mesynthes

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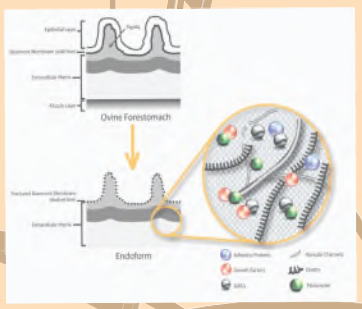
Abstact: We have generated a new biologically-derived extracellular matrix (ECM) termed 'Endoform', to meet existing needs within wound care and emerging needs for tissue regeneration scaffolds. A novel tissue processing procedure has been developed to render the ECM of ovine forestomachs acellular, while conserving its inherent biological and physical properties. As such, Endoform retains a biologically rich collagen matrix, and importantly, a host of co-factors that are critical for cellular growth and tissue regeneration. Small angle X-ray scattering (SAXS) demonstrated that OFM retains a native collagen architecture (dspacing=63.5±0.2 nm, orientation index=20°). The biophysical properties of OFM were further defined using ball-burst, uniaxial and suture retention testing, as well as a quantification of aqueous permeability. OFM biomaterial was relatively strong (yield stress=10.15±1.81 MPa) and elastic (modulus=0.044±0.009 GPa). Lamination was used to generate new OFM-based biomaterials with a range of biophysical properties. The resultant multi-ply OFM biomaterials had suitable biophysical characteristics for clinical applications where the grafted biomaterial is under load.



Introduction

Synthetic meshes (e.g. polypropylene) have historically been used in a number of clinical applications where the graft must support load, for example, herniaplasty. However, the suitability of synthetic meshes has been under scrutiny in light of the increased infection and rejection rates associated with these materials. Consequently, there has been a move towards native biomaterials, particularly those composed of decellularized extracellular matrix (dECM), for example human acellular dermis (HAD, Alloderm®, LifeCell Corporation - New Jersey, USA) and acellular small intestinal submucosa (SIS, Cook Biotech – Indiana, USA). The clinical uptake of dECM-based biomaterials has been such that it is expected that these biomaterials will account for more than 50% of the market value of implantable meshes by 2011 1. These dECM-based biomaterials are prepared from suitable source tissues that are decellularized and delaminated, typically by exposure to detergents, to yield an intact dECM 2. dECM-based biomaterials retain the complexity of native tissue ECM, with an intact collagen framework consisting of structural proteins, as well as, associated cell signaling and adhesion molecules 3. In this way, exogenous dECM can stimulate, support and host cell colonization of the tissue deficit leading to regeneration. No chronic inflammatory response is observed with native dECM-based biomaterials instead, the observed inflammatory response is one associated with constructive remodeling, whereby the biomaterial is degraded and replaced by a new collagenous framework as part of normal tissue remodeling 4,5. Increased vascularization, as well as, reduced scar tissue and capsule formation have been noted with the use of dECM-based biomaterials following implantation. Importantly, due to their biophysical properties, either inherent or engineered, these biomaterials can physically bridge large tissue deficits to allow tension free repair of adjacent tissues and therefore replace synthetic meshes in certain clinical applications.

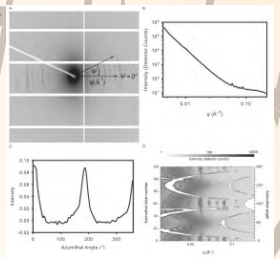
Biomaterials must be engineered with suitable biophysical properties to allow successful clinical use. For example, hernia grafts must withstand the load bearing stresses exerted by the contents of the abdominal cavity, retain sutures used to secure the graft, and provide sufficient elasticity to mimic natural tissue movement. Numerous technologies have been developed to modify the biophysical properties of collagen-based biomaterials to match certain clinical applications. Chemical crosslinking (e.g. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) or glutaraldehyde) is one such approach that covalently crosslinks adjacent collagen fibers to increase matrix density, strength and persistence. However, confidence in this approach has decreased due to the chronic inflammatory response that is associated with crosslinked biomaterials 4,5. Other approaches have attempted to introduce new biophysical properties to collagen scaffolds by combining or layering biomaterials such that the properties of the hybrid biomaterial benefits from the properties of the individual components. For example, composite biomaterials comprising urinary bladder matrix/PGA 6, heparinized poly(vinyl alcohol)/small intestinal submucosa 7, collagen/hydroxyapitate 8, collagen/hyaluronan/chitosan 9, collagen/silica 10 and collagen/polypropylene 11, have been reported. While the introduction of synthetic components to tune the physical properties of collagen-based biomaterials can be effective, these advantages may be offset by the risk of introducing non-native components that may not undergo constructive remodeling. Additionally, in order for these hybrid biomaterials to properly serve as templates for regeneration they must adequately recapitulate structural features of native ECM.

