remove one of the protective plastic films from the wound dressing and apply the exposed side of the dressing to the wound. Remove the other protective plastic film from the wound dressing and apply a self-adhesive secondary dressing (i.e., thin film), compression wrap, or other appropriate covering over the exposed surface of the dressing(s) so that it extends at least one inch beyond the XCell[®] Dressing on all sides.
 - a. When using the XCell[®] Dressing in the rope configuration, gently place it into the wound, layering it by folding it onto itself, taking care not to pack the material tightly within the wound. Cover and secure the rope as described above
 - b. If dressing a sacral ulcer, place the XCell[®] Dressing Sacral pad into the gluteal fold, and then smooth outwards to ensure complete coverage. Cover and secure the sacral pad as described above
7. Change the dressing as needed.
 - a. The frequency of dressing change is affected by the nature and condition of the wound.
8. The dressing may not be used for more than seven days.

To remove the primary wound dressing:

Remove the secondary dressing(s) as appropriate.

1. Carefully remove the XCell[®] Dressing by lifting one edge and gently peeling the dressing from the wound site
2. Properly dispose of the dressing. Do not reapply after removal.
3. Prior to re-dressing, cleanse the wound as directed by a health care professional
4. Repeat the application procedure outlined above as needed

Made in the USA for:

XYLOS[™] Corporation
838 Town Center Drive
Langhorne, PA 19407
www.xcellwoundcare.com

Distributed by:

PDI Medical Devices & Diagnostics
10 Mountainview Road, Suite C200
Upper Saddle River, NJ 07458
1-888-GET-XCELL (1-888-438-9235)

Product Ordering Information:

Reorder #: Description/Size:	Dressings/box	Boxes/case
04-001-10 3.5" x 3.5" (9cm x 9cm)	10	10
04-002-10 5.5" x 8" (14cm x 20cm)	5	10
04-003-10 8" x 7" (15cm x 18cm) Sacral	5	10
04-004-10 0.75" x 8.5" (2cm x 21cm) Rope	10	10

[™] Trade Mark of XYLOS Corporation. ® Registered Trade Mark in the USA
U.S. Patent Nos. 4,588,400, 4,655,759, 4,788,146 and other patents pending

Document Control ID: LA114.002

APPENDIX C

STABILITY TESTING PROTOCOL

(b)(4) Protocol

(b)(4) Protocol

(b)(4) Protocol

(b)(4) Protocol

APPENDIX D

BIOCOMPATIBILITY TESTING

- | | |
|------------|--------------------------------|
| D.1 | Cytotoxicity |
| D.2 | Dermal Sensitization |
| D.3 | Irritancy |
| D.4 | Hemolysis |
| D.5 | Acute Systemic Toxicity |

APPENDIX D.1

CYTOTOXICITY

(b)(4) Protocol

(b)(4) Protocol

(b)(4) Test Data

(b)(4) Protocol

(b)(4) Protocol

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(b)(4) Test Data

APPENDIX D.2

DERMAL SENSITIZATION

(b)(4) Protocol

(b)(4) Protocol

(b)(4) Test Data

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APPENDIX D.3

IRRITANCY

(b)(4) Protocol

(b)(4) Protocol

(b)(4) Test Data

(b)(4) Protocol

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APPENDIX D.4

HEMOLYSIS

(b)(4) Protocol

(b)(4) Protocol

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APPENDIX D.5

ACUTE SYSTEMIC TOXICITY

(b)(4) Protocol

(b)(4) Protocol

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APPENDIX E

PERFORMANCE TESTING

(b)(4) Protocol

(b)(4) Protocol

(b)(4) Test Data

(b)(4) Test Data

(b)(4) Test Data

APPENDIX F
510(K) SUMMARY

(b)(4) Draft

(b)(4) Draft

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

October 24, 2005

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

ORGANOGENESIS, INC.
150 DAN RD.
CANTON, MA 02021
ATTN: PATRICK BILBO

510(k) Number: K051647
Product: FORTADERM
ANTIMICROBIAL
WOUND DRESSING

The additional information you have submitted has been received.

We will notify you when the processing of this submission has been completed or if any additional information is required. Please remember that all correspondence concerning your submission MUST be sent to the Document Mail Center (HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html. On August 12, 2005 CDRH issued the Guidance for Industry and FDA Staff: Format for Traditional and Abbreviated 510(k)s. This guidance can be found at <http://www.fda.gov/cdrh/oivd/guidance/1567.html>. Please refer to this guidance for assistance on how to format an original submission for a Traditional or Abbreviated 510(k).

The Safe Medical Devices Act of 1990, signed on November 28, states that you may not place this device into commercial distribution until you receive a letter from FDA allowing you to do so. As in the past, we intend to complete our review as quickly as possible. Generally we do so 90 days. However, the complexity of a submission or a requirement for additional information may occasionally cause the review to extend beyond 90 days. Thus, if you have not received a written decision or been contacted within 90 days of our receipt date you may want to check with FDA to determine the status of your submission.

If you have procedural or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,

Marjorie Shulman
Supervisory Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health

CDRH PREMARKET REVIEW SUBMISSION COVER SHEET

Date of Submission 10/21/2005	User Fee Payment ID Number (b)(4)	FDA Submission Document Number (if known) K051647
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SECTION A TYPE OF SUBMISSION				
PMA ☐ Original Submission ☐ Premarket Report ☐ Modular Submission ☐ Amendment ☐ Report ☐ Report Amendment ☐ Licensing Agreement	PMA & HDE Supplement ☐ Regular (120 day) ☐ Special ☐ Panel Track (PMA Only) ☐ 30-day Supplement ☐ 30-day Notice ☐ 135-day Supplement ☐ Real-time Review ☐ Amendment to PMA & HDE Supplement ☐ Other	PDP ☐ Original PDP ☐ Notice of Completion ☐ Amendment to PDP	510(k) ☐ Original Submission: ☐ Traditional ☐ Special ☐ Abbreviated (Complete section 1, Page 5) ☒ Additional Information ☐ Third Party	Meeting ☐ Pre-510(K) Meeting ☐ Pre-IDE Meeting ☐ Pre-PMA Meeting ☐ Pre-PDP Meeting ☐ Day 100 Meeting ☐ Agreement Meeting ☐ Determination Meeting ☐ Other (specify):
IDE ☐ Original Submission ☐ Amendment ☐ Supplement	Humanitarian Device Exemption (HDE) ☐ Original Submission ☐ Amendment ☐ Supplement ☐ Report ☐ Report Amendment	Class II Exemption Petition ☐ Original Submission ☐ Additional Information	Evaluation of Automatic Class III Designation (De Novo) ☐ Original Submission ☐ Additional Information	Other Submission ☐ 513(g) ☐ Other (describe submission):

Have you used or cited Standards in your submission? ☐ Yes ☐ No (If Yes, please complete Section I, Page 5)

SECTION B SUBMITTER, APPLICANT OR SPONSOR			
Company / Institution Name Organogenesis, Inc.		Establishment Registration Number (if known) 1221816	
Division Name (if applicable) NA		Phone Number (including area code) (781) 401-1155	
Street Address 150 Dan Road		FAX Number (including area code) (781) 575-1570	
City Canton	State / Province MA	ZIP/Postal Code 02021	Country US
Contact Name Patrick Bilbo			
Contact Title Vice President, Regulatory and Clinical Affairs		Contact E-mail Address pbilbo@organo.com	

SECTION C APPLICATION CORRESPONDENT (e.g., consultant, if different from above)			
Company / Institution Name N/A			
Division Name (if applicable)		Phone Number (including area code) ()	
Street Address		FAX Number (including area code) ()	
City	State / Province	ZIP/Postal Code	Country
Contact Name			
Contact Title		Contact E-mail Address	

SECTION D1			REASON FOR APPLICATION - PMA, PDP, OR HDE		
☐ Withdrawal ☐ Additional or Expanded Indications ☐ Request for Extension ☐ Post-approval Study Protocol ☐ Request for Applicant Hold ☐ Request for Removal of Applicant Hold ☐ Request to Remove or Add Manufacturing Site	☐ Change in design, component, or specification: ☐ Software / Hardware ☐ Color Additive ☐ Material ☐ Specifications ☐ Other (specify below)	☐ Location change: ☐ Manufacturer ☐ Sterilizer ☐ Packager			
☐ Process change: ☐ Manufacturing ☐ Sterilization ☐ Packaging ☐ Other (specify below)	☐ Labeling change: ☐ Indications ☐ Instructions ☐ Performance ☐ Shelf Life ☐ Trade Name ☐ Other (specify below)	☐ Report Submission: ☐ Annual or Periodic ☐ Post-approval Study ☐ Adverse Reaction ☐ Device Defect ☐ Amendment			
☐ Response to FDA correspondence:		☐ Change in Ownership ☐ Change in Correspondent ☐ Change of Applicant Address			
☐ Other Reason (specify):					
SECTION D2			REASON FOR APPLICATION - IDE		
☐ New Device ☐ New Indication ☐ Addition of Institution ☐ Expansion / Extension of Study ☐ IRB Certification ☐ Termination of Study ☐ Withdrawal of Application ☐ Unanticipated Adverse Effect ☐ Notification of Emergency Use ☐ Compassionate Use Request ☐ Treatment IDE ☐ Continued Access	☐ Change in: ☐ Correspondent / Applicant ☐ Design / Device ☐ Informed Consent ☐ Manufacturer ☐ Manufacturing Process ☐ Protocol - Feasibility ☐ Protocol - Other ☐ Sponsor ☐ Report submission: ☐ Current Investigator ☐ Annual Progress Report ☐ Site Waiver Report ☐ Final	☐ Repose to FDA Letter Concerning: ☐ Conditional Approval ☐ Deemed Approved ☐ Deficient Final Report ☐ Deficient Progress Report ☐ Deficient Investigator Report ☐ Disapproval ☐ Request Extension of Time to Respond to FDA ☐ Request Meeting ☐ Request Hearing			
☐ Other Reason (specify):					
SECTION D3			REASON FOR SUBMISSION - 510(k)		
☒ New Device	☐ Additional or Expanded Indications	☐ Change in Technology			
☐ Other Reason (specify):					

SECTION E ADDITIONAL INFORMATION ON 510(K) SUBMISSIONS									
Product codes of devices to which substantial equivalence is claimed						Summary of, or statement concerning, safety and effectiveness information ☒ 510 (k) summary attached ☐ 510 (k) statement			
1	KMF	2	MGQ	3				4	
5		6		7				8	
Information on devices to which substantial equivalence is claimed (if known)									

#	510(k) Number	#	Trade or Proprietary or Model Name	#	Manufacturer
1	K011026	1	FortaDerm™ Wound Dressing	1	Organogenesis Inc.
2	K024054	2	Xcell® Antimicrobial Cellulose Wound Dressing	2	Xylos Corporation
3		3		3	
4		4		4	
5		5		5	
6		6		6	

SECTION F PRODUCT INFORMATION - APPLICATION TO ALL APPLICATIONS

Common or usual name or classification
FortaDerm™ Antimicrobial Wound Dressing

#	Trade or Proprietary or Model Name for This Device	#	Model Number
1	FortaDerm™ Antimicrobial Wound Dressing	1	N/A
2		2	
3		3	
4		4	
5		5	

FDA document numbers of all prior related submissions (regardless of outcome)					
1	2	3	4	5	6
7	8	9	10	11	12

Data Included in Submission
☐ Laboratory Testing ☐ Animal Trials ☐ Human Trials

SECTION G PRODUCT CLASSIFICATION - APPLICATION TO ALL APPLICATIONS

Product Code KMF	C.F.R. Section (if applicable) 880.5090	Device Class ☐ Class I ☐ Class II ☐ Class III ☒ Unclassified
Classification Panel General, Plastic and Reconstructive Surgery		

Indications (from labeling)
 The FortaDerm Antimicrobial PHMB Wound Dressing is intended for the management of wounds and as an effective barrier to resist microbial colonization within the dressing and reduce microbes penetrating through the dressing. FortaDerm Antimicrobial PHMB Wound Dressing may be used for the management of: partial and full thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grafts, post-Moh's surgery, post-laser surgery, podiatric, wound dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, and skin tears) and draining wounds.

Note: Submission of this information does not affect the need to submit a 2891 or 2891a Device Establishment Registration form.		FDA Document Number (if known)	
SECTION H MANUFACTURING / PACKAGING / STERILIZATION SITES RELATING TO A SUBMISSION			
☒ Original ☐ Add ☐ Delete	FDA Establishment Registration Number 1221816	☒ Manufacturer ☐ Contract Manufacturer	☐ Contract Sterilizer ☐ Repackager / Relabeler
Company / Institution Name Organogenesis, Inc.		Establishment Registration Number 1221816	
Division Name (if applicable) N/A		Phone Number (including area code) (781) 401-1155	
Street Address 150 Dan Road		FAX Number (including area code) (781) 575-1570	
City Canton		State / Province MA	ZIP/Postal Code 02021
		Country US	
Contact Name Patrick Bilbo		Contact Title Vice President, Regulatory and Clinical Affairs	Contact E-mail Address pbilbo@organo.com

(b)(4) Vendor Information

☐ Original ☐ Add ☐ Delete	FDA Establishment Registration Number	☐ Manufacturer ☐ Contract Manufacturer	☐ Contract Sterilizer ☐ Repackager / Relabeler
Company / Institution Name		Establishment Registration Number	
Division Name (if applicable)		Phone Number (including area code) ()	
Street Address		FAX Number (including area code) ()	
City		State / Province	ZIP/Postal Code
		Country	
Contact Name		Contact Title	Contact E-mail Address

SECTION I**UTILIZATION OF STANDARDS**

Note: Complete this section if your application or submission cites standards or includes a "Declaration of Conformity to a Recognized Standard" statement.

1	Standards No.	Standards Organization	Standards Title	Version	Date
2	Standards No.	Standards Organization	Standards Title	Version	Date
3	Standards No.	Standards Organization	Standards Title	Version	Date
4	Standards No.	Standards Organization	Standards Title	Version	Date
5	Standards No.	Standards Organization	Standards Title	Version	Date
6	Standards No.	Standards Organization	Standards Title	Version	Date
7	Standards No.	Standards Organization	Standards Title	Version	Date

Please include any additional standards to be cited on a separate page.

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Organogenesis Inc.

L I V I N G T E C H N O L O G Y

October 21, 2005

Food and Drug Administration
Center for Devices and Radiological Health
CDRH Document Control Center (HFZ-401)
9200 Corporate Blvd.
Rockville, MD 20850
PRSB/DGRND/ODE/CDRH

**Re: FortaDerm Antimicrobial Wound Dressing
K051647**

Dear Sir/Madam:

(b)(4) Deficiencies

(b)(4) Deficiencies

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MATERIAL SAFETY DATA SHEET

(b)(4) Vendor Information

(b)(4) Vendor Information



P11 S0142-9612(96)00186-8

Biomaterials 18 (1997) 95-105
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0142-9612/97/\$17.00

REVIEW

Methods for the treatment of collagenous tissues for bioprostheses

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Collagenous tissue as a biomaterial possesses many favourable characteristics and advantages over synthetic materials. The resemblance to human tissue suggests that it has a performance advantage over alternative materials. This advantage has been exploited to produce clinical devices that have been implanted in patients for more than a quarter of a century. The method of treating collagenous tissue for bioprostheses has developed from crude exposure of tissue to chemicals to a sophisticated level of considering the biochemical, chemical, engineering and clinical aspects of the process. This review focuses on the various chemical and physical treatments that have made the bioprostheses possible, highlighting the chemical agents and the cross-linking mechanism involved. © 1996 Elsevier Science Limited

Keywords: Collagenous tissue, bioprostheses, porcine aortic valve, bovine pericardium, cross-linking

Received 1 July 1995; accepted 12 July 1996

In the late 1980s, the tissue-based heart valve came into existence in response to a need for an alternative to circumvent the clinical complications experienced with the mechanical heart valve¹. Beginning with human tissue and progressing to animal tissue, the bioprosthetic heart valve became a reality². The term bioprosthesis was introduced by Carpentier¹ to refer to devices derived from biological tissue, invariably collagenous in nature, that is treated to impart *in vivo* durability. In this process, the treatment invalidates the regenerative capabilities of the tissue. The treatment of collagenous materials for bioprostheses has been practised for more than 25 years. This review provides a general survey of the methods that have been reported, highlighting the chemical aspects, and concludes with the author's view for the future.

BIOPROSTHESES

There are many devices that can be classified as bioprostheses. In this review, by virtue of its overwhelming proliferation, reference pertains predominantly to the bioprosthetic heart valve. Wherever possible, examples of other bioprostheses, such as vascular grafts, biohybrid vascular grafts, ligament substitutes and pericardial patches, will be cited under the respective treatment methods. The collagenous tissues treated with the various processes are non-viable. This distinguishes bioprostheses from treatments which are meant to retain the cell-regenerative capability of the tissue, such as cryopreservation

of homografts, which is outside the scope of this review³.

COLLAGENOUS TISSUE

Biological tissue used to fabricate the bioprosthesis is connective tissue rich, the main component being collagen. When animal tissue is used it is termed a xenograft, while with human tissue it is either an autograft or a homograft. The main types of collagenous tissue that have been used for bioprostheses are the porcine aortic valve (PAV), bovine pericardium (BP),

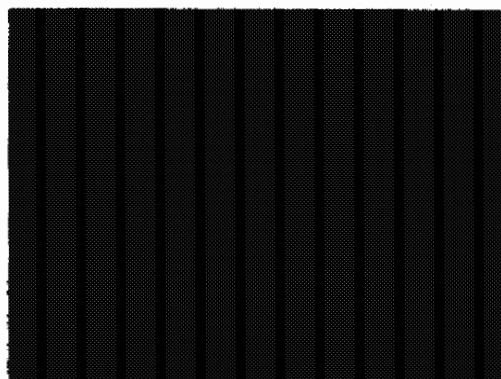


Figure 1 The porcine aortic valve root after trimming and glutaraldehyde-fixation (courtesy of St. Vincent's Meditech Pte. Ltd.).

dura mater, fascia lata and autologous pericardium. Figure 1 shows a typical porcine aortic valve root, which resembles the human aortic heart valve in form and function. Various degrees of success have been achieved with each type of collagenous tissue as a bioprosthetic material. Initially, human tissues were used extensively with sterilization methods. However, the supply problem and their subsequent failure led to the emergence and subsequent dominance of the xenograft. In turn, the problems that are associated with the xenograft have now prompted some researchers to re-evaluate the autograft. There is no denying that autografts are the best choice, followed by homografts and finally xenografts⁴⁻⁷. However, the difficulty of procuring and preserving autografts and homografts makes the alternative of the abundant heterograft sensible. Ultimately, the choice of tissue type rests in complex considerations which include patient profile, prior experience and prejudice.

