Review Article
Biochemical and biomechanical characterization of porcine small intestinal submucosa (SIS): a mini review

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Abstract: Porcine small intestinal submucosa (SIS [Oasis®]) is an acellular, biological extracellular matrix (ECM) that has been found to significantly improve the healing of difficult-to-heal or chronic wounds in humans. Like dermal ECM, SIS contains collagen, elastin, glycosaminoglycans, proteoglycans, and growth factors that play important roles in healing. Preclinical studies have shown that numerous cell types attach to SIS, proliferate and migrate into the matrix, and differentiate. In addition, SIS can reduce the activity of matrix *metalloproteinases* (MMPs)—endogenous proteolytic enzymes whose levels and activities are increased in chronic wounds. Compared to the original single-layer SIS, multi-layer SIS has stronger mechanical properties and is more slowly degraded in wounds. Together, these SIS products provide flexibility in the selection of biologically-active ECMs that may be useful for the repair of diverse wound types.

Keywords: Small intestinal submucosa, SIS, extracellular matrix, burn, chronic wounds

Introduction

Chronic wounds affect millions of Americans and cost the country more than $50 billion annually [1, 2]. These wounds can dramatically reduce quality of life, causing pain, impairing mobility, interfering with everyday activities, and, in some cases, leading to amputation [3-5]. Although standard of care is effective for some patients, 40-60% of chronic ulcers remain unhealed after 3 or more months of treatment [6, 7].

The molecular environment of chronic wounds exhibits numerous changes that impede healing. High levels of inflammatory cytokines are associated with chronic inflammation [8], and high levels of matrix *metalloproteinases* (MMPs) degrade extracellular matrix (ECM) [9, 10]. Degradation of the ECM interferes with the dynamic and reciprocal cell-ECM interactions that are essential in every step of wound healing.

Porcine small intestinal submucosa (SIS)—an acellular, biological ECM (see **Figure 1**)—has been applied to chronic wounds in an attempt to address the ECM deficits and stimulate the cell-ECM interactions that are necessary for healing to occur. In randomized, controlled trials, SIS has been found to significantly increase the percentage of wounds healed and healing rate compared to standard of care alone [11-13]. These clinical findings are buttressed by a plethora of preclinical research investigating the biochemical and biomechanical features of SIS. In this review, we summarize the preclinical findings related to the basic properties of SIS, comparing the single-layer and triple-layer SIS constructs where data are available.

Structural and biochemical properties of SIS

The structural properties and biochemical composition of SIS have been well studied, as documented in **Table 1**. Porcine SIS ranges in thickness from 0.05 to 0.22 mm [14] and has a variable, porous microstructure with pores ranging from 20 to 30 μm [15, 16] that enables the oxygen diffusion necessary for maintaining cell proliferation and viability. Androjna *et al*. determined the oxygen diffusion coefficients of SIS to be 7×10^{-6} - 2×10^{-5} cm²/s [17]. The same analyses showed that the oxygen diffusion coefficients of engineered ECMs, human dermis (Alloderm®) and canine fascia lata were

Figure 1. SIS material stretched after being wetted.

Table 1. Structural Properties and Biochemical Composition of Porcine SIS

Property	Description
Thickness	0.05 to 0.22 mm [14]
Ultrastructure	Pores varying from 20 to 30 μm [17]
Mechanical strength [40]	1 layer: 20.6 MPa (dry) and 7.2 MPa (wet)
	2 layer: 24.7 MPa (dry) and 18.8 MPa (wet)
	3 layer: 31.2 MPa (dry) and 22.0 MPa (wet)
	4 layer: 36.7 MPa (dry) and 22.4 MPa (wet)
Suture Retention [40]	1 layer: 0.5 N (dry) and 0.7 N (wet)
	2 layer: 1.3 N (dry) and 2.6 N (wet)
	3 layer: 2.1 N (dry) and 4.1 N (wet)
	4 layer: 2.8 N (dry) and 5.2 N (wet)
Oxygen diffusion coefficient	7×10^{-6} - 2×10^{-5} cm^2/s [17]
Fiber structure	Single, continuous preferred fiber direction, parallel to the long axis of the intestine [41]
Biochemical composition	Collagen [18, 21]
	Elastin [18]
	Fibronectin [19, 20]
	Laminin [20]
	Glycosaminoglycans and proteoglycans [21, 22]
	Fibroblast growth factor-2 (FGF-2) [27]
	Transforming growth factor-beta1 (TGF-ß1) [26]
	Vascular endothelial growth factor (VEGF) [27]

