Regenerative Medicine:
Harnessing the Intrinsic Power of the Human Body

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The concept of harnessing the intrinsic biological power of the human body goes back to the first half of the 20th century; however, it was only in the 1990s that significant research activities emerged. These were the "go-go" years, and although in the first part of this century there was a downturn, activities have rebounded with research not only continuing to advance, but with the state of our knowledge accelerating. As far as what has been translated to the "bedside," i.e. to patients, the cell-based products/treatments are largely the skin substitutes; however, there are a number of acellular approaches. Today there are 100s of products/treatments in various stages of development by companies. These range from ones that are cell-based to scaffolds without cells to genetic engineering approaches. What has emerged as critical to any approach is the ability to deliver the right biological signals to the right place and at the right time. The real question is how to do this? Also, what are right signals, not a single biological signal, but the combination necessary to foster repair and regeneration? Once the community achieves this understanding, the applications are limitless.

Role of Matrix and Cell Dynamics in Heart Valve Health and Disease

This presentation summarizes our evolving understanding of heart valve dynamics in both health and disease, using the aortic valve as the paradigm. Heart valve tissue is composed of architecturally and functionally organized, mutually interacting, and biomechanically responsive extracellular matrix (ECM) and specialized cells, which actively regulate valve structure and behavior. Two types of cells predominate: superficial *valve endothelial/endocardial cells* (VECs), and mesenchymal *valve interstitial cells* (VICs), deep to the surface. These cells have some similar and some unique properties relative to endothelial and mesenchymal cells in other locations. The cells and ECM which VICs secrete and are surrounded by are distributed in three histologically and mechanically distinct layers: the *fibrosa* (containing densely packed collagen providing strength through locking of rigid fibrils in the closed phase) on the outflow side, the *ventricularis* (containing prominent elastin, which provides the contraction of the valve cusps during opening) on the inflow side and the *spongiosa* (containing glycosaminoglycans [GAGs] and proteoglycans (providing a cushioning and lubricating layer for rapid ECM reorganization when the valve opens and shuts), separating the other layers. Since individual collagen fibers are incompressible, cuspal shape and size changes throughout the cardiac cycle are accommodated by extension and restoration of collagen fibril crimping (i.e., pleating) and changes in alignment. In diastole, the crimp of the fibrosa collagen fibers is flattened, and the fibers align, thereby locking the structure with the cusps apposed to avoid regurgitation; simultaneously, elastin in the ventricularis is stretched. In systole, when the cusps open, the elastin fibers contract (i.e., return to their resting state), and the collagen fibers in the fibrosa restore their crimp and reduce their alignment. Importantly, aortic valve cusps have approximately 30% greater area in diastole than systole. Thus, the valvular microstructure is dynamic and highly sensitive to biomechanical forces throughout the cardiac cycle.

Valve structure and properties are also dynamic over an extended time scale, and cell-ECM interactions regulate valve structure function in development, homeostasis and disease. During cardiac development the endocardial cells lining the lumen surface become activated and express special properties that regulate their invasion into underlying tissue through a process called *endothelial (*or *endocardial) to mesenchymal transformation* (EMT) as VECs transform into VICs involving key growth factor and transcription factor molecules, including bone morphogenetic protein 2 (BMP2), transforming growth factor-beta (TGF-β), fibroblast growth factor-beta (FGF-β), Notch transcription factors and Wnt/β-catenin. During fetal development and intrauterine maturation, human valves have indistinct ECM layers with a rudimentary trilaminar architecture emerging in the 3rd trimester; moreover, the adult configuration only fully develops after birth. Mutations of critical genes involved in regulating cardiac development are associated with congenital valve abnormalities in experimental animals and humans. In addition, the cell and matrix composition of valve tissue function continue to evolve throughout life, largely regulated by biomechanical and hemodynamic forces. Indeed, valvular responses to stress and other injury likely involve both down- and up-regulation of various genes and their products in the valve cells (including many of those which were active during development).

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Development of a SIS Regenerative Heart Valve; From Benchtop to Clinical Trial

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Tissue engineering has offered the promise of developing a perfect replacement heart valve. This valve would avoid the problems associated with current forms of mechanical and biologic replacements such as the need for anticoagulation, thrombus formation, or structural deterioration.

The deficiencies in recent tissue engineered approaches will be highlighted while underscoring the biologic mechanisms required for the formation of a normal functioning living valve replacement.

The preclinical development of a SIS engineered valve required an altered strategy that relied not only on flow loops but on combining embryologic principles and finite element analysis. These studies will be reviewed with an emphasis on the failures and successes in light of an understanding of the cellular mechanisms involved in regenerating a valve substitute.

The preclinical animal studies exposed other deficiencies that required an alteration in the structure while maintaining basic principles of ECM remodeling. The result is a valve substitute that undergoes adaptive regeneration.

The clinical experience has been substantial and a review of this as well as the results of the current clinical trials will be presented.

Development of chemically stabilized acellular cardiac valve scaffolds and in vivo testing in a sheep right ventricular outflow tract model

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Background and Objectives: The main treatment for valvular disease consists in their surgical replacement with artificial devices. Current alternatives are either mechanical or biological valves, both of which have limited life spans and are associated with thrombogenicity (mechanical valves) or degeneration due to inflammation and calcification (biological valves). Acellular xenogeneic valves have been tested in large animals with promising outcomes. However recent reports showed that these scaffolds are invariably infiltrated by host cells, including activated myofibroblasts which initiate chronic fibrotic reactions within the cusp matrix and induce cusp retraction and valvular stenosis, limiting their long-term in vivo performance.
The goal of this study was to evaluate whether chemical stabilization of acellular valve scaffolds would alleviate these shortcomings. We chose penta-galloyl glucose (PGG), a polyphenolic agent which binds strongly to collagen and elastin components via strong, non-covalent bonds and reduces their in vivo degradation and calcification and diminishes cell infiltration.
Methods: We first generated fully acellular porcine aortic roots in a purpose-designed, automated cyclical perfusion system using detergents and enzymes. We also separately decellularized fresh porcine pulmonary valve roots using an immersion technique. All valve scaffolds were then stabilized with penta-galloyl glucose (PGG). Decellularization efficacy and extracellular matrix integrity were validated using biochemical and histological methods, biaxial biomechanical tests and valve hemodynamics evaluated in an Aptus Valve bioreactor. Valves were then mounted within PGG-treated acellular pericardial conduits and implanted in the right ventricular outflow tract (RVOT) of juvenile sheep as RV to PA shunts with full pulmonary valve ligation (n=6 per group). Sheep were followed up for 6 months by echocardiography and histologic analysis was performed on explanted valves. Two additional sheep were allowed to survive beyond 6 months and are currently being monitored for long-term outcomes (>1 year).
Results and Discussions: All animals had favorable post-operatory evolution with rapid recovery and without immediate complications or early deaths. All sheep underwent normal physiologic growth without signs of pulmonary or cardiac failure and valve leaflets were found with preserved structure and mobility and minimal regurgitation, lacking macroscopic signs of fibrous overgrowth, thrombi, inflammation or calcification. Histological and immunohistochemical analysis of explanted cusps showed complete absence of infiltrating cells and lack of calcification or micro-thrombi at 6 months in juvenile sheep. These results are very promising since historically glutaraldehyde-fixed valves calcify heavily at 6 months in this model and non-stabilized acellular valve scaffolds undergo fibrotic cusp retraction.
Conclusions: Acellular, PGG-stabilized, xenogeneic cardiac valves are reliable, non-immunogenic, non-thrombogenic and non-calcifying scaffolds with excellent hemodynamics and long-term stability. These could represent excellent alternatives to existing artificial valves.
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Acellular Cardiac Extracellular Matrix-Silk Patches for Cardiac Repair post-Myocardial Infarction