Tissue morphology

The porcine aortic valve and bovine pericardium exemplify typical tissue types and their morphological features are described briefly. The porcine aortic valve comprises three cusps, the aortic valve annulus and aortic sinuses of valsalva⁸. The cusps are the most important features and consist of three regions, the ventricularis, the spongiosa and the fibrosa. The quantity and orientation of collagen in these layers are complex and varied, the ventricularis and fibrosa having the most collagen. The collagen assembly in bovine pericardium is different to the porcine aortic valve, being predominantly comprised of multiple overlapping layers of collagen aligned in different directions⁹. There are again three layers, the serosal, the fibrosa and the epipericardial connective tissue, all of which contain varying amounts of collagen. The features of these tissues after fixation and implantation, and of dura mater and fascia lata, have been described¹⁰⁻¹⁷.

Collagen

The primary constituent of these tissues is collagen, a generic term for a family of extracellular proteins which are essentially polymers of amino acids. Eleven types of collagen have been identified¹⁸⁻²². At the most basic level, a collagen molecule consists of three chains of poly(amino acids) (or polypeptides) arranged in a trihelical configuration ending in non-helical carboxyl and amino terminals, one at each end. These non-helical ends are believed to contribute to most of the antigenic properties of collagen. Collagen molecules assemble to form microfibrils, which in turn form fibrils to give the collagen fibre. In their natural state, the collagen trihelical configurations are held in place by direct chemical bonds, hydrogen bonds and water-bridged cross-links. Associated with collagen in tissue are elastin and proteoglycans, mucopolysaccharides attached covalently to protein cores. They are believed to modulate collagen fibrillogenesis, fill space, bind and organize water, and repel negatively charged molecules in these collagenous tissue fibres. The amino acids in collagen contain pendant groups such as amines (NH₂), acids (COOH) and hydroxyls (OH) and, together with the amide bond of the polymer, are points for possible chemical reactions on collagen.

Additionally, water molecules surrounding the collagen molecules form another source of entry for reaction, since they can be displaced upon dehydration, exposing previously concealed groups for potential cross-linking.

CROSS-LINKING

Collagenous tissues obtained from the abattoir, cadaver or patient begin to degrade immediately. Therefore, in the exploitation of tissue as clinical material this deterioration must be arrested and deferred, preferably beyond the recipient's natural life. The aim is to prolong the materials' original structural and mechanical integrity and remove or at least neutralize the antigenic properties attributed to these materials. Methods typically concentrate on creating new additional chemical bonds between the collagen molecules. These supplementary links reinforce the tissue to give a tough and strong but non-viable material that maintains the original shape of the tissue.

Physical and chemical methods for the treatment of collagenous tissue are available. The process of stabilizing tissue involves the chemical agent or the physical process initiating, ideally, irreversible and stable intra- and intermolecular chemical bonds between collagen molecules. Preferably, the agent promotes bonds between the functional groups of the amino acids. Chemical methods typically utilize bifunctional chemicals that interact with collagen at two different sites. The functional groups of the chemical agent react with those on the amino acid residues of collagen, such as the ϵ -amino function on lysine and hydroxylysine or the carboxyl function on aspartic and glutamic acids, to give rise to 'cross-links' between the collagen molecules. A drawback of chemical agents is the potential toxic effects a recipient may be exposed to from residues and/or chemicals resulting from a reversal of the cross-links. Physical methods include drying, heating or exposure to ultraviolet or gamma radiation. Unlike chemical cross-linking, these methods do not introduce toxic chemicals into the tissue, but this does not preclude undefined side-effects that may arise due to these processes.

The efficiency and extent of these reactions depend on the thickness of the layers of collagenous tissue which defines the magnitude of penetration and are a function of parameters such as the concentration of the reagent, and the time and the temperature of exposure. It is generally accepted that the more exogenous bonds that are generated in the natural biomaterial, the better the durability. Ideally, the treatment should also maintain much of the original character of the tissue, such as its flexible mechanical properties, and should not shrink significantly. Hence the necessity of keeping the tissue near neutral pH, ensuring an aqueous media environment and minimizing denaturation of the collagen. Therefore, a balance must be achieved for attaining enough reliable cross-links for the biomaterial to last the lifetime of the recipient yet permit the biomaterial to perform as it would in its natural state. Therein lie the contradicting requirements of treating collagenous tissue for bioprostheses. The methods that have been developed do not and probably cannot satisfy the dual

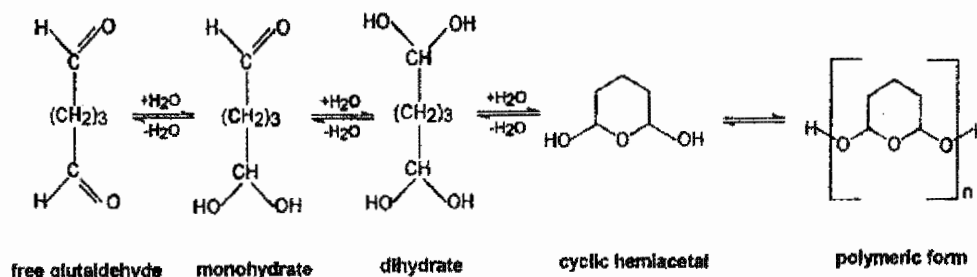


Figure 2 Glutaraldehyde and the forms it takes in aqueous media. Adapted from Korn *et al.*²⁴.

requirements and what is used commercially is the best compromise.

CHEMICAL TREATMENTS

The predominant chemical agents that have been investigated for the treatment of collagenous tissue for bioprostheses are glutaraldehyde, formaldehyde, polyepoxy compounds, acyl azide, carbodiimides and hexamethylene diisocyanate. These treatments give a biomaterial that is non-viable, the intended advantage being resistance to *in vivo* degradation. In addition, methods that were originally developed to preserve the tissue for possible host regeneration, as in the use of antibiotic sterilants in combination with cryopreservation using glycerol, have also been included when such treatments result in the destruction of the tissue's regenerative capabilities.

Glutaraldehyde treatment

Glutaraldehyde is the most successful chemical agent and is the only commercially viable process that has received widespread acceptance. No new method has yet been able to replace it effectively and, consequently, glutaraldehyde treatment dominates this topic, exemplified by a recent review²³. Glutaraldehyde was first applied successfully for bioprostheses in the late 1960s by Carpentier *et al.*². Since then many variations and conditions have been applied to optimize its efficiency and, consequently, entrenching xenografts as the tissue of choice. Glutaraldehyde is easily available, inexpensive and forms aqueous solutions that can effectively cross-link tissue in a relatively short period. Commercial bioprosthetic heart valves

fabricated from porcine aortic valves (PAVs) are usually treated with a low concentration glutaraldehyde solution (typically around 0.5%) for more than 24 h to ensure optimum fixation.

Glutaraldehyde is a five-carbon aliphatic molecule with an aldehyde at each end of the chain, rendering it bifunctional (Figure 2)²⁴. The aldehyde is able to interact chemically with amino groups on collagen to form chemical bonds, as depicted in Figure 3. Since it is bifunctional, it links two different collagen molecules, hence its mode of cross-linking²⁵. However, the situation is complicated by the fact that it polymerizes and is rarely in its monomeric form in aqueous solutions²⁶. The mode of action has been the focus of many investigations²⁷⁻²⁹. Lower concentrations have been found to be better in bulk tissue cross-linking compared to higher concentrations. Cheung *et al.*²⁹ proposed that high concentrations of glutaraldehyde promote rapid surface cross-linking of the tissue, generating a barrier that impedes or prevents the further diffusion of glutaraldehyde into the tissue bulk. There is also some dispute as to whether fixation is complete as the depth of tissue increases, hence glutaraldehyde's cross-linking effectiveness is suspect³⁰. The manner in which tissue is exposed to the reagent is also important. Studies have shown that low- or zero-pressure fixation is best in retaining the original character of tissue³¹⁻³³. It is now also known that glutaraldehyde-fixed biological tissue is not as durable as once thought. While the debate is still ongoing, it is quite evident that mechanical failure such as tears in the cusps of the tissue at points which are the most stressed are noted in a number of retrieved valves^{34,35}. Furthermore, depolymerization of the glutaraldehyde cross-links has been reported³⁶⁻³⁸. This releases glutaraldehyde into the recipient. However, the toxic effects may be minimal or at a level that is tolerable. Other factors including the mechanical properties have also been the subject of much deliberation.

Calcification

Calcification is the predominant cause in the failure of PAV and BP bioprostheses that has been attributed to the glutaraldehyde process³⁹⁻⁴². Calcification and ways to control or circumvent it have been the focus of most research in the past 15 years³⁶. Calcification is a multi-effect event which is caused by a number of factors such as the presence of phospholipids in tissue that can attract calcium ions, or voids and cavities in the tissue created by the removal of proteoglycans

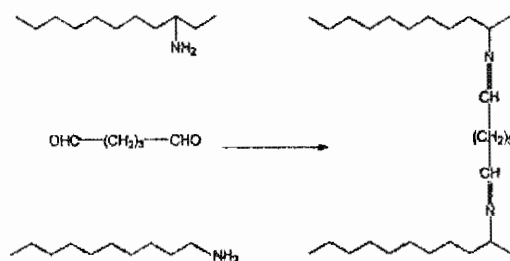


Figure 3 Simplified representation of monomeric glutaraldehyde reaction with amino groups on collagen to form cross-links.

during processing or cellular degradation. This predisposes, in the glutaraldehyde-fixed tissue, potential points that can trap foreign particles that may lead to nucleation centres for calcium. Increased calcium uptake leads to a build-up of calcium phosphate, which in time mineralizes into calcium hydroxyapatite.

Attempts to control or mitigate calcification in glutaraldehyde-fixed biological tissue have yielded many possible candidates that have been shown to be effective at the small animal model level. Their effectiveness is based on interrupting or retarding one or more steps in the calcification process. For example, the surfactant sodium dodecyl sulphate is believed to control calcification by removing phospholipids, and has been commercially applied⁴⁵. Other agents include diphosphonates (synthetic analogues of natural calcification inhibitors), amino-oleic acid and metal ions (Al^{3+} and Fe^{3+})⁴⁴⁻⁵¹. In the case of the diphosphonates, rather than contacting tissue with the agent, it has been incorporated into a silicone drug delivery reservoir and attached to the sewing ring. Anticalcification agent release has been shown to inhibit calcification in animal studies. More recently, the use of chloroform and dimethyl sulphoxide (DMSO) with glutaraldehyde-fixed biological tissue has also been reported^{46,60}. The results of the DMSO work have important implications for calcification. Khor *et al.*⁵² proposed that high concentrations of DMSO denature tissue collagen to some degree. However, the overall integrity of tissue structure is retained because of the prior treatment with glutaraldehyde⁵². Therefore, neat DMSO in effect changes the three-dimensional structure in tissue, eliminating or at the very least minimizing the oriented Ca^{2+} attracting points, such as carboxyl and residual amines, in tissue. Combined with the inherent tissue shrinkage caused by DMSO exposure, sites along the fibrils that are nucleation sites for calcium crystal formation as proposed by Glimcher⁵³ are reduced. This explanation, combined with the hypothesis that glutaraldehyde can also be a nucleating site for Ca^{2+} , probably accounts for the complete picture of *in vivo* dystrophic calcification of bioprosthetic tissue⁵⁴.

New approaches to the utilization of glutaraldehyde

It is evident from the preceding discussion that glutaraldehyde-fixation of xenografts has probably reached a plateau. However, the chemical itself appears to have

been given an extension of service-life. In recent years, shrinkage temperature determinations of glutaraldehyde on biological tissue have led researchers to conclude that short time fixation may be advantageous⁵⁵. In one such procedure, autologous pericardium is exposed to short durations (~10 min) of low concentrations of glutaraldehyde solutions⁵⁶. This protocol appears to impart to tissue the required stiffness for it to be shaped into a valve for implantation⁵⁷. Preliminary results showing good tissue performance with no calcification are encouraging and may lead to a revival of the homograft as a viable alternative to the xenograft.

Formaldehyde

In the 1960s, formaldehyde, a common preservative for biological tissue, was also considered alongside glutaraldehyde as a candidate fixative⁵⁸⁻⁶⁰. Aqueous solutions of formaldehyde ranging from 0.5 to 10% were found to provide the desired tissue stability as determined by shrinkage temperature. However, the long-term durability was found to be inferior to glutaraldehyde. This was attributed to unstable and reversible fixation by formaldehyde. This is not surprising considering the single functionality of this reagent, which only permits reaction with a single collagen molecule (Figure 4). When exposed to the dynamic situation that exists in storage as well as *in vivo*, reversal to formaldehyde is the natural consequence, thereby negating the stability achieved initially²⁶. Furthermore, formaldehyde treatment results in cusp stretching and deformations, extreme reduction of the collagen matrix and increased immunological responses⁶⁷. The use of formaldehyde in tissue fixation was discontinued soon after. However, the use of formaldehyde as a sterilant remains a primary post-treatment step for glutaraldehyde-fixed bioprostheses, with thorough rinsing being necessary prior to implant to remove residual formaldehyde.

OTHER CHEMICAL CROSS-LINKING AGENTS

After the establishment of glutaraldehyde as the fixative of choice, the problems associated with the process gradually became known. Anticalcification has already been mentioned as one approach to improve on the situation. Alternatively, other researchers embarked on attempts to replace glutaraldehyde. The obvious strategy would be to use other bifunctional cross-

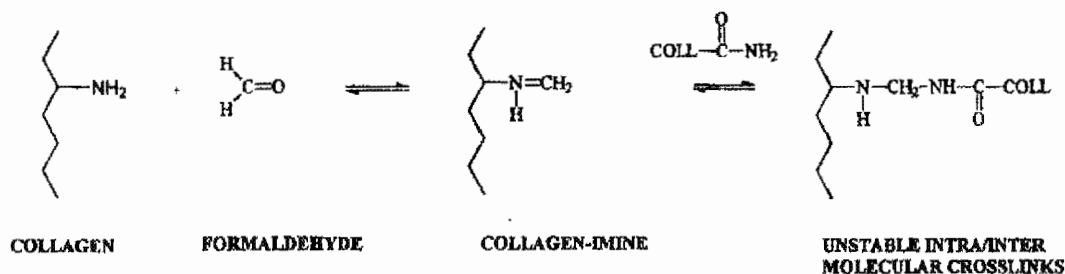


Figure 4 Simplified representation of formaldehyde reaction with amino groups on collagen to form cross-links.

linking agents to react with the functional groups on collagen. Epoxy and diisocyanate compounds dominate this approach. Another strategy has been to perform chemical reactions on the functional groups of amino acids on collagen to generate reactive sites for cross-linking with each other, the basis for the acyl azide and carbodiimide reactions. The apparent advantages of all these methods over glutaraldehyde are comparable or better tissue stability, lower calcification and, presumably, lower toxicity.

Polyether oxide

One of the more promising approaches, first reported in the late 1980s, is the use of polyether or polyepoxy compounds⁶¹. This reagent has been applied to a variety of tissues including bovine pericardium and artery, and porcine aortic valve cusps and tendons⁶²⁻⁶⁷. Figure 5 shows two typical structures, the common feature being the presence of two and three points of bonding on the molecule for the diglycidyl ether and triglycidyl ether, respectively. The structure is based on glycerol, where the hydroxyl groups have been replaced by short- to medium-length polymers terminating in an epoxy functionality. The three-atom epoxy ring in chemistry terms is very strained and favours ring opening to relieve this strain, the driving force for the cross-linking reaction. The epoxy functionality predominantly reacts with the amine group on lysine, much like glutaraldehyde. Additionally, the possibility to react with the nitrogen on the pyrrole ring on the amino acid histidine as well as acid groups exists, thereby increasing the versatility of cross-linking. Figure 6 shows the proposed mechanism of cross-linking reactions using this reagent with lysine in collagen. The amine group on lysine acts as the nucleophile, substituting the oxygen bond on the terminal carbon to give a carbon-nitrogen bond stable to acid hydrolysis⁶².

The profile of the molecule is large compared to the straight-chain five-carbon glutaraldehyde. The three-carbon backbone can have branches that vary from 3 to 46 carbons in length. This profile, however, does not appear to affect the diffusion of polyepoxy compounds

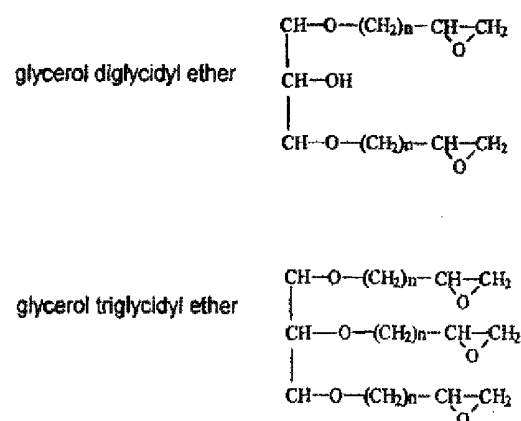


Figure 5 Structure of two polyepoxy compounds. The $[n]$ number can vary from 1 up to 46 for presently known compounds. Adapted from Imamura *et al.*⁶¹.