matrix, have been identified in SIS. Additionally, SIS contains glycosaminoglycans and proteoglycans [21, 22] that provide cell attachment and growth factor binding sites, sequester matrix-degrading enzymes, and enhance cellular infiltration into injured tissue [23-25]. SIS has also been reported to release growth factors including fibroblast growth factor-2 (FGF-2), transforming growth factor-beta1 (TGF-β1), and vascular endothelial growth factor (VE-GF) [21, 26, 27].

Mechanisms of SIS effects on cellular growth and differentiation

In vivo, cells and ECM exhibit continuous, bidirectional interactions that have been termed dynamic reciprocity [28]. These interactions occur in response to alterations in the cellular microenvironment and under homeostatic conditions, as well as during tissue repair, angiogenesis, embryogenesis, and malignant transformation [29]. For example, changes in the microenvironment (e.g., due to tissue damage) may induce changes in cellular gene expression which then alter the extracellular matrix leading to further alterations in gene expression and cytoskeletal properties.

Cells interact with endogenous ECM largely through integrin binding. The mechanism by which cells interact with SIS presumably also involves integrin binding. Hodde *et al.* evaluat-
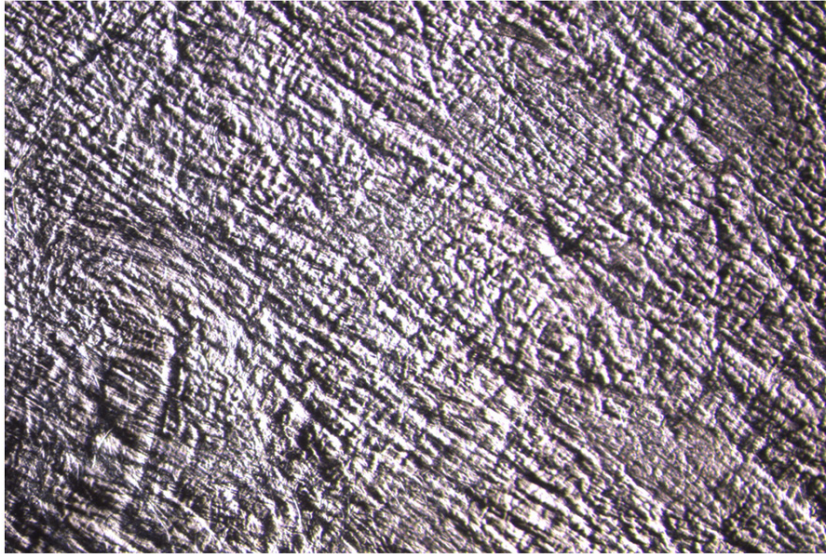
1.9 - 3.1×10^{-5} cm^2/s and 1.6 - 4×10^{-5} cm^2/s [17].

Like dermal extracellular matrix, SIS is primarily composed of type I collagen fibers (see the fibrous construct in **Figure 2**), but also contains minor amounts of elastin and collagen types III, IV, and VI [18]. Multidomain glycoproteins such as fibronectin [19, 20] and laminin [20], which mediate cell adhesion to the extracellular

Figure 2. Microstructure of SIS material (Optical microscopy, 10X, dry).
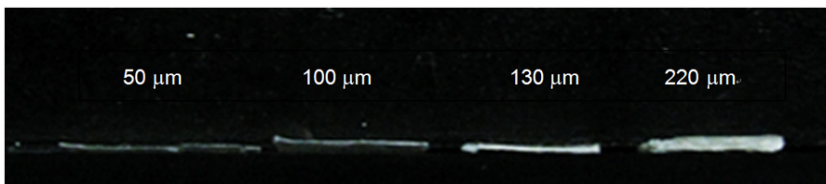

50 µm   100 µm   130 µm   220 µm

Figure 3. Section pictures showing thickness comparison for one, two, three, and four layer SIS constructs (dry).