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Over the last few decades, a number of different scaffold formulations and configurations have been developed to repair the heart in both young and old patients. Natural biomaterials offer important cues to the cells in tissues through integrin-mediated signaling, but they are often more difficult to control in terms of their mechanical properties and structural organization, both of which are important in the heart. Moreover, the use of singular extracellular matrix (ECM) proteins in the context of cardiac repair may be too simplistic given the complex mixture of proteins present in the ECM in vivo. Recently, adult porcine left ventricular ECM has demonstrated the ability to promote functional repair upon injection following myocardial infarction (M) via both stem cell homing and increased vascularization and it is currently in Phase I clinical trials. However, the ability to tune the mechanics and degradation time of solubilized cardiac ECM is limited. In addition, while the use of complex ECM is an important step towards promoting a more physiological signaling environment to the cells present in the scar tissue during post-MI remodeling, the use of cardiac ECM (cECM) from the homeostatic adult organ may not promote the most robust regenerative response. Indeed, our group has previously shown that there are significant changes to the complex composition of cECM during normal and pathological cardiac development and that these changes alter the ability of cells to sense the stiffness of their environment while also modulating important cell functions including the proliferation of cardiomyocytes and paracrine signaling of stem and progenitor cells. In addition, we have previously developed hybrid silk-cECM sponges for cardiac repair that demonstrated improved cardiac cell phenotypes and better cell infiltration in vivo when compared to silk-collagen sponges or silk scaffolds alone. In this study we sought to assess whether the use of an acellular hybrid silk-cECM patch could minimize pathological remodeling and maintain cardiac function following MI. We also wanted to determine whether cECM derived from an earlier developmental time point (e.g. fetal), would lead to a better response as compared to adult cECM. Our data indicate that silk patches are able to be generated with fetal porcine cECM and that these patches are able to be sutured onto the heart. Functional data thus far demonstrates that fetal cECM containing silk patches are able to prevent further functional decline following MI, which does not occur with any other group (adult cECM-silk patches, silk only patches or sham controls). Ongoing studies will continue functional analysis out to 10 weeks post repair (including hemodynamic analysis) and subsequent tissue analysis via immunohistology, LC-MS/MS proteomics and PCR/ Western blot to determine how the hybrid silk ECM patches influence the remodeling response across the different experimental groups.

Metabolic End Products of Absorbable Bioscaffolds for Soft Tissue Repair; Are They Helping or Hurting Us?

Robert G. Martindale, MD, PhD

Material used for reconstruction soft tissue defects has recently become a major focus in surgery. In the USA nearly 400,000 non-inguinal hernia repairs were done in 2013.Many done in high risk patients with known risk for major complications such as infection, fistula and recurrence. As the risk and cost of complications of involving permanent mesh is becoming more apparent the entire landscape is changing with resorbable bioscaffold materials gaining interest for use in the repair of soft tissue defects. Recently not only has the absorption characteristics of mesh being considered but the actual biochemical makeup and local metabolic consequences of these materials has become of interest. In most cases these resorbable materials have shown reliable bio-compatability, mechanical strength and stability in in-vivo human trials. In the clinical setting the most commonly used bioabsorbable mesh materials are polyglycolic acid:trimethylene carbonate (PGA:TMC), Glycolide, lactide, trimethylene carbonate, and Poly-4-hydroxybutyrate (P4HB). The metabolic end products of these compounds have various effects on the native tissue. In the case of P4HB with the metabolic end product of butyrate which has recently been reported to alter macrophage class differentiation with the shift from M1 to M2. This shift may explain some of the current finding of a low infection rates and rapid incorporation of this mesh in clinical studies.

The synthetic bioabsorbable meshes offer compelling early clinical results and may offer a significant advantage over the biological matrix materials currently in use for soft tissue reconstruction in the high risk patient. Taking advantage of the biologic end products ability to enhance remodeling and decrease the risk of complication has yet to be fully explored but offers an exciting avenue for exploration.

Use of Biodesign after chest wall resection in children: Our experience in two cases

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Objective

To the describe our experience with chest wall replacement using an 8-ply Biodesign® mesh after tumor resections in children.

Methodology

A retrospective description of two young children operated for a Hamartoma of infancy/pleuropulmonary blastoma and a local recurrence of pleuropulmonary blastoma using Biodesign as replacement of the defect in the thoracic wall.

Result

Patient 1: A girl, were a solid mass on the right side of thorax is diagnosed at birth. Biopsy is preformed showing a benign lesion (Hamartoma of infancy or Infantile fibromatosis). Resection of the intrathoracic component was performed at 2 months of age due to rapid growth. She is reoperated 60 days later due to fast recurrence and one rib is resected together with part of latissimus dorsi, serratus anterior and subcutaneous tissue. To get tissue between the skin and the lung, a 8-ply biodesign mesh were sewn over the defect. The abdominal rectus muscle, which was released during the resection was attached to it´s lower edge. She recovered well, the histology showed suspected pleuropulmonary blastoma and chemotherapy was given.
After three years of follow-up she is desease free and she has no scoliosis.
Her chest wall on the right side is not moving on flouroscopy, but her parents consider her healthy.

Patient 2: A 3 year old boy is diagnosed with a tumor in his right lung after a period of cough and dyspnea. He was operated with a lobectomy of his right lower lobe, were the tumor was situated. After finishing his chemotherapy, a mass appeared on the inside of his chest wall which was positive on PET.
A chest wall resection of about 10 x 10 cm next to the spine was performed. The gap was covered with titan ribs and an 8-ply Biodesign® mesh, before the latissimus dorsi and skin was closed.
After 6 months he began to develop scoliosis, concave to the operated side. After 9 months a recurrence in the posterior mediastinum developed which was treated with chemotherapy and resection through a sternotomy. At the operation, the tissue in the replaced part of the chest wall was firm.
6 Months after the second operation the patient is without clinical recurrence and his scoliosis is operated with growing rods.

Conclusion

Tumors in the chest wall, often require large resections in order to be radical. When more than two ribs are resected, the gap has to be replaced to avoid flail chest.
Replacement with Biodesign, has in these two cases developed into firm connective tissue, which seems to grow with the patients.
Biodesign has not shown to promote tumor growth in an animal study. Allthough there has been one recurrence, it was not situated in the replaced area. The resection was performed on a recurrent malignant desease, which elevates the risk of further micrometastases and can give rise to further macroscopic recurrenses in the future.
The replaced part seems to grow with the patients which makes it attractive to use in growing individuals.
Further development of biomatrixes should aim for the reformation of functional tissue.

Title: The Use of Urinary Bladder Matrix for Body Wall Repair in Multiple Preclinical Models

Authors: Adam Young, Nicole Ehrhart, Juan Martin Riganti, Alejandro Nieponice, Thomas Gilbert

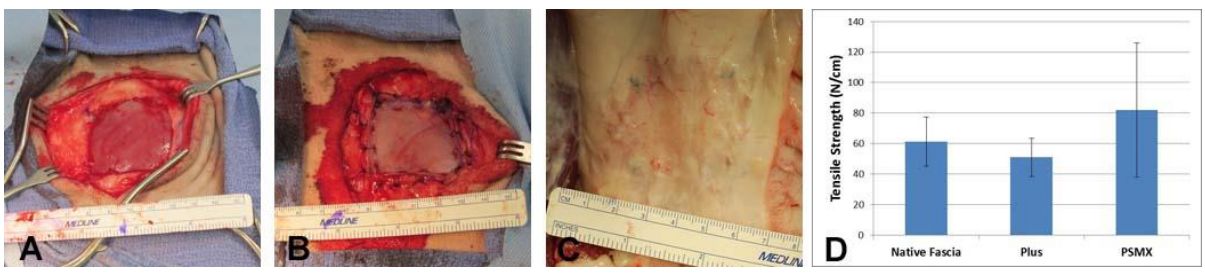
Urinary bladder matrix, or UBM, is a form of decellularized extracellular matrix isolated from the luminal layers of the porcine bladder. This ECM-based material contains an epithelial basement membrane and has been shown to facilitate cellular and vascular ingrowth, thereby encouraging site-appropriate constructive remodeling of the surrounding tissue. A multilaminate, vacuum pressed version of UBM has been developed to act as a surgical mesh for the reinforcement of soft tissue where weakness exists. The purpose of this work was to evaluate the remodeling and mechanics of 6-layer (MatriStem® Surgical Matrix PSMX) and 8-layer (MatriStem® Surgical Matrix Plus) UBM devices in various preclinical models of body wall repair.

