A New Biomaterial Derived from Small Intestine Submucosa and Developed into a Wound Matrix Device

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Abstract: A biomaterial derived from porcine intestinal submucosa has been used in the development of a new biologic wound matrix. This review describes the origin of the wound matrix device, including the discovery of the biomaterial and its properties, to the development of the commercial product currently in clinical use. The composition and structure of this biomaterial are described and considered as mechanisms that contribute to its effectiveness in wound management. The wound matrix developed from this unique biomaterial was evaluated in a pilot study of human partial-thickness dermal wounds and was found to be beneficial, especially in its dehydrated form. Other applications of this biomaterial, as well as its limitations, are also discussed.

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too active enzymatically to retain sutures. Subsequent implantation studies used intestine with various layers removed. In the end, the most successful graft was composed solely of the thin, translucent, but resilient, submucosal layer that remained after removing the mucosal and muscular layers. The submucosal layer of the small intestine is approximately 0.15 to 0.25 mm thick and consists primarily of a collagen-based extracellular matrix (ECM) containing relatively few resident connective tissue cells. This layer provides structural support, stability, and biochemical signals to the rapidly regenerating mucosal cell layer. The naturally cross-linked collagen network of the submucosal layer also gives strength to the whole intestine. For these reasons, SIS biomaterial was derived from this intestinal layer and used initially for vascular graft studies. SIS biomaterial is harvested from a porcine source and minimally processed to lyse all resident cells and remove cellular debris as described elsewhere. SIS biomaterial is sterilized using a proprietary method that includes treatment with ethylene oxide. This sequential processing method allows long-term storage of the acellular sheet of ECM without destroying its ability to support wound healing and tissue repair.

Following the initial discovery and evaluation, this naturally occurring ECM-based biomaterial was tested in a number of pre-clinical studies to evaluate its biocompatibility and persistence upon implantation. The SIS biomaterial is biocompatible in all host species tested. In addition, the biomaterial was remodeled gradually into new tissue by the host. This phenomenon was particularly remarkable because the new tissue generated by the host was specific to the site of implantation rather than a generalized fibrotic tissue. For example, when SIS was implanted in place of a blood vessel, within four months the biomaterial had been incorporated and replaced by new tissue, which appeared nearly identical to the original vessel. Even though the conduit had been formed from a single layer of the thin SIS biomaterial, a multilayered vessel was formed, which was several times thicker than the original SIS. The implanted graft supported the development of new artery tissue with an intimal lining of endothelial cells and a supporting outer layer of muscle tissue. This regeneration of tissue structures following implantation has been termed “smart remodeling” by researchers. A biomaterial with such properties was anticipated to provide a suitable covering for dermal wounds, and a pre-clinical animal study has specifically demonstrated the potential effectiveness of SIS as a biological-derived dressing in the clinical setting.

In this review, we report the results of a pilot study showing the initial clinical experiences with the wound matrix device (WMD) developed from the SIS biomaterial. A discussion of the need for and advantages associated with biological-derived dressings as compared to synthetic dressings is followed by a brief review of the discovery and development of the SIS biomaterial. The significant properties revealed in pre-clinical studies and the mechanisms behind the effective tissue restorative properties of the biomaterial are detailed. Finally, the initial clinical experiences with the WMD designed for dermal application and the development of other healthcare products from the SIS biomaterial are discussed.

Synthetic and Biological-Derived Wound Dressings

Accepted goals of state-of-the-art wound care include wound hydration, thermal insulation, protection from infection and desiccation, and facilitation of healing. Additionally, wound care dressings must be devoid of toxins and infectious agents, be easily placed and replaced, and be an aid to wound drainage. The wound healing process, in dermal or other tissue sites, is a complex process of tissue restoration. The wound healing process can lead either to fibrotic tissue replacement (scarring) with limited functional restoration or to natural tissue restoration, particularly in healing by second intention. Very few, if any, current wound care products have the capacity to direct the healing process towards tissue restoration.

The hundreds of wound care dressings presently available can be divided into two broad categories: synthetic (including biosynthetic) and biologic (tissue origin). Synthetic wound dressings are typically inexpensive, have long shelf life, induce minimal inflammatory reaction, and lack the risk of disease agent transmission. Such synthetics/biosynthetics include textiles, polyurethane films, foams, hydrogels, hydrocolloids, and collagen/alginate combina-
### Table 1. Summary of pre-clinical studies with SIS biomaterial