GF blocked the ability of SIS to induce tube formation from human microvascular endothelial cells in a fibrin-based angiogenesis assay [33].

Matrix metalloproteinases interacting with SIS

Various studies have reported the presence of high MMP levels and activity in chronic wound fluid [35, 36]. Excess levels of MMPs found in chronic wounds are believed to be a key contributor to wound chronicity; thus, reducing elevated levels of MMPs in chronic wounds may promote healing.

Clinical evidence supporting this hypothesis was provided by Trengove and colleagues

ed the peptide sequences within fibronectin that may be important in adherence of cells to SIS [30]. Results with human microvascular endothelial cells showed that the RCG and REDV played a role in the attachment to SIS, suggesting that the binding of integrins to these sequences in SIS may mediate cell attachment. Badylak *et al*. found that attachment of human microvascular endothelial cells to SIS depended on both its composition and architecture [31]. In addition, Hurst *et al*. reported that neutralization of beta 1, beta 4, and alpha 6 integrins altered the adhesion of bladder cancer cells to SIS [32], suggesting the importance of these integrin subunits in cell attachment.

Hodde *et al*. has conducted several studies to investigate the release of growth factors from SIS and resultant effects on cell differentiation and angiogenesis *in vitro* [33, 34]. A neutralizing antibody against FGF-2 inhibited the differentiation of PC12 cells induced by SIS, [34] raising the possibility that FGF-2 may contribute to the biological response to SIS *in vivo*. Similarly, neutralizing antibodies against VE-

[35], who reported that levels of proteases decreased as chronic venous ulcers began to heal, and by Ladwig and colleagues [37], who reported that optimal healing of chronic pressure ulcers correlated with low values of the ratio of MMP-9/tissue inhibitor of metalloproteinases-1 (TIMP-1). Thus, control of MMP levels may improve the poor healing conditions found in chronic wounds.

Shi *et al*. conducted several studies to investigate the interaction between SIS and MMPs [38]. MMP-1, MMP-2, and MMP-9 displayed different binding affinities, indicated by a loss of activity in solution upon incubation with SIS of 53.8%, 85.9%, and 36.9%, respectively, over 24 hours. A human keratinocyte cell migration study was conducted to evaluate the effects of MMPs and SIS-treated MMP solutions on cell migration. These data indicated that MMPs inhibit keratinocyte migration in vitro, and that the inhibition can be significantly reduced by pre-incubating the MMP solution with SIS. Similar activity was seen *in vivo* using a diabetic mouse wound healing model. Biopsy samples

were collected on days 3 and 7 following wounding for analysis of MMP levels by gelatin zymography. MMP activity was found to be maximally attenuated by SIS treatment on Day 3. On Day 7 the attenuation became less significant, indicating that the MMP binding ability of SIS had become saturated [38]. Overall, results with these models indicate that SIS reduces MMP activity immediately after application and reduces the inhibitory effects of MMP-1, MMP-2, and MMP-9 on keratinocyte migration.

Enzymatic degradation study of SIS with different thicknesses

To enhance the utility of SIS in different clinical situations, the SIS constructs have been designed with different thicknesses. These constructs consist of 1, 2, 3, or 4 layers of SIS matrix (see **Figure 3**). An *in vitro* study was designed to determine the relative degradability of the four SIS thicknesses in a proteolytic solution containing collagenases, neutral protease, and other proteases designed to mimic a wound environment [14]. After incubation for two hours at 37 °C, the residue dry weight of SIS was used for calculating the level of degradation.