An ovine model of a full thickness fascial defect was first utilized to examine the mechanics of remodeled tissue after 3 months *in vivo*. Briefly, a 4 cm x 4 cm defect was created in in the left tunica flava abdominis fascia and the left fascia lata (**A**) and replaced with a similarly sized piece (**B**) of MatriStem Surgical Matrix (either PSMX or Plus). After three months *in vivo,* each device had been replaced by vascularized connective tissue that largely resembled the surrounding fascial tissue (**C**) and had been completely infiltrated by cells. There was no evidence of inflammation, swelling, or seroma at the site of device placement. Histological analysis showed no fibrosis, encapsulation, or chronic inflammatory response. The tensile strength of the remodeled tissue at the defect site was similar to native fascial tissue (**D**). A slight trend toward increased thickness was also noted for remodeled tissue compared to native fascial tissue, however, no statistical difference was observed (data not shown).

Additional preclinical studies were conducted to specifically evaluate the remodeling of UBM in more clinically-relevant models. The first porcine model created a surgical defect in the diaphragm to mimic a hiatal hernia that was then repaired with sutures. A piece of MatriStem Surgical Matrix PSMX was then used to reinforce the primary repair. After two months *in vivo*, these animals were seen to have much more robust, vascularized tissue deposition at the defect site compared to control animals that only received a primary repair without the reinforcement device. Mechanical analysis showed that reinforcement of the primary repair also resulted in an increased load at failure and increased tissue stiffness compared to non-reinforced controls.

A second pilot preclinical model was developed to evaluate parastomal hernia repair in pigs. In this model, a colostomy was created and additional bowel was pulled through the surgical opening to simulate a parastomal hernia. The hernia was then repaired and reinforced with MatriStem Surgical Matrix Plus using either a keyhole technique or a Sugarbaker technique. Interestingly, in the animals that received Sugarbaker repairs, the device was completely replaced with newly formed connective tissue over the stoma site with no evidence of stricture in the bowel at one month. The keyhole repairs also showed constructive remodeling of the device around the stoma site with no evidence of stricture, although one animal did have a hernia recurrence at one week post-op requiring repair.

As seen in these studies, the urinary bladder matrix devices were constructively remodeled into site appropriate tissue and maintained the mechanical integrity of the native tissue during remodeling, thus highlighting the positive impact of UBM devices in the reinforcement of body wall repairs.



Characterization of a Biologically Derived Graft for Nipple-Areolar Complex Reconstruction

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There exists a need for a reproducible off-the-shelf ready graft for Nipple – Areolar Complex (NAC) reconstruction. There are more than 2.8 million breast cancer survivors in the United States, many of who have undergone reconstructive surgery. Approximately 36% of patients with early stage diagnoses and 60% of patients with late stage diagnoses undergo mastectomies. Moreover, immediate breast reconstruction following mastectomies has become more common, significantly increasing at from 20.8% in 1998 to 37.8% in 2008. This increasing trend is not surprising as there is evidence to suggest that NAC reconstruction affects psychological wellbeing of female patients who have undergone mastectomies. Evidence also suggests that woman are more comfortable with getting a mastectomy if the nipple can be spared during the mastectomy procedure, or if nipple reconstruction is possible if a nipple-sparing mastectomy is not an option. Current strategies for NAC reconstruction are limited to surgical techniques that create a NAC-like structure from existing local tissue, secondary site grafting, or 3D tattooing, or a combination of surgical reconstruction with tattooing. Generating a tissue engineered, biocompatible NAC graft for use in place of surgically created NAC structures is a promising approach to NAC reconstruction following mastectomies.

To date, no tissue engineering strategies have been targeted towards the regeneration of the NAC structure. By isolating a biological-derived collagen-rich acellular NAC scaffold, the NAC native micro and macro structural components are maintained. Once derived, NAC scaffolds would be onlay engrafted onto the patient, allowing for host resident-skin cells to repopulate the NAC scaffolds.

Using a Rhesus Macaque Non- Human Primate tissue model, it has been demonstrated that biological scaffolds were reproducibly derived from native tissues with maintenance of extracellular matrix (ECM) proteins and cellular adhesion molecules, with effective removal of nuclear material—significant reduction of DNA. This study details the in vitro material and biological characterization of ECM and cell-ECM interactions through: gene expression of stem cell differentiation profile and percentage of proliferation/ apoptosis of reseeded cells, histological analysis, and protein quantification/analysis.

Neuroprotective Potential of Biologic Scaffolds in Acute Stroke and Human Translational Feasibility: A Neurosurgeon's Perspective

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Despite decades of promising research, there is no medical therapy for restoring functionality of human brain tissue affected by stroke. Injectable extracellular matrix (ECM) gel delivered to the site of cerebral infarction in rat brains decreases the neuroinflammatory response to acute stroke and may afford neuroprotective potential for the penumbra by influencing the microenvironment. While the timing and dosage of therapy requires more study, this therapeutic approach has strong potential for clinical benefit and is clinically feasible for humans suffering from acute stroke.

Mechanisms of action of a myocardial matrix hydrogel for treating myocardial infarction

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The incidence of heart failure following myocardial infarction (MI) has been on the rise in recent decades due to increased use of revascularization therapies, necessitating the development of therapies to prevent progression to heart failure following MI. Our lab previously developed an injectable hydrogel derived from decellularized porcine myocardial extracellular matrix (ECM) and showed that injection of this biomaterial alone is capable of halting the post-infarction progression of negative left ventricular remodeling and the decline in cardiac function in both small and large animal MI models, which led to recent initiation of a clinical trial in post-MI patients. While these results makes the myocardial matrix hydrogel a promising therapy for patients post-MI, the process by which the therapeutic benefit occurs is not well understood. To better understand the mechanisms of action, we performed an unbiased whole transcript microarray analysis on the gene expression changes within the infarcted myocardium three and seven days post injection of the myocardial matrix hydrogel compared to saline in a rat ischemia-reperfusion MI model. These time points were chosen since there is significant cell infiltration into the material, yet they are prior to complete material degradation at approximately three weeks. Quantitative PCR confirmed expression of key genes and histological quantification further confirmed activation in altered pathways. Principal component analysis of the transcriptomes showed that samples collected from myocardial matrix injected infarcts were distinct and clustered separately from saline injected controls by one week post-injection. Pathway analysis indicated that these differences are due to changes in several tissue processes that may contribute to improved cardiac healing post-MI. Matrix injected infarcted myocardium exhibited an altered inflammatory response, reduced cardiomyocyte apoptosis, enhanced infarct neovascularization, diminished cardiac hypertrophy and fibrosis, altered metabolic enzyme expression, increased cardiac transcription factor expression, and progenitor cell recruitment. The results indicate that the myocardial matrix alters several key pathways post-MI creating a pro-regenerative environment, and further demonstrates its promise as a potential post-MI therapy.

A Role for Versican in Engineered Tissues: Modulating Elasticity and Inflammation

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Versican is a large chondroitin sulfate (CS) proteoglycan that is found in the extracellular matrix (ECM) of most soft tissues. Through its negatively charged CS chains, its interaction with a number of ECM components and its ability to act as a reservoir for cytokines and growth factors, versican influences the ability of cells to proliferate, migrate, adhere and assemble an ECM. Normally, versican is present in low amounts, but increases dramatically when tissues become inflamed. There are at least four different alternatively spliced variants of versican which differ in the size of the core protein and the number of attached CS chains. While the V0 and V1 variants bear CS chains and are the major forms that accumulate and promote events associated with inflammation and disease, the V3 variant lacks CS chains and is not elevated in disease to any significant extent. We and others have found that V3 acts as a dominant negative by reducing the CS-carrying isoforms of versican that accumulate in tissue, thus inhibiting the effects of V0/V1 on cell phenotype and the inflammatory response. In particular, we have shown that blocking the accumulation of V0/V1 in vascular smooth muscle cell (SMC) ECM, via blocking antibodies, antisense or over-expression of the V3 isoform, has pleiotropic effects on cell phenotypes, including the reduction of proliferation and migration *in vitro* and reduction of neointimal thickening *in vivo*. Reduction of V0/V1 promotes production of an ECM enriched in organized elastic fibers and depleted in hyaluronan (HA), which resists monocyte adhesion *in vitro* and macrophage accumulation *in vivo*. We have specifically demonstrated that V3 expression in SMCs regulates transforming growth factor β (TGFβ) signaling to promote elastogenesis, and blocks epidermal growth factor receptor (EGFR) and nuclear factor κ B (NFκB) activation to suppress HA accumulation and vascular cell adhesion molecule 1 (VCAM1) expression. Using microarray analysis, we found that V3 expression in SMCs affected expression of 521 genes by more than 1.5-fold. Among the most upregulated genes were components of actin cytoskeletal networks which also serve as markers of contractile SMCs, and the most downregulated genes included components of the complement system and chemokines crucial for regulating inflammatory processes. Successfully modulating tissue elasticity and inflammation are major challenges in tissue engineering making the manipulation of versican a potentially valuable tool. Indeed, we have shown that engineered blood vessels expressing V3 have enhanced structural integrity and higher resistance to burst. In a bi-layer skin graft application, forced expression of V3 or versican antisense slowed growth, decreased versican V1 expression, enhanced elastin expression and/or deposition, and increased mechanical integrity of the graft *in vitro*. Studies are currently planned to determine the *in vivo* inflammatory response to engineered tissues in which versican expression has been modulated. Taken together, our findings suggest that reduction of V0/V1 versican isoform accumulation in tissues reprograms cell phenotypes to promote pro-elastogenic/contractile and anti-inflammatory pathways and will be a useful approach in the development of cellularized biological scaffolds for use in tissue engineering.

