A Comparison of Human and Porcine Acellularized Dermis: Interactions with Human Fibroblasts In Vitro

Alexis D. Armour, M.D., M.Sc.
Joel S. Fish, M.D., M.Sc.
Kimberly A. Woodhouse, P.Eng., Ph.D.
John L. Semple, M.D., M.Sc.

Toronto, Ontario, Canada

Background: Dermal substitutes derived from xenograft materials require elaborate processing at a considerable cost. Acellularized porcine dermis is a readily available material associated with minimal immunogenicity. The objective of this study was to evaluate acellularized pig dermis as a scaffold for human fibroblasts.
Methods: In vitro methods were used to evaluate fibroblast adherence, proliferation, and migration on pig acellularized dermal matrix. Acellular human dermis was used as a control.
Results: Pig acellularized dermal matrix was found to be inferior to human acellularized dermal matrix as a scaffold for human fibroblasts. Significantly more samples of human acellularized dermal matrix (83 percent, $n = 24$; $p < 0.05$) demonstrated fibroblast infiltration below the cell-seeded surface than pig acellularized dermal matrix (31 percent, $n = 49$). Significantly more ($p < 0.05$) fibroblasts infiltrated below the surface of human acellularized dermal matrix (mean, 1072 ± 80 cells per section; $n = 16$ samples) than pig acellularized dermal matrix (mean, 301 ± 48 cells per section; $n = 16$ samples). Fibroblasts migrated significantly less ($p < 0.05$) distance from the cell-seeded pig acellularized dermal matrix surface than in the human acellularized dermal matrix (78.8 percent versus 38.3 percent cells within 150 μm from the surface, respectively; $n = 5$). Fibroblasts proliferated more rapidly ($p < 0.05$) on pig acellularized dermal matrix ($n = 9$) than on the human acellularized dermal matrix (7.4-fold increase in cell number versus 1.8-fold increase, respectively; $n = 9$ for human acellularized dermal matrix). There was no difference between the two materials with respect to fibroblast adherence (8120 versus 7436 average adherent cells per section, for pig and human acellularized dermal matrix, respectively; $n = 20$ in each group; $p > 0.05$).
Conclusion: Preliminary findings suggest that substantial differences may exist between human fibroblast behavior in cell–matrix interactions of porcine and human acellularized dermis. (*Plast. Reconstr. Surg.* 117: 845, 2006.)

The dermis is responsible for the skin's strength and elasticity and provides support and durability to the overlying epidermis. Despite the functional and aesthetic importance of the dermis, dermal replacement presents an ongoing challenge in the treatment of full-thickness wounds. The ideal dermal replacement achieves rapid vascularization and infiltration of host cells with minimal scarring. Cellular infiltration of a dermal substitute material is essential to achieving effective closure of the wound, thereby minimizing wound contraction and hypertrophic scarring.[1] The greatest clinical success with skin replacement for full-thickness cutaneous wounds has been achieved with materials composed of the natural dermal protein collagen. Cadaveric allograft dermis can be acellularized to remove antigenic cellular components, producing an acellular dermal matrix of collagen and elastin. The clinical effectiveness but high cost of the commercial product Allo-

From the Division of Plastic Surgery, Department of Surgery, and the Institute of Biomedical Engineering, Department of Chemical Engineering and Applied Chemistry, Faculty of Engineering, University of Toronto; and the Ross Tilley Burn Center and the Division of Plastic Surgery, Sunnybrook and Women's College Health Science Center.
Received for publication October 22, 2002; revised September 27, 2004.
Presented at the 55th Annual Meeting of the Canadian Society of Plastic Surgeons Meeting, in Jasper, Alberta, Canada, June 6 through 9, 2001.

DOI: 10.1097/01.prs.0000204567.28952.9d

Derm (LifeCell Corporation, Inc., The Woodlands, Texas) has driven the development of new acellularization techniques and the evaluation of acellularized materials derived from xenogenic sources.

Porcine dermis has long been used as a temporary wound covering for partial-thickness and full-thickness burn wounds.[2–5] Its greater availability, low cost, ability to be harvested in large quantities, and excellent handling properties are compelling reasons to now investigate acellularized porcine dermis as a potential xenograft dermal substitute. Preliminary testing of acellularized porcine dermis in a xenograft in vivo model suggests that it may be an effective material for preventing contraction and hypertrophic scarring in full-thickness burns.[6,7] However, successful infiltration by human dermal fibroblasts and endothelial cells has never been demonstrated in porcine dermis. In this study, acellularized porcine dermis is investigated as a scaffold for human fibroblast ingrowth in vitro. Acellularized human dermis is used as a control.

MATERIALS AND METHODS

The use of pigs for this study was conducted according to protocols approved by the Animal Research Ethics Board of the University of Toronto. Porcine dermis was obtained from freshly killed York pigs (Riemens Fur Ranch, Guelph, Ontario, Canada) used for surgical demonstration. The pigs were either 7 weeks or 3 months of age. Surgical specimens were obtained from breast reduction procedures according to a protocol approved by the Ethics Review Board at the University of Toronto. All chemicals used for acellularization were purchased from Sigma-Aldrich Canada (Oakville, Ontario). All cell culture experiments were performed in a biohazard level 2 tissue culture facility approved by the University of Toronto Biohazard Safety Committee. Cell culture dishes and media were obtained from Becton Dickinson (Franklin Lakes, N.J.). Passaged human dermal fibroblasts were isolated by explant culture[8] from the dermis of surgical specimens. Patient age ranged from 18 to 37 years.

Dermal Harvest and Acellularization

After cleaning and depilation, an electrodermatome (Padgett Instruments, Kansas City, Mo.) was used to harvest 0.02-inch-thick layers of porcine dermis from the paravertebral areas. After discarding the epidermis-bearing layer, five layers were serially harvested for acellularization. Human dermis was sharply dissected from the subcutaneous fat. The skin was then clamped to a cutting board and tangentially cut with a dermatome blade into four layers approximately 0.02 inch thick, after discarding the top 0.02-inch-thick, epidermis-bearing layer. The level of dermis, according to its depth from the epidermal surface, was recorded. The dermal strips were acellularized through a series of rinses in Tris buffer, 1% Triton-X, DNase and RNase-containing buffers, and 1% sodium dodecyl sulfate.[8,9]

Sample Preparation and Sterilization

Biopunches, 8 mm in diameter, were used to make disks of human and porcine acellularized dermis. The acellularized dermal matrix disks were sterilized by serial rinses in ethanol and then freeze-dried. Before fibroblast seeding, the acellularized dermal matrix disks were placed in a 96-well culture plate and soaked in cell culture media overnight.