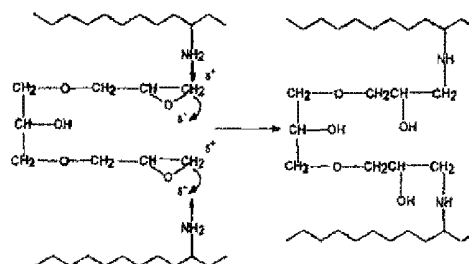


Figure 6 Proposed mechanism of cross-linking for polyepoxy compounds to collagen, showing the amino group on collagen undertaking a nucleophilic displacement of the epoxy function to form cross-links. Adapted from Tu *et al.*⁶².

into the tissue if fixation time is long and may even be desirable for a wider variety of cross-links. The mechanical properties of the resulting bioprosthetic tissue are not compromised and are invariably better than fresh tissue^{63,64}. Furthermore, polyepoxy compounds, regardless of their side-chain complexity, can attain a similar extent of cross-linking if allowed to react for up to 17 months⁶⁵. This has been attributed, in the case of smaller polyepoxy molecules, to polymerization with time resulting in the formation of larger polyepoxy molecules. Tu *et al.*⁶³, in a kinetic study of the fixative on bovine thoracic arteries, have demonstrated by amino acid analysis assay that the entire tissue can be cross-linked. As expected, the usual influence of temperature, pH and concentration of reagent plays a role in the final material property. A catalyst and accelerator system at high pH for more than 48 h appears to be the best fixation condition⁶⁵. Finally, the aqueous-based reaction format has been maintained and the resultant material appears to be more hydrophilic than glutaraldehyde.

Tissue cross-linked by this method has been shown to mitigate calcification significantly compared to glutaraldehyde-treated tissue⁶⁶⁻⁷⁰. For example, one study using PAV cusps reported that after 3 months implantation in the rat model, polyepoxy-treated tissues were found to have an average of 1 μg of calcium per mg of dry tissue compared to glutaraldehyde-treated samples with an average of 140 μg per mg⁶⁶. The cytotoxicity of the polyepoxy compounds has also been evaluated and shown to be acceptable^{71,72}.

Hexamethylene diisocyanate and polyurethane

Hexamethylene diisocyanate (HMDI) is another difunctional molecule where the terminal isocyanate groups can react with amines of lysine on collagen to form the urea bond. This chemical has been used by Chvapil and co-workers^{73,74} to cross-link extracted collagenous materials intended for temporary implantable support devices. Materials so cross-linked provided the necessary strength at the site of implant for up to 8 months to permit the regeneration of the recipient's tissues. The implant slowly degrades to non-toxic products. HMDI is a straight-chain molecule having a 10-atom spread between the linking points. Naimark *et al.*⁷⁵ have used HMDI to cross-link bovine pericardium. Figure 7 shows the sequence of the cross-

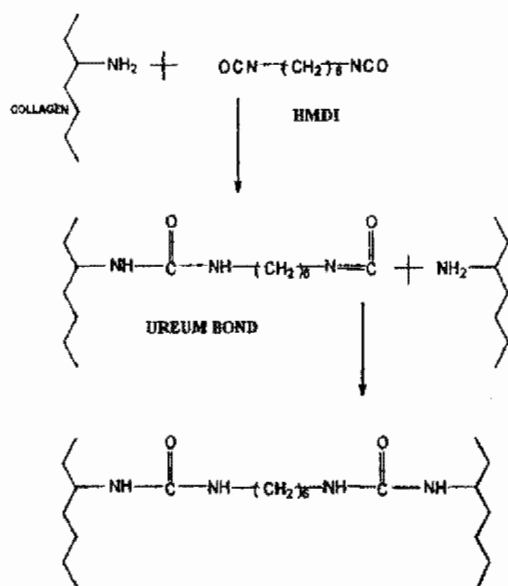


Figure 7 Representative reaction of hexamethylene diisocyanate with the amino group on collagen to form ureum cross-links. Adapted from Naimark *et al.*⁷⁵

linking reaction. The process of cross-linking is unique because it has been found that the best results are obtained when cross-linking is carried out under anhydrous conditions using 2-propanol. This is necessary to circumvent the high reactivity of the isocyanate to water, destroying the isocyanate functionality before it can react with collagen. The shrinkage temperature of HMDI-treated tissue of 85°C is comparable to the 86°C for glutaraldehyde-fixed bovine pericardium⁷⁶. Similar results were obtained for mechanical properties and enzyme degradation resistance. The cross-links are reported to be stable in water⁷⁷. The cytotoxic effects and mechanical properties of collagen and tissue treated with HMDI have been investigated^{78,79}. The cytotoxic effects of HMDI appear to be tolerable and much better than glutaraldehyde. Furthermore, Chvapil⁷³ states that the advantage of HMDI is its presumed degradation to hexamethylene diamine, which is cited to be minimally cytotoxic.

In a similar approach, Loke *et al.*⁸⁰ have also used non-aqueous reactions to cross-link porcine pericardium. Instead of 2-propanol, dimethyl sulphoxide (DMSO) was used as the reaction solvent. An isocyanate-terminated polyurethane oligomer was the cross-linking agent. A grey surface layering, which does not delaminate, was formed on the surface of the porcine pericardium and is attributed to the cross-linking of surface amino functionalities with polyurethane. The predominantly surface cross-linking is possibly attributed to the size of the polyurethane oligomers, which may be a hindrance for diffusion into the tissue⁸¹. The use of polyurethane offers a wider dimension because the chain and cross-linking points could be predetermined to optimize the final material's properties.

The introduction of non-aqueous environments

presents a new dimension in the cross-linking of collagenous materials. First, it is likely to disturb the three-dimensional arrangement of native collagen and this can lead to cross-linking by virtue of its dehydrating effect, which can alter the side-chain chemistry of collagen⁷⁵. Furthermore, it may remove fats, proteoglycans and, in regions where the solvent contacts residual water, upsets the solubility of the reagent, precipitating the cross-linking agent. Finally, it may impart unexpected results. For example, with DMSO, Khor *et al.*⁸² have reported improved calcification mitigation properties. Nevertheless, careful construction of the experimental approach may lead to a profitable outcome in terms of material properties.

Acyl azide and carbodiimides

In this approach to the chemical fixation of biological tissue, the use of a traditional bifunctional cross-linking agent is obviated⁸². Instead, the carboxyl functional groups of aspartic and glutamic amino acids on collagen undergo a three-step chemical reaction, and in the process are converted to acyl azide functionalities. Figure 8 summarizes the reaction scheme of this process. The carboxyl group is first esterified, followed by reaction with hydrazine to form a hydrazide. Finally, the hydrazide is reacted with sodium nitride to give the acyl azide. These acyl azide functionalities in turn react and couple with adjacent amino groups on other amino acids in collagen to give the cross-linked tissue⁸². Apparently, the sequence of chemical reactions does not give rise to any deleterious effects on the tissue. Tissue cross-linked by this method was found to have a shrinkage temperature slightly lower than that of glutaraldehyde-fixed tissue (~2°C), but was comparable in tissue stability as determined by cyanogen bromide cleavage and enzyme degradation studies. A newer variation of this reaction using

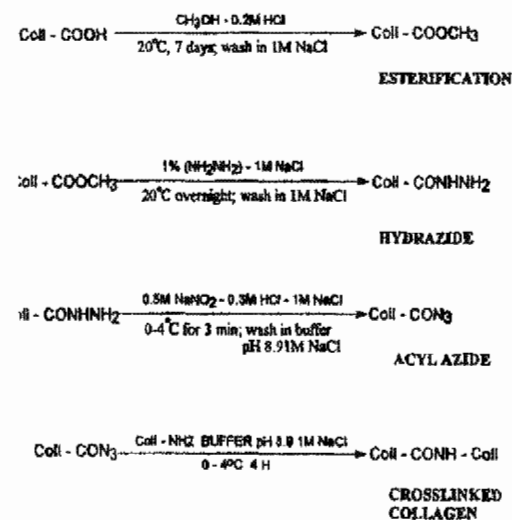


Figure 8 The sequence of reactions of the acyl azide method to cross-link tissue. Carboxyl groups of aspartic and glutamic acid residues of collagen are converted to acyl azide, which in turn reacts with amino groups on collagen to form the urea cross-links. Adapted from Petite *et al.*⁸²

diphenylphosphoryl azide (DPPA) has also been reported⁶³. DPPA provides a simpler and faster cross-linking performance compared to its predecessor, although it is a solvent-based reaction. In a comparison study of the cytotoxic effects of these reagents, DPPA was found to be less cytotoxic than acyl azide and both were better than glutaraldehyde⁶⁴. Finally, bovine pericardium treated by the acyl azide procedure demonstrated marked reduction in calcification compared to glutaraldehyde-fixed controls⁶⁵.

The carbodiimide reagent offers another method for generating cross-links between corresponding reaction sites, without itself being incorporated. In an early application, the reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride was used as an activating agent in the promotion of attaching acrylic polymers to glutaraldehyde-fixed biological tissue⁶⁶. The process was reported to reduce calcification. The wider application of the carbodiimide reaction is in the promotion of cross-links in tissue, the mechanism being similar to the acyl azide reaction^{67,68}. Figure 9 shows the sequence of events in a typical reaction. The carbodiimide is first protonated and reacts with the carboxyl function on collagen to form the *o*-isourea. This is followed by nucleophilic attack of the amine functions on the adjacent collagen, generating the cross-link and simultaneously releasing the urea derivative of the carbodiimide reagent. Proper post-treatment removal of the activating reagents gives a treatment with no residual chemicals. In practice, most procedures usually involve a combination of physical and chemical processes. In a typical procedure collagen is first dehydrated by a combination of thermal and vacuum drying and then treated with the chemical reagent cyanamide for final cross-linking⁶⁹. This method has been applied to collagenous tissue. Initial results as indicated by shrinkage temperature measurements suggested poor cross-linking, but new refinements using more sophisticated carbodiimide reagents have improved the results⁷⁰⁻⁷⁵. One point to note is that the contribution of heating and vacuum drying has not been accounted for⁶⁴.

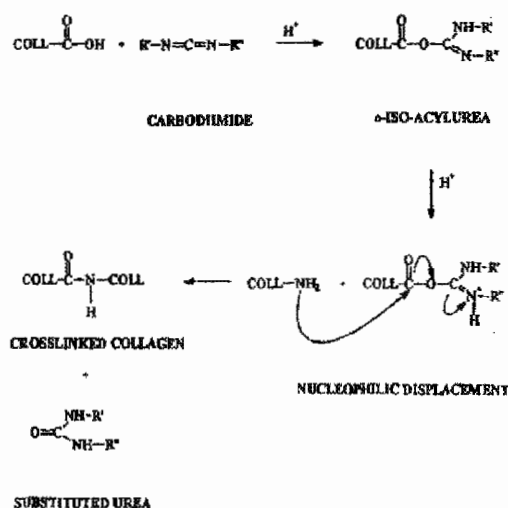


Figure 9 The sequence of reactions for the carbodiimide method to cross-link tissue.

One feature of these reagents is the relatively short length of the cross-links. The cross-link is two atoms long. This limits the 'spread' of the cross-links to adjacent collagen molecules, unlike PEO or the diisocyanates, where the length can be quite long. However, this may be an advantage. As long as mechanical studies prove that the material can be used, the short chain may be beneficial in terms of its anticalcification properties by limiting calcium binding sites, as discussed earlier^{47,90}.

Glycerol

Glycerol is a small three-carbon molecule with three hydroxyl functional groups. These hydroxyl groups do not contribute to any cross-linking reactions. This method was originally conceived as one of a myriad of methods to sterilize and preserve homografts for host cell regeneration capabilities⁹¹. An overview of the preservation and sterilization is described by Bodnar and Ross⁹². The role of the reagent is not clearly understood but at the concentration used, 98%, the tissue is rendered non-viable and is justifiably bioprosthetic. Presumably, in the process of preserving the tissue, the high level of glycerol displaces the water in the cells and consequentially leads to cell death⁹³. The tissue used in most studies has been the dura mater^{97,98}. One such procedure is the room temperature preservation of freshly retrieved human dura mater preserved in 98% glycerol. After fabrication into a valve, preservation was resumed in 98% glycerol until implantation, when a final rinse in an antibiotic solution was effected⁹⁹. Reported clinical results of this approach have been promising. In a comparative study to glutaraldehyde-fixed bovine pericardium, glycerol-treated dura mater was found to degrade and calcify slower^{100,101}. Recently, bovine pericardium treated with glycerol for more than 2 weeks was reported to be biocompatible and significantly lowered levels of calcium in explants were found¹⁰².

PHYSICAL METHODS

The primary advantage of physical methods is that they do not introduce chemicals that may cause potential harm as there are no residues in this approach. Typical physical processes such as drying, heating and irradiation have been applied to collagen¹⁰³.

These studies highlight the interaction and types of cross-links that can form using these physical methods¹⁰⁴. Extending this knowledge to collagenous tissue is inevitable. The limitation of physical methods appears to be in trying to achieve a balance between attaining the desired amount of cross-linking but preventing the onset of degradation of tissue as a consequence of the long exposure times inevitable for physical processes. With irradiation, there is also the need to determine the depth of penetration of the radiation.

Dye-mediated photo-oxidation

Recently, dye-mediated photo-oxidation of biological tissue has been reported as a viable method for cross-linking tissue for bioprostheses¹⁰⁵. Pericardial tissue stabilized using ultraviolet irradiation was found to be

a suitable biomaterial for fabrication into a bioprosthesis. In a typical procedure tissue is placed in a reaction vessel, each piece $\sim 2\text{ cm}^2$. The reaction solution comprises buffered NaCl solution containing 1% (w/v) methylene green. Irradiation with light for up to 22 h in the presence of dissolved oxygen gives the fixed biomaterial.

Cross-linking has been verified by biochemical studies¹⁰⁶. Dye-mediated photo-oxidized tissue subjected to protein extraction and enzyme (pepsin) digestion shows that the longer the irradiation period the less protein is recovered/extracted, indicating that cross-linking of the tissue had occurred. The shrinkage temperature of the photo-oxidized tissue was found to be similar to untreated tissue, suggesting that the tissue behaves like the original (i.e. cross-linking does not affect the tissue character). Subcutaneous implants retrieved after 60 d *in vivo* showed that the photo-oxidized and glutaraldehyde implants were still intact, while in contrast the untreated control was not found. Preliminary results of the performance of pericardial valves, using bioprosthetic tissue from dye-mediated photo-oxidation, implanted in juvenile sheep are promising⁶⁴.

Microwave irradiation to initiate cross-links on collagen has also been reported¹⁰⁷. This can probably be extended to collagenous tissue. Another physical method that has become popular is dehydrothermal treatment^{108,109}. It is reported that the process of slowly removing water under a high vacuum and a temperature of around 110°C leads to cross-linking of collagen. Preliminary data show that the process increased the strength of collagen. It is probably only a matter of time before this method is extended to tissue.

COMPARISONS

An interesting anomaly appears to be the lack of a comprehensive comparison between the different treatments. Most studies compare the individual treatment to glutaraldehyde with respect to tissue morphology and/or calcification. More comprehensive studies are few; an example is that by Pereira *et al.*⁹⁴ on the effects on the mechanical properties of pericardium after chemical and physical treatments. In this work, the chemical treatments were glutaraldehyde, cyanamide and polyepoxy compounds. The physical treatments were heat-drying and freeze-drying. Glutaraldehyde- and polyepoxy-fixed tissues were found to have the best cross-linking as determined by shrinkage temperature measurements, while the drying methods were found to be similar to fresh tissue, but cyanamide treatment appears to be slightly detrimental. The mechanical properties as determined by tensile measurements showed that heat-drying gives the best tissue modulus and ultimate modulus.

Another comparison of the treatments relates calcification to cytotoxicity, cellular interactions and cross-linking. The chemicals surveyed were HMDI, acyl azide and cyanamide compared to fresh and glutaraldehyde-treated collagen. The results suggest that proper removal of residual chemicals reduces the cytotoxic effect and improves the potential of the bioprosthetic material⁹⁵.

SUMMARY

The principal methods for the treatment of collagenous tissues for bioprostheses have been reviewed. The methods rely on the chemical agent interacting with functional groups on collagen to cross-link tissue to produce a biomaterial that, in most instances, is durable but non-viable. All methods have their advantages and disadvantages. The most successful method, glutaraldehyde-fixation, is now known to have questionable durability and also results in calcification of the bioprosthesis. However, to date, no new method has been demonstrated to successfully replace it. The limitations of the requirements for treating biological tissue for the case of the porcine aortic valve and bovine pericardium suggests that this practice has reached its limits. The associated durability, antigenicity and toxicity problems will always persist. Anticalcification strategies are promising and the newer methods of cross-linking tissue have been shown to be useful alternatives to glutaraldehyde. However, most of the results are preliminary and more development has to follow before clinical applications can be realized. Whether this will be achieved in timely fashion remains unknown.

Finally, the long-held concept of treating xenografts to resist long-term degradation appears to be threatened. There appears to be a resurgence of the use of homografts. Present research directions point to the use of short time exposure to low concentrations of glutaraldehyde with autologous pericardium. This can be extended to other tissue types. Competition will also emanate from fledgling tissue engineering strategies¹¹⁰. It is the opinion of this author that the best treatment will be one that revitalizes, even to a limited extent, the graft in the host. Perhaps it is fitting to end by quoting Buch *et al.*⁵⁸, who in 1970 stated that 'Only by preserving the viability of the donor tissue to ensure subsequent cellular replication and neocollagen formation can satisfactory long term function be realized'.

ACKNOWLEDGEMENTS

The author is grateful to Goh Khoo Seng for his unyielding enthusiasm for the duration of the bioprosthetic heart valve R&D programme in Singapore, and to the National University of Singapore, the National Science and Technology Board, Singapore, and St. Vincents Meditech Pte. Ltd for financial sponsorship of the programme.

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BIOMAT., MED. DEV., ART. ORG., 11(4), 293-318 (1983-84)

EVALUATION OF COLLAGEN CROSSLINKING TECHNIQUES

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ABSTRACT

The properties of collagen films crosslinked by physical and chemical techniques were compared to the properties of films crosslinked with glutaraldehyde (GTA). Physical techniques studied include exposure to short wave (254 nm) u.v. irradiation and severe dehydration. Chemical techniques studied include immersion of collagen films in aqueous solutions of cyanamide or GTA. Collagen films exposed to combinations of aqueous solutions of cyanamide and severe dehydration had moduli of elasticity, swelling ratios and resistance to bacterial collagenase similar to films crosslinked with GTA. Theoretical calculations based on amino acid composition indicate that approximately seven times as many amino acid residues are capable of forming crosslinks using cyanamide or severe dehydration procedures as compared to GTA crosslinking. In addition, using severe dehydration or cyanamide forms crosslinks involving both amino and carboxyl residues which may allow these procedures to act synergistically. Based on our studies this two-step procedure effectively crosslinks collagen-based biomaterials while the only by-product of this reaction is water-soluble urea. Preliminary biocompatibility studies suggest that this crosslinking procedure may allow for pronounced tissue ingrowth.