Defining the Device to Tissue Transition in Fetal Bovine Acellular Dermal Matrix

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Introduction

Extracellular matrix (ECM) scaffolds derived from decelluarized tissue are being researched for tissue engineering, and a subset are currently finding use in human clinical applications. SurgiMend, derived from fetal/neonatal bovine dermis, is commonly employed in plastic and reconstructive surgeries and abdominal wall reconstructions. Pre-clinical and clinical studies have demonstrated that under standard conditions, this ECM biomaterial does not remodel by the known standard mechanisms, as defined by the tissue engineering community at large. It is neither quickly degraded via hydrolysis or macrophage-mediated mechanisms, nor encapsulated like permanent synthetic materials. This unique integration process remains ill-defined, and the parameters that affect it have yet to be fully elucidated. Therefore, the goal of this study was to better understand the mechanisms by which this scaffold interacts with host tissues in an animal model, and to apply this understanding to clinical case examples.

Methods

Clinical case reports were retrospectively reviewed under IRB exemption from the author's clinical experience in complex underlay ventral hernia repair in cancer patients. Patients with more than 4 year follow-up that received routine CT-imaging as part of cancer surveillance were included. Pre-clinical data were collected via a rat intraperitoneal implant model consisting of 2 x 2 cm squares of SurgiMend placed intraperitoneally and secured to the abdominal wall with permanent suture. Animals were randomized to undergo injury (debrided peritoneum) or control (uninjured) conditions and further subdivided into tight or loose approximation with the abdominal wall. Gross observation and histological evaluations were conducted at 5 week explantation.

Results: Clinical Case Series

CT-imaging and follow-up of more than 4 years was available on 3 patients. A thick tissue was still present on follow-up imaging in all patients for several years, confirming that rapid degradation did not occur. This tissue shape and thickness was modulated with time in all patients suggestive of matrix reorganization. In one patient, these tissues were not observed to be adherent to the abdominal wall lateral to the suture line. In another patient, a biopsy was obtained during reoperation for cancer recurrence at 4 years post-op. Pathological evaluation determined it to have features of dense connective tissue including fibroblasts and collagen.

Results: Intraperitoneal Implant Model

The area of adherence between the SurgiMend and host tissue increased with both injury and with approximation. The area and rate of revascularization increased with injury but not approximation. Vascularization preferentially invaded the matrix in areas of injury, being proximal to the sutures without peritoneal debridement, or in in all planar areas where SurgiMend remained in contact with injury (debrided peritoneum). The revascularization continues uniformly into the matrix based on as yet uncharacterized signaling. Host collagen degradation and deposition occurs following repopulation with host cells and vasculature.

Conclusions

Integration of SurgiMend to the abdominal wall in this animal model requires three distinct processes: adherence, vascularization, and remodeling/replacement. Additionally, these integration processes appear to correlate with limited long-term patient examples. Further understanding of the contributions of each one of these process may improve our ability to use these scaffolds in regenerative medicine applications.

A novel bioactive component of biologic scaffolds: Implications for tissue repair and regeneration.

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Biological scaffold materials derived from the extracellular matrix (ECM) have been successfully used in a variety of tissue engineering/regenerative medicine applications both in preclinical studies and in clinical applications. Although it is recognized that ECM-scaffolds materials have constructive remodeling properties, the biological mechanisms by which functional tissue restoration is achieved are not completely understood. Separate from the mechanical and structural functions of ECM-scaffolds, the molecular components of ECM have been extensively investigated for their ability to confer bioactivity. These molecular components include the "ligand landscape" or the repertoire of growth factors and cytokines stored within the matrix; structural molecules such as collagen and laminin that provide a substratum for cell attachment; and the formation of matricryptic peptides derived from the degradation products of the parent ECM. However, there is legitimate controversy concerning not only the relevant importance of these biochemical and structural components, but also the potential influence that the decellularization, disinfection, and sterilization methods used during the manufacturing process confer on their biological activity.

Here, we provide a new perspective on the biological activity of ECM-scaffolds by introducing the novel concept of ECM-embedded microvesicles. Microvesicles (exosomes) are 50-100nm cell-derived membranous particles that act as protective carriers for biologically active signaling molecules (e.g. microRNAs, proteins, enzymes and lipids), thereby shielding their cargo from degradation and denaturation. Formerly identified almost exclusively in body fluids, the presence of microvesicles within the interstitial matrix of connective tissue has not been previously reported. Using transmission electron microscopy we have identified microvesicles embedded within decellularized ECM-scaffolds, and also reveal that these ECM-embedded microvesicles are incorporated directly into the collagen network of the matrix itself. Importantly, we demonstrate that ECM-embedded microvesicles can be only be separated from the matrix after enzymatic digestion of the ECM-scaffold material, a process similar to the functional activity attributed to the degradation of implanted biologic scaffolds used in clinical applications. These findings suggest that ECM-embedded microvesicles are only available for cellular uptake during matrix remodeling events, such as those that occur during normal physiologic processes like wound healing and mechanical stress, or during degradation initiated by the host response to implanted biologic scaffolds. The technique of enzymatic digestion coupled with high speed ultracentrifugation has subsequently allowed for isolation and purification of microvesicles from biologic scaffolds and downstream interrogation of their molecular cargo. Next generation sequencing and proteomic profiling have revealed that ECM-embedded microvesicles contain miRNA and protein molecules capable of exerting dramatic phenotypical and functional effects as assessed by *in vitro* cell culture studies on macrophage polarization and stem cell differentiation. These preliminary findings strongly suggest that microvesicles are an integral and functional component of the extracellular matrix and are impervious to the deleterious effects of the decellularization process. Furthermore, we hypothesize that given their location within the interstitial matrix, ECM-embedded microvesicles may represent a unique class of exosomes with biological properties and functions distinct from circulating exosomes. Based upon these findings, we postulate novel perspectives for the implementation of ECM-embedded microvesicles in tissue engineering and regenerative medicine applications.

Extracellular Matrix Organization and Function

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The mechanical and functional properties of any tissue are largely determined by the extracellular matrix (ECM) network laid down during development. The ECM is designed to function as a large heteropolymeric network that provides structural support and informational signals to its associated cells. To organize structures as large and complex as the ECM, cells have developed a unique assembly process that is built around moving relatively large polymeric building blocks through the intracellular compartment to sites of assembly in the pericellular space. Synthesis and assembly of the ECM is under tight developmental control where cells produce a limited set of ECM components appropriate for their place and time in development. The instructional signals for controlling ECM protein expression are inherent in the ECM itself, where the composite material interacts directly with specific signaling receptors, regulates growth factor availability and provides mechanical signals that influence gene expression networks. Our work uses mouse genetics and high resolution electron microscopy to elucidate how the ECM is synthesized, organized and how the individual ECM components work with each other to maintain tissue integrity and function.