Acellular Matrix Characterization of Acellularity and Microstructure

Samples of freshly acellularized pig and human dermis were formalin-fixed for analysis of acellularity. Samples from each batch of acellularized dermis were stained with hematoxylin and eosin, Movats, and antibodies to vimentin. Freeze-fracture scanning electron microscopy and transmission electron microscopy were performed on 10 samples each of pig and human acellularized dermal matrix. Each sample was examined at low (50× and 100×) and high (500× and 1000×) magnification for collagen bundle size and arrangement and acellularized dermal matrix surface characteristics. Specimens from different levels of dermis were also compared by hematoxylin and eosin staining and by high-magnification scanning electron microscopy.

Fibroblast Seeding and Culture

For all experiments, the day of cell seeding was considered day 0. Primary adult human fibroblasts at passages 2 through 4 were seeded onto the surface of the acellularized dermal matrix disks in the following manner. Fibroblast cultures were maintained in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and penicillin/streptomycin. Fibroblasts cultured on T75 tissue culture flasks were trypsinized using 0.25% trypsin/ethylenediaminetetraacetic acid for 5 minutes. The concentration of cells in suspension was

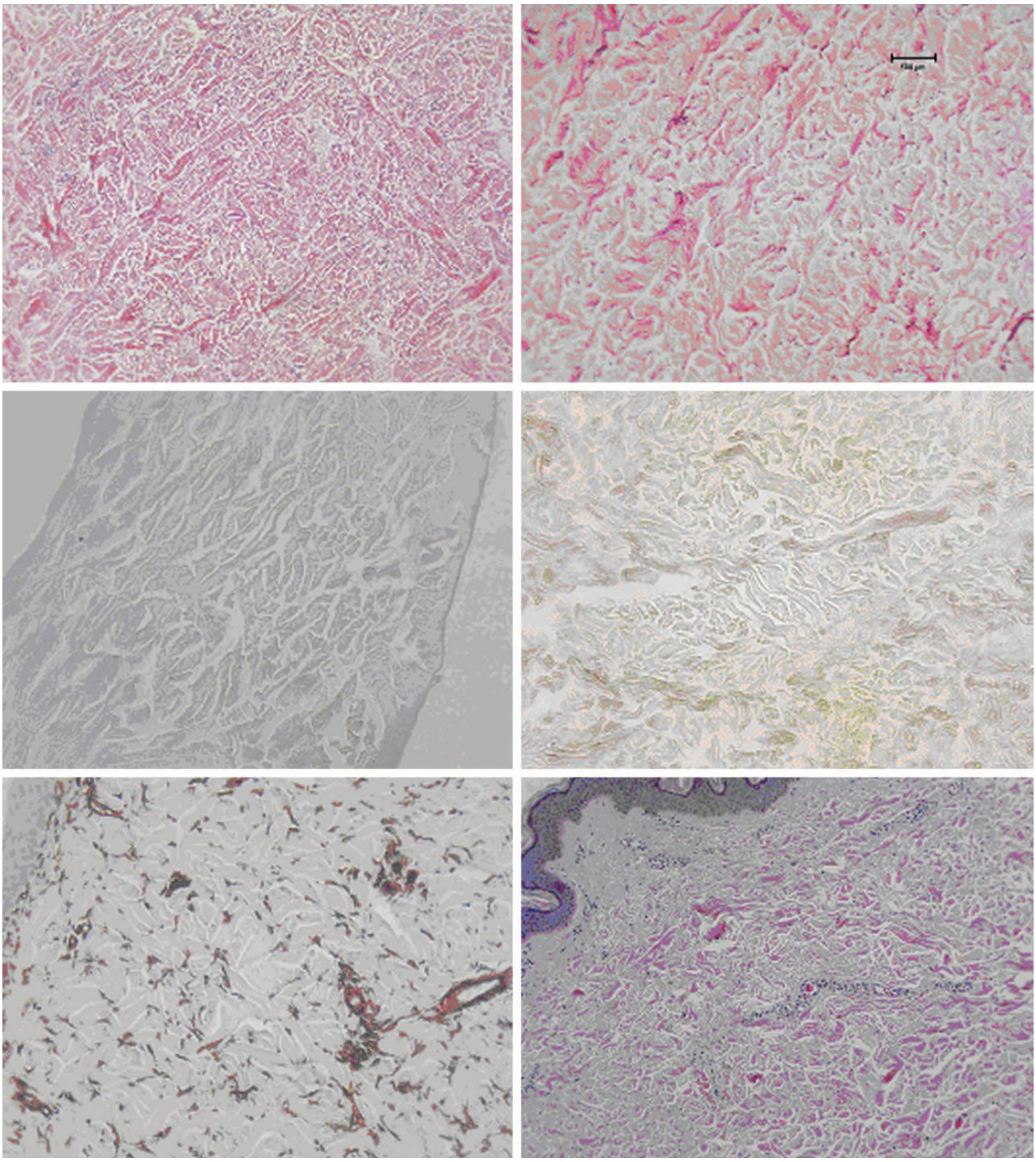

Fig. 1. (*Above*, *left*) Pig acellularized dermal matrix (hematoxylin and eosin; original magnification, ×10). (*Above*, *right*) Human acellularized dermal matrix (hematoxylin and eosin; original magnification, ×10). (*Center*, *left*) Pig acellularized dermal matrix (vimentin; original magnification, ×20). (*Center*, *right*) Human acellularized dermal matrix (vimentin; original magnification, ×20). (*Below*, *left*) Pig dermis (vimentin; original magnification, ×10). (*Below*, *right*) Human dermis (hematoxylin and eosin; original magnification, ×10).

determined through cell counting with a hemocytometer. Cells were seeded at different densities for different assays (see below). The desired volume of cell suspension was slowly added to the surface of the acellularized dermal matrix not in contact with the culture dish. After allowing 16 to 24 hours for the cells to attach to the acellularized dermal matrix surface, the volume in each well was

gently made up to 150 μl with media. The media was subsequently changed every 2 to 3 days.