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INTRODUCTION

Collagen is the primary structural component of mammalian tissues. The fiber forming collagens, collagen types I, II and III, give shape to tissues as well as limit tissue deformation in response to applied loads. Although the amount and types of collagen vary from tissue to tissue and species to species, the primary sequence has been shown to be highly conserved. In addition, because of the low antigenicity of collagen, type I collagen has been used as the structural component of many biopolymeric based prostheses such as heart valves, blood vessels, preparations for tissue augmentation and wound dressings (1). Although collagen based materials in general stimulate only a mild inflammatory response, they are still recognized as "foreign" by the tissue and initiate a sequence of cellular events which include mobilization and activation of granulocytes, macrophages and fibroblasts (2). It is unclear whether a component of this inflammatory response is due to an inherent antigenicity of the collagen, the presence of residual crosslinking agents, or the formation of collagen peptides during radiation or gas sterilization (3). Nonetheless, the end result is still the degradation of the implant by collagenase and other enzymes released from fibroblasts and inflammatory cells (4).

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Crosslinking is an effective means of controlling the biodegradation rate of collagen-based biomaterials, preventing

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rapid elution of the material into wound fluids and increasing the tensile properties of the material to the level where they can be handled or sutured conveniently. Crosslinked collagen has a greater modulus of elasticity (Youngs modulus), greater resistance to proteases and a lower degree of swelling than uncrosslinked collagen (4, 5, 6). Present crosslinking procedures often use bifunctional reagents containing reactive groups which form bridges between two amino acid side chains of adjacent protein molecules. Such reagents include aldehydes, isocyanates, alkyl and aryl halides, imidoesters, carbodiimides, N-substituted maleimides and acylating compounds (7). Intermolecular crosslinks are covalent and can support stresses to the same degree as the bonds in the polymer backbone (8). With optimal pH, concentration of reagent and temperature, the crosslinking efficiency of any reagent is a function of the number of amino acid residues which can react with the reagent, the stability of the crosslink, and the bond energy associated with each crosslink.

Often, the reagents previously mentioned are difficult to work with because of toxicity or low water solubility. They may also leach out of the material, polymerize or form unstable crosslinks (4). Aldehydes, particularly glutaraldehyde (GTA), are effective crosslinking reagents and are most often chosen as reagents for crosslinking collagen-based biomaterials used specifically for implantation. At low concentrations GTA

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produces intramolecular crosslinks in collagen, while at higher concentrations GTA forms long polymeric chains which produce intermolecular crosslinks (9).

The reaction of GTA at high concentrations with collagen is reversible (9). After implantation of GTA crosslinked collagenous biomaterials, large polymers of GTA are continuously hydrolysed (10) and this results in the release of free monomeric GTA into surrounding tissue (11). Tissue culture (12) and implantation studies in animals (13) have shown that cytotoxicity and slow growth of granulation tissue are associated with materials crosslinked with GTA. Other deleterious characteristics of GTA crosslinking are heterogenous crosslinking (9) and inability to incorporate other macromolecules into the material (14).

Obviously, where cellular ingrowth into the implant is desirable, alternative crosslinking methods should be employed. These methods should effectively crosslink collagen-based materials without resultant cytotoxicity. Previous investigations have demonstrated the use of short-wave ultraviolet irradiation (15), carbodiimides (16) and severe dehydration to crosslink gelatin and collagen (17). These procedures have not been shown to produce any by-products which may induce cytotoxicity. This study evaluates the in vitro resistance to bacterial collagenase, stress-strain properties, swelling ratios and in vivo biocompatibility of collagen using these alternative crosslinking procedures.

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MATERIALS AND METHODS

Characterization of Starting Material

The collagen was prepared from fresh uncured bovine hide using a procedure similar to that reported by Komanowski (18). This procedure involves separating the reticular dermis (corium) from the papillary dermis and cutting the corium into strips. The corium is fragmented, ground, washed in distilled water, swollen in a dilute acid solution, precipitated and freeze dried.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) based on the method of Laemmli (19) and amino acid analysis on HCl-hydrolyzed aliquots were used to characterize the collagen. Gels were run on the collagen before and after limited pepsin digestion so that the presence of noncollagenous proteins as well as the distribution of types I and III could be established. Gels were also run after reduction with mercaptoethanol.

The lyophilized collagen samples were dissolved in SDS-PAGE sample buffer and incubated with this buffer for 30 minutes at 65°C. These samples were then loaded onto 7.5% SDS polyacrylamide slab gels (1.5 mm). The gels were initially run at 10 mA/gel until the dye front reached the bottom of the gel. Following electrophoresis, the gels were fixed (10% w/v trichloroacetic acid, 75% v/v methanol, 13% v/v acetic acid), stained (0.25% w/v coomassie brilliant blue in 45.4% v/v

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methanol and 4.2% v/v acetic acid), and destained (7.5% v/v acetic acid and 5% v/v isopropanol).

Material Preparation

Lyophilized collagen was swollen in HCl pH 2.0 and dispersed at a concentration of 1% w/v by grinding for 2 minutes in a Waring blender. For film preparation, 30 ml of this dispersion was poured into a plastic tray, covered with aluminum foil and allowed to air dry under a hood for two days at 22°C. Collagen films were then removed from the trays, wrapped in aluminum foil and stored at room temperature in a dessicator prior to crosslinking. For collagen sponge preparation, 60 ml of the dispersion were poured into a tray, frozen at -20°C and then freeze dried at -65°C. The sponges were then ready for crosslinking.

Crosslinking Procedures

Crosslinking by ultraviolet irradiation. Collagen sheets were placed on aluminum foil and set 6 inches directly below a short wave (254 nm) ultraviolet lamp (Mineralight, Model UVS-11). The sheets were irradiated for 1, 3 and 5 hours.

Dehydrothermal crosslinking (DHT). Collagen films were placed in a vacuum oven at 22°C and subjected to a vacuum of

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less than 100 mtorrs for 1 hour as previously described (17). The temperature within the vacuum oven was then increased to 110°C. Dehydrated collagen films were removed from the oven after DHT treatment of 1, 2 and 3 days. The oven temperature was kept below 50°C before the vacuum was released and collagen films removed.

Carbodiimide crosslinking. Collagen films were immersed in a 1% w/v pH 5.5 solution of cyanamide (a carbodiimide, H-N=C=N-H), and removed after 1, 2 and 3 days. After removal the films were rinsed in distilled water for 1 day (materials used for implantation studies were washed in a 1% solution of glycine for 24 hours). All films were air dried at 22°C for 1 day.

Glutaraldehyde crosslinking. Collagen films were immersed in a 0.25% v/v pH 5.5 solution of glutaraldehyde (GTA) for 1, 2 and 3 days at room temperature. Films were then rinsed in distilled water for 1 day. Materials used for implantation studies were washed in a 1% solution of dimedone for 24 hours to remove free aldehyde groups. All films were air dried at 22°C for one day.

Combinations of dehydrothermal and carbodiimide crosslinking treatments. Collagen films were treated with carbodiimide (C) for one day, followed by DHT treatment for 1, 2 and 3 days. In some cases the films were first DHT treated for 1, 2 and 3 days followed by 1 day of exposure to the

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carbodiimide solution. These procedures will be described by the following nomenclature; C(1)DHT(3) refers to carbodiimide treatment for 1 day followed by DHT treatment for 3 days, or DHT(3)C(1), 3 days of DHT treatment followed by 1 day of carbodiimide treatment.

Mechanical Testing

Mechanical properties of films crosslinked by exposure to short-wave ultraviolet light (254 nm), glutaraldehyde, severe dehydration, and carbodiimide were determined as a function of extent of crosslinking. Collagen films were cut into strips (4.0 cm X 1.0 cm) and immersed in distilled water 15 minutes prior to testing. The mechanical properties of these strips were analyzed in a hydrated state by applying a tensile force to the specimen at 22°C and a constant strain rate (10% / minute) using an Instron Tester Model 1122. The ends of the strips were placed between pneumatic grips (40 psi grip pressure) allowing a gage length of 20 mm. The crosshead speed was 2 mm/minute.

Stress-strain curves obtained from this analysis were used to calculate Young's moduli in both low and high strain regions. Young's modulus was reported as the ratio of stress (force / cross-sectional area) to strain ((final length - initial length) / (initial length)) and was obtained from the

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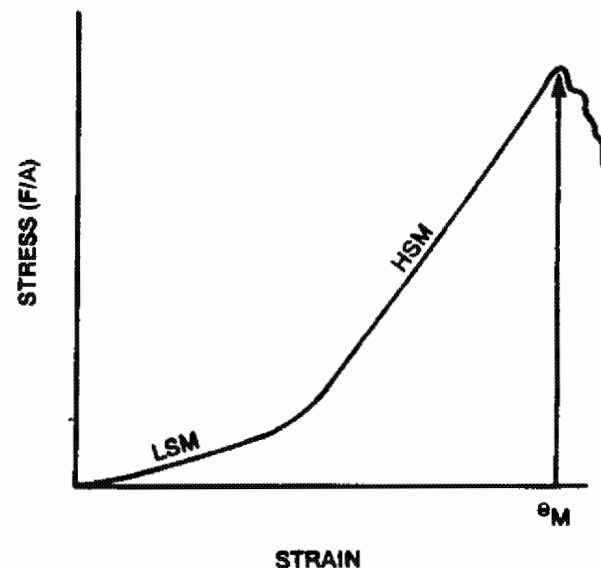


FIG. 1. Stress-strain curve illustrating LSM (low strain modulus), HSM (high strain modulus) and e_M (strain at which the material failed).

slope of the stress-strain curve. The strain at which the material failed was designated e_M (see FIG. 1).

Collagenase Assay

The resistance of collagen films crosslinked using a variety of techniques to biodegradation was assessed by immersion in a bacterial collagenase solution (Sigma Chemical Company, St. Louis, Mo.). A collagenase solution containing 1000 units/ml was prepared by adding 1000 units of bacterial collagenase (1 unit will liberate peptides from collagen

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equivalent to 1.0 micromole L-leucine in 5 hours at pH 7.4 and 37°C in the presence of calcium ions) to 1.0 ml of stock buffer solution (10 mM Tris, 25 mM CaCl₂, in 100 ml distilled H₂O, pH 7.4).

A 1.0 mg collagen sample to be assayed was added to 2.0 ml stock buffer solution along with 100 units of collagenase (100 microliters of the 1000 units/ml collagenase solution) and placed in a 37°C environment. Each sample was checked for degradation every 15 minutes and the time was recorded when the collagenase-buffer solution became clear. The duration of collagenase activity at 37°C was checked by placing samples with known collagenase resistance times into collagenase solutions that had been previously incubated for periods of 1, 2, 3 and 4 days at 37°C.

Swelling Ratio Determination

The swelling ratio of denatured collagen films was measured to assess the extent of crosslinking. Denatured collagen has been shown to have similar behavior to that of ideal rubbers (17). The swelling ratio r^* is a measure of the extent of crosslinking in randomly coiled polymer networks. Samples of collagen were boiled for 3 minutes and then placed between two dry pieces of filter paper. A 1.0 kg mass was placed on top of the filter paper covering the collagen for 20

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seconds. The collagen was then weighed and this weight recorded as " wet weight " or WW.

The collagen was then placed on aluminum foil and put into an oven maintained at 110°C for 2 hours. The collagen was then weighed and this weight recorded as "dry weight" or DW. The swelling ratio r^* is defined as the inverse of the volume fraction of dry collagen (V_f) and is given by equations (1) and (2):

$$r^* = 1 / V_f \quad (1)$$

$$V_f = (DW/p_c) / ((DW/p_c) + (WW-DW)/p_{H_2O}) \quad (2)$$

where p_c = density of collagen (1320 mg/cc).

Biocompatibility Tests

Five white female 250 gram rats received sterile (5 Mrad radiation) 1.0 gram subcutaneous sponge implants of the following materials; (a) uncrosslinked collagen (b) glutaraldehyde crosslinked collagen (c) C(1)DHT(3) crosslinked collagen and (d) DHT(3)C(1) crosslinked collagen. Animals were sacrificed at 7, 14 and 21 days post implantation, the implants were excised and processed using routine histological procedures. Sections were stained with hematoxylin and eosin and trichrome. The sections were qualitatively analysed with

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RESULTS

Our studies were conducted to compare the efficiency of physical and chemical processes for crosslinking collagen films. The collagen used in these studies (based on amino acid analysis) was composed of about 1/3 glycine (330 residues per thousand) with proline and hydroxyproline accounting for about 210 residues. The collagen was judged to be primarily type I since only small amounts of cysteic acid were present. SDS-PAGE also showed that the material used in these experiments was primarily type I collagen.

In Vitro Analysis

Six different crosslinking procedures were assessed. These procedures are listed below:

- 1) exposure to short-wave ultraviolet light for periods of 1, 3 and 5 hours.
- 2) severe dehydration at 110°C for periods of 1, 2 and 3 days.
- 3) exposure to cyanamide for 1, 2 and 3 days at pH 5.5 and 22°C.
- 4) exposure to glutaraldehyde for 1, 2 and 3 days at pH 7.4 and 22°C.
- 5) exposure to a combination of cyanamide for 1 day followed by severe dehydration at 110°C for 1, 2 and 3 days.

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- 6) exposure to severe dehydration for 3 days and cyanamide for 1, 2 and 3 days.

Materials crosslinked according to the six protocols above were evaluated via use of physical, enzymatic and in vivo tests. Table 1 presents the results obtained from these investigations. All values are the average of 3-6 individual observations. A one-tail hypothesis test was applied so that the level of significance could be analysed (20). Only a confidence interval of 0.95 ($p < 0.05$) was considered a significant result.

The mechanical tests involved measurement of the modulus in the low and high strain regions (LSM and HSM, respectively) as well as the strain at failure (e_M). As shown in Table 1, exposure to ultraviolet light (protocol A) for 1, 3 or 5 hours as well as exposure to cyanamide (protocol C) for 1, 2 or 3 days resulted in materials with moduli that were significantly lower than that observed using glutaraldehyde (protocol D) or severe dehydration (protocol B) alone or in combination with exposure to cyanamide (protocols E and F). The stiffness to a first approximation is proportional to the crosslink density (21) suggesting that the crosslink density is higher for materials crosslinked using protocols D, E and F compared to protocols A, B and C.

Swelling ratios calculated as the inverse of the volume fraction of polymer (V_f) were less than 4.0 for procedures D, E

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TABLE I

Comparison of the Properties of Crosslinked Collagen Films using Physical and Chemical Techniques.*

CROSSLINKING PROCEDURE	r*	COLLAGENASE RESISTANCE TIME(min)**	MECHANICAL DATA***		
			LSM g/mm ²	HSM g/mm ²	e _M
Control	22.22	72	111	508	0.37
A	UV(1 hr)	23.81	100	539	0.39
	UV(3 hr)	17.86	107	614	0.30
	UV(5 hr)	13.51	135	820	0.33
B	DHT(1)	6.99	123	849	0.25
	DHT(2)	4.11	213	1058	0.19
	DHT(3)	4.17	380	1468	0.22
C	C(1)	5.89	105	662	0.42
	C(2)	4.48	123	869	0.24
	C(3)	4.69	185	990	0.30
D	G(1)	3.30	1080	1611	0.23
	G(2)	2.52	1440	1836	0.20
	G(3)	2.50	1440	2148	0.16
E	C(1)DHT(1)	2.75	720	1186	0.15
	C(1)DHT(2)	2.16	960	1441	0.16
	C(1)DHT(3)	2.62	1440	1872	0.12
F	DHT(3)C(1)	2.75	960	1959	0.14
	DHT(3)C(2)	3.03	960	1833	0.16
	DHT(3)C(3)	3.06	960	1637	0.11

* The following abbreviations have been used: r*, swelling ratio; LSM, low strain modulus; HSM, high strain modulus; e_M, maximum strain. All measurements shown are the average of 3 to 6 individual observations and typical standard deviations are as follows; r* +/- 10%, collagenase resistance time +/- 5%, LSM +/- 15%, e_M +/- 15% and HSM +/- 10%.

** conducted using 100 units of collagenase per mg of collagen at 37°C.

*** mechanical data obtained at a strain rate of 10% per minute.

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and F while they were greater than 4.0 for procedures A, B and C. In general, the swelling ratio is known to decrease with increasing crosslink density (22). The swelling ratio r* is inversely proportional to the crosslink density and can be calculated from equations (1) and (2). The swelling ratio is determined via analysis of denatured collagen, which behaves similarly to a random coiled polymer network (23).

The resistance to degradation by collagenase was assayed for each crosslinking procedure as a function of crosslinking time. It has been demonstrated (5) that the resistance to collagenase increases with increased crosslink density. Collagenase resistance time for procedures D, E and F were significantly longer than the resistance times for procedures A, B and C.

In summary, the physical and degradation tests indicate that the crosslink density obtained using procedures D, E and F was significantly higher than for procedures A, B and C.

In Vivo Analysis

Uncrosslinked collagen implants were degraded at a greater rate than uncrosslinked materials. Many neutrophils were seen at day 7, indicative of an inflammatory response. Fibroblast infiltration and native collagen deposition were apparent by day 14.

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Implants crosslinked with glutaraldehyde were only partially degraded by day 21. These implants were encapsulated by native collagen deposition and were void of any cellular ingrowth until day 21, when fibroblasts could be observed at the periphery of the implant. Implants crosslinked with combinations of cyanamide and severe dehydration were degraded at a slightly greater rate than glutaraldehyde crosslinked implants. Fibroblasts and native collagen deposition were observed within the implant by day 7 and by day 14 many capillaries were seen within the implant. There was no apparent difference in host tissue reaction to C(1)DHT(3) or DHT(3)C(1) crosslinked implants.