<table>
<thead>
<tr>
<th>Key Property</th>
<th>Study</th>
<th>Result</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smart remodeling:</strong> SIS scaffolds regenerate into host tissue structures, with SIS biomaterial responding to site-specific stressors</td>
<td>Evaluated implanted SIS scaffold as a bladder wall patch with pressurized static flow</td>
<td>SIS scaffold remodeled into bladder wall having measurable contractile activity and active nerve receptors comparable to normal bladder</td>
<td>25, 26</td>
</tr>
<tr>
<td></td>
<td>Evaluated implanted SIS scaffolds in tendonous and ligamentous sites</td>
<td></td>
<td>31–33</td>
</tr>
<tr>
<td></td>
<td>Evaluated implanted single layer of SIS as a blood vessel</td>
<td>SIS scaffolds remodeled, at four months, into multilayer vessel having intimal lining of endothelial cells and supporting outer layer of muscle tissue</td>
<td>3, 4, 19, 23</td>
</tr>
<tr>
<td><strong>Rapid capillary ingrowth:</strong> thought critical to successful tissue replacement</td>
<td>Evaluated implanted SIS scaffolds in the vascular, urological, and muscular systems</td>
<td>Rapid cellular infiltration and angiogenesis was observed with capillary ingrowth occurring after four days, and the scaffolds remodeled into host tissues</td>
<td>3, 5, 19, 20–22, 24, 28–30, 34</td>
</tr>
<tr>
<td><strong>Greater resistance to infection</strong></td>
<td>Evaluated and compared resistance to bacterial infection, SIS vs. synthetic graft materials</td>
<td>SIS implants were less likely to harbor infection than synthetic graft implants. Thought to be due to 1) rapid and high levels of vascularization induced by SIS and 2) degradation of material unable to provide a nidus for infection</td>
<td>4, 5, 40, 41</td>
</tr>
<tr>
<td><strong>Lack of adverse immunological reaction</strong></td>
<td>Evaluated implanted SIS scaffolds in a full-thickness rat skin replacement study; in a dural replacement study; in a bone regeneration study</td>
<td>SIS produced less contracture than control wounds with good epithelialization; no evidence of infection or acute or delayed hypersensitivity reactions at any sites</td>
<td>9, 35–37, 44</td>
</tr>
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</table>
Synthetics, such as these, have been designed primarily around the moist wound theory of wound healing put forth by Winter and function well as short-term moisture barriers. However, others have challenged the theory as being insufficient to the promotion of regenerative wound healing.

Biological-derived wound dressings have been advocated for their ability to more effectively promote granulation and epithelialization of dermal wounds than synthetic dressings. In addition, biological-derived wound dressings effectively regulate evaporation and exudation and effectively protect the wound site from bacterial infection. Biological-derived wound dressings are not new, but their effectiveness has increased greatly with recent innovative developments. Early biological-derived dressings include a collagen-based dressing made from porcine skin in the 1960s with the first reported clinical use of a porcine skin dressing in 1973. Sheets of collagen, laboriously harvested from sheep intestine, have also been used as wound dressings. More specialized biological-derived dressings have been developed since these early studies were reported. These products include a product derived from human cadaver skin, which is treated to be acellular and deepidermalized to provide a near natural neodermis for skin re-growth; a product prepared by seeding dermal fibroblasts on a biodegradable matrix; and a tissue-engineered human skin equivalent made by layering a sheet of stratified human epithelium onto a bovine collagen matrix impregnated with human foreskin fibroblasts (HSE). Such technologically advanced products are approaching the equivalent of human skin replacement; however, they are still limited by the extensive preparation time (17–20 days for HSE), the high cost of manufacture, and the short shelf life. Autologous skin grafts probably present the optimal wound dressing when considered in terms of healing alone. However, additional site morbidity and limited supply dramatically compromise the benefits of these wound dressings. Cellular xenograft dressings remain immunologically incompatible for human use.

An ideal wound dressing would be one made from a readily available biomaterial that requires minimal processing and, after sterilization and storage, retains the biological characteristics that promote wound healing. Such an acellular biologic dressing would incorporate both the advantages typical of synthetic dressings (low cost, long shelf life, and low risk of immunological reaction) and those typical of biological-derived dressings (regulated fluid flow, increased resistance to bacterial contamination, and enhanced wound healing). SIS biomaterial, derived as a by-product from porcine small intestine, has demonstrated these characteristics.

**Development of SIS Biomaterial into a Wound Matrix: Pre-Clinical Studies with SIS Biomaterial**

The development of the new SIS biomaterial began at Purdue University with a search for suitable vascular graft materials. SIS biomaterial was observed to have excellent implant healing characteristics when implanted as a vascular replacement, so the idea of its potential application to other sites of tissue destruction, including healing of dermal wounds.
wounds, was nurtured in the context of numerous pre-clinical studies. These studies repeatedly demonstrated the ability of SIS biomaterial to regenerate as host tissue, induce rapid capillary ingrowth, be resistant to infection, and induce little or no immunologic reaction (Table 1). The mechanisms behind these physiological phenomena are beginning to be understood as inherent properties of the architecture and composition of the SIS biomaterial.