Determination of Fibroblast Adherence, Proliferation, and Infiltration

Fibroblast adherence was determined qualitatively through phalloidin staining and confocal microscopy, and quantitatively through a fluorescent cell viability assay (CyQuant, Molecular Probes, Ore.) 24 hours after cell seeding. Fibroblasts were seeded on human acellularized dermal matrix, pig acellularized dermal matrix, and empty culture dish wells at a density of 10,000 cells/cm^2. Four samples of each group were seeded, and the experiment was repeated five times for the quantitative assay. One sample of each group in one experiment was stained with phalloidin for visualization of fibroblast adherence to the surface.[8]

The CyQuant assay was used to determine the number of viable cells present on the pig and human acellularized dermal matrix samples, and in the control wells, 24 hours after seeding.[8] Fluorescence was measured by using a spectrofluorometer with filters suited for 485-nm excitation and 538-nm emission maxima. Calibration curves for the CyQuant assay were generated by seeding samples of collagenase-digested human acellularized dermal matrix; pig acellularized dermal matrix; and empty wells with human fibroblasts at densities of 0, 5000, 10,000, and 20,000 cells/cm^2. The appropriate calibration curve was used to determine cell number values. The results were analyzed for statistically significant differences between groups using the *t* test.

Fibroblast proliferation was determined by using the CyQuant assay as well. Pig acellularized dermal matrix, human acellularized dermal matrix, and empty wells were seeded with 10,000 cells/cm^2 and cultured for 1, 2, 3, and 4 weeks. The experiments were repeated three times, with triplicate samples at each time point. The fluorescence of the nine replicate samples, at each time point, was expressed as a percentage of the fluorescence obtained for equivalent samples at 24 hours postseeding. The percentage fluorescence results were analyzed using the analysis of variance test, looking for a statistically significant association between group and fluorescence. Because the cells measured at each of the four time points came from different wells and were therefore independent, the repeated measures analysis of variance was not used. For graphical representation, the results at each time point were averaged for each group.

Fibroblast infiltration into the acellularized dermal matrix samples was determined histologically by examining fibroblast-seeded samples of pig and human acellularized dermal matrix in cross-section. Fibroblasts were seeded onto one surface of the acellularized dermal matrix, at a density of 30,000 cells/cm^2, and cultured for 4 weeks. At the end of the experiment, the cell-seeded acellularized dermal matrix samples were removed from the plate and fixed in formalin for 24 hours. The samples were then carefully sectioned with a no. 10 scalpel blade into three pieces. The three tissue pieces were embedded, on edge, in a paraffin block. Two histologic slides were made of the paraffin blocks, featuring three cross-sectional views each. The slides were stained

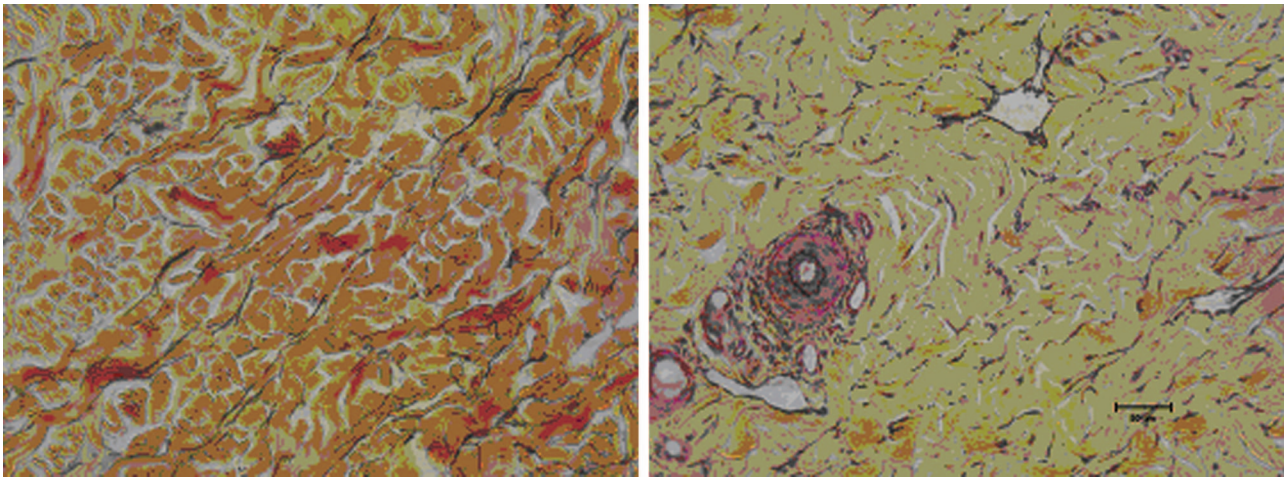

Fig. 2. Movats staining shows the preserved collagen (*orange*) and elastin (*black*) matrix structure of the pig acellularized dermal matrix (*left*), with pig dermis (*right*) as a reference.