Where the Wild Things Are: Perivascular Regulation of Disseminated Tumor Cell Dormancy and Chemoresistance

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In a significant fraction of breast cancer patients, distant metastases emerge after years or even decades of latency. How disseminated tumor cells (DTCs) are kept dormant, and what wakes them up, are fundamental problems in tumor biology. To address these questions, we use metastasis assays in mice and zebrafish and have determined that the perivascular niche of distant sites like the lung, bone marrow, liver and brain regulate DTC dormancy. We have developed organotypic microvascular niches to specify that endothelial cells regulate breast cancer cell growth, and applied proteomics to identify endothelial-derived mediators of DTC dormancy. More recently, we have begun to explore whether the perivascular niche confers therapeutic resistance to DTCs. I will present data that suggests strongly that the perivascular niche regulates therapeutic resistance of DTCs in a manner that is independent from its role in regulating DTC growth. Our goal is to uncover these mechanisms to guide strategies to eradicate dormant DTCs without affecting their growth status. We believe this will result in a viable strategy to eliminate these metastatic seeds and prevent metastatic recurrence.

Urinary Bladder Extracellular Matrix Inhibits Tumor Formation

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Every year, approximately 1.5 million individuals are diagnosed with cancer in the U.S. Many of the most prevalent cancer types, such as breast, colon, and melanoma, are treated by surgical tumor resection in greater than 95% of cases. However, the deficits generated by these surgeries impair tissue function and cosmesis. Therefore, therapies that promote both tissue restoration and inhibit cancer recurrence are necessary. Tissue derived extracellular matrix materials provide a pro-regenerative environment, however their effect on cancer growth has not been fully explored.

A commercially available ECM particulate composed of porcine urinary bladder matrix (UBM) was obtained from ACell Inc. (MicroMatrix) to characterize the effect of ECM on tumor formation *in vivo*. B16-F10 mouse metastatic melanoma cells were mixed with UBM particles or saline only, and injected subcutaneously into C57BL/6 mice. Tumor growth was monitored, with histologic evaluation after 7 days and at tumor volumes of 200 mm^3 and 3,000 mm^3. Immune phenotype of tumors and nearby lymph nodes was evaluated by flow cytometry and qRT-PCR, respectively. B16-F10 tumors spontaneously developed by 7-9 days with saline delivery, and rapidly grew to 3,000 mm^3 by 21 days. Injection with UBM significantly delayed initial growth and increased survival time from 21.2 days to 28.8 days ($P<0.05$). Although initial tumor growth lagged with UBM injection, growth rate was not affected once tumors reached approximately 100 mm^3. Histologically, UBM elicited a robust mononuclear cell response after 7 days, without B16-F10 proliferation clusters seen in saline controls. Tumor clusters eventually formed at the UBM border (16-18 days). UBM affected the local immune environment in lymph nodes with increased expression of *Il4* (4.7 fold) and *Tnfa* (1.4 fold) relative to saline. UBM co-injected tumors also had reduced IL4ra expression on regulatory T cells, which are implicated in suppressing immune activation and promoting tumor growth. Contact between tumor cells and UBM was necessary for delayed growth, and intraperitoneal UBM injection, rather than subcutaneous co-injection, did not affect tumor formation. However, subcutaneous UBM injection 4 days after B16-F10 inoculation delayed tumor formation in 60% of animals.

In summary, UBM was found to have an inhibitory role in tumor formation *in vivo* corresponding with an alteration of local immune environment. Close proximity between the tumor cells and UBM was necessary for this phenomenon, indicating that it is suitable for tissue reconstruction following tumor resection.

MSCs: How They Work and Why (Some Surprises)

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Adult marrow-derived Mesenchymal Stem Cells (MSCs) are able to differentiate into a number of skeletal phenotypes when properly induced in cell culture. These characteristics caused many investigators, me included, to consider MSCs to be multipotent and that this reflected their in vivo functionality. Quite the contrary, for the most part, MSCs do not function as skeletal tissue progenitors. Indeed, EVERY tissue has its own tissue specific progenitor that is, in part, controlled by MSCs at sites of tissue injury. The new and surprising logic is that MSCs are derived from perivascular cells, pericytes, and function at sites of injury and inflammation to initiate and direct the regenerative process. Moreover, these sentinels of injury and invasion have powerful effects on cells of the immune system and, unexpectedly, MSCs produce powerful anti-bacterial proteins to further protect sites of injury. The total shocker is our recent discovery that pericytes/MSCs PULL melanoma cells into the stroma of marrow; the molecular details this sequence of events can be used as platform to select drugs that inhibit the process of metastasis. Time, thousands of published scientific papers, and pursuit of information all team up for a new view of the MSCs.

Developing a standard approach to evaluating the decellularization of biomaterial ECMs

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Considering the growing use of decellularized extracellular matrix (dECM) products for medical use, there is a related need to develop a standard approach to evaluating decellularization processes and/or the products of decellularization. A draft document describing an approach to decellularization assessment has been developed in ASTM committee F04 by a group of industry and academic professionals. Due to varied and often superficial decellularization assessments, the wealth of publications using dECM scaffolds can be difficult to compare. In addition, limited conclusions can be drawn to precisely link the effect of decellularization with the intended result of reducing adverse immune responses. The content and localization of DNA or cell nuclei are often reported, but often without justification of appropriateness and without the context of other cell remnants, damage to ECM, or the persistence of detergents and other reagents.

The approach presented is intended to guide both industry and academic decellularization efforts. It includes recommendations for critical quality attributes, instructions for analyzing the process steps, guidance on developing decellularization specifications, and descriptions of common testing methods. The described evaluation allows the assessment of three objectives: removal of cellular materials, preservation of ECM, and limiting the persistence of reagents or contaminants. While quantification and localization of DNA are recommended measurements of decellularization, analyses of other cell remnants, such as phospholipids or intracellular proteins, are also suggested. The document does not attempt to define decellularization limits or thresholds; not only is there insufficient evidence for such parameters, but these would also require consideration of the intended use. Instead, guidance is provided which discusses important considerations in the establishment of decellularization targets.

The Agony and the Ecstasy of Getting into the Clinic

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The journey of taking a new idea from its initial inception, through to clinical trials with good efficacy, is exciting, surprising, and oftentimes fraught. Biological arterial replacements have been under study for roughly 40 years, but few concepts for replacement arteries have survived pre-clinical testing to be subsequently studied in patients. In developing any new therapeutic tissue, critical attention must be paid to target tissue composition, mechanics, remodeling capacity, and immunogenicity. Through the development process for the tissue product, paying careful attention to where prior technologies have failed, and to the real-life medical realities of the disease under treatment, are paramount. This talk is a description of a journey to take an idea that was – initially – completely clinically inapplicable, and to then turn it into something that may one day be useful for humans.

Tissue engineered vascular grafts are grown in vitro and then decellularized. These vessels have been tested in a set of preclinical models of arterial replacements, including coronary artery bypass grafting and arteriovenous conduits. The acellular vessels exhibit excellent mechanical properties prior to implant, comparable to those of native human vein and artery. Furthermore, the mechanical properties of the grafts (suture retention and burst pressure) strengthened after implantation. Based on the successful and robust preclinical data sets, regulatory bodies both in Europe and the United States have approved first-in-man testing of these bioengineered blood vessels for hemodialysis access. To date, 60 grafts have been successfully implanted in patients as a vascular access graft for use in hemodialysis, with most patients out past two years of implant, and some past 3 years. All implanted grafts have been suitable for hemodialysis access with excellent flow rates, with no evidence of structural degeneration or immunologic rejection. Four to eight weeks after implantation, vessels are cannulated with large-bore needles thrice per week, for regular dialysis therapy. Despite frequent cannulation, grafts maintain structural stability, implying an ability to remodel and “heal” within the patient after injury.

This emerging technology is poised to be a real alternative to conventional synthetic vascular prostheses, particularly for large-diameter (eg. 6-mm or greater) applications. For smaller-diameter applications, novel covalent approaches to shield luminal collagen from blood elements may render these grafts suitable for lower blood flow applications, such as distal peripheral artery and coronary grafting.

Immune Control of Cardiac Repair

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The adult mammalian heart does not retain the robust repair capacity of the neonate and gradually loses its regenerative potential, generally attributed to the loss of adequate cell replacement and persistent inflammation. Although stem cells hold promise for treating chronic diseases and contributing to tissue replacement in acute cardiac injury, the regenerative capacity of stem cells in many tissues is influenced by regulatory networks orchestrated by the immune system, which are responsible for the removal of damaged cells and providing a cohort of important growth factors and signaling molecules. Immune cells modulate new vessel growth, supply essential nutrients to damaged tissue and mediate scarring by regulating inflammation and fibroblast action.