**SIS biomaterial regenerates as host tissue.** The results of numerous pre-clinical studies have demonstrated that SIS biomaterial is capable of inducing host tissue proliferation and replacement when implanted in various tissue sites (Table 1). SIS accomplishes this through smart remodeling. That is, the SIS biomaterial provides a scaffold for regeneration of the host tissue with the SIS biomaterial responding to natural, site-specific stressors (Figure 1). SIS biomaterial induction of host tissue proliferation and replacement has been demonstrated in many tissues, including blood vessels, lower urinary tract, body wall, tendon, ligament, dura, and bone. Tissue regeneration upon implantation of SIS biomaterial as diaphragmatic prosthesis also has been observed. Upon implantation of the SIS biomaterial into these sites, the biomaterial was remodeled by the host into replacement tissue with site-specific structural and functional properties. For example, following placement of SIS as an arterial vessel with pressurized, pulsatile flow, the biomaterial was remodeled into tissue with identifiable smooth muscle layers organized along lines of stress. Similarly, when implanted in a bladder site with pressurized static flow, SIS was remodeled into a tissue with measurable contractile activity. In addition, SIS-regenerated bladder wall contained active nerve receptors comparable to normal bladder tissue. In contrast, SIS biomaterial implanted into a venous location did not evidence any actin-containing spindle cells. Similar results were reported for implantation of SIS into tendonous and ligamentous sites. Newly deposited connective tissue replacing the SIS was highly organized along lines of stress. The poorly organized fibrous granulomas surrounding implanted synthetic materials observed in comparisons in abdominal wall sites contrasted greatly with the remodeling of SIS biomaterial.

**SIS biomaterial induces rapid capillary ingrowth.** Pre-clinical studies also demonstrated rapid capillary ingrowth after implantation of SIS scaffolds. This rapid cellular infiltration and angiogenesis, thought critical to successful tissue replacement, was observed following implantation of SIS scaffolds in the vascular, urological, and muscular systems. In vascular implants, capillary ingrowth was present after just four days.

**SIS biomaterial shows resistance to bacterial infection.** In pre-clinical implantation studies, wounds treated with SIS have demonstrated greater resistance to bacterial infection compared to those treated with synthetic graft materials. A subsequent study involving direct inoculation of abdominal wall graft sites with *Staphylococcus aureus* resulted in persistent infection in the control group grafted with synthetic mesh, while effective graft remodeling and absence of infection were observed in the SIS biomaterial group one month after implantation. One mechanism thought responsible for this infection resistance is the rapid and high levels of vascularization induced by implantation of SIS biomaterial. A second possible mechanism is the fact that the SIS scaffold degrades as host tissue replaces it and therefore the tissue is unable to provide a long-term nidus for infection.

**SIS biomaterial does not induce an adverse immunologic reaction.** In none of the SIS pre-clinical implantation studies was there evidence of the pronounced, chronic, foreign body reaction characterized by a high density of mononuclear macrophages and often seen with synthetic implants. The lack of adverse immunological reaction is thought to be related to the acellular condition and significant collagen composition of the SIS biomaterial. Collagen proteins are highly conserved across species and appear to be readily degraded and/or incorporated into the new tissue with minimal antigenicity even when the implant source material is xenogeneic. A high density of fibroblasts present in the neotissue 26 weeks after SIS implantation into a ligamentous tissue site also suggested that collagen production and tissue remodeling can continue without adverse immune response for an extended time until full regeneration is achieved. Furthermore, mice that showed chronic inflammation and graft rejection when
implanted with xenogeneic tissue had only an acute inflammatory response and active tissue remodeling when implanted with SIS biomaterial grafts. The type of immune response induced by the SIS biomaterial was consistent with graft accommodation based upon antibody and cytokine analysis.44

In a full-thickness, rat skin replacement study, no acute or delayed hypersensitivity reactions were observed.9 Additionally, many aspects of the tissue regenerative healing response to implantation of SIS were reproduced in this animal study directed toward dermal application. The SIS biomaterial significantly reduced the contraction observed in control wounds. SIS-treated wounds developed a healthy epithelial layer covering and had no signs of infection.

These pre-clinical implantation studies provided the basis for developing a clinical wound care product from the SIS biomaterial. Additional research on the basic properties of SIS biomaterial have provided a clearer understanding of the fundamental mechanisms by which such a wound care product could function.

Studies on the Mechanisms Behind the Tissue Repair Properties of SIS Biomaterial

The working hypothesis has been that both the composition and the architecture of the SIS biomaterial contribute to its induction and enhancement of tissue remodeling.4,5 The actual mechanisms behind this tissue restoring property of SIS biomaterial have been investigated at biochemical, structural, and cell biological levels (Table 2).

Biochemical composition of SIS biomaterial. Studies on the composition of SIS have revealed that the biomaterial has a water content of approximately 90 percent in its fully hydrated form. This high water content is consistent with having originated from a natural tissue source. The nonwater portion of the biomaterial is approximately 90-per cent protein with the remainder comprised of mainly carbohydrate and some lipid. The high protein content consists primarily of collagens, with types I, III, and V predominating.45 In tissue, these collagen proteins form the scaffolding of the robust three-dimensional network of the ECM. This three-dimensional matrix provides a suitable structural environment for the resident cells of a tissue. In the absence of resident cells, such a collagen matrix provides an immediately stable environment for infiltrating host cells.