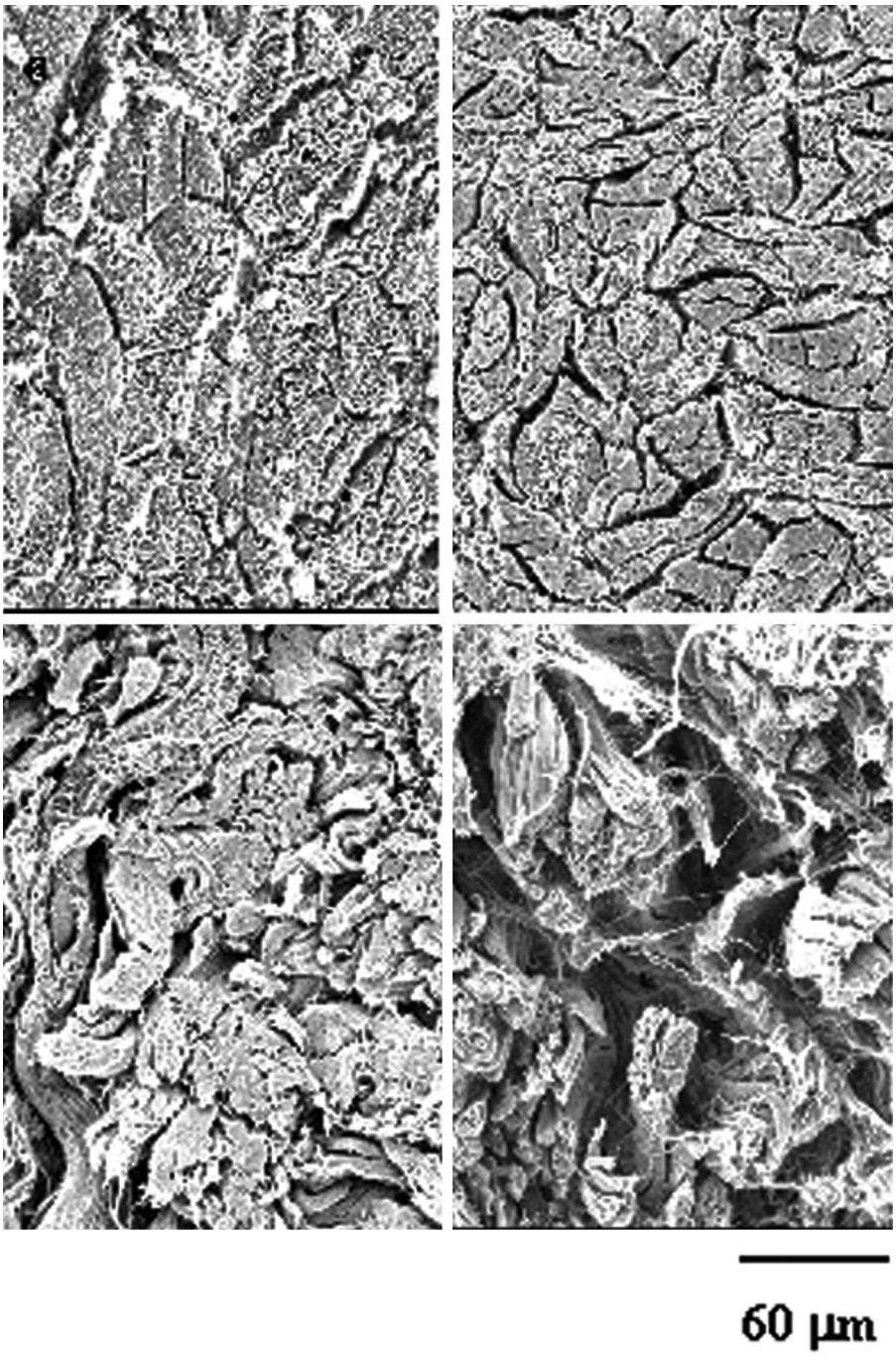

Fig. 3. Pig (*above*) and human acellularized dermal matrix (*below*) at day 0 and day 28 after cell seeding. Cross-sectional scanning electron microscopy of the matrix structure just below the cell-seeded surface (original magnification, ×500). Scale bar = 60 μm.

with either hematoxylin and eosin, or vimentin. The slides were then reviewed to quantify the extent of fibroblast infiltration in both pig and human acellularized dermal matrix. Only slides featuring a monolayer of cells at one side of the specimen were included, thus ensuring that the tissue was cut perpendicular to the surface.

Cell infiltration was assessed in three ways. First, the percentage of samples in each experimental group with at least 50 cells seen, at least 100 μm below the monolayer surface on a section, were compared. The results were statistically analyzed using the chi-square test. Second, the cells on each section demonstrating cell infiltration (as

defined by the first criterion) were counted. Cells within 50 μm of the monolayer were excluded from the count. The number of cells per section was averaged for all sections within each group and analyzed statistically for significant differences using the Wilcoxon test for two independent samples. Third, the distance of each cell from the surface was recorded using digital photography and the MCID image analysis software package (Imaging Research, Inc., St. Catharines, Ontario). Distance measurements were recorded for three microscopic fields per section at 10× magnification. Again, the cells of the surface monolayer were not included in the analysis. Migration distances were compared between the pig acellularized dermal matrix and the human acellularized dermal matrix using the chi-square test. The effect of dermal level on fibroblast infiltration was analyzed using the Fisher's exact test.

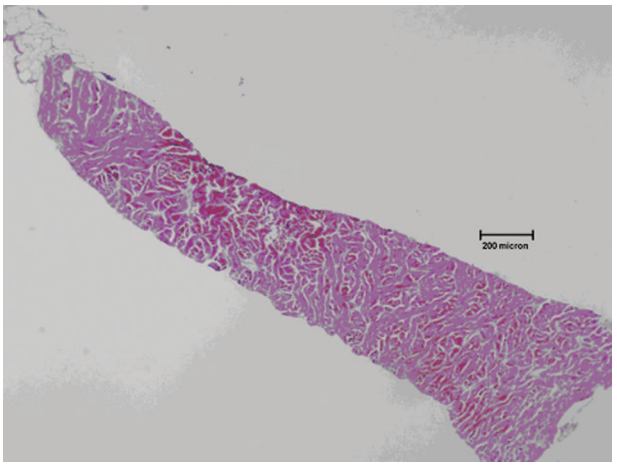

Fig. 4. Full-thickness pig acellularized dermal matrix (hematoxylin and eosin; original magnification, ×5). The epidermal surface is at the lower right-hand corner and the fat surface is at the upper left-hand corner.

RESULTS

Material Characterization

Our acellularization protocol had not been used to acellularize human dermis before this study. Samples of freshly acellularized pig and human dermis were therefore formalin-fixed for analysis of acellularity. Each stain demonstrates the complete removal of dermal cells from both pig and human acellularized dermal matrix (Fig. 1). Staining with Movats demonstrates the persistence of the normal collagen and elastin dermal matrix structure following acellularization of pig dermis (Fig. 2).

A comparison of pig and human acellularized dermal matrix by scanning electron microscopy reveals that both have a tightly packed network of thick collagen bundles (Fig. 3). After cell seeding and prolonged cell culture, however, the human acellularized dermal matrix appears to have a more loosely packed surface than the pig acellularized dermal matrix. This suggests that human acellularized dermal matrix may have less collagen per volume than pig acellularized dermal matrix.

The different levels of dermis were compared histologically and by scanning electron microscopy. For both pig and human acellularized dermal matrix, the papillary dermis appears to have been removed with the epidermis, leaving only reticular dermis for the levels used in the cell-seeding experiments. The deeper dermis is characterized by larger collagen bundles and the presence of subcutaneous fat interspersed between the collagen bundles (Fig. 4). In the pig dermis, the more superficial levels show a greater density of hair follicles than the deeper dermis, although hair follicles are found at all levels. Scanning electron microscopic examination revealed similar information about structural differences between the layers (data not shown).