DISCUSSION

The use of bifunctional reagents for crosslinking collagen-based bioprotheses often imparts deleterious characteristics to the protheses as well as host tissue. Ideally, the crosslinking procedure should not be responsible for elution of toxic substances from the protheses while still effectively crosslinking the material. Physical crosslinking procedures are suitable for this purpose as well as chemical procedures which have non-toxic water soluble by products. This work involves development of an efficient crosslinking technique which does not produce toxic products which may leach

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into host tissues, enables the material to be of sufficient mechanical strength to support loads and yields an increased resistance to proteolytic environments.

In Vitro Analysis

Crosslinking efficiency of both physical (severe dehydration and ultraviolet irradiation) and chemical (cyanamide, a water soluble carbodiimide) crosslinking treatments were evaluated and compared to glutaraldehyde by performing mechanical tests, collagenase tolerance assays, and swelling ratio determinations on 1% bovine hide collagen (BHC) films before and after crosslinking treatment. Combinations of physical and chemical crosslinking techniques (cyanamide and severe dehydration) were also evaluated.

Rubin (15) demonstrated that collagen membranes could be crosslinked with short wave (254 nm) ultraviolet irradiation. Ultraviolet irradiation produces radicals in the form of unpaired electrons located predominately on the nuclei of the aromatic residues such as tyrosine and phenylalanine. Crosslinks are introduced by bonding between these radicals (22). Of the 3156 residues on the collagen molecule, about 51 aromatic residues are available for this crosslinking mechanism (see Table II). Ultraviolet irradiation was evaluated as a means of crosslinking collagen because it is a physical means

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TABLE II

Theoretical Distribution of Potential Reactive Amino Acid Residues for Various Crosslinking Procedures.

PROCEDURE	REACTIVE RESIDUES	REACTIVE RESIDUES PER ALPHA CHAIN		SUM OF REACTIVE RESIDUES
		$\alpha 1(I)$	$\alpha 2(I)$	
short-wave u.v. light	tyrosine	4	3	51
	phenyl- alanine	12	16	
Dehydro- thermal treatment or cyanamide	lysine	32	21	745
	arginine	50	57	
	aspartic acid	45	47	
	glutamic acid	77	71	
	serine	34	24	
	threonine	16	17	
glutar- aldehyde	lysine	32	21	85

Amino acid compositions from K. Kuhn, (30).

of crosslinking and no toxic products are introduced into the material. However, irradiation times of greater than five hours may fragment the collagen molecule with subsequent denaturation (15). This property is unfortunate because the crosslink density increased significantly after five hours of irradiation with respect to uncrosslinked collagen films, but was significantly less than crosslink densities obtained through

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other procedures. This may be a result of the relatively low amount of residues available for such a crosslinking mechanism. Also, the stability of the radicals or radical-induced crosslinks may not be adequate.

Dehydrothermal treatment (DHT) increases the helix-to-coil transition temperature (denaturation temperature) for collagen by removing water (17). Removal of water from collagen results in the formation of interchain crosslinks which are the result of condensation reactions either by esterification or amide formation. Amino acid residues which may be involved in such a reaction are those of aspartic acid, glutamic acid, serine, threonine, arginine and lysine and constitute about 745 of the 3156 residues on the collagen molecule (see Table II). Silver, et al have shown that the number of free carboxyl and amino groups decreased with prolonged DHT treatment (23). After DHT treatment of 72 hours, collagen films had significantly higher values for high strain modulus (HSM) and collagenase resistance time (CRT) while having a significantly lower value for r^* as compared to uncrosslinked collagen films and collagen films exposed to ultraviolet irradiation for 5 hours. Dehydrothermal treatment has been shown to maintain the intactness of the collagen triple helix even for prolonged periods (24), and has been proposed as a possible agent for incorporating glycosaminoglycans into a collagen-based dermal equivalent

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(14). Since DHT treatment is a physical mode of crosslinking, toxic substances would not be introduced into host tissues.

Carbodiimides are used to crosslink compounds containing many types of functional groups, including carboxylic acids, amines, phosphates, alcohols and thiols, with the formation of amides and esters. Carboxyl and amino groups from the same residues available to DHT treatment are bound to the carbodiimide with the release of water-soluble urea as a by product (25). One per cent BHC films exposed to a 1% solution of cyanamide for 72 hours had a crosslink density comparable to DHT treatment for the same period. Note that cyanamide, $H-N=C=N-H$, has a limited crosslinking ability because of its short length and inability to polymerize. Larger water-soluble carbodiimides (26) may be able to crosslink residues with longer separation distances and thus yield materials with a greater crosslink density.

Since both DHT and carbodiimide treatments proved to be effective crosslinking procedures, the two were combined. Materials crosslinked $C(n)DHT(n)$ or $DHT(n)C(n)$ showed a significant increase in crosslink density as compared to either procedure carried out independently. Cyanamide derived crosslinks may modify residues or position collagen molecules so as to enhance the action of DHT treatment. Accordingly, the action of cyanamide may be enhanced when the material is first exposed to DHT treatment. Crosslinking sites for each procedure

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may be different, thus the increased crosslink density may be a result of two independent crosslinking mechanisms. This hypothesis is further supported by the fact that there was no significant difference between materials crosslinked $C(1)DHT(3)$ or $DHT(3)C(1)$. Actual elucidation of the crosslinking mechanism would require analytical methods such as cyanogen bromide assays or spectrophotometry.

Glutaraldehyde (GTA) at high concentrations reacts with the ϵ -amino groups of lysyl residues of collagen forming intermolecular crosslinks (27). Of the 3156 residues on the collagen molecule, about 85 are available for this reaction. Collagen films crosslinked with GTA had a high strain modulus (HSM) and collagenase resistance time (CRT) significantly greater than those obtained for any single crosslinking procedure evaluated in this study. The swelling ratio was also significantly lower than for any other procedure carried out independently. The efficiency of this reagent may be due to its tendency to polymerize, yielding a heterogenous population of crosslink lengths. Any advantages gained by this effective crosslinking agent must be weighed against its deleterious effects. The long chain polymers that GTA forms are hydrolysed and may leach out into tissues during wound healing processes (11). Another consequence of GTA polymerization is heterogenous crosslinking. Long GTA polymers cannot penetrate further into the material because they are "blocked out" by previous

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crosslinks (9). Therefore, only the surface of the material is crosslinked. This presents obvious problems for any collagen-based prosthesis, especially heart valves. Glutaraldehyde tanned materials have also been shown to impede cellular ingrowth in both tissue culture assays and in vivo experiments (11,12).

The increased stiffness of crosslinked collagen films is due to an increase in the degree of intermolecular crosslinking. Extensive crosslinking does not allow for reorientation of the collagen fibers and the material may then behave in a brittle manner. Crosslinking may inhibit the activity of proteases by sterically restricting them from the site of enzyme-substrate interaction. The crosslinks may also inhibit penetration of the enzyme into the material. When cleavage occurs, crosslinks may serve to retain most of the structure, retarding further degradation by other proteolytic enzymes (many proteases require some denaturation of collagen before they become active (28)). An increase in the number of crosslinks may impede the ability of the material to bind with water thus decreasing the degree of swelling when the material is placed in an aqueous environment.

In Vivo Analysis

Uncrosslinked implants were degraded at a faster rate than crosslinked implants because of the increased susceptibility to

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proteases whose activity is diminished by the presence of crosslinks (28). The inflammatory response observed in the absence of crosslinking may be due to the rapid cleavage of collagen molecules by tissue collagenase. Once the collagen molecule is cleaved by collagenase it is rapidly degraded by general proteases. Gelatin and gelatin peptides have been shown to be chemotactic for neutrophils and may therefore induce an acute inflammatory response (2). Glutaraldehyde crosslinked implants had delayed cellular ingrowth. This may be associated with leaching of glutaraldehyde monomers into surrounding tissues. Materials crosslinked with combinations of cyanamide and severe dehydration were relatively resistant to proteolytic degradation and had pronounced cellular infiltration and native collagen deposition. These preliminary results indicate that this two-step method is an effective means of crosslinking collagen while still allowing native tissue ingrowth. More extensive biocompatibility studies are in progress.

The two-step crosslinking procedure involving a water-soluble carbodiimide and dehydrothermal treatment yields collagenous materials with equivalent swelling ratios, stiffnesses and collagenase resistance times to collagenous materials crosslinked with glutaraldehyde. This procedure does not produce the potential for any toxic substances to leach into host tissues for the only by-product is urea, which is water-soluble and easily washed from the material. The latter

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characteristic of this two-step procedure may allow pronounced cellular infiltration and subsequent tissue formation.

ACKNOWLEDGEMENTS

The authors would like to acknowledge technical assistance from Rita Hahn and Jane Brokaw. We would also like to thank Dr. David Birk for his constructive discussions.

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BIOMAT., MED. DEV., ART. ORG., 11(4), 319 (1983-84)

BOOK REVIEW

"Macromolecular Biomaterials" edited by Garth W. Hastings and Paul Ducheyne Ph.D., CRC Press, Inc., 1984, \$89.00, 125 pgs.

The first part of this volume deals with some basic considerations of structure and its relation to function. After outlining some basic principles in the first chapter, including manufacturing effects, structural determination is discussed in detail. This is important since adequate characterization in terms of molecular size is necessary for a proper understanding and use. The theme of interrelationships between structure and properties is developed in the next chapters.

The second part of the volume deals with selected applications in which the development and use of macromolecular materials in specific applications is described. The selection of applications includes synthetic and natural polymers and the range of medical areas involved include orthopedics, dialysis, drug release (macromolecular pharmacology) blood contact, and plastic surgery. Since the list could not be completely comprehensive, these represent areas of special development or of continuing problems.

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Zero-Length Crosslinking Procedure with the Use of Active Esters¹

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Received July 24, 1989

A two-step zero-length crosslinking procedure for studying protein-protein complexes has been developed. One component of a complex is briefly incubated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in the presence of *N*-hydroxysuccinimide resulting in the conversion of some of the protein carboxyls into succinimidyl esters. The reaction is stopped by addition of β -mercaptoethanol and other interacting proteins are then added. Crosslinking arises from substitution of lysine ϵ -amino groups of these proteins for the succinimidyl moieties during a 1- to 2-h incubation period. The advantage of this method versus one-step zero-length crosslinking is that only one component of the complex is exposed to the crosslinker, which eliminates complications arising from the formation of crosslinks among several proteins of a multicomponent complex. Furthermore, crosslinks can be formed even in the presence of reagents, such as dithiothreitol and EDTA, that would interfere with direct crosslinking with EDC. © 1990 Academic Press, Inc.

N-substituted carbodiimides are widely used for peptide synthesis. The water soluble 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)² is capable of inducing isopeptide bonds between amino acid side chains in proteins in aqueous solution (1) and has found application as one of the so-called zero-length crosslinkers (2).

Since no spacer is introduced, zero-length crosslinking provides a better chance that linked residues are located in the actual site(s) of protein-protein interaction—an important factor when crosslinking is used for identifying interaction sites in the primary structure (2). The mechanism of crosslinking with EDC involves formation of an adduct—an *O*-acylisourea derivative—between the EDC and the carboxyl group of Glu or Asp followed by a nucleophilic substitution with an amino group, most often the ϵ -NH₂ of lysine (3,4). In the absence of amino groups the active *O*-acylisourea undergoes rapid hydrolysis regenerating the original carboxyl and an *N*-substituted urea.

In some cases the use of EDC in crosslinking may present serious difficulties or may not lead to crosslinking at all. In the case of multicomponent protein complexes a polymer containing many subunits is usually formed. In the presence of some reagents such as β -mercaptoethanol, DTT, or EDTA side reactions preclude formation of a crosslinked complex. To overcome these difficulties we were seeking a two-step procedure in which only one component would be exposed to the crosslinking reagent. In their two-step procedure Morinet *et al.* (5) used a 5- to 10-fold dilution, following activation by EDC of the first protein component, prior to the addition of the second component. We found, however, that even submillimolar concentrations of EDC were sufficient to induce crosslinking, making dilution ineffective in preventing the reaction of the second component with EDC. Moreover the *O*-acyl derivatives undergo fast hydrolysis; the estimated rate constant at pH 4.75 is of the order of $2-3 \text{ s}^{-1}$ (3). Thus in the absence of carbodiimide the "active carboxyls" become deactivated within a fraction of a second—most likely before the complex is formed. For the procedure to be successful the active form of a protein would have to be more stable than the *O*-acylisourea derivative. Such a condition is fulfilled by the so-called "active esters," in particular the *N*-hydroxysuccinimidyl esters (NHS), developed for

¹ A preliminary account of this work has been presented: Grabarek, Z., and Gergely, J., 1989, *Biophys. J.* 53, 892a.

² Abbreviations used: Tn, troponin, the three-component regulatory protein of striated muscle thin filaments; TnC, calcium binding component of troponin; TnI, inhibitory component of troponin; TnT, tropomyosin binding component of troponin; Tm, tropomyosin; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS, *N*-hydroxysuccinimide; CPM, 7-diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin; Mes, 2(*N*-morpholino)ethanesulfonic acid; DTT, dithiothreitol; SDS, sodium dodecyl sulfate.

peptide synthesis (6), and their sulfonated derivatives (7). NHS and sulfo-NHS active esters have been used for covalent attachment of small molecules to proteins (8-11). A number of active esters of dicarboxylic acids have been synthesized for protein-protein crosslinking (7,12-14). Cuatrecasas and Parikh (15) described synthesis of an NHS-activated derivative of agarose for immobilization of proteins. Kishida *et al.* (16) applied an essentially identical method to link ferritin to antibodies. In their method amino groups are first succinylated and then both the carboxyls originally present in the protein and the newly introduced ones are converted into NHS active esters. While this procedure assures a high yield of crosslinking and eliminates intramolecular crosslinking it is not suitable for identification of protein-protein interaction sites because of the extensive modification of the protein.

We have developed a two-step zero-length crosslinking procedure utilizing the transient formation of active esters of carboxyls in a protein molecule. Initially one component of the complex is activated with EDC in the presence of NHS, leading to the formation of *N*-succinimidyl esters of some glutamic and aspartic acid carboxyls. Then the activation step is terminated by addition of β -mercaptoethanol which rapidly reacts with excess EDC, and other components of the protein complex are added. Crosslinking occurs during a 1- to 2-h incubation period at room temperature. The presence of β -mercaptoethanol in the reaction mixture prevents activation of components of the complex other than that initially activated. The active groups that do not form crosslinks undergo hydrolysis regenerating the original carboxyls. Crosslinking can be interrupted at any time, if desired, by addition of millimolar concentrations of hydroxylamine. We have applied the new technique to a two-component complex (TnC-TnT complex) and to a multi-component system—the regulated thin filament of skeletal muscle containing actin, tropomyosin, TnI, TnT, and TnC.

MATERIALS AND METHODS

Materials. Buffers and other common reagents were purchased from Sigma Chemical Co. CPM was from Molecular Probes, Inc. (Eugene, OR). EDC and NHS were from Pierce (Rockford, IL).

Protein preparation. All proteins were obtained from rabbit back and leg muscles. Actin was prepared according to Drabikowski and Gergely (17) from acetone-dried muscle powder. Troponin was prepared from ether-dried muscle powder and separated into subunits according to Greaser and Gergely (18). Tropomyosin was prepared according to Bailey (19).

Protein labeling. Actin (1-2 mg/ml) in G-buffer (2 mM HEPES, pH 7.5, 0.2 mM CaCl_2 , 0.2 mM ATP) was labeled at Cys-374 by incubation for 2 h at room tempera-

ture with 2 mol of CPM (added as a concentrated solution in dimethylformamide) per mole of actin. Labeling was terminated by addition of 10 mM DTT and actin was polymerized by addition of 2 mM MgCl_2 and 0.1 M KCl. CPM-F-actin was separated by ultracentrifugation for 20 min at 220,000g in a Beckman TL 100 ultracentrifuge. The pellet was depolymerized in G-buffer followed by ultracentrifugation under the same conditions to remove insoluble material. The supernatant containing CPM-G-actin was collected and polymerized by addition of 2 mM MgCl_2 and 0.1 M KCl.

Lysine determination. The extent of modification of lysine residues in proteins incubated with EDC was determined from the reaction with fluorescamine (20) according to Lai (21).

Protein concentrations. Protein concentrations were estimated using published absorbance coefficients: TnC, $A_{280}(0.1\%, 1 \text{ cm}) = 0.18$; TnT, $A_{280}(0.1\%, 1 \text{ cm}) = 0.46$; actin, $A_{280}(0.1\%, 1 \text{ cm}) = 0.63$. For the fluorescently modified proteins the colorimetric method of Bradford (22) was used.

Electrophoresis. Electrophoresis was performed in the presence of SDS on 10% polyacrylamide slabs in 80 mM Tris-glycine buffer, pH 8.6, or on 15% polyacrylamide slabs according to Thomas (23).

RESULTS AND DISCUSSION

Carbodiimide-catalyzed formation of amide bonds involves as an intermediate step the formation of an *O*-acylisourea derivative (3). This intermediate can then react with an amino group to form an isopeptide bond or with a water molecule to regenerate the original COOH. When used in protein crosslinking the fast hydrolysis of the *O*-acylisourea derivative reduces the yield of crosslinking and precludes the use of EDC in a two-step procedure. Generating an activated complex with a slower hydrolytic rate would make it possible to activate one protein and subsequently add one or more components to form a complex. Since succinimidyl esters are relatively stable (6,13,24), we have activated carboxyls of one protein via conversion to active esters with NHS (Scheme 1) before complex formation with a second protein.