Other critical components of the ECM are naturally embedded within or attached onto the collagen scaffold of the biomaterial (Table 2). Some of the more important components are complexes of protein and carbohydrate: glycoproteins, proteoglycans, and glycosaminoglycans (GAG). Many glycoproteins and proteoglycans contain specific sites on their protein portion that help cells to attach and settle within the matrix.46,47 By providing such cell attachment sites in the matrix, these molecules contribute to the re-population of the matrix and to the regulation of cell migration, proliferation, and differentiation. Each of these cellular processes is necessary for remodeling tissue to mature into fully functional tissue.

Glycosaminoglycans are integral to the matrix architecture and consist of a core protein molecule extensively decorated with long complex carbohydrate chains. Many of the carbohydrates are electrostatically charged, and when linked together they form highly charged, space-filling complexes. These complexes are responsible for the high water retention properties and the compressibility of the matrix. Five types of GAG molecules have been identified in SIS biomaterial: heparan sulfate, hyaluronic acid, chondroitin sulfate A, dermatan sulfate, and heparin.48 Preliminary evidence also exists for several essential proteoglycans and glycoproteins in the SIS biomaterial, including the important cell-regulating molecule fibronectin.49

Another component of the SIS biomaterial critical to its mechanism of action is its growth factor content. Growth factors are typically small protein molecules found in limited quantities in cells and cell environments. These highly potent molecules regulate many aspects of cellular activity, including stimulating growth and cell division, migration, and differentiation. These activities are essential to the regenerative aspect of wound healing.11 Two important molecules, identified as the major growth factor components of SIS, are fibroblast growth factor-2 (FGF-2) and a transforming growth factor-β (TGF-β) related protein.50 Both of these proteins are known to participate in wound repair, particularly in vascular development. In addition, vascular
### Table 2. Mechanisms of tissue repair properties of SIS biomaterial

<table>
<thead>
<tr>
<th>Property</th>
<th>Component</th>
<th>Functional Effect</th>
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<tbody>
<tr>
<td>Biochemical Composition</td>
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<tr>
<td>Proteins</td>
<td>• Water content 90%, remainder proteins primarily collagens</td>
<td>• Three-dimensional collagen matrix provides immediately stable environment for infiltrating host cells</td>
</tr>
<tr>
<td></td>
<td>• Predominately types I, III, V</td>
<td>• Collagens are highly conserved across species</td>
</tr>
<tr>
<td>Protein-carbohydrate complexes</td>
<td>• Glycoproteins (including fibronectin)</td>
<td>• Provide cell attachment sites in the matrix</td>
</tr>
<tr>
<td></td>
<td>• Proteoglycans</td>
<td>• Regulation of cell migration, proliferation, and differentiation necessary for maturation of developing tissue</td>
</tr>
<tr>
<td></td>
<td>• Glycosaminoglycans (including heparin sulfate, hyaluronic acid, chondroitin sulfate A, dermatan sulfate and heparin)</td>
<td>• Structural organizers for tissues as well as signaling molecules for cells</td>
</tr>
<tr>
<td></td>
<td>• Fibroblast growth factor-2 and transforming growth factor-β related protein</td>
<td>• High water retention properties and compressibility of the matrix</td>
</tr>
<tr>
<td></td>
<td>• Vascular endothelial growth factor</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Other growth factors still being identified</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Structural Properties</td>
<td></td>
<td></td>
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<tr>
<td>Porosity</td>
<td>• Microscopic pores and tunnels</td>
<td>• May aid cellular infiltration and rapid new vessel in-growth</td>
</tr>
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<td></td>
<td>• Limited porosity; hydrated form has porosity between nonporous ureter and relatively porous caron mesh</td>
<td>• Limited water flow suggests that the material may serve as an effective barrier to wound bed dehydration while preventing sub-dressing seroma formation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• More compliant than venous or synthetic graft materials</td>
</tr>
<tr>
<td>Strength and flexibility</td>
<td>• Intervenous pattern of collagen fibers of varying sizes</td>
<td></td>
</tr>
<tr>
<td>Cell Growth Properties</td>
<td>• Substrate for multiple cell types in culture</td>
<td>• Stimulation of cell proliferation and differentiation was observed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Various cell types exhibited their natural phenotype and morphology when grown on the SIS biomaterial</td>
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</table>
endothelial growth factor (VEGF) has been identified as a component of SIS. VEGF is also an important regulatory molecule that induces the migration of endothelial cells. Several other known growth factors have been tentatively identified in the SIS biomaterial and are thought to contribute to the development of a healthy epithelial cell layer.

Several of the bioactive components of ECM tissues are highly labile when isolated in solution. This is particularly true of growth factors. However, these components show remarkable stability when bound to the matrix, even when exposed to fairly harsh chemical treatments. This appears to be true for the SIS biomaterial as well, where growth factor activity remained detectable after terminal sterilization. SIS biomaterial used in pre-clinical studies was routinely disinfected before implantation. Treatments such as 0.05-percent gentamycin, 10-percent neomycin, or 0.1-percent peracetic acid were without effect on the remodeling properties of the biomaterial. In addition, various sterilization methods for the biomaterial were evaluated and their effect on biological activity was found to be treatment dependent.