Fibroblast–Matrix Interactions

Adherent fibroblasts can be seen on the surface of both pig and human acellularized dermal matrix (Fig. 5). The morphologic appearance of the fibroblasts on the surface of the pig acellularized dermal matrix, and their density, is indicative of good adherence of human fibroblasts to the surface of pig acellularized dermal matrix. Quantitatively, human fibroblasts adhere equally well to the pig acellularized dermal matrix as to the human acellularized dermal matrix (Fig. 6). There was no difference ($p = 0.35$, t test) between the two materials with respect to fibroblast adherence after 24 hours in culture (8120 ± 4259 versus 7436 ± 4941 average adherent cells per section, for pig and human acellularized dermal matrix, respectively; $n = 20$ in each group). Fibroblasts proliferated as well on the pig acellularized dermal matrix as on the polystyrene culture dish (7.4-fold increase in cell number versus 7.3-fold increase, respectively; $n = 9$ for each group). However, they proliferated more slowly on the human acellularized dermal matrix (Fig. 7). Fibroblasts proliferated much more rapidly ($p < 0.05$, analysis of variance) on the pig acellularized dermal matrix than on the human acellularized dermal matrix (7.4-fold increase in cell number versus 1.8-fold increase, respectively; $n = 9$ for each group).

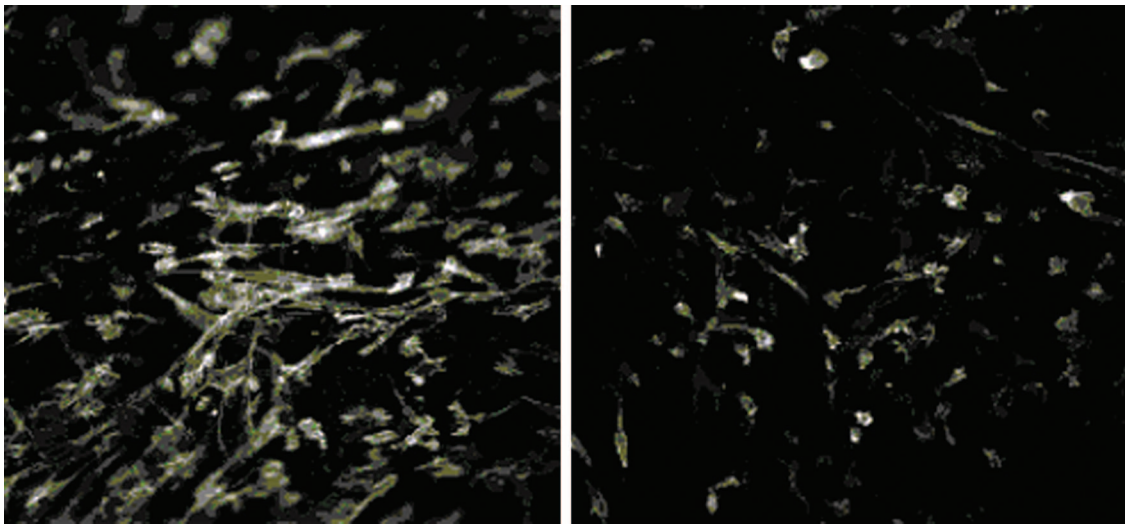

Fig. 5. Confocal microscopy of passaged human dermal fibroblasts stained for phalloidin, cultured on pig acellularized dermal matrix (*right*) and human acellularized dermal matrix (*left*), at low magnification (original magnification, ×10).

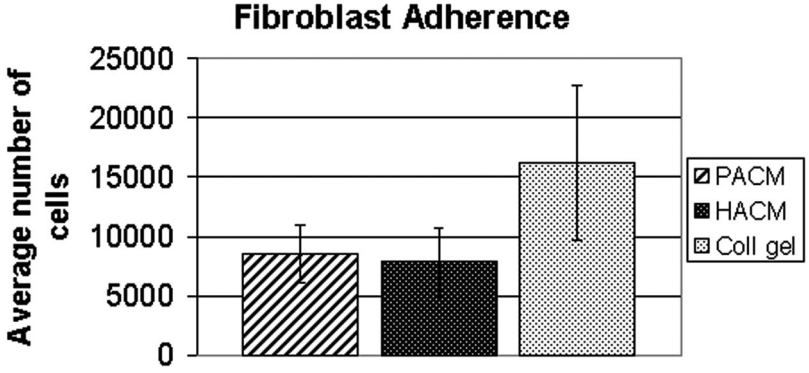

Fig. 6. Fibroblast adherence to pig and human acellularized dermal matrix as determined by the CyQuant assay. Error bars represent standard error. No difference was found between fibroblast adherence on the pig acellularized dermal matrix versus the human acellularized dermal matrix ($p = 0.35$, *t* test; $n = 20$ per group). *PACM*, pig acellularized dermal matrix; *HACM*, human acellularized dermal matrix; *Coll gel*, collagen gel.

Minimal fibroblast infiltration was seen in the pig acellularized dermal matrix samples, as shown by hematoxylin and eosin or vimentin staining (Fig. 8). The human acellularized dermal matrix samples had significantly more fibroblast infiltration (Fig. 9 and Table 1). Significantly more ($p < 0.05$, chi-square test) samples of human acellularized dermal matrix (83 percent of samples with fibroblast infiltration; $n = 24$) demonstrated fibroblast infiltration below the cell-seeded surface than pig acellularized dermal matrix (31 percent with fibroblast infiltration; $n = 49$). A greater number of cells were seen below the surface of human acellularized dermal matrix samples than of pig acellularized dermal matrix (Fig. 10). Significantly more ($p < 0.05$, Wilcoxon test) fibroblasts infiltrated below the surface of human acellularized dermal matrix (mean, 1072 ± 80 cells per section; $n = 16$ samples, three sections per sample) than pig acellularized dermal matrix (mean, 301 ± 48 cells per section; $n = 16$ samples, three sections per sample). The distance of each cell from the surface was also measured using imaging software. As shown in Table 2, fibroblasts in the pig acellularized dermal matrix migrated a significantly shorter ($p < 0.05$, chi-square test) distance from the cell-seeded surface than in the human acellularized dermal matrix (average, 78.8