Using combination of genetic manipulation and pharmacological intervention we have modified the profile of immune cell infiltration, which can facilitate or prevent cardiac regeneration in mouse and axolotl, an efficiently regenerating member of the urodele amphibian family. Transition from wound healing to regeneration and restoration of tissues relies on complex interactions between stem cell progenitors and immune cell subsets that confer both growth potential and immune tolerance. We are currently correlating the dramatic variability in cardiac regenerative capacity across recombinant inbred mouse strains with distinct differences in immune composition and response, to uncover new cell targets for clinical intervention.

Multiscale Properties of ECM Scaffolds

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All metazoan cells have relied upon an extracellular matrix scaffold to guide and support development, growth, regeneration, and repair. At the largest scale, the ECM determines overall body form and function through the development of biomaterial properties ranging from 1 to $1x10^9$ pascals. Collagens, proteoglycans, elastic components, mineral crystals and matricellular proteins interact with one another and constituent cells to facilitate a fabulous range of functional adaptations, many of which are determined by the proportions, arrangements, and crosslinking of the various components. The combination and arrangement of scaffold ECM molecules is part of the continuum between the nuclear transcriptional machinery and the physical environment. During development, complex interactions between cell populations and morphogens determines the fine-scale arrangement of the ECM scaffold in an organ- and tissue-specific manner. The combined effects of ECM chemistry, morphology and mechanical properties dictate many aspects of cell fate. During regeneration, participating cells are able to recapitulate scaffold reorganization. In contrast, repair of damaged scaffolds often leads to an imperfect, fibrotic template that prioritizes mechanical stability over physiological renewal. The native ECM scaffold is both a reservoir and a buffer for soluble mediators of repair that can be mobilized from their ECM-bound state by micron-scale mechanical and proteolytic activity. ECM scaffolds are used widely in wide medical applications with an eye towards a regenerative response; however the designation of scaffolds as devices (for the present) has retarded our understanding of how much or how little of the organization and biochemical composition of the ECM is critical to each application. Manufacturing/processing of ECM can markedly affect the content, mechanical properties, pore structure, antigenicity, durability, binding activity, and biocompatibility of commercial ECM scaffolds. Future work must clarify the scale at which, if any, of the aspects of the native ECM must be preserved to achieve optimal biological response.

Developing *in vitro & in vivo* models to study tissue reactions to biologic scaffolds

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Healing processes are a highly regulated series of biological activities in the so-called regenerative niche to restore structural and functional integrity, and involve the coordinated and sequential release of numerous biological signals from various cell types. Understanding these processes is a pre-requisite for regenerative medicine. Based on the working hypothesis that cells are Nature's prime signal delivery systems, we have endeavoured to understand the underlying mechanisms of cellular crosstalk by establishing suitable human co-culture models with a view to delineating tissue reactions to both synthetic and biologic scaffolds. The model systems have concentrated on an essential component of healing, namely vascularization. Thus, in studying bone regeneration human osteoblasts or mesenchymal stromal cells, which have been differentiated along the osteogenic lineage, demonstrate characteristic molecular interactions in co-culture with human microvascular or progenitor endothelial cells (EC) and yield microvascular structures as a result of mutual stimulation [1,2]. The latter can also be formed on a 3D biomaterial scaffold *in vitro*, the resulting microvessels being rapidly inosculated on implantation *in vivo* [3]. More recent research involves the role of the initial inflammatory reaction, indicating that pro-inflammatory macrophages, can accelerate this vascularization process [4]. Preliminary *in vivo* studies confirm that macrophages pre-cultivated on ceramics can stimulate the vascularization response following implantation. Further co-culture models have been established for the lung, e.g. the air-blood barrier [5,6], which is of interest for nanomedicine applications in which nanoparticles could be transported into the body by an inhalational route [7-9]. The most complex of these air-blood barrier models involves the incorporation of macrophages, as these cells are present in the alveoli [10]. In addition, co-cultures of respiratory basal epithelial progenitor cells with lung fibroblasts have been established as a model of upper respiratory tract regeneration and demonstrate formation of an intact, functional respiratory mucosa [11-12]. Current studies are focusing on cellular colonization of decellularized upper airways [13]. Other co-culture models have been developed to simulate the blood-brain and skin barriers.

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1. Kirkpatrick CJ, Fuchs S, Unger RE. Adv Drug Deliv Rev 2011; 63: 291-299
2. Kirkpatrick CJ. Tissue Engineering Part A 2014; 20: 1355-1357
3. Ghanaati S et al. J Tissue Eng Reg Med 2011; 5/6: e136-143
4. Dohle E et al. E Cells & Mater J 2014; 27:149-164
5. Hermanns MI et al. Lab Invest 2004; 84, 736-752
6. Hermanns MI et al. J R Soc Interface 2010; 7, S41-S54
7. Kasper J et al. Part Fibre Toxicol 2011; 8: 6
8. Kasper J et al. Eur J Pharm Biopharm 2013; 84: 275-287
9. Kasper J et al. Beilstein J Nanotechnol 2015; 6: 517-528
10. Kasper J et al. JTERM 2015 ; doi: 10.1002/term.2032
11. Pohl C et al. Eur J Pharm Biopharm 2009; 72: 339-349
12. Pohl C et al. J Biotechnol 2010; 148: 31-37
13. Melo E et al. Tissue Engineering Part C 2015; 21: 909-921

Macrophages. The Chicken and the Egg in Immune Responses to Injury or Biologic Scaffolds

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Macrophages are the central initiating and directing element for both constructive (repair) and destructive (kill) immune processes throughout the animal kingdom. This new knowledge is causing a revolution immunology. Long-standing immunologic thinking has been that macrophages require T or B lymphocytes to function. Instead, both these adaptive responses and innate responses (e.g., neutrophils) depend on macrophages. Immunology had it backward. Macrophages can exhibit either repair or kill-dominant activity, in part, because they have a unique ability to produce growth-promoting or inhibiting molecules as needed. Such polar-opposite–type responses (referred to as M1 or M2) directly influence inflammatory outcomes in profoundly different ways. Examples include responses to pathogens; but also responses when 'foreignness' is not apparent, such as in cancer, atherosclerosis, injury or against implanted biomaterials. Macrophages also indirectly determine what other innate or adaptive responses occur. For example, M1/kill or M2/repair-dominant responses stimulate Th1 or Th2-type activity in lymphocytes, respectively. Macrophages are the Chicken and the Egg in immunology.

Th2 T cells are required for extracellular matrix-mediated functional muscle regeneration

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Volumetric muscle loss injuries due to trauma, tumor resection, or degenerative disease result in significant morbidity. Extracellular matrix (ECM) scaffolds have been successfully used clinically for soft tissue reconstruction including treatment of volumetric muscle loss with restoration of muscle function. Concomitant with these promising outcomes in tissue regeneration, ECM scaffolds promote recruitment of M2-macrophages. M2 macrophages are anti-inflammatory, compared to classically activated M1 macrophages, and are important in wound healing and scaffold remodeling. Here, we determined that the pro-regenerative nature of ECM scaffolds and M2 macrophage polarization is dependent upon Th2 polarized $CD4^+$ T cells. Knife-milled porcine tissue (decalcified bone and ventricular cardiac muscle) was treated with peracetic acid, Triton X-100/EDTA, and DNase. The resulting material was lyophilized and cryogenically milled into a fine particulate. Hydrated particulate ECM scaffolds were then injected into volumetric muscle wounds in wild type and immunodeficient mouse strains. After 1, 3 and 6 weeks we evaluated the scaffold immune microenvironment through flow cytometry and qRT-PCR, and muscle regeneration through histology and functional testing (treadmill exhaustion assays). ECM scaffolds induced an M2-macrophage phenotype ($CD86^{lo}CD206^{hi}$*Arg1*^{hi}*Retnla*^{hi}*Cebpb*^{hi}) that was similar between both cardiac and bone tissue sources. Additionally, scaffolds recruited T cells with significantly increased expression (Wilcoxon Rank Sum $P \leq 0.05$) of several Th2 genes including *Il4* and *Jag2* and decreased Th1 gene expression, *Tbx21* and *Ifng*, compared to a saline treated control. In *Rag*$^{-/-}$ mice, which lack mature T and B cells, functional tissue regeneration is hindered and macrophages lose their M2 phenotype. Post-operative muscle in *Rag*$^{-/-}$ mice has higher levels of ectopic adipogenesis and fibrous tissue formation suggesting an imbalance in fibro-adipogenic lineage commitment. This deficiency is also present in *Cd4*$^{-/-}$ mice, specifically linking the regenerative potential of ECM scaffolds to $CD4^+$ T cells. When *Rag*$^{-/-}$ mice are repopulated with wild type $CD4^+$ T cells, we rescue the regeneration deficiency and M2-macrophage ($CD206^{hi}$) phenotype. However, if the donor T cells are *Rictor*$^{-/-}$, which lack a key component of the mTORC2 complex and cannot polarize toward Th2, there is no rescue. Additionally, in *Il4ra*$^{-/-}$ mice, there is a severe decrease in regeneration and M2 phenotype, defining scaffold-associated macrophages more specifically as M(IL-4) macrophages and confirming IL-4 as an important cytokine in scaffold-mediated muscle regeneration. These findings suggest a major role of Th2 $CD4^+$ T cells, dependent upon mTORC2-Rictor signaling, in formation of a pro-regenerative scaffold immune microenvironment.