These many biologically important components together with the matrix of collagen as a scaffold, in a natural architectural configuration, appear to offer a remarkable environment for induction and regulation of tissue regeneration, particularly for a dermal wound.

Structural properties of SIS biomaterial. The microstructure of the SIS biomaterial has been studied using high-resolution microscopy. Such studies have determined that the natural three-dimensional architecture of the tissue ECM is retained in the SIS biomaterial (Figure 2). The orientation changes across the thickness ranging from a definite cross-hatch pattern to a random weave pattern. In lower power optical images, remnant structures of blood vessels are observed as regularly interspersed throughout the patterns of collagen fibers (Figure 3). Such microscopic pores and tunnels give the biomaterial a limited porosity and may aid in the cellular infiltration and the rapid new vessel in-growth observed upon implantation of the SIS biomaterial.

Several additional structural properties of this biomaterial also have been investigated. The measured porosity values of hydrated SIS were lower from the luminal (mucosal) side than the abluminal (serosal) side due to the oriented architecture of the biomaterial. These values were intermediate between those of nonporous bovine ureter and relatively porous, synthetic Dacron mesh used for vascular grafts. These results indicated that the biomaterial has a limited capacity for water flow through. This suggests that the material might serve as an effective barrier to wound bed dehydration while preventing sub-dressing seroma formation; both of which are critical to the effective clinical management of acute and chronic skin wounds.

Fully hydrated SIS biomaterial exhibits strength and flexibility values similar to the intestinal tissue from which it is derived, even after being lyophilized for long-term storage. The nominal burst force of SIS biomaterial was determined to be between the lower values of bladder wall, aorta,
3). Most patients were nonsmokers and were nondiabetic. Several partial-thickness wound types were present in the evaluation, including pressure ulcers, venous ulcers, trauma wounds, and drug-induced ulcers (hydroxyurea, a chemotherapeutic agent). Wounds were measured, photographed, and traced for accurate surface area determination. The average wound area measured 3.96 cm² (range 0.42–15.48) at time of initial treatment. One patient (87-year-old man) expired due to congestive heart failure (CHF) eight days after the initial treatment. This left 14 patients to follow up for wound healing evaluation.

The lyophilized (i.e., dry) WMD was easily placed and hydrated without difficulty in all cases and was preferred due to the greater ease of handling. The hydrated form was found to be less useful on highly exudative wounds as it resisted adherence to the wound bed. At various times in the course of healing, the dressing became translucent in appearance on the wound bed or became incorporated into the granulating bed (Figure 4). In most wounds the absorption of the dressing was observed to be primarily in the central region of the wound. Where the dressing had become translucent or absorbed, new dressing was applied directly on top of the region without attempting to remove that portion of the previous dressing which typically presented with attachment to the wound margin. Wounds were epithelialized with minimal to no scar formation (Figure 4).

Time to complete epithelialization was evaluated for each wound and divided into three categories: 1) less than four weeks, 2) five to eight weeks, and 3) greater than eight weeks (Table 4). Three wounds (two treated with WMD stored lyophilized and one treated with the stored hydrated form) were completely epithelialized within four weeks. Six more wounds (four with lyophilized and two with hydrated) were observed to be completely epithelialized within the five-to-eight-week duration. One wound (treated with the dressing stored lyophilized) persisted for more than eight weeks, but did completely epithelialize in ten weeks. Of the remaining four wounds, all of which had been treated with WMD stored hydrated, three were switched to calcium alginate and one was switched to the lyophilized form. The wound switched to the lyophilized form of WMD at week two was completely epithelialized at week three.

The patient (32-year-old man) that was switched to the lyophilized form of WMD presented with a venous ulcer of the lower leg (Figure 5A). The patient had recurring ulcerations due to congenital venous abnormalities, and the present ulcer had persisted for one month. At the initial visit, the wound was managed with WMD stored hydrated, and a compression bandage was applied and used throughout. At the one-week visit, the wound was evaluated (Figure 5B). After cleansing, the wound area was determined to have decreased in size by more than 50 percent (Figure 5C), and a second application of WMD stored hydrated was made (Figure 5D). At the second visit (14 days) the wound was again evaluated

<table>
<thead>
<tr>
<th>Form of Wound Matrix</th>
<th>Patients Available for Follow Up</th>
<th>Time to Wound healing</th>
<th>Complete Healing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyophilized</td>
<td>7</td>
<td>4 weeks or less</td>
<td>7/7 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 to 8 weeks</td>
<td>3/7 (40%*)</td>
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<tr>
<td></td>
<td></td>
<td>8 weeks or more</td>
<td></td>
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<tr>
<td>Hydrated</td>
<td>7</td>
<td>1</td>
<td></td>
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<td></td>
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<td>2</td>
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</table>