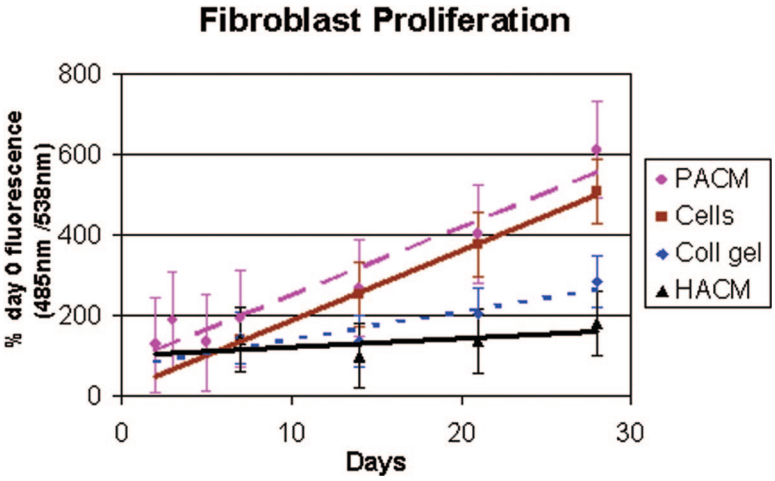

Fig. 7. Fibroblast proliferation on different substrates as determined by the CyQuant assay. See text for significant differences, by analysis of variance (n = 9 per group). *PACM*, pig acellularized dermal matrix; *HACM*, human acellularized dermal matrix; *Coll gel*, collagen gel; *Cells*, cells on culture dish.

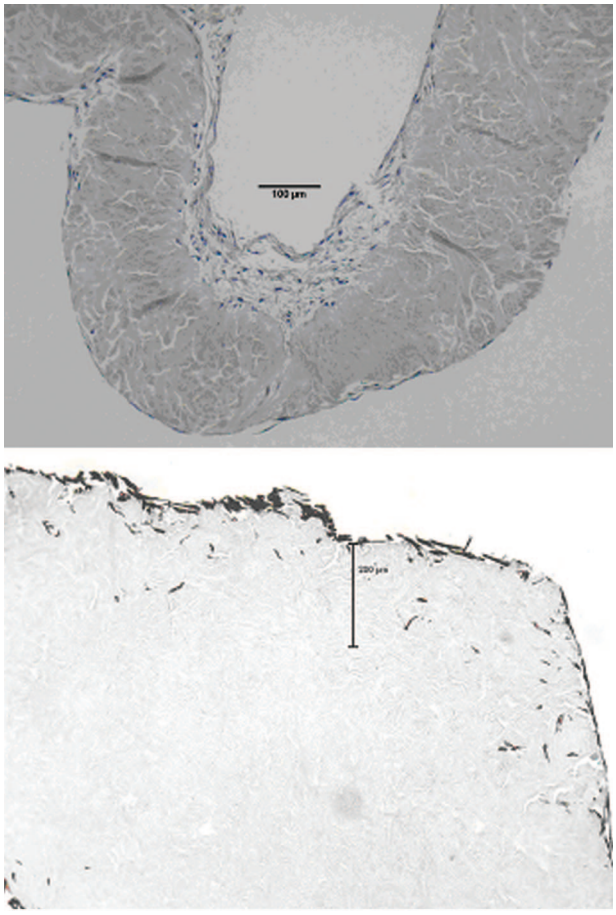

Fig. 8. Pig acellularized dermal matrix seeded with human fibroblasts, after 4 weeks (hematoxylin and eosin, original magnification, ×10; vimentin, original magnification, ×10). There is considerable proliferation of the fibroblasts at the surface (*arrow*), with few cells present within the matrix.

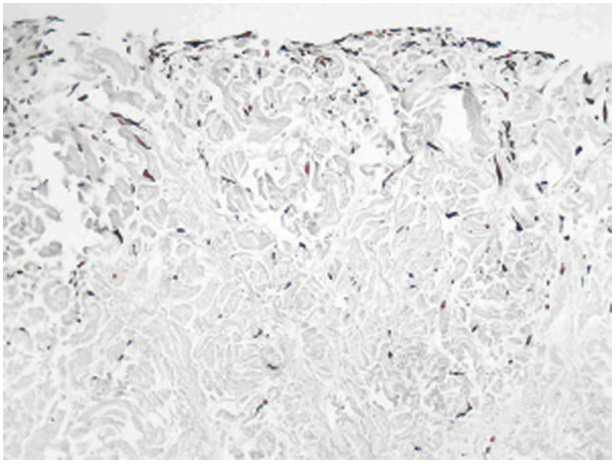

Fig. 9. Human acellularized dermal matrix seeded with human fibroblasts, after 4 weeks in culture (vimentin; original magnification, ×10).

percent versus 38.3 percent of cells within 150 μm from the surface, respectively; five samples per group). The total number of cells per section ranged from 300 to 1300, and three sections were examined per acellularized dermal matrix sample.

The level of dermis used in each experiment was recorded for the pig and human acellularized dermal matrix samples; it was therefore possible to determine its effect on fibroblast infiltration. There was no statistically significant effect ($p > 0.05$, Fisher's exact test) of dermal level on fibroblast infiltration for either pig or human acellularized dermal matrix (Table 3).

Table 1. Comparison of Human Fibroblast Infiltration in Fresh Pig Acellularized Dermal Matrix, Freeze-Dried Pig Acellularized Dermal Matrix, and Freeze-Dried Human Acellularized Dermal Matrix

ACM	Total No. of Samples	No. of Samples with Cell Infiltration in at Least One Section	% of Samples (χ^2)
Pig	49	15	31*
Human	24	20	83*

*Significant difference ($p < 0.05$) between the groups according to the chi-square test.
ACM, acellularized dermal matrix.

Table 2. Distance of Cells from Monolayer Surface, in Representative Fields of Vimentin-Stained Sections of Cell-Seeded Acellularized Dermal Matrix Samples at 3 Weeks*

	Pig ACM ($n = 5$)	Human ACM ($n = 5$)
Range of cell distance from surface	15–434 μm	34–492 μm
Infiltrated cells, %		
0–150 μm	78.8 ± 8.3	38.3 ± 6.5
151–300 μm	14.2 ± 7.9	29.1 ± 6.2
301–500 μm	8.2 ± 1.2	33.0 ± 6.6

ACM, acellularized dermal matrix.
*Distances of over 100 cells per field were recorded.

DISCUSSION

The replacement of lost dermis is important to controlling wound contraction and scar formation in full-thickness wounds.[10] Acellularization technology has allowed significant progress toward overcoming the immunologic challenges of allograft and xenograft materials. The clinical success of acellularized allograft dermis, however, is hampered by its limited supply and high cost.[11] The ongoing goal of dermal engineering is to design a readily available dermal substitute, derived from synthetic or xenograft materials, that works as well as acellularized human dermis. The similarity to human dermis, the mature collagen bundles, and the porous nature of porcine acellularized dermal matrix are all favorable features for a potential dermal substitute. However, the use of acellularized porcine dermis as a dermal replacement is currently limited by its inadequate vascularization in human wounds.[5] Even in rat wounds, the vascularization of acellularized porcine dermis is controversial.[6,7] The reasons for the inadequate vascularization of porcine skin in human wounds are unknown.