Results demonstrated that degradation of the product was inversely proportional to the number of layers [14]. The 1-layer matrix was about 82% digested under the test conditions. The 2-layer matrix lost 75% of its weight, slightly less than the one layer matrix when tested under the same conditions. Significantly less degradation was found for the 3-layer (49%) and 4-layer (47%) matrices, indicating that the higher number of layers will likely increase the material's durability and reduces its susceptibility to enzymatic degradation. However, the thickness effect became less significant as seen between 3 and 4 layer materials, indicating that at certain levels of thickness, the degradation will saturate.

Durability of SIS in pig wounds and diabetic mouse wounds

SIS materials become incorporated into the wound bed over time, with some wounds requiring repeated applications to achieve closure. Based on the results obtained in proteolytic solutions, multiple-layer SIS would be expected to persist longer in the wound bed than single layer SIS.

The incorporation of different-thickness SIS materials in vivo was evaluated in porcine and mouse wound pilot studies [39]. In the pig model, 1-layer SIS and 3-layer SIS were applied to burn wounds after debridement. Four days after application, the 1-layer SIS was visibly incorporated while substantial unincorporated 3-layer SIS matrix remained. All wounds formed a soft eschar surface layer over the course of the study making accurate visual evaluation of incorporation impossible later in the study.

Biopsy samples were taken from two wounds for each treatment, 10 and 17 days after SIS application. Histology demonstrated that 1-layer SIS had incorporated at the wound surface by day 10, while 3-layer SIS samples still exhibited distinct layers not yet incorporated into the wound bed. At day 17, all four SIS-treated samples displayed some degree of reepithelialization, while one of two control samples exhibited no reepithelialization [39].

In the diabetic mouse model, 1-layer SIS and 3-layer SIS were applied to full-thickness wounds on the day of wounding and 3 days later. Inspection of the wounds over time demonstrated that the 1-layer SIS largely or fully incorporated into treated wounds, while the 3-layer SIS had significant unincorporated layers [39]. These studies suggest that one benefit of increasing SIS matrix thickness may be the need for less frequent application.

Comparison of mechanical properties between SIS with different matrix layers

Mechanical properties of the 1-, 2-, 3-, and 4-layer SIS constructs were also compared [40]. The thickness of one layer is approximately 50 μm. The ultimate tensile strength, elastic modulus, elongation, and suture retention strength were determined in both wet and dry conditions. When tested in dry conditions, peak strength increased with the number of layers (**Table 1**). However, as the number of layers increased, the more the material elongated. The elastic modulus differed little between the SIS materials, suggesting that they all have similar material stress-strain characteristics.

Compared to the dry test, the wet peak stress was lower for all materials, with the 1-layer SIS

sample being significantly lower than the multi-layer samples [40]. However, no statistically significant difference was found between the multi-layer samples. Under wet conditions, elongation was inversely related to number of layers and peak stress. The 1-layer SIS elongated the most (~130%), while all multi-layer SIS elongated 30-40%.

Elastic modulus under wet conditions also differed from the dry results. A higher modulus was measured for each increasing layer. For the suture retention test, the wet conditions provided higher retention strength. Both wet and dry samples displayed the same trend that more layers in the material resulted in greater suture retention strength (**Table 1**).

Conclusions

SIS is a well-studied biomaterial that significantly improves the healing of chronic wounds [11]. As a naturally-occurring ECM, SIS supports the adherence, proliferation, migration, and differentiation of numerous cell types. The bioactivity of SIS includes releasing growth factors, minimizing the destructive activity of MMPs, and inducing angiogenesis to support new blood vessel in growth. SIS is biodegradable and can be well incorporated into tissue. Recently, studies have demonstrated that the new multi-layer SIS has stronger mechanical properties and is more slowly degraded in wounds than single-layer SIS. Thus, the multi-layer SIS combines the known beneficial properties of SIS with increased durability to meet the needs for repair of diverse tissues and wounds.

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Disclosure of conflict of interest

None.

Trademark statement

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