* Patient 10—switched to calcium alginate after eight weeks of treatment.
Patient 11—switched to calcium alginate after three weeks of treatment.
Patient 12—switched to lyophilized WMD after two weeks of treatment; wound healed within one week of treatment with lyophilized WMD.
Patient 14—switched to calcium alginate after eight weeks of treatment.
and colon and the higher value of abdominal body wall.\textsuperscript{57} The compliance (expandability) of SIS biomaterial configured as a small-diameter graft is about 50 percent of that of the dog carotid artery. However, SIS biomaterial is much more compliant (four times) than a typical vein graft, and more than an order of magnitude compliant than synthetic vascular grafts.\textsuperscript{58} In studies of tensile strength, force measurements were made of the maximum force required to tear a multilayer SIS device used for ligament replacement.\textsuperscript{33} The strength of the multilayer SIS biomaterial greatly increased from before implantation to after 26 weeks of remodeling as a ligament. The suture retention strength for single-layer SIS biomaterial also was significantly higher than the calculated physiologic forces exerted by tissues in several common implantation sites including bladder wall, aorta, colon, and abdominal body wall.

Cell biological properties of SIS biomaterial. The unusual tissue remodeling ability of the SIS biomaterial prompted \textit{in-vitro} cell culture studies into the responses of various cell types to this extracellular matrix material. \textit{In-vitro} cell culture, using SIS biomaterial as a substrate, provides an environment very similar to wounded tissue in which to study cellular responses. Cells in this environment can migrate, proliferate, attach to the substrate, and differentiate in a liquid medium with serum and cell stimulating factors present. Such studies have involved isolated cell lines, both primary cultures and established cultured lines, grown in the presence of the SIS biomaterial.\textsuperscript{7,59,60} Two basic questions were evaluated in these studies: 1) Could the apparent stimulation of cell proliferation and differentiation, observed in SIS implantation studies, be observed in cell culture? and 2) Would different cell types respond differently to the SIS biomaterial? SIS biomaterial supported cell morphologies that resembled the morphologies observed in the tissue of origin for the four different cell types tested: squamous epithelial (pulmonary artery), fibroblast (embryo), smooth muscle-like (urinary bladder), and glandular epithelial (adenocarcinoma). This propensity for maintaining or restoring phenotypic morphology was in contrast to two other materials used for growing cells in culture: a pure collagen based gel substance (Vitrogen, Cohesion Technologies) and a basement membrane extract formed into a gelled matrix (Matrigel\textsuperscript{\textregistered}, Becton, Dickinson and Co.). The pure collagen gel allowed cell proliferation without significant differentiation. In contrast, the gelled basement membrane extract greatly limited cell proliferation and induced an altered morphology not common to the cells in their tissue of origin. These results supported the hypothesis that SIS biomaterial retains a unique structure and composition that enables cells to develop and maintain a natural tissue morphology. These results also are in agreement with the theory put forth by Bissell,\textsuperscript{61} that the architecture and structural properties of the extracellular matrix are equally as important to cell response as the composition of the matrix. These observations were confirmed using a different selection of cells that included primary human keratinocytes, human microvascular endothelial cells (HMECs), and an established rat osteosarcoma (ROS) cell line.\textsuperscript{59} Each of these cell types also maintained the ability to attach and proliferate on the SIS biomaterial. The keratinocytes migrated more freely into the three-dimensional scaffold of the SIS matrix, whereas the ROS cells and the HMECs remained on the surface. In addition, a study of cell attachment properties of SIS biomaterial using HMECs demonstrated that the adherence of HMECs to hydrated SIS was greater than to several other ECM components tested individually.\textsuperscript{62}

These studies of cell attachment, proliferation, and migration in and through the SIS biomaterial further demonstrated its ability to orchestrate physiological cell behavior even in culture. Since the SIS biomaterial retains the matrix architecture and com-

\textbf{Figure 3. Lower power optical image of SIS showing the collagen fiber matrix regularly interspersed with the remnant structures of blood vessels.}
position of healthy tissue, it encourages cell growth and development towards a normal tissue state. This orchestration of cellular response is believed to be a significant mechanism behind the healing properties observed in preclinical studies. Taken together, all of the \textit{in-vitro} studies of the SIS biomaterial fully supported the development of a clinical wound care product particularly oriented toward dermal repair.

**Initial Clinical Experience with the Wound Matrix**

WMD, developed from SIS biomaterial, was subjected to biocompatibility testing prior to its use in a clinical setting. Following completion of standard \textit{in-vitro} and \textit{in-vivo} biocompatibility testing, WMD was cleared by the Food and Drug Administration (FDA) for the intended use of management of partial-thickness wounds.