The focus of this preliminary study is a comparison of porcine and human acellularized dermal matrix as a scaffold for potential infiltration by human cells. Our simplistic study of in vitro cell–matrix interactions raises the possibility of ultrastructural differences between pig and human dermis. These differences may explain, at least in part, the challenge to pig dermis vascularization by human cells. Future work involving in vitro endothelial cell culture techniques and in vivo animal models will be necessary to definitively understand this problem.

Several researchers have emphasized the morphologic similarities of porcine and human skin.[12] Porcine and human acellularized dermal matrix have similar handling properties, as would be expected from their primarily collagenous composition. Histologically, the similarities between porcine and human acellularized dermal matrix include the interlacing network of collagen bundles of varying thickness and the apparent spaces between the collagen bundles. These spaces appear to be large enough in both species to accommodate the migration of fibroblasts into the matrix.[13]

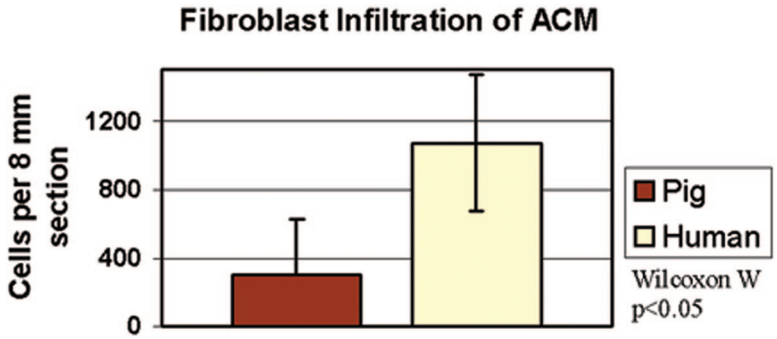

Fig. 10. Fibroblast infiltration of pig and human acellularized dermal matrix at 4 weeks, determined by automated cell counting. The number of cells per section was normalized for section size and then averaged for 16 samples, with three sections each. The bars represent SD. Fibroblast infiltration was significantly higher in the pig acellularized dermal matrix samples, as determined by the Wilcoxon signed rank test.

Table 3. Effect of Dermal Level on Fibroblast Infiltration

	No. of Samples with Fibroblast Infiltration on at Least One Section	
Level	Pig (%)	Human
1	3/8 (38)	5/6 (83)
2	4/12 (33)	7/8 (88)
3	3/10 (30)	5/6 (83)
4	3/10 (30)	3/4 (75)
5	2/9 (22)	X
Fisher's exact test	NS	NS

NS, not significant.

In both surface and cross-sectional scanning electron microscopy, the porcine acellularized dermal matrix appears denser than the human acellularized dermal matrix. Also, the human acellularized dermal matrix surface appears more rough and uneven than that of the porcine acellularized dermal matrix. An obvious variable in this comparison of pig and human dermal acellularized dermal matrix could be their discrepant anatomical locations. Back and breast dermis may differ in thickness, mechanical properties, and ultrastructure.

The objectives of the acellularization were to completely remove all cells from the dermis and to preserve the original extracellular matrix structure. The acellularization protocol developed in this study proved to be effective in producing cell-free dermis of both human and pig origin (Fig. 1).

The CyQuant assay was selected to determine cell viability over time on the acellularized dermal matrix. The difference in proliferation rates of fibroblasts on the porcine and human acellularized dermal matrix suggests differences in the cell–matrix interactions between the two materials. Although the fibroblasts proliferate more on porcine acellularized dermal matrix, this did not translate to better scaffold properties. The greater number of surface fibroblasts is associated with fewer fibroblasts infiltrating deeper into the porcine matrix (Table 4).

Table 4. Comparison of Fibroplasia in Pig Acellularized Dermal Matrix with That in Human Acellularized Dermal Matrix*

Fibroblast Behavior	Human ACM	Pig ACM
Adherence	+++	+++
Proliferation	+	+++
Infiltration	+++	+

ACM, acellularized dermal matrix.
*Symbols refer to quantitative results presented in the article.

By all three measures of fibroblast infiltration (i.e., number of samples with infiltration, number of cells per cross-section, and distance of cells from surface), human acellularized dermal matrix supported significantly more fibroblast infiltration than porcine acellularized dermal matrix. Furthermore, it was observed that in the porcine acellularized dermal matrix, fibroblasts appeared on the surface of the empty hair follicle more frequently than among the extracellular matrix collagen bundles. This was not seen in the human acellularized dermal matrix, because of the paucity of hair follicles. This observation suggests that the number of cells that actually migrated into the porcine extracellular matrix from the cell-seeded surface was less than that measured on cross-sectional histology for porcine acellularized dermal matrix.

One concern was the level of dermis as a variable influencing cell behavior on the pig and human acellularized dermal matrix. In this study, no significant effect of dermal level was seen on fibroblast infiltration (Table 3). However, a more rigorous methodology would be required, with greater standardization of harvesting and processing techniques, before the importance of dermal level could be conclusively determined. As it is known that fibroblasts do not infiltrate the dermal basement membrane in the absence of keratinocytes,[14] all dermis used in this study was second-cut dermis, with the papillary dermis discarded in the top epithelial layer.