Macrophage phenotype profile regulated by tissue matrices for Screening of Biomaterials

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There are sufficient evidences support the post-injury inflammation influences outcome of tissue regeneration. Activation of sub-type macrophages plays an important role during the course of normal healing. Traditional method to evaluate the biological response to acellular dermal matrices (ADM) was through the in vivo animal studies, which is inefficient economically and time consuming. We have developed an in vitro system to screen ADM responding to macrophages. This approach can provide valuable information effectively to estimate biological response of ADM implanted in vivo. We have cultured monocytes on a variety of tissue matrices for 3-7 days. RNA was isolated and analyzed by using quantitative PCR. Gene expression of macrophage polarization and induction of genes involved in tissue remodeling were tested and analyzed. The results indicated that certain tissue matrices activated pro-inflammatory cytokine and chemokine genes while others up-regulated genes related to M2 phenotype macrophage. These results were correlated to tissue regeneration in vivo in a porcine wound model. Tissue matrices that exhibited a significant up-regulation of the M2 genetic profile in vitro showed advanced wound healing stages demonstrated by histological analysis of the wounds and gene array profile. In conclusion, gene expression of macrophage phenotype profile regulated by tissue matrices is a powerful, in vitro, screening tool.

Effect of Source Animal Age upon Macrophage Response to ECM Scaffolds

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Successful remodeling of extracellular matrix-based biomaterials (ECM) has been linked to a shift in the polarization of the host macrophage response from an M1 (pro-inflammatory) to a unique M2 (anti-inflammatory)-like ECM-mediated phenotype. Shifts of the phenotype of macrophages participating in the host response to ECM scaffold materials harvested from aged animals towards a more pro-inflammatory phenotype have been observed and are correlated with a reduction in functional tissue remodeling downstream. The objective of the present study was to isolate ECM from animals of increasing age and investigate their effects on macrophage polarization and function in vitro.

ECM was produced from the small intestinal submucosa of 12 week, 26 week and 52 week-old pigs. DNA, collagen, GAG, and growth factor content were assessed. Bone marrow derived macrophages from 8 week-old mice were exposed to M1 (IFN-γ/LPS) or M2 (IL-4)-polarizing cytokines or ECM degradation products for 24h. Additionally, cells were exposed to ECM for 24h then challenged with M1/M2 stimulus. Macrophage phenotype was assessed using a combination of immunolabeling and gene expression for multiple M1 and M2 markers. Macrophage function was assessed for phagocytosis, nitric oxide production and metabolic profile .

DAPI staining and gel electrophoresis showed effective decellularization of all tissues. Increases in collagen and decreases in GAG and growth factor content were observed with increasing source animal age. ECM exposure alone had minimal effect on macrophage phenotype, though increased ECM age was found to result in increased pro-inflammatory surface marker labeling, gene expression, and nitric oxide production with decreased phagocytosis. Metabolism in ECM-exposed macrophages shifted from respiratory (M2-like) to glycolytic (M1-like) phenotype with increased ECM age. ECM-exposed macrophages which were then challenged with an M1 stimulus showed increased iNOS and decreased MHC-II expression with increasing ECM age, as well as increased phagocytosis, decreased NO production and decreased glycolytic metabolism. ECM-exposed macrophages challenged with an M2 stimulus showed decreased Arginase and increased Fizz1 expression with increased ECM age.

These results show a compromised ability of macrophages to polarize towards an M2, anti-inflammatory phenotype with increasing ECM age. Metabolic data show impaired glycolysis in M1 macrophages with aging of the ECM, suggesting a reduced ability to secrete cytokines and perform other functions. Ongoing studies suggest a link between the observed differences in macrophage response to ECM and the accumulation of advanced glycation end products (AGEs) and lipids within tissues over time. These results have implications for sourcing of biological ECM scaffolds and suggest potential methods to remove age-related changes such as lipids and glycation from these scaffolds during fabrication. Additionally, these results demonstrate that the primary effects of ECM based materials upon the host response following implantation may be to alter the response to subsequent stimuli. Lastly, of note, few if any changes in macrophage polarization or functional profile were observed when cells were harvested from the bone marrow of aged mice. This suggests that the ECM microenvironment from aged subjects may represent a cell-extrinsic factor which contributes to the dysfunctions in macrophage polarization which have been observed following exposure to pathogen or tissue injury in aged individuals.

Regulation of macrophage function by engineered biopolymer scaffolds

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Macrophages are essential regulators of the innate immune system, and play an important role in advancing and resolving inflammation during wound healing. To perform their functionally diverse roles, these cells can rapidly change their function in response to cues in their surrounding microenvironment. For example, inflammatory stimuli such as interferon-gamma and toll like receptor ligands lead to macrophage polarization toward a classically activated phenotype and production of cytokines and reactive species to promote inflammatory signaling. However, exposure to Th2 cytokines including IL-4 in a wound healing environment causes the same cells to polarize toward an alternatively activated phenotype, which promotes tissue healing and regeneration. While much is known about how soluble cues in the environment regulate macrophage phenotype, less is understood about how physical cues modulate their behavior. In particular, the effects of changes in adhesive environment caused by matrix remodeling during wound healing have not been clearly elucidated. Our laboratory recently showed that the geometry of cell adhesion plays an important role in macrophage polarization; specifically cell elongation induced by micropatterned substrates promotes the expression of markers associated with an alternatively activated, pro-healing phenotype. In current work, we investigate how adhesion in three-dimensional fibrillar matrices regulate macrophage cell shape and function. We developed three-dimensional matrices composed of natural biopolymers including collagen and fibrin, which are present at varying concentrations and proportions during the wound healing process. Fibril dimensions were characterized by second harmonic and backscattering imaging techniques, which revealed that the addition of fibrin reduced the fiber length and thickness. In addition, while macrophages remained mostly rounded when seeded on three-dimensional matrices, the presence of fibrin enhanced cell spreading and the formation of cell protrusions into the matrix. Current work is focused on examining how adhesion within engineered fibrillar matrices impacts the inflammatory versus wound healing behavior of macrophages. A better understanding of how physical and adhesive cues regulate macrophage behavior will ultimately aid in the design of biopolymer scaffolds for tissue engineering and regeneration.

Biologic Scaffold Treatment for Volumetric Muscle Loss: Results of a Thirteen Patient Cohort Study

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Volumetric muscle loss is a severe and debilitating problem with significant clinical and economic consequences. Such injuries can occur as the result of traumatic injury, excessive exercise, tumor ablation, or degenerative disease. It is estimated that 35-55% of all sports injuries and 53% of battlefield extremity injuries involve damage to soft tissue and myofibers. This results in approximately 4.5 million reconstructive surgical procedures annually which contributes to billions of dollars in health care expenses. Current standards of care include physical therapy or orthotics, which do not correct underlying strength deficits, and surgical tendon transfers or muscle transfers, which often result in donor site morbidity and fall short of restoring function.