WMD was evaluated in a pilot study for effectiveness in treating partial-thickness skin wounds. The dressings used in this study were supplied as 7cm x 10cm sheets having a thickness of approximately 0.15mm. The sterile dressings, intended for one time use, were stored either at room temperature in a lyophilized (i.e., dry) state or refrigerated in a fully hydrated state. At the time of application, the WMD was cut to size, slightly larger than the wound, and the lyophilized form was hydrated with sterile saline after placement. The lyophilized form had preferred handling characteristics to the fully hydrated form. However, both forms were evaluated to determine if their effects on wound management differed in any way from each other. Wounds presenting with clinical signs of infection were treated with antibiotic therapy prior to or coincident with the initial application of WMD. A secondary absorbent film dressing was applied after placement of the WMD to further protect the healing environment and to maintain good contact with the wound bed, although the WMD, particularly the dry form, was immediately adherent to the wound. Necessity of repeat applications of the WMD was determined for each wound based on the amount of dressing observed on the surface of the wound and the extent of epithelialization at each change of secondary dressing.

The simplicity of application of the WMD allowed for patients enrolled in this pilot study to be treated at a variety of clinical sites, including long-term care facilities, a wound care facility treating outpatients, or in home care. Occasionally patients were instructed to reapply the dressing by themselves between visits.

Patients were selected based on the following broad inclusion criteria: greater than 18 years of age and presence of at least one partial-thickness wound. Exclusion criteria included life expectancy less than five months, concurrent adjunct treatment modalities, such as whirlpool treatment or electrical stimulation to target wound, uncontrolled diabetes, and known allergy or cultural/religious objections to porcine products. Wound assessments were made at baseline and weekly or more frequent intervals depending upon wound severity, frequency of dressing changes, and stage of healing until wounds were considered healed. Additionally, the patient’s response to treatment and the condition of the WMD were evaluated.

A total of 15 patients were evaluated (8 men, 7 women) with an average age of $72 \pm 19$ years (Table 3).

<table>
<thead>
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<th>Table 3. Patient demographics and wound characteristics</th>
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<td><strong>Patient Demographics</strong></td>
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<td>Women</td>
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<td><strong>Wound Characteristics</strong></td>
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<td>Trauma or Drug-Induced</td>
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<td>Foot</td>
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<td>Lower Leg</td>
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<td>Wound area (cm$^2$)</td>
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(Figure 5E) and switched to the stored dry form of WMD because of the obvious exudate accumulation beneath the dressing and because of the greater ease of application for the patient. During the evaluation in the third week, the wound was observed to be completely epithelialized (Figure 5F).

With respect to clinical events observed during the course of the study, two patients treated with WMD stored hydrated developed infections; one of these required hospitalization. Both of these patients had venous ulcers with a history of recurrent cellulitis. There was no sign of infection in patients treated with WMD stored dry, but a second patient had CHF (in addition to the death due to CHF mentioned above), and one patient had a chemotherapy related drug toxicity which, on further exploration by the oncologist, was determined to be the cause of the initial leg ulceration. However, there was no evidence of dressing-induced toxicity, clinical signs of rejection of the biomaterial-based dressing, or sub-dressing seroma as the wounds progressed to full healing.

**Discussion**

This pilot study of the use of a new dermal wound dressing made from SIS biomaterial demonstrated that the WMD was easy to apply (particularly the dry storage form), was nontoxic, and did not induce an adverse immunological reaction even in patients given repeated applications. The results also demonstrated that placement of WMD on various nonhealing skin wounds and ulcers resulted in initiation and complete epithelialization of the wound. The complete epithelialization of wounds treated with dry stored WMD versus the partial epithelialization with the hydrated stored form indicated that, for dermal applications, the dry form was more effective. These observations and initial clinical findings are consistent with the known properties of the biomaterial, derived from the submucosa of porcine small intestine, and from which the dressing was prepared. Results indicate that WMD can be used to successfully manage acute and chronic partial-thickness wounds due to its excellent protective properties and ability to act as a natural template for tissue regrowth.

Dressings derived from acellular ECM tissues are likely to provide environments well suited for the body's wound repair mechanisms. The mechanism of action of this ECM biomaterial appears to be linked to its basic tissue-like composition and architecture. SIS biomaterial retains the three-dimensional architecture created by the fibroblasts in vivo. ECM architecture has been demonstrated to be a critical component of tissue development and necessary for regenerative wound healing. The three-dimensional architecture of the SIS biomaterial is built upon the complex composition of various collagens, proteoglycans, and glycosaminoglycans, which provide the structural integrity, flexibility, and elasticity appropriate for dermal wound covering and subsequent epithelialization. In wound healing studies, the SIS biomaterial served as a scaffold for rapid vascularization and cellular invasion. Both of these processes are necessary to provide nutrients and signals in support of dermal regeneration and epithelial cell proliferation. In addition, the presence of multiple growth factors, each having a significant role on the stimulation and regulation of tissue regeneration, is likely an important component contributing to the wound healing properties of the biomaterial.

With ECM biomaterials, the structural integrity of the matrix provides a barrier to dehydration and infection, the regulatory factors provide the signals necessary for the propagation of new and healthy tissue, and the native tissue architecture provides a stable structure for cell attachment, proliferation, and differentiation. Together these elements were expected to combine to provide an exceptional environment to apply to dermal wounds. In light of this, the initial clinical results demonstrating the utility of the ECM based dressing are completely consistent with this expectation.