We have tested this approach on TnC and TnT, which are the components of the regulatory complex in the thin filament of skeletal muscles. Several pieces of evidence indicate that the two proteins interact with each other in solution and are in contact in the regulated thin filament (for review see Leavis and Gergely (25)). TnC was incubated with EDC in the presence of NHS. A 15-min incubation terminated by the addition of β -mercaptoethanol resulted in the incorporation of approximately three NHS groups per molecule of TnC as calculated from the increase in A_{280} (Fig. 1). The NHS derivative of TnC has the ability to crosslink TnT in the absence of

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active carbodiimide (Fig. 1, inset, Scheme 2). Although for analysis reagents in excess have been removed by rapid filtration on a Sephadex G-25 column, this step is not necessary in the routine crosslinking procedure since the addition of β -mercaptoethanol rapidly inactivates EDC. If β -mercaptoethanol was added prior to the addition of EDC no complex formation between TnC and TnT was observed regardless of the presence of NHS. On the other hand β -mercaptoethanol did not interfere with the crosslinking step, i.e., substitution of an amino group of the second protein for the succinimidyl moiety. Carraway and Triplett (26) have found that β -mercaptoethanol reacts with EDC with a pseudo-first-order rate constant $k = 0.029 \text{ s}^{-1}$ (0.1 M EDC, 0.013 M β -mercaptoethanol, 25°C, pH 5.0). The product of the reaction is stable in solution and is resistant to 1 M hydroxylamine or 2 M NH_3 . Thus addition of β -mercaptoethanol appears to be an effective way to quench the unreacted EDC. The NHS-TnC derivative undergoes slow hydrolysis (Fig. 1, Scheme 2) as indicated by a decrease in A_{280} of the protein fraction ($T_{1/2} = 40 \text{ min}$ at pH 6.0). The decrease in A_{280} is paralleled by a decrease in the formation of crosslinks between TnC and TnT (Fig. 1). A similar rate of hydrolysis of NHS-activated protein has recently been reported (27), while a slightly slower hydrolysis rate was found for sulfo-NHS active esters (24). On the basis of the half-life of the *N*-hydroxysuccinimidyl active esters we have routinely used a 2-h incubation time to induce protein-protein crosslinking. The data of Lomant and Fairbanks (18) suggest that the reaction with NH_3 may be faster especially at pH values higher than 6.0, thus requiring shorter incubation times.

A relatively long half-life of the active ester raises the question whether crosslink formation is specific, i.e., whether it occurs at the interaction interface or whether it results from random collision of molecules that do not form stable complexes. While random collisions underlie crosslinking of low molecular weight compounds to proteins (8-11) it usually requires more drastic conditions than those used for protein crosslinking in our experiments. Upon protein complex formation the lysine residues located at the interaction interface may be expected to react preferentially with their carboxyl counterparts since no diffusion is involved. This expectation is supported by our observation that in the presence of sodium dodecyl sulfate no crosslinked TnC-TnT complex was formed. Thus although the activation of the carboxyl groups of Asp and Glu is random, crosslinking occurs at the interaction interfaces and those active groups that cannot make contact with $\epsilon\text{-NH}_2$ groups of Lys undergo

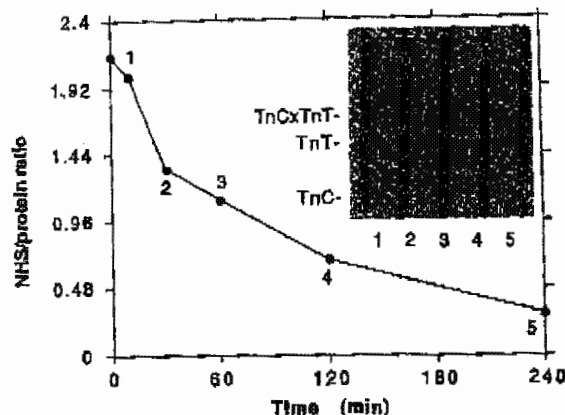
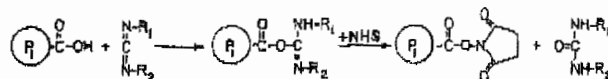


FIG. 1. Stability of NHS-activated TnC. TnC (1 mg/ml) was incubated for 15 min at 25°C in a solution containing 0.1 M Mee, pH 6.0, 0.5 M NaCl, 2 mM EDC, and 5 mM NHS. The reaction was terminated by addition of β -mercaptoethanol (final concentration 30 mM) and the solution was further incubated at the same temperature. At various times samples of the solution were withdrawn and passed through a Sephadex G-25 column (0.6 $\times$ 5 cm) equilibrated with 0.5 M NaCl and 0.1 M Mee, to remove low molecular weight components. By centrifugation of the columns on a clinical centrifuge it was possible to decrease the time required for this step to $\sim 3 \text{ min}$. One portion of the effluent was combined with TnT in a 1:1 molar ratio in 0.5 M NaCl, 0.1 M Mee and further incubated for 2 h followed by polyacrylamide gel electrophoresis (inset) to monitor the ability of TnC to crosslink TnT. The other portion was used to record the uv spectrum. Since both the protein and the protein-bound succinimidyl moieties contribute to the absorption at 280 nm the number of NHS groups bound per molecule of TnC was calculated after subtracting the contribution of the protein whose concentration was measured colorimetrically with the Bio-Rad reagent based on the procedure of Bradford (22). We used the molar absorption coefficient of $\epsilon_{280} = 8200$ for NHS (35) and $A_{280}(0.1\%, 1 \text{ cm}) = 0.17$ for TnC.

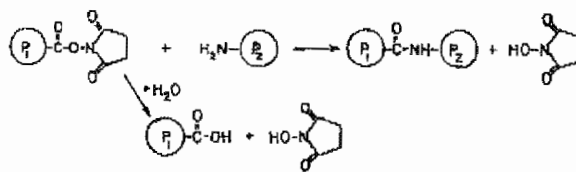
hydrolysis. We have also found that the active carboxyls can be rapidly neutralized, most likely by hydrolysis, upon treatment with dilute (10 mM) hydroxylamine further decreasing the possibility of unspecific crosslinking.

Polarity of the Interaction at the Protein-Protein Interface

Since carboxyls are the targets for the nucleophilic attack of the carbodiimide our method provides an opportunity to probe the protein-protein interface with respect to the distribution of the negatively and positively



SCHEME 1



SCHEME 2

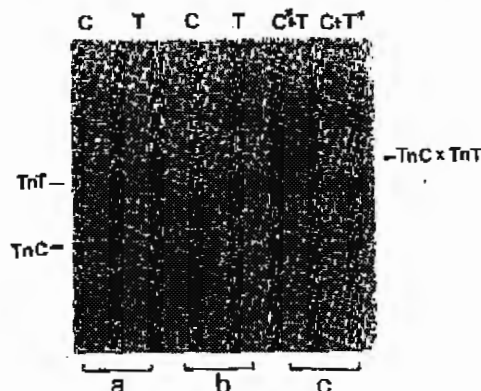


FIG. 2. Polarity of the TnC-TnT interaction. Either TnC or TnT in 0.5 M NaCl, 0.1 M Mes, pH 6.0, was incubated for 15 min at 25°C with 2 mM EDC and 5 mM NHS. The activation step was terminated by addition of β -mercaptoethanol (final concentration 20 mM) and the other component of the complex was added. Protein solutions were incubated for 1 h at 25°C and the products were analyzed by polyacrylamide gel electrophoresis in the presence of SDS. Key: (a) proteins, no crosslinker present; (b) proteins incubated with EDC-NHS; (c) complexes of TnC with TnT. C and T stand for TnC and TnT, respectively. Asterisks indicate the component that was activated with EDC and NHS prior to the formation of the complex.

charged side chains. We have treated either one or the other of the interacting components with EDC and NHS (Fig. 2). The crosslinked TnC-TnT complex was formed only when TnC was activated. When TnT was activated we observed small amounts of a TnT dimer but no TnC-TnT complex was formed. This result indicates that the interaction between the two proteins involves COOH groups on TnC and NH₂ groups on TnT. Small amounts of the dimer probably reflect the known tendency of TnT for aggregation.

Crosslinking in a Multicomponent System

We have also tested our method on a complex of F-actin with the regulatory proteins tropomyosin, TnC, TnI, and TnT. Since these proteins can assemble *in vitro* into an active complex containing these components in the ratio found *in vivo*, viz. 7:1:1:1, it seems likely that the interactions between the components in the reconstituted complex are the same as those in the native complex. In the experiment shown in Fig. 3 actin was labeled with a fluorescent probe to facilitate identification of the actin containing crosslinked complexes. Since the label was located at the C-terminus of actin it was further possible to identify the crosslinking site (not shown) using the method of Sutoh (28). Figure 3 shows that actin-TnI and actin-tropomyosin crosslinked complexes are formed if the complex is assembled after either F-actin or tropomyosin is activated with EDC-NHS. A number of high molecular weight species are found indicating formation of crosslinked complexes

with various actin to tropomyosin ratios. We have previously determined that TnI crosslinks to the N-terminal 12 residue segment of actin in a F-actin-TnI complex (29). The same segment of actin was crosslinked with TnI in the complex containing all the components of the thin filament. A detailed account of this work will be published elsewhere. Tropomyosin was also found to crosslink to the N-terminal segment of actin if actin was activated; however, activation of tropomyosin resulted in crosslinking to the C-terminal segment of actin (30).

Side Reactions

The use of EDC in protein crosslinking requires consideration of possible side reactions. While the solvent-exposed COOH groups are the primary targets for EDC some other side chains can also react. Carraway and Triplett (26) have demonstrated that SH groups react rapidly, and virtually irreversibly, with EDC. Exposed tyrosines can be modified by EDC although the reaction can be reversed by treatment with 0.5 M hydroxylamine (31). The imidazole moiety of histidine was shown to interfere with coupling most likely by accelerating the hydrolysis of the NHS ester (15). A possible side reaction may involve a rearrangement of the *O*-acylisourea derivative to a stable *N*-acylurea (4,32). Such modification in proteins is rather selective and seems to be limited to carboxyls that are partially buried in a hydrophobic environment (32). Another complication that should be considered is intramolecular crosslinking. In the case of zero-length crosslinking with carbodiimides intramolec-

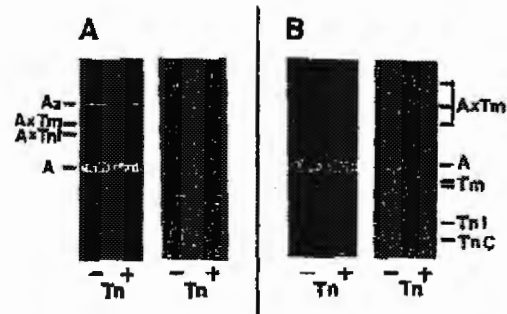


FIG. 3. Crosslinking between actin and tropomyosin (Tm) in reconstituted thin filaments. Actin was labeled with a fluorescent probe (CPM) at Cys-374. Either actin (A) or tropomyosin (B) was activated with 2 mM EDC, 5 mM NHS for 15 min; then the reaction was terminated by addition of β -mercaptoethanol which was followed by a 2-h incubation at 25°C with tropomyosin (A) or actin (B). The three-component tropomyosin complex was added where indicated. Samples were analyzed on 15% polyacrylamide gels in a modified Laemmli system according to Thomas (23). Both the picture taken under uv light and the Coomassie blue-stained SDS-gels are shown. Note the formation of a number of high molecular weight fluorescent bands corresponding to crosslinked actin-tropomyosin (A-Tm) complexes of various stoichiometry. The actin-TnI complex (A-TnI) and the actin trimer (A₃) are formed only when actin is activated.

ular crosslinking can occur if there are $\text{NH}_3^+ \text{--} \text{COO}^-$ interactions within the protein. Such interactions are known to be involved in stabilization of the 3D structure of proteins as, for example, in the "i to i + 4" type interaction between Lys and Glu (or Asp) side chains in α -helical segments (33). We have observed some intramolecular crosslinking in TnC upon incubation with EDC and NHS (34) although the extent of such crosslinking was rather small under the conditions normally employed for protein-protein crosslinking. Depending on the conditions of the activation, between zero and two lysine residues out of nine in TnC were lost as a result of treatment with EDC and NHS. Under the same conditions 50-100% crosslinking with TnT or TnI could be obtained (data not shown) suggesting a preference for intermolecular rather than intramolecular crosslinking.

CONCLUSIONS

We have shown that protein-protein crosslinking can be achieved in a two-step procedure in which carboxyls of one of the components are activated by formation of an ester with *N*-hydroxysuccinimide. Our method has several advantages over the one-step crosslinking procedure: (i) It can be used in a multicomponent system. Only one component of the complex is exposed to the carbodiimide which greatly simplifies the analysis of the products of crosslinking. (ii) The relative stability of the active ester provides an opportunity for changing the conditions of crosslinking, e.g., an introduction of EDTA or DTT. (iii) Information can be obtained about distribution of charged groups at the interaction interface. (iv) Generally higher crosslinking yields are obtained with less exposure to the crosslinker.

ACKNOWLEDGMENTS

We thank Gizela Valez and Yasuko Mabuchi for excellent technical assistance. This work was supported by grants from NIH (R-37-HL-05949) and the Muscular Dystrophy Association.

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(b)(4) Vendor Information

(b)(4) Vendor Information

Indications for Use

510(k) Number (if known): K051647

Device Name: FortaDerm™ Antimicrobial PHMB Wound Dressing

Indications for Use:

The FortaDerm™ Antimicrobial PHMB Wound Dressing is intended for the management of wounds and as an effective barrier to resist microbial colonization within the dressing and reduce microbes penetrating through the dressing. FortaDerm™ Antimicrobial PHMB Wound Dressing may be used for the management of: partial and full thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grafts, post-Moh's surgery, post-laser surgery, wound dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, and skin tears) and draining wounds.

The device is intended for one-time use.

Prescription Use ✓
(Part 21 CFR 801 Subpart D)

AND/OR

Over-The-Counter Use _____
(21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE
OF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Page __ of __

(Posted November 13, 2003)

PROPOSED LABELING
PROPOSED FORTADERM™ ANTIMICROBIAL PHMB WOUND DRESSING
INSTRUCTIONS FOR USE AND PACKAGE INSERT

(b)(4) Draft Labeling

(b)(4) Draft Labeling

(b)(4) Draft Labeling

(b)(4) Draft Labeling

Organogenesis Inc.

Document Number: (b)(4)

Revision Number: 2.0

Design Control Number: (b)(4)

Date: 10/10/05

Page 1

Research Report

(b)(4) Protocol

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(b)(4) Test Data

(b)(4) Test Data

〈 51 〉 ANTIMICROBIAL EFFECTIVENESS TESTING

(b)(4) Protocol

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FINAL REPORT

(b)(4)

(b)(4) Protocol

(b)(4) Protocol

(b)(4) Protocol

(b)(4) Test Data

(b)(4) Test Data

(b)(4) Test Data

510(K) SUMMARY

Submitted by:

Organogenesis Inc.
150 Dan Road
Canton, Massachusetts 02021

Contact

Patrick R. Bilbo
Telephone: (781) 401-1155
Facsimile: (781) 575-1570

Date: October 21, 2005

Device:

Trade Name:	FortaDerm™ Antimicrobial PHMB Wound Dressing coated with 0.1% Polyhexamethylene Biguanide Hydrochloride (PHMB)
Common/Usual Name:	Topical Wound Dressing, Wound Management Biomaterial
Classification Name:	Dressing, Wound (KMF)
Classification:	Unclassified

Predicate Devices:

The relevant predicate devices are FortaDerm™ (PuraPly™) Wound Dressing (K011026) manufactured by Organogenesis, Inc. and Xcell Antimicrobial Cellulose Wound Dressing (K024054) manufactured by Xylos® Corporation.

Statement of Substantial Equivalence:

The FortaDerm™ Antimicrobial PHMB Wound Dressing is similar with respect to intended use, technological characteristics, materials and physical construction to the predicate devices in terms of section 510(k) equivalency.

Intended Use:

The FortaDerm™ Antimicrobial PHMB Wound Dressing is intended for the management of wounds and as an effective barrier to resist microbial colonization within the dressing and reduce microbes penetrating through the dressing. FortaDerm™ Antimicrobial PHMB Wound Dressing may be used for the management of: partial and full thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grafts, post-Moh's surgery, post-laser surgery, wound

dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, and skin tears) and draining wounds.

The device is intended for one-time use.

Device Description:

FortaDerm™ Antimicrobial PHMB Wound Dressing consists of two layers, crosslinked sheet of fenestrated sheet of porcine intestinal collagen coated with 0.1 % Polyhexamethylene Biguanide hydrochloride (PHMB). FortaDerm Wound Dressing is supplied dry in sheet form in sizes ranging from 4 x 4 cm to 12 x 36 cm. The device is packaged in sterile, sealed single pouches.

Performance Data:

FortaDerm™ Antimicrobial PHMB Wound Dressing was subjected to a number of tests to assess biocompatibility and performance. The device passed the requirements of all tests.