The increased fibroblast infiltration in the human acellularized dermal matrix suggests that human and pig dermis must differ in either structure or their interactions with human fibroblasts. Infiltration of acellular porcine dermis by human fibroblasts in vitro has in fact never been reported. Two previous articles found proliferation of human fibroblasts at the surface of the pig dermis only.[15,16] It is therefore promising that any human fibroblast infiltration was seen at all in a few samples in this study. This finding suggests that there is no xenogenic barrier to human fibroblast migration on porcine collagen but possibly a variable structural obstacle to widespread infiltration. Given the manual methods of dermal harvesting, it is quite possible that the structure of the pig acellularized dermal matrix may not have been consistently preserved in every sample. In fact, slight variability in the degree of cell infiltration was noted between batches of pig acellularized dermal matrix.[8] As demonstrated by the scanning electron microscopic findings, the structure of human acellularized dermal matrix becomes less

Table 5. Comparison of Structural Features of Pig and Human Acellularized Dermal Matrix Developed in This Study

	Pig ACM	Human ACM
Source	Paravertebral area of back	Medial and lateral poles of breast
Processing	Electric dermatome	Hand-held dermatome blade
Gross (qualitative)	Conformable More rigid than human ACM	Conformable More elastic than pig ACM
Histology	Densely packed network of interwoven collagen and elastin bundles, with frequent hair shaft spaces	Loosely packed network of interwoven collagen and elastin bundles
Surface SEM	Smooth, cleanly cut surface with residual hairs	Irregular surface with loose collagen bundles, no hair
Cross-sectional SEM	Densely packed network of interwoven collagen bundles	Loosely packed network of interwoven collagen bundles

ACM, acellularized dermal matrix; SEM, scanning electron microscopy.

tightly packed after 4 weeks in culture with fibroblasts. The structure of porcine acellularized dermal matrix, however, remained tightly packed under cell culture conditions (Fig. 3). The more densely packed nature of the pig dermal collagen matrix may delay its infiltration by surrounding cells (Table 5).

Our ongoing research in this area will focus on the modification of the acellular porcine dermal matrix structure, to improve its scaffold properties. The in vitro methods developed in this study will facilitate future evaluations of acellularized dermal matrix structure modification. With the development of a xenogenic guinea pig model, in vivo correlation of our in vitro findings will be pursued. Questions regarding host immunologic responses to acellularized porcine dermis can then also be addressed.

CONCLUSIONS

Acellularized porcine dermis is compared with acellularized human dermis in terms of its ability to support fibroblast infiltration and other aspects of fibroplasia. Porcine acellularized dermal matrix is not equivalent to human acellularized dermal matrix with respect to human fibroblast infiltration in vitro. Our results suggest that previously unrecognized differences in cell–matrix interactions between the porcine and human acellularized dermal matrices should be investigated as a possible obstacle to the success of porcine dermis as a xenograft dermal substitute in humans.

John Laurie Semple, M.D., M.Sc.
Division of Plastic Surgery
Sunnybrook and Women's Health Sciences Center
2075 Bayview Avenue, M1-522
Toronto, Ontario M4N 3M5, Canada
john.semple@sw.ca

ACKNOWLEDGMENTS

This study was funded by the Natural Science and Engineering Research Council, and Materials and Manufacturing Ontario operating and salary grants. The authors acknowledge the assistance of R. Levine, M.D., at St. Joseph's Health Center for kindly providing breast reduction surgical specimens. They also acknowledge the assistance of Trudey Nicklee at Princess Margaret Hospital with the automated histologic analysis of cell infiltration. Histologic processing of the acellularized dermal matrix was skillfully performed by members of the pathology and immunohistochemistry departments at Sunnybrook and Women's College Health Science Center and at Princess Margaret Hospital. Trygve Armour is gratefully acknowledged for objective statistical analysis of all data.

REFERENCES

1. Berthod, F., and Rouabhia, M. Exhaustive review of clinical alternatives for damaged skin replacement. In M. Rouabhia (Ed.), *Skin Substitute Production by Tissue Engineering: Clinical and Fundamental Applications.* Austin, Texas: R. G. Landes, 1997.
2. Gao, Z.-R., Hao, Z. Q., Li, Y., Im, M. J., and Spence, R. J. Porcine dermal collagen as a wound dressing for skin donor sites and deep partial skin thickness burns. *Burns* 18: 492, 1992.
3. Basile, A. A comparative study of glycerinized and lyophilized porcine skin in dressings for third-degree burns. *Plast. Reconstr. Surg.* 69: 969, 1982.
4. Breach, N., Davies, D., and Yiacoumettis, A. Study of effects of porcine skin and bovine dermis on the healing of split-skin graft donor sites in humans. *Plast. Reconstr. Surg.* 63: 546, 1979.
5. Artz, C. P., Rittenbury, M. S., and Yarbrough, D. R. An appraisal of allografts and xenografts as biological dressings for wounds and burns. *Ann. Surg.* 175: 934, 1972.
6. Fang, C. H., Robb, E. C., Yu, G. S., Alexander, J. W., and Warden, G. D. Observations on stability and contraction of composite skin grafts: Xenodermis or allodermis with an isograft onlay. *J. Burn Care Rehabil.* 11: 538, 1990.
7. Wang, H.-J., Chen, T.-M., and Cheng, T.-Y. Use of a porcine dermis template to enhance widely expanded mesh autolo-

gous split-thickness skin graft growth: Preliminary report. *J. Trauma* 42: 177, 1997.
8. Armour, A. Evaluation of Porcine Acellularized Dermal Matrix as a Biomaterial: In Vitro Fibroplasia. Toronto: University of Toronto, Department of Surgery, Institute of Medical Sciences, 2001. P. 144.
9. Courtman, D., Pereira, C. A., Kashef, V., McComb, D., Lee, J. M., and Wilson, G. J. Development of a pericardial acellular matrix biomaterial: Biochemical and mechanical effects of cell extraction. *J. Biomed. Mater. Res.* 28: 655, 1994.
10. Rudolph, R., and Ballantyne, D. L. Skin grafts. In J. G. McCarthy (Ed.), *Plastic Surgery.* Philadelphia: Saunders, 1990. P. 244.
11. Wainwright, D. Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. *Burns* 21: 243, 1995.
12. Meyer, W., Schwarz, R., and Neurand, K. The skin of domestic mammals as a model for the human skin, with special reference to the domestic pig. *Curr. Probl. Dermatol.* 7: 39, 1978.
13. Yannas, I. V. In vivo synthesis of tissues and organs. In R. P. Lanza, R. Langer, and W. L. Chick (Eds.), *Principles of Tissue Engineering.* Austin, Texas: R. G. Landes, 1997. Pp. 169-177.
14. Ghosh, M. M., Boyce, S., Layton, C., Freedlander, E., and MacNeil, S. A comparison of methodologies for the preparation of human epidermal-dermal composites. *Ann. Plast. Surg.* 39: 390, 1997.
15. Matouskova, E., Vogtova, D., and Konigova, R. A recombined skin composed of human keratinocytes cultured on cell-free pig dermis. *Burns* 19: 118, 1993.
16. Oliver, R., Barker, H., Cooke, A., and Grant, R. A. Dermal collagen implants. *Biomaterials* 3: 38, 1982.