As part of efforts directed at developing materials as biomimetics of native ECM, a dECM termed ovine forestomach matrix (OFM) has been developed for applications in tissue regeneration, including the treatment of chronic and acute wounds and in the form of implantable grafts for soft tissue reconstruction. OFM comprises the decellularize propria submucosa isolated from ovine forestomach tissue. Previous studies 12 have shown that OFM is primarily composed of collagens I and III, and retains the collagen microarchitecture of native ECM. Additional ECM-associated macromolecules (e.g. fibronectin and fibroblast growth factor basic) were also present in OFM, as were remnant basement membrane components (e.g. laminin and collagen IV) on the luminal surface and in native vascular channels. These secondary molecules, working in concert with the collagenous framework, were shown to support cell attachment, infiltration and stimulate cell differentiation [11]. OFM was developed as a means of addressing shortcomings with existing dECM-based biomaterials. Namely, the disease risk associated with porcine, bovine and human-sourced dECM, the cultural and religious objections to collagens sourced from these raw materials, and the limited biophysical properties of current dECM-based biomaterials. The aim of the current study was to understand the physical properties of OFM, and a series of new laminated OFM biomaterials whose properties were tunable through a process of lamination.

Small Angle X-ray Scattering (SAXS)

Lyophilized samples of OFM were characterized using small angle X-ray scattering (SAXS) according to the method described in Basil-Jones et al 13. Single SAXS images were taken from a spot 80 x 250 µm in size. SAXS diffraction patterns were recorded on the Australian Synchrotron SAXS/WAXS beamline, utilizing a high-intensity undulator source.

SAXS analysis of OFM provided insights into the structure and orientation of the collagen fibrils. The periodicity in the collagen overlaps is apparent with the rings in the scattering pattern (Figure 1.A) which, when integrated around all azimuthal angles showed distinct peaks representing the collagen overlap spacing (Figure 1.B). d Period spacing is the measurement of the collagen fibril repeating unit, termed axial periodicity. The average d period was obtained from the q position of the peaks in Figure 1.B, and was measured in OFM as 63.5±0.2 nm. Information about larger collagen structures was gained from the lower q region (<0.03 Å-1). The slope at low q for the OFM SAXS profile (Figure 1.B) is q-3.7 which corresponds quite closely with a Porod's law slope (of q-4) and indicates that distinct boundaries exist between the collagen fibrils, which is another indicator of native collagen structure. The scattering pattern observed from the higher q ranges (0.04 – 0.1 Å-1) showed the non-isotropic nature of the collagen fibrils present in OFM (Figure 1.B). This non-isotropy is apparent in the significantly higher intensities of the Bragg peaks in the azimuthal region Ψ=150°–210°, and 330°–30°compared to Ψ=60°–120° and 240°–300° showing the orientation approximately through 0° to 180°. The range of orientations in the collagen fibrils was determined by plotting the azimuthal angle versus relative intensities for a Bragg peak. A single 5° meridional arc at q0.06 Å-1 represents only one Bragg peak, and the variation in intensity of this peak through 360° provides information on the orientation of collagen (Figure 1.C). OFM showed only one preferred fibril orientation that goes through approximately 0° and 180°. The fibril orientation index (OI), defined as the minimum angle which contains 50% of the fibrils, was calculated as 20°.



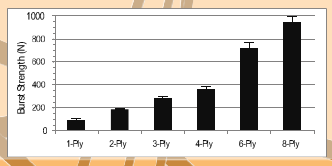
Lamination

Thicker OFM biomaterials were created by lamination either via lyophilization, with or without additional sewing, or through adhesion of lyophilized OFM sheets using a collagen gel. All lamination procedures used a perforated stainless steel tray that allowed adequate vapor flow from the multi-ply laminates during lyophilization. Lamination via lyophilization proceeded as follows: a sheet of wet OFM was laid flat on a perforated stainless steel surface. Additional sheets of wet OFM were added to the top of the first to create a multi-laminate stack. Care was taken to remove any air bubbles between each of the sheets. The stack of wet OFM sheets, up to 8 sheets in total, was freeze-dried according to proprietary procedures. The lyophilized laminates were sewn using polyglycolic acid (PGA) absorbable suture, 4-0 or 5-0 gauge (Foosin Medical Supplies - Shandong, China). OFM laminates were additionally created using a collagen gel prepared from powdered OFM. OFM was powdered using a spice grinder and liquid nitrogen. The powdered material was placed in phosphate buffered saline (PBS) 10% w/v and heated at 90 °C for 1.5 hours, then centrifuged at 4K rpm for 20 minutes. The supernatant was shown to reversibly gel on cooling, and thus the gel was maintained at 37 °C during application. The collagen gel was applied as a continuous layer (approximately 25-100 µm thick) to a single sheet of lyophilized OFM. A second layer of lyophilized OFM was applied to the layer of gel and this procedure repeated to build up a laminate of the desired thickness (up to 8-ply). Care was taken to remove any air bubbles between each of the sheets. The multi-