Form Approved: OMB No. 0910-511 Expiration Date: August 31, 2005. See Instructions for OMB Statement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES FOOD AND DRUG ADMINISTRATION MEDICAL DEVICE USER FEE COVER SHEET		PAYMENT IDENTIFICATION NUMBER: Write the Payment Identification number on your check.	(b)(4)
A completed Cover Sheet must accompany each original application or supplement subject to fees. The following actions must be taken to properly submit your application and fee payment:			
1. Electronically submits the completed Cover Sheet to the Food and Drug Administration (FDA) before payment is sent. 2. Include printed copy of this completed Cover Sheet with a check made payable to the Food and Drug Administration. Remember that the Payment Identification Number must be written on the check. 3. Mail Check and Cover Sheet to the US Bank Lock Box, FDA Account, P.O. Box 956733, St. Louis, MO 63195-6733. (Note: In no case should payment be submitted with the application.) 4. If you prefer to send a check by a courier, the courier may deliver the check and Cover Sheet to: US Bank, Attn: Government Lockbox 956733, 1005 Convention Plaza, St. Louis, MO 63101. (Note: This address is for courier delivery only. Contact the US Bank at 314-418-4821 if you have any questions concerning courier delivery.) 5. For Wire Transfer Payment Procedures, please refer to the MDUFMA Fee Payment Instructions at the following URL: http://www.fda.gov/cdrh/mdufma/faqs.html#3a . You are responsible for paying all fees associated with wire transfer. 6. Include a copy of the complete Cover Sheet in volume one of the application when submitting to the FDA at either the CBER or CDRH Document Mail Center.			
--> 1. COMPANY NAME AND ADDRESS (include name, street address, city state, country, and post office code) ORGANOGENESIS INC 150 DAN ROAD CANTON MA 02021 US 1.1 EMPLOYER IDENTIFICATION NUMBER (EIN) 042871690		2. CONTACT NAME Patrick Bilbo 2.1 E-MAIL ADDRESS pbilbo@organo.com 2.2 TELEPHONE NUMBER (include Area code) 781-401-1155 2.3 FACSIMILE (FAX) NUMBER (Include Area code) 781-575-1570	
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/dc/mdufma)			
<u>Select an application type:</u> ☒ Premarket notification(510(k)); except for third party ☐ Biologics License Application (BLA) ☐ Premarket Approval Application (PMA) ☐ Modular PMA ☐ Product Development Protocol (PDP) ☐ Premarket Report (PMR)		3.1 Select one of the types below ☒ Original Application <u>Supplement Types:</u> ☐ Efficacy (BLA) ☐ Panel Track (PMA, PMR, PDP) ☐ 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) ☐ 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:			
5. 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, parents, and partner firms ☐ 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			
6. 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 ☒ NO			
7. USER FEE PAYMENT AMOUNT SUBMITTED FOR THIS PREMARKET APPLICATION (FOR FISCAL YEAR 2005) (b)(4)			
17-Jun-2005			

Form FDA 5601 (08/2003)

Close Window

(b)(4)

SK04

DEPARTMENT OF HEALTH AND HUMAN SERVICES FOOD AND DRUG ADMINISTRATION CDRH PREMARKET REVIEW SUBMISSION COVER SHEET				Form Approval OMB No. 9010-0120 Expiration Date: May 31, 2007. See OMB Statement on page 5.	
Date of Submission 6/20/2005		User Fee Payment ID Number (b)(4)		FDA Submission Document Number (if known)	

SECTION A TYPE OF SUBMISSION				
PMA ☐ Original Submission ☐ Premarket Report ☐ Modular Submission ☐ Amendment ☐ Report ☐ Report Amendment ☐ Licensing Agreement	PMA & HDE Supplement ☐ Regular (120 day) ☐ Special ☐ Panel Track (PMA Only) ☐ 30-day Supplement ☐ 30-day Notice ☐ 135-day Supplement ☐ Real-time Review ☐ Amendment to PMA & HDE Supplement ☐ Other	PDP ☐ Original PDP ☐ Notice of Completion ☐ Amendment to PDP	510(k) ☒ Original Submission: ☒ Traditional ☐ Special ☐ Abbreviated (Complete section I, Page 5) ☐ Additional Information ☐ Third Party	Meeting ☐ Pre-510(K) Meeting ☐ Pre-IDE Meeting ☐ Pre-PMA Meeting ☐ Pre-PDP Meeting ☐ Day 100 Meeting ☐ Agreement Meeting ☐ Determination Meeting ☐ Other (specify):
IDE ☐ Original Submission ☐ Amendment ☐ Supplement	Humanitarian Device Exemption (HDE) ☐ Original Submission ☐ Amendment ☐ Supplement ☐ Report ☐ Report Amendment	Class II Exemption Petition ☐ Original Submission ☐ Additional Information	Evaluation of Automatic Class III Designation (De Novo) ☐ Original Submission ☐ Additional Information	Other Submission ☐ 513(g) ☐ Other (describe submission):

Have you used or cited Standards in your submission? ☐ Yes ☐ No (If Yes, please complete Section I, Page 5)

SECTION B SUBMITTER, APPLICANT OR SPONSOR			
Company / Institution Name Organogenesis, Inc.		Establishment Registration Number (if known) 1221816	
Division Name (if applicable) NA		Phone Number (including area code) (781) 401-1155	
Street Address 150 Dan Road		FAX Number (including area code) (781) 575-1570	
City Canton	State / Province MA	ZIP/Postal Code 02021	Country US
Contact Name Patrick Bilbo			
Contact Title Vice President, Regulatory and Clinical Affairs		Contact E-mail Address pbilbo@organo.com	

SECTION C APPLICATION CORRESPONDENT (e.g., consultant, if different from above)			
Company / Institution Name N/A			
Division Name (if applicable)		Phone Number (including area code) ()	
Street Address		FAX Number (including area code) ()	
City	State / Province	ZIP/Postal Code	Country
Contact Name			
Contact Title		Contact E-mail Address	

SECTION D1**REASON FOR APPLICATION - PMA, PDP, OR HDE**

- ☐ Withdrawal
- ☐ Additional or Expanded Indications
- ☐ Request for Extension
- ☐ Post-approval Study Protocol
- ☐ Request for Applicant Hold
- ☐ Request for Removal of Applicant Hold
- ☐ Request to Remove or Add Manufacturing Site

- ☐ Change in design, component, or specification:
- ☐ Software / Hardware
- ☐ Color Additive
- ☐ Material
- ☐ Specifications
- ☐ Other (specify below)

- ☐ Location change:
- ☐ Manufacturer
- ☐ Sterilizer
- ☐ Packager

- ☐ Process change:
- ☐ Manufacturing
- ☐ Sterilization
- ☐ Packaging
- ☐ Other (specify below)

- ☐ Labeling change:
- ☐ Indications
- ☐ Instructions
- ☐ Performance
- ☐ Shelf Life
- ☐ Trade Name
- ☐ Other (specify below)

- ☐ Report Submission:
- ☐ Annual or Periodic
- ☐ Post-approval Study
- ☐ Adverse Reaction
- ☐ Device Defect
- ☐ Amendment

- ☐ Response to FDA correspondence:

- ☐ Change in Ownership
- ☐ Change in Correspondent
- ☐ Change of Applicant Address

- ☐ Other Reason (specify):

SECTION D2**REASON FOR APPLICATION - IDE**

- ☐ New Device
- ☐ New Indication
- ☐ Addition of Institution
- ☐ Expansion / Extension of Study
- ☐ IRB Certification
- ☐ Termination of Study
- ☐ Withdrawal of Application
- ☐ Unanticipated Adverse Effect
- ☐ Notification of Emergency Use
- ☐ Compassionate Use Request
- ☐ Treatment IDE
- ☐ Continued Access

- ☐ Change in:
- ☐ Correspondent / Applicant
- ☐ Design / Device
- ☐ Informed Consent
- ☐ Manufacturer
- ☐ Manufacturing Process
- ☐ Protocol - Feasibility
- ☐ Protocol - Other
- ☐ Sponsor

- ☐ Report submission:
- ☐ Current Investigator
- ☐ Annual Progress Report
- ☐ Site Waiver Report
- ☐ Final

- ☐ Repose to FDA Letter Concerning:
- ☐ Conditional Approval
- ☐ Deemed Approved
- ☐ Deficient Final Report
- ☐ Deficient Progress Report
- ☐ Deficient Investigator Report
- ☐ Disapproval
- ☐ Request Extension of Time to Respond to FDA
- ☐ Request Meeting
- ☐ Request Hearing

- ☐ Other Reason (specify):

SECTION D3**REASON FOR SUBMISSION - 510(k)**

- ☒ New Device

- ☐ Additional or Expanded Indications

- ☐ Change in Technology

- ☐ Other Reason (specify):

SECTION E ADDITIONAL INFORMATION ON 510(K) SUBMISSIONS								
Product codes of devices to which substantial equivalence is claimed							Summary of, or statement concerning, safety and effectiveness information ☒ 510 (k) summary attached ☐ 510 (k) statement	
1	KMF	2	MGQ	3		4		
5		6		7		8		
Information on devices to which substantial equivalence is claimed (if known)								
	510(k) Number		Trade or Proprietary or Model Name		Manufacturer			
1	K011026	1	FortaDerm™ Wound Dressing	1	Organogenesis Inc.			
2	K024054	2	Xcell® Antimicrobial Cellulose Wound Dressing	2	Xylos Corporation			
3		3		3				
4		4		4				
5		5		5				
6		6		6				
SECTION F PRODUCT INFORMATION - APPLICATION TO ALL APPLICATIONS								
Common or usual name or classification FortaDerm™ Antimicrobial Wound Dressing								
	Trade or Proprietary or Model Name for This Device				Model Number			
1	FortaDerm™ Antimicrobial Wound Dressing			1	N/A			
2				2				
3				3				
4				4				
5				5				
FDA document numbers of all prior related submissions (regardless of outcome)								
1	2	3	4	5	6			
7	8	9	10	11	12			
Data Included in Submission ☐ Laboratory Testing ☐ Animal Trials ☐ Human Trials								
SECTION G PRODUCT CLASSIFICATION - APPLICATION TO ALL APPLICATIONS								
Product Code KMF	C.F.R. Section (if applicable) 880.5090			Device Class ☐ Class I ☐ Class II ☐ Class III ☒ Unclassified				
Classification Panel General, Plastic and Reconstructive Surgery								
Indications (from labeling) The FortaDerm Antimicrobial PHMB Wound Dressing is intended for the management of wounds and as an effective barrier to resist microbial colonization within the dressing and reduce microbes penetrating through the dressing. FortaDerm Antimicrobial PHMB Wound Dressing may be used for the management of: partial and full thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grfts, post-Moh's surgery, post-laser surgery, podiatric, wound dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, and skin tears) and draining wounds.								

Note: Submission of this information does not affect the need to submit a 2891 or 2891a Device Establishment Registration form.		FDA Document Number (if known)	
SECTION H MANUFACTURING / PACKAGING / STERILIZATION SITES RELATING TO A SUBMISSION			
☒ Original Add ☐ Delete	FDA Establishment Registration Number 1221816	☒ Manufacturer ☐ Contract Manufacturer	☐ Contract Sterilizer ☐ Repackager / Relabeler
Company / Institution Name Organogenesis, Inc.		Establishment Registration Number 1221816	
Division Name (if applicable) N/A		Phone Number (including area code) (781) 401-1155	
Street Address 150 Dan Road		FAX Number (including area code) (781) 575-1570	
City Canton	State / Province MA	ZIP/Postal Code 02021	Country US
Contact Name Patrick Bilbo	Contact Title Vice President, Regulatory and Clinical Affairs	Contact E-mail Address pbilbo@organo.com	

(b)(4) Vendor Information

☐ Original ☐ Add ☐ Delete	FDA Establishment Registration Number	☐ Manufacturer ☐ Contract Manufacturer	☐ Contract Sterilizer ☐ Repackager / Relabeler
Company / Institution Name		Establishment Registration Number	
Division Name (if applicable)		Phone Number (including area code) ()	
Street Address		FAX Number (including area code) ()	
City	State / Province	ZIP/Postal Code	Country
Contact Name	Contact Title	Contact E-mail Address	

SECTION I**UTILIZATION OF STANDARDS**

Note: Complete this section if your application or submission cites standards or includes a "Declaration of Conformity to a Recognized Standard" statement.

1	Standards No.	Standards Organization	Standards Title	Version	Date
2	Standards No.	Standards Organization	Standards Title	Version	Date
3	Standards No.	Standards Organization	Standards Title	Version	Date
4	Standards No.	Standards Organization	Standards Title	Version	Date
	Standards No.	Standards Organization	Standards Title	Version	Date
6	Standards No.	Standards Organization	Standards Title	Version	Date
7	Standards No.	Standards Organization	Standards Title	Version	Date

Please include any additional standards to be cited on a separate page.

Public reporting burden for this collection of information is estimated to average 0.5 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing 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:

Food and Drug Administration
CDRH (HFZ-342)
9200 Corporate Blvd.
Rockville, MD 20850

agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control

**510(k) PREMARKET NOTIFICATION
FOR
ORGANOGENESIS INC.
FORTADERM™ ANTIMICROBIAL PHMB WOUND DRESSING**

June 20, 2005

**ORGANOGENESIS INC.
CANTON, MASSACHUSETTS**

**510(k) PREMARKET NOTIFICATION
FOR
ORGANOGENESIS INC.
FORTADERM™ ANTIMICROBIAL PHMB WOUND DRESSING**

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SCREENING CHECKLIST FOR ALL PREMARKET NOTIFICATION [510(k)] SUBMISSIONS

510(k) Number: _____

The cover letter clearly identifies the type of 510(k) submission as **(Check the appropriate box)**

Special 510(k) - Do Sections 1 and 2

Abbreviated 510(k) - Do Sections 1, 3 and 4

Traditional 510(k) or no identification provided - Do Sections 1 and 4

Section 1: Required Elements for All Types of 510(k) submissions:

	Presentor Adequate	Missing or Inadequate
Cover letter, containing the elements listed on page 3-2 of the Premarket Notification [510] Manual.		
Table of Contents.		
Truthful and Accurate Statement.		
Device's Trade Name, Device's Classification Name and Establishment Registration Number.		
Device Classification Regulation Number and Regulatory Status (Class I, Class II, Class III or Unclassified).		
Proposed Labeling including the material listed on page 3-4 of the Premarket Notification [510] Manual.		
Statement of Indications for Use that is on a separate page in the premarket submission.		
Substantial Equivalence Comparison, including comparisons of the new device with the predicate in areas that are listed on page 3-4 of the Premarket Notification [510] Manual.		
510(k) Summary or 510(k) Statement.		
Description of the device (or modification of the device) including diagrams, engineering drawings, photographs or service manuals.		
Identification of legally marketed predicate device. *		
Compliance with performance standards. * [See Section 514 of the Act and 21 CFR 807.87 (d).]		
Class III Certification and Summary. **		
Financial Certification or Disclosure Statement for 510(k) notifications with a clinical study. * [See 21 CFR 807.87 (i)]		
510(k) Kit Certification ***		

* - May not be applicable for Special 510(k)s.

** - Required for Class III devices, only.

*** - See pages 3-12 and 3-13 in the Premarket Notification [510] Manual and the Convenience Kits Interim Regulatory Guidance.

Section 2: Required Elements for a SPECIAL 510(k) submission

	Present	Inadequate or Missing
Name and 510(k) number of the submitter's own, unmodified predicate device.		
A description of the modified device and a comparison to the sponsor's predicate device.		
A statement that the intended use(s) and indications of the modified device, as described in its labeling are the same as the intended uses and indications for the submitter's unmodified predicate device.		
Reviewer's confirmation that the modification has not altered the fundamental scientific technology of the submitter's predicate device.		
A Design Control Activities Summary that includes the following elements (a-c):		
a. Identification of Risk Analysis method(s) used to assess the impact of the modification on the device and its components, and the results of the analysis.		
b. Based on the Risk Analysis, an identification of the required verification and validation activities, including the methods or tests used and the acceptance criteria to be applied.		
c. A Declaration of Conformity with design controls that includes the following statements:		
A statement that, as required by the risk analysis, all verification and validation activities were performed by the designated individual(s) and the results of the activities demonstrated that the predetermined acceptance criteria were met. This statement is signed by the individual responsible for those particular activities.		
A statement that the manufacturing facility is in conformance with the design control procedure requirements as specified in 21 CFR 820.30 and the records are available for review. This statement is signed by the individual responsible for those particular activities.		

Section 3: Required Elements for an ABBREVIATED 510(k)* submission:

	Present	Inadequate or Missing
For a submission, which relies on a guidance document and/or special control(s), a summary report that describes how the guidance and/or special control(s) was used to address the risks associated with the particular device type. (If a manufacturer elects to use an alternate approach to address a particular risk, sufficient detail should be provided to justify that approach.)		
For a submission, which relies on a recognized standard, a declaration of conformity [For a listing of the required elements of a declaration of conformity, SEE Required Elements for a Declaration of Conformity to a Recognized Standard]		

is posted with the 510(k) boilers on the H drive.]		
For a submission, which relies on a recognized standard without a declaration of conformity, a statement that the manufacturer intends to conform to a recognized standard and that supporting data will be available before marketing the device.		
For a submission, which relies on a non-recognized standard that has been historically accepted by FDA, a statement that the manufacturer intends to conform to a recognized standard and that supporting data will be available before marketing the device.		
For a submission, which relies on a non-recognized standard that has <u>not</u> been historically accepted by FDA, a statement that the manufacturer intends to conform to a recognized standard and that supporting data will be available before marketing the device <u>and</u> any additional information requested by the reviewer in order to determine substantial equivalence.		
Any additional information, which is not covered by the guidance document, special control, recognized standard and/or non-recognized standard, in order to determine substantial equivalence.		

- * - When completing the review of an abbreviated 510(k), please fill out an Abbreviated Standards Data Form (located on the H drive) and list all the guidance documents, special controls, recognized standards and/or non-recognized standards, which were noted by the sponsor.

Section 4: Additional Requirements for ABBREVIATED and TRADITIONAL 510(k) submissions (If Applicable):

	Present	Inadequate or Missing
a) Biocompatibility data for all patient-contacting materials, OR certification of identical material/formulation:		
b) Sterilization and expiration dating information:		
i) sterilization process		
ii) validation method of sterilization process		
iii) SAL		
iv) packaging		
v) specify pyrogen free		
vi) ETO residues		
vii) radiation dose		
viii) Traditional Method or Non-Traditional Method		
c) Software Documentation:		

Items with checks in the "Present or Adequate" column do not require e additional information from the sponsor. Items with checks in the "Missing or Inadequate" column must be submitted before substantive review of the document.

Passed Screening ____ Yes ____ No

Reviewer: _____

Concurrence by Review Branch: _____

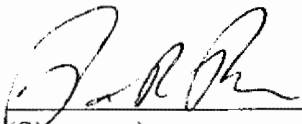
Date: _____

The deficiencies identified above represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed. In developing the deficiencies, we carefully considered the statutory criteria as defined in Section 513(i) of the Federal Food, Drug, and Cosmetic Act for determining substantial equivalence of your device. We also considered the burden that may be incurred in your attempt to respond to the deficiencies. We believe that we have considered the least burdensome approach to resolving these issues. If, however, you believe that information is being requested that is not relevant to the regulatory decision or that there is a less burdensome way to resolve the issues, you should follow the procedures outlined in the "A Suggested Approach to Resolving Least Burdensome Issues" document. It is available on our Center web page at: <http://www.fda.gov/cdrh/modact/leastburdensome.html>

(b)(4) Draft

**PREMARKET NOTIFICATION
TRUTHFUL AND ACCURATE STATEMENT**
[As Required by 21 CFR 807.87(k)]

I certify that, in my capacity as Vice President of Regulatory and Clinical Affairs of Organogenesis 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.


(Signature)

Patrick R. Bilbo
(Typed Name)

6/20/05
(Date)

K051647
*(Premarket Notification [510(k)] Number)

510(k) PREMARKET NOTIFICATION
FOR
ORGANOGENESIS INC.
FORTADERM™ ANTIMICROBIAL PHMB WOUND DRESSING

1. COMPANY NAME, ADDRESS AND CONTACT PERSON

Sponsor/Manufacturer

Organogenesis Inc.
150 Dan Road
Canton, Massachusetts 02021

Contact

Patrick Bilbo
Vice President, Regulatory and Clinical Affairs
Organogenesis, Inc.