Several additional characteristics of the SIS biomaterial provide an advantage to any wound care product developed from it. First, the biomaterial is prepared from a porcine tissue, which is abundantly available being a by-product of the meat packing industry. Second, the SIS is minimally processed to provide a cell-free, sterile biomaterial, which retains many biological properties. The absence of severe, complicated, or highly technological processing means that production costs will be lower. Development of lower cost wound care alternatives is becoming increasingly important due to projections, which indicate that the patient population over 65
years of age will increase much faster than the general population. These patients require longer treatments for poorly healing wounds. Third, the facilitated epithelialization with the biomaterial results in a tissue that has minimal scarring. This is especially relevant to dermal wounds where cosmetically acceptable appearance is often as important as functional restoration. In fact, in two recent studies in which SIS biomaterial was placed into in-vivo models of growing animals, the SIS biomaterial was able to provide functional tissue replacement even as the tissue was growing with the host animal.38,66 Such

**Figure 4.** Healing of partial-thickness skin wound using WMD. A) Wound measurement at initial visit. B) Placement of lyophilized WMD onto the ulcer. The matrix adheres easily to the wound. C) Partial incorporation of the WMD into the center of the wound. There was no need to remove the matrix after incorporation had begun. D) The wound is prepared for a second application of lyophilized WMD. E) Reapplication of the matrix. F) The wound was completely epithelialized by week four.
preliminary reports suggest that SIS biomaterial based wound care products can have even broader applications to restoring functional tissue. Finally, because the biomaterial is almost completely composed of proteins and carbohydrates, all of which are basic components of the extracellular matrix of all species, there are no anticipated problems with biocompatibility. Therefore, the wound care and other medical products derived from this biomaterial are likely to rapidly become accepted among

Figure 5. Case study of patient treated initially with WMD stored hydrated and switched after two weeks to the lyophilized form. A) Initial observation of nonhealing venous ulcer (1.5cm x 0.5cm) on the lower leg. B) Evaluation after one week of treatment. C) After cleansing the wound area was measured. D) Second application of hydrated WMD to the upper wound. The lyophilized WMD that was placed onto the lower wound ulcer is also visible. E) Second week evaluation before wound cleansing and switching upper wound to WMD stored lyophilized. F) Final visit, one week later, the wound was completely epithelialized.
healthcare professionals.

SIS biomaterial has been recently developed into several other medical products, which are sterile biomaterial devices‡ intended for use as soft tissue reinforcements. These products are implanted into low-stress and high-stress body systems, respectively, and provide the additional strength and support necessary to proper functioning of body tissue. Other nondermal applications for the SIS biomaterial, which are already in clinical evaluation, include urological, gynecological, and orthopedic uses.

This initial clinical study had several limitations related to it being a pilot study. Progress in wound management was monitored only out to 12 weeks, and none of the wounds were large in surface area. Patients were not defined by any specific pathophysiology (e.g., diabetes), and only partial-thickness wounds were addressed in this study. WMD has recently been cleared by the FDA for full-thickness wounds, and fully controlled clinical studies for partial- and full-thickness wounds are now in progress.²⁶,⁶⁸

There are few limitations to the WMD produced from SIS biomaterial, one of which is that a patient with known sensitivity or with cultural and religious objections to porcine materials will not be able to be treated with the product. A second limitation is that even though the biomaterial has limited porosity and provides a well-hydrated environment for wound healing, it is not a moisture barrier. Therefore, the wound covered by WMD must be protected by an appropriate secondary dressing to avoid wound dehydration.

Conclusion

Criteria that define dressings optimal for the treatment of wounds with the goal of facilitating rapid, pain-free, regenerative healing are directing researchers and clinicians towards biological-derived dressings. The development of effective biological-derived dressings previously has been limited by complications of immunologic rejection and risk of disease/infection transfer. Problems of insufficient structural integrity or, in contrast, insufficient elasticity and flexibility have also hampered the use of biomaterials as wound dressings. A new biomaterial derived from the submucosal portion of porcine small intestine is not limited with these complications and has been used successfully in pre-clinical studies of wound healing. This SIS biomaterial now has been developed into WMD. In this pilot clinical study, this new wound matrix was found to have similar outcomes as the biomaterial when applied to partial-thickness wounds in humans. These promising results justify further evaluation of this biological-derived matrix for its effectiveness toward the treatment of full-thickness wounds.

*OASIS® Wound Matrix (Cook Biotech, Inc., West Lafayette, Indiana)
** Alloderm® (LifeCell Corp., Branchburg, New Jersey)
*** Dermagraft® (Smith & Nephew Inc., Largo, Florida)
† Apligraf® (Novartis Pharmaceuticals Corp., East Hanover, New Jersey)
‡ SURGISIS® and SURGISIS® ES (enhanced strength) (Cook Biotech, Inc., West Lafayette, Indiana)

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