Ball-Burst Strength

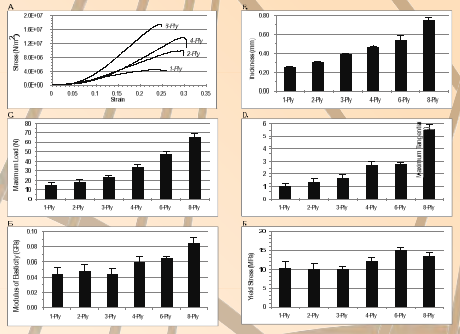
Materials were tested using an adaption to the ball burst method described in ASTM D 3787-07 "Standard Test Method for Bursting Strength of Knitted Goods, Constant-Rate-of-Traverse (CRT) Ball-burst Test" 14. A materials testing machine was equipped with a 1 kN load cell and fitted with a custom built ball-burst compression apparatus consisting of an orifice and a 25.4 mm stainless steel ball. Test samples measuring approximately 10 x 10 cm were centered over the orifice and clamped in place. The stainless steel ball was pushed through the test material at a constant feed-rate of 305 mm/min and the compression load of failure was recorded.

The ball-burst test measured resistance to force applied equally in all directions. When considering implanted biomaterials, the ball-burst test may be considered more predictive than the uniaxial strength test, as the test load is distributed in all directions across the surface of the biomaterial. In comparison, uniaxial strength determines load at failure in one direction only. Under ball-burst testing, the 8-ply OFM biomaterial had a maximum burst strength of 941.89±48.75 N, while single-ply OFM was significantly weaker, with a maximum burst strength of 92.84±12.73 N



Uniaxial Strength

Materials were cut with a die to 'dumbbell' shaped samples with widths of 6 mm and 25 mm along the gauge length and specimen ends, respectively. The specimen ends were fixed to the grips of a materials testing machine, ensuring a grip-to-grip distance of 75 mm. The samples were tested to failure, whilst the tensile load was measured using a 100 N load cell and a constant feed-rate of 25.4 mm/min. Maximum tangential stiffness was calculated from the slope of the load (N) versus elongation (mm). The load versus elongation curve was transformed to a stress (N/m2) versus strain curve, using the cross-sectional area calculated from the thickness and the width of the sample. The slope of this latter curve at its linear transition was used to calculate the modulus of elasticity, or Young's modulus (GPa).



A comparison of the uniaxial strength properties of the OFM biomaterials is presented in Figure 3, and Supplementary Table 1. A representative bimodal stress-strain curve derived from the elongation versus force curve is shown in Figure 3.A. Stress-strain curves were characterized by a 'toe region', a transition through a linear elastic deformation region and a well-defined yield point. There was good agreement between the relative strengths of biomaterials tested under uniaxial and ball-burst methods. As expected, increasing the thickness (Figure 3.B) of the biomaterial by increasing the lamination state significantly increased the maximum load (Figure 3.C). For example, the maximum load at failure of 8-ply material was 65.13±4.37 N, while the single-ply material was 15.07±2.68 N. Yield stress (Figure 3.F) was determined by normalizing the maximum load to the cross sectional area of the sample. Yield stress was approximately 10 MPa, irrespective of the thickness. This is to be expected given that the material composition of the laminates did not change with increasing the lamination state. There was no increase in the observed yield stress of the 2-ply (9.77±1.68 MPa) material relative to the single-ply (10.75±1.81 MPa) material suggesting that the OFM determined the strength of the biomaterial rather than the collagen gel used during lamination.

Lamination did not increase the maximum elongation (approximately 20 mm), but the maximum tangential stiffness increased between the single- and multi-ply laminates (Figures 6.D). The modulus of elasticity (Young's modulus) is an intrinsic property and describes the tendency of a material to be deformed elastically. The modulus increased between the single and multi-ply biomaterials (Figure 6.E). For example, the modulus of elasticity of the single-ply material was 0.044±0.009 GPa, while the 8-ply material was 0.085±0.006 GPa. The observed increase in the modulus and the increase in the maximum tangential stiffness suggest that the thicker biomaterials (e.g. 6- and 8-ply) prepared with collagen gel would be less elastic than thinner OFM biomaterials laminated using this approach.