Telephone: (781) 401-1155
Facsimile: (781) 575-1570

2. DEVICE NAME

<u>Proprietary Name:</u>	FortaDerm Antimicrobial PHMB Wound Dressing
<u>Common/Usual Name:</u>	Topical Wound Dressing, Wound Management Biomaterial
<u>Classification Name:</u>	Unclassified (KMF)

3. ESTABLISHMENT REGISTRATION NUMBER

The product will be manufactured at Organogenesis, Inc. in Canton, Massachusetts. The establishment registration number for Organogenesis Inc., located at 150 Dan Road in Canton, Massachusetts is 1221816.

The product will be sterilized at (b)(4)
(b)(4) The establishment registration number for (b)(4) is (b)(4)

4. DEVICE CLASSIFICATION

Classification Name: Dressing, Liquid

Product Code: KMF

Regulatory Class: Unclassified

5. PERFORMANCE STANDARDS

No performance standards applicable to this device have been adopted under Section 514 of the Act. Organogenesis Inc. has applied the use of voluntary standards to FortaDerm Antimicrobial PHMB Wound Dressing, including current United States Pharmacopeia (USP) standards for biocompatibility tests. FortaDerm Antimicrobial PHMB Wound Dressing conforms with the requirements of these voluntary standards.

6. PROPOSED LABELING

Advertising and promotional materials have not yet been developed for this device. Draft proposed labeling for FortaDerm Antimicrobial PHMB Wound Dressing, which is a prescription device, includes a package insert and instructions for use, as provided in APPENDIX A.

7. SUBSTANTIAL EQUIVALENCE COMPARISON

The FortaDerm Antimicrobial PHMB Wound Dressing is comparable to the predicate device FortaDerm™ (PuraPly™) Wound Dressing (K011026) with the addition of the antimicrobial agent, an additional layer of collagen and crosslinking with carbodiimide. In the antimicrobial version of this product, the aforementioned collagen wound dressing is coated with (b)(4) % polyhexamethylenebiguanide hydrochloride (PHMB) solution. FortaDerm Antimicrobial PHMB is substantially equivalent to Xylos Xcell Antimicrobial Cellulose Wound Dressing (K024054), in that both products are non-interactive wound dressings coated with PHMB as an antimicrobial agent.

The FortaDerm Antimicrobial PHMB Wound Dressing is similar with respect to indications for use, materials, technological characteristics, physical construction and performance to the predicate devices in terms of section 510(k) equivalency. TABLE 1 below compares the device characteristics of FortaDerm (currently only marketed by Royce Medical as PuraPly™) Wound Dressing with FortaDerm Antimicrobial PHMB Wound Dressing and Xylos Xcell Antimicrobial Cellulose Wound Dressing.

Table 1. Comparison of FortaDerm Antimicrobial PHMB Wound Dressing to Predicate Devices

Device Characteristics	Devices		
	<i>FortaDerm™ (PuraPly™) Wound Dressing</i>	<i>FortaDerm™ Antimicrobial PHMB Wound Dressing</i>	<i>Xylos® XCell® Antimicrobial Cellulose Wound Dressing</i>
Same Intended Use	Yes	Yes	Yes
Design	Single-layer Collagen Sheet, Fenestrated	(b)(4)	Single-layer hydrophilic, cellulose sheet that contains PHMB
Materials	Porcine Intestinal Collagen	Porcine Intestinal Collagen and PHMB	Cellulose and PHMB
Performance	Effective for the management of wounds in an animal model	Effective for inhibiting microbial growth <i>in vitro</i>	Effective for the management of wounds in an animal model
Sterilization Process	Gamma Irradiation	Gamma Irradiation	Electron Beam irradiation
Biocompatibility & Safety	Biocompatible and Safe	Biocompatible and Safe	Biocompatible and Safe
Thickness	0.05 – 0.07 mm	0.05 – 0.15 mm	0.75" (19 mm)
Available Sizes	4cm x 6 cm to 12 cm x 36 cm	4 cm x 4 cm to 12 cm x 36 cm	2 in x 2 in (5 cm x 5 cm)

8. DEVICE DESCRIPTION

8.1 Intended Use

The FortaDerm™ Antimicrobial PHMB Wound Dressing is intended for the management of wounds and as an effective barrier to resist microbial colonization within the dressing and reduce microbes penetrating through the dressing. FortaDerm™ Antimicrobial PHMB Wound Dressing may be used for the management of: partial and full thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grafts, post-Moh's surgery, post-laser surgery, podiatric, wound dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, and skin tears) and draining wounds.

The device is intended for one-time use.

FortaDerm Antimicrobial PHMB Wound Dressing is a sterile single-use sheet dressing made of a collagen matrix and is coated with polyhexamethylene biguanide hydrochloride (PHMB). This product is available in sizes within a range of 4 cm x 4 cm and 12 x 36 cm and is available fenestrated for moderate to heavy exudate wounds. The dressing may also be used on minimally exuding, non-exuding and dry wounds as stated in the Instructions-for-use. The wound dressing also protects against abrasion, desiccation, and external contamination. Under the supervision of a health care professional, the FortaDerm Antimicrobial PHMB Wound Dressing is intended for the local management of exuding wounds, including infected and non-infected pressure ulcers, venous ulcers, diabetic ulcers, arterial ulcers, donor sites and other bleeding surface wounds, dermal lesions, trauma injuries or incisions, superficial cuts, minor scalds and burns, minor skin irritations, lacerations and abrasions. The device is intended for one-time use.

8.2 Design and Materials

FortaDerm Antimicrobial PHMB Wound Dressing consists of (b)(4) (b)(4) of porcine intestinal collagen, approximately 0.05 to 0.15mm in thickness. The device is cross-linked with (b)(4) (b)(4) coated with a (b)(4) solution of polyhexamethylene biguanide (PHMB). The product consists primarily of Type I porcine collagen (b)(4) in its native form, with less than (b)(4) lipids and undetectable levels of Glycosaminoglycans (b)(4) and DNA (b)(4). The porcine intestinal collagen is free of cells and cell remnants. FortaDerm Antimicrobial PHMB Antimicrobial Wound Dressing is supplied in sheet form in sizes ranging from 4 cm x 4 cm to 12 cm x 36 cm.

8.3 Information for Animal-Derived Tissue

Species and tissue from which the animal material is derived:

- Porcine small intestine submucosa (predominantly Type I collagen).

How the health of the herd is maintained and monitored:

-
-
-
-
-

(b)(4)

How the health of each animal is maintained and monitored:

-
-
-

(b)(4)

8.4 Device Manufacture

FortaDerm Antimicrobial PHMB Wound Dressing is prepared from the small intestine of large swine ((b)(4) (b)(4))

(b)(4)

(b)(4)

8.5 Packaging and Sterilization

(b)(4) Vendor Information

8.6 Viral Inactivation Validation

Information on viral inactivation validation is provided in K011026.

8.7 Final Product Specifications

In Process and Final Product Specifications for critical parameters will be finalized during the ongoing manufacturing process validations and sterilization validations. The parameters that will be evaluated include histology (to verify the chemically cleaned material is (b)(4)), thermal stability (b)(4), bioburden (b)(4), endotoxin levels (< (b)(4)) and sterility.

8.8 Product Expiration Dating

The product expiration date for the device has not yet been determined. The expiration date will be established according to the protocol provided in APPENDIX C. Data will be collected from at least three production lots of the finished packaged device.

9. BIOCOMPATIBILITY TESTING

(b)(4) Test Data

FortaDerm Antimicrobial PHMB Wound Dressings used for this testing were (b)(4) Protocol

(b)(4) Protocol

(b)(4) Protocol

CYTOTOXICITY TESTING

(b)(4) Test Data

DERMAL SENSITIZATION

(b)(4) Test Data

PRIMARY SKIN IRRITATION

(b)(4) Test Data

HEMOCOMPATIBILITY

(b)(4) Test Data

ACUTE SYSTEMIC TOXICITY

(b)(4) Test Data

BIOCOMPATIBILITY TESTS CONDUCTED FOR SIMILAR FORTAFLEX PRODUCTS

PYROGENICITY

(b)(4) Test Data

SUBCHRONIC TOXICITY

(b)(4) Test Data

GENOTOXICITY

(b)(4) Test Data

ANTIGENICITY

(b)(4) Test Data

10. PERFORMANCE TESTING

(b)(4) Test Data

11. 510(k) SUMMARY

As required by The Safe Medical Devices Act of 1990 and 21 CFR 807.87(h), the 510(k) Summary for this submission is provided in APPENDIX F.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

July 14, 2005

Food and Drug Administration
Center for Devices and
Radiological Health
Office of Device Evaluation
Document Mail Center (HFZ-401)
9200 Corporate Blvd.
Rockville, Maryland 20850

ORGANOGENESIS, INC.
150 DAN RD.
CANTON, MA 02021
ATTN: PATRICK BILBO

510(k) Number: K051647
Product: FORTADERM
ANTIMICROBIAL
WOUND DRESSING

We are holding your above-referenced Premarket Notification (510(k)) for 30 days pending receipt of the additional information that was requested by the Office of Device Evaluation. Please remember that all correspondence concerning your submission MUST cite your 510(k) number and be sent in duplicate to the Document Mail Center (HFZ-401) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at www.fda.gov/cdrh/ode/a02-01.html.

The deficiencies identified represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed. In developing the deficiencies, we carefully considered the statutory criteria as defined in Section 513(i) of the Federal Food, Drug, and Cosmetic Act for determining substantial equivalence of your device. We also considered the burden that may be incurred in your attempt to respond to the deficiencies. We believe that we have considered the least burdensome approach to resolving these issues. If, however, you believe that information is being requested that is not relevant to the regulatory decision or that there is a less burdensome way to resolve the issues, you should follow the procedures outlined in the "A Suggested Approach to Resolving Least Burdensome Issues" document. It is available on our Center web page at: <http://www.fda.gov/cdrh/modact/leastburdensome.html>

If after 30 days the requested information, or a request for an extension of time, is not received, we will discontinue review of your submission and proceed to delete your file from our review system. Please note our guidance document entitled, "Guidance for Industry and FDA Staff FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Performance Assessment". The purpose of this document is to assist agency staff and the device industry in understanding how various FDA and industry actions that may be taken on 510(k)s should affect the review clock for purposes of meeting the Medical Device User Fee and Modernization Act. You may review this document at <http://www.fda.gov/cdrh/mdufma/guidance/1219.html>. Pursuant to 21 CFR 20.29, a copy of your 510(k) submission will remain in the Office of Device Evaluation. If you then wish to resubmit this 510(k) notification, a new number will be assigned and your submission will be considered a new premarket notification submission.

Please remember that the Safe Medical Devices Act of 1990 states that you may not place this device into commercial distribution until you receive a decision letter from FDA allowing you to do so.

If you have procedural or policy questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301) 443-6597 or at their toll-free number (800) 638-2041, or contact me at (301) 594-1190.

Sincerely yours,

Marjorie Shulman
Supervisor Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and
Radiological Health

Organogenesis Inc.
LIVING TECHNOLOGY

K051647

June 20, 2005

Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center (HFZ-401)
9200 Corporate Boulevard
Rockville, Maryland 20850

VIA FEDERAL EXPRESS

Re: Traditional 510(k) Premarket Notification for FortaDerm™ Antimicrobial PHMB Wound Dressing

Dear Sir/Madam:

Enclosed are two copies of a 510(k) Premarket Notification submitted in accordance with 21 CFR 807, Subpart E, for Organogenesis Inc.'s FortaDerm™ Antimicrobial PHMB Wound Dressing. The device is indicated for use as a wound dressing and is reviewed by the Plastic and Reconstructive Surgery Devices Branch in the Division of General, Restorative and Neurological Devices. The Common/Usual Name is Topical Wound Dressing, Wound Management. The Biomaterial/Classification Name is Unclassified (KMF).

FortaDerm™ Antimicrobial PHMB Wound Dressing is a modification of the FortaDerm™ Wound Dressing previously cleared for marketing (K011026). The principal modifications include an increase in the number of collagen layers, addition of a crosslinking agent and the addition of a chemical antimicrobial agent polyhexamethylenebiguanide hydrochloride (PHMB). The product will be manufactured at Organogenesis Inc., 150 Dan Road, Canton, MA, and sterilized at (b)(4) Vendor Information. The establishment registration

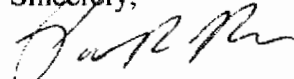
number for Organogenesis Inc. is 1221816. The establishment registration number for (b)(4) Vendor Information

(b)(4) Vendor Information

This submission includes extensive trade secrets and commercial information which is privileged or confidential, and which, in accordance with 21 CFR 20.61, is not available for public disclosure.

We believe that this submission includes all of the necessary information to allow FDA to make a determination of substantial equivalence for this device, however, should you have any questions regarding the submission or require additional information, please contact Patrick Bilbo, Vice President, Regulatory and Clinical Affairs at (781) 401-1155.

Sincerely,



Patrick R. Bilbo

Vice President, Regulatory and Clinical Affairs

Enclosures

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
9200 Corporate Boulevard
Rockville MD 20850

NOV - 8 2005

Patrick R. Bilbo, Vice President
Regulatory and Clinical Affairs
Organogenesis, Inc.
150 Dan Road
Canton, Massachusetts 02021

Re: K051647

Trade/Device Name: FortaDerm™ Antimicrobial PHMB Wound Dressing
Regulatory Class: Unclassified
Product Code: FRO
Dated: October 21, 2005
Received: October 25, 2005

Dear Mr. Bilbo:

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.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such 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); 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.

Page 2- Patrick R. Bilbo, Vice President

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of Compliance at (240) 276-0115. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its Internet address <http://www.fda.gov/cdrh/industry/support/index.html>

Sincerely yours,

Handwritten signature of Barbara Buckner in cursive script, with the word "for" written below it.

Mark N. Melkerson
Acting Director
Division of General, Restorative
and Neurological Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): K051647

Device Name: FortaDerm™ Antimicrobial PHMB Wound Dressing

Indications for Use:

The FortaDerm™ Antimicrobial PHMB Wound Dressing is intended for the management of wounds and as an effective barrier to resist microbial colonization within the dressing and reduce microbes penetrating through the dressing. FortaDerm™ Antimicrobial PHMB Wound Dressing may be used for the management of: partial and full thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers, chronic vascular ulcers, tunneled/undermined wounds, surgical wounds (donor sites/grafts, post-Moh's surgery, post-laser surgery, wound dehiscence), trauma wounds (abrasions, lacerations, second-degree burns, and skin tears) and draining wounds.

The device is intended for one-time use.

Prescription Use ✓
(Part 21 CFR 801 Subpart D)

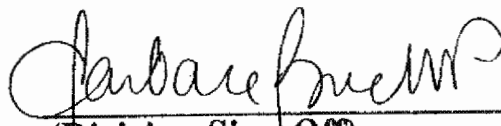
AND/OR

Over-The-Counter Use _____
(21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE
OF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

(Posted November 13, 2003)


(Division Sign-Off)

Division of General, Restorative,
and Neurological Devices

Page ___ of ___

510(k) Number K051647

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Memorandum

November 4, 2005

From: Reviewer(s) - Name(s)

David Krause, PhD

Subject: 510(k) Number

K051647/s

To: The Record - It is my recommendation that the subject 510(k) Notification:

(b)(5) FDA Reviewer Notes

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Memorandum

July 12, 2005

From: Reviewer(s) - Name(s)

David Krause, PhD

Subject: 510(k) Number

K051647

To: The Record - It is my recommendation that the subject 510(k) Notification:

(b)(5) FDA Reviewer Notes

(b)(5) FDA Reviewer Notes

**510(k) Memorandum
K051647**

To: The Record

**From: David Krause, PhD, Expert Biologist
ODE/DGRND/Plastic & Reconstructive Surgery Devices Branch**

Date: July 13, 2005

**Re: Device Name: FortaDerm™ Antimicrobial PHMB Wound Dressing
Sponsor: Organogenesis Inc. of Canton, Massachusetts
Sponsor Contact: Patrick Bilbo at 781-401-1155**

(b)(5) FDA Reviewer Notes

(b)(5) FDA Reviewer Notes

(b)(5) FDA Reviewer Notes

(b)(5) FDA Reviewer Notes

(b)(5) FDA Reviewer Notes

(b)(5) FDA Reviewer Notes

(b)(5) FDA Reviewer Notes

Krause, David

From: Krause, David
Sent: Wednesday, July 13, 2005 2:27 PM
To: 'pbilbo@organo.com'
Subject: 510(k) for FortaDerm Antimicrobial Dressing

Dear Mr. Bilbo,

We have reviewed your 510(k), K051647, for the FortaDerm Antimicrobial PHMB Dressing. We cannot complete the review until you have addressed the following issues. Please submit these in hard copy to the CDRH Document Control Center, HFZ-401, 9200 Corporate Blvd., Rockville, MD 20850.

(b)(4) Deficiencies

(b)(4) Deficiencies

Thank you,

David Krause, PhD
Expert Biologist
PRSB/DGRND/ODE/CDRH

Internal Administrative Form

(b)(5) FDA Reviewer Notes

**"SUBSTANTIAL EQUIVALENCE" (SE) DECISION MAKING DOCUMENTATION
K051647**

(b)(5) FDA Reviewer Notes

(b)(5) FDA Reviewer Notes

**510(k) Memorandum
K051647/S01**

To: The Record

**From: David Krause, PhD, Expert Biologist
ODE/DGRND/Plastic & Reconstructive Surgery Devices Branch**

Date: November 4, 2005

**Re: Device Name: FortaDerm™ Antimicrobial PHMB Wound Dress
Sponsor: Organogenesis Inc. of Canton, Massachusetts
Sponsor Contact: Patrick Bilbo at 781-401-1155**

(b)(5) FDA Reviewer Notes

(b)(5) FDA Reviewer Notes

(b)(5) FDA Reviewer Notes

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(b)(5) FDA Reviewer Notes

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(b)(5) FDA Reviewer Notes

Internal Administrative Form

(b)(5) FDA Reviewer Notes