The results of a thirteen patient cohort study are described herein and involve a regenerative medicine approach for treatment of volumetric muscle loss. Acellular bioscaffolds composed of mammalian extracellular matrix (ECM) were implanted in thirteen patients with VML representing seven different anatomic sites and who had exhausted all available standard-of-care options. Immunolabeling of ultrasound guided tissue biopsies and MRI or CT imaging were performed to evaluate the cellular remodeling response and the macroscopic three dimensional formation of new muscle tissue, respectively. Force production, range of motion, and functional task performance were quantified, and electrodiagnostic testing was conducted to evaluate the extent of innervation.

In vivo remodeling of ECM bioscaffolds was associated with mobilization of perivascular stem cells; formation of new, vascularized, innervated skeletal muscle within the implantation site; increased force production; and improved functional task performance when compared to pre-operative performance. Implantation of acellular bioscaffolds derived from ECM can promote site-appropriate formation of functional skeletal muscle in patients with volumetric muscle loss.

9th Symposium on Biologic Scaffolds for Regenerative Medicine, Napa, CA, 28-30 April 2016

Decellularized Allogeneic Neurovascular Bundles for Reinnervation and Revascularization in Soft and Hard Tissue Reconstruction, the Rehabilitation of Massive Scarring, and Engineered Tissues

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ABSTRACT:

OBJECTIVES: In traumatic injuries (eg, large craniofacial defects) and severe scarring (eg, massive burns or chest irradiation), quality of life is dependent on restoring form and function. Regeneration requires robust vascular supply and sensory-motor reinnervation. While amenable to transplantation, two critical barriers remain: (1) transplant rejection / devastating effects of immunosuppression (eg, 40% of pediatric heart transplants have acute rejection episodes in the 1st year [Kirk 2009]); and (2) significant donor tissue shortages (50% of US patients sit on waiting lists for >5 years [UNOS 2013]). Decellularized tissues aim to overcome these barriers. Dermis and nerve grafts are commercially available for smaller defects. However, these are not sufficient for regeneration through large 3D volumes of tissue (graft take requires <5 mm proximity to vascular supply), or over long nerve gaps (autografts >10 cm should be vascularized). Therefore, we propose a *novel allograft: decellularized neurovascular bundles (NVBs)* that can be recellularized with recipient endothelial cells and Schwann cells for implantation into large areas of relatively avascular, asensate and/or paralyzed scar tissue, to allow reconstruction by "local" techniques that otherwise would only be suited to small defects. Furthermore, these NVBs may be suitable for rehabilitating scar tissue, as well as for innervating and vascularizing engineered tissues such as myocyte sheets - to finally create true muscle.

METHODS: Femoral NVBs have been harvested from both rat (~2.0 cm) and rabbit (~3.5 cm) species, comprising femoral artery and vein, and femoral/saphenous nerve. Animals were heparinized premortem. A laparotomy was performed to expose the abdominal aorta and inferior vena cava which were cannulated (18G) inferior to the renal vessels, to offer large vessel access for subsequent re-endothelialization, post-decellularization. The vessels were dissected distally to free the femoral NVB as a single structure within the femoral canal. The NVB was then divided and cannulated distally too. Tissues were decellularized (SDS and Triton-X) in two groups for each species: perfusion and immersion decellularization. The value of each technique was assessed by characterizing the tissues: histological staining (H&E, Masson's trichrome), immunohistochemistry (fibronectin, laminin, GAGs, collagen), PicoGreen® DNA assay, collagenase assay, and differential scanning calorimetry (DSC). These samples will now undergo recellularization using perfusion re-endothelialization and microinjections of Schwann cells for the nerves.

RESULTS: The larger rabbit tissues were found to be far easier to dissect and cannulate. Although cannulation is still recommended at harvest (for subsequent recellularization), all tissues decellularized well with either technique (perfusion or immersion) and no significant differences in characterization were found. Characterization results will be presented.

SIGNIFICANCE: We have developed techniques for harvesting and decellularizing NVBs in 2 species. Currently this is being expanded to primates, and work for recellularization of all 3 species has begun. We believe this is the first time this application of NVBs has been described. These novel "autologous" off-the-shelf NVBs aim to play an important role in reinnervation and revascularization for soft and hard tissue reconstruction, rehabilitation of massive scarring, and engineered tissues.

[2,998 char; 484 words]

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Miromatrix Medical Inc. and the Mayo Clinic

Engineering a Clinically Relevant Transplantable Liver with Sustained In-Vivo Perfusion

In the United States, more than 17,000 people are currently awaiting a liver transplant. The demand for a transplant far exceeds the approximately 6,500 livers that are available annually, as there are no existing treatments capable of long-term restoration of liver function except transplantation which leads to many patients dying while waiting to receive a liver. To address this critical need, we are developing a clinically relevant perfusable, revascularized liver graft to treat chronic liver failure. The liver graft is based on perfusion decellularization technology capable of creating a scaffold of extracellular matrix (ECM) that is biologically complex, architecturally correct, and that retains the native environmental cues of the liver that are essential for successful recellularization. Adolescent whole porcine livers were successfully isolated and perfusion decellularized. The whole liver scaffolds were re-endothelialized using Human Umbilical Cord Endothelial Cells (HUVEC) or Porcine Umbilical Cord Endothelial Cells (PUVEC) to optimize the initial seeding conditions to achieve >90% re-endothelialization of the native vasculature. The PUVEC re-endothelialized liver grafts were successfully transplanted into a porcine model via anastomosis to the renal vein and the inferior vena cave. The degree of continuous perfusion was characterized via Doppler for over 3 days before the liver grafts were explanted and imaged via an angiogram to further characterize the patency vessels and capillaries within the transplanted grafts. The re-endothelialized liver grafts demonstrated continuous and sustained blood perfusion over the three days with evidence of capillary perfusion. Non-revascularized livers were acutely transplanted into the porcine model and were fully occluded after only 30 minutes. The successful re-endothelialization and long term transplantation of the liver grafts now enables the next step of liver recellularization consisting of hepatocyte seeding to engineering a functional liver for transplantation. Optimization and characterization of primary hepatocyte seeding and hepatocyte function will also be presented.

Decellularized Organs: Whole Organ Construction with Stem Cells

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Whole organ decellularization generates a native three-dimensional scaffold that preserves the architecture and spatial and temporal distribution of extracellular proteins. These 3D scaffolds can be regenerated to functional organs with the help of stem cells. Here we will show the feasibility of whole organ bioengineering with pluripotent stem cells towards the regeneration of kidney. The worldwide shortage of donor kidneys for transplantation underlines the pressing need for a tissue engineered kidney, for which whole organ engineering is a viable approach. Decellularized kidneys with native microstructure with extracellular matrix proteins enabled the bioengineering of kidney with human induced pluripotent stem cells derived renal progenitor cells and endothelial cells. Endothelial cells play a critical role in the regeneration of the functional kidney. In the repopulated organ, the presence of endothelial cells widely increased the expression level of genes related to renal development. Implantation of the cellularized native scaffolds in SCID mice further supports the requirement of endothelial cells in the whole kidney bioengineering. Apart from the vascularization of the implants in the presence of endothelial cells, the most striking observation was the regeneration of the glomeruli. Regeneration of the glomeruli was noticed to occur only in the presence of endothelial cells. Functional assessment of the bioengineered kidney was carried out in a simulated bio-reactor set-up with urea, creatinine and albumin. The albumin absorption efficiency and the excretion efficiency of urea and creatinine were noticed higher in the presence of endothelial cells. Furthermore, the use of isogenic cells derived from a common iPSC source represents an important step for clinical translation of the technology. To the best of our knowledge this is the first study to report the generation of a functional whole kidney.

Application of bioartificial dermal regeneration templates for skin restoration in combat casualty injuries

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ABSTRACT

Introduction:

Bioartificial dermal regeneration templates (DRTs) have been employed extensively in the treatment of burns, but fewer studies have reported their use in the care combat wounds. Com at injuries represent devastating wounds that present a therapeutic challenge to not only restore the protective skin barrier function but also to preserve functions such as tendon and muscle excursion, protective padding around nerves, and joint motion. Accordingly, regenerative medicine modalities that can facilitate such goals are of great interest. The use of bioartificial DRTs in these complex traumatic combat soft tissue injuries can provide initial wound coverage while also aiding in establishment of a well-vascularized wound bed that is suitable for definitive soft tissue coverage measures. This study reports on the impact and outcomes of a subset of DRTs in the reconstruction of traumatic combat extremity wounds.

Methods: An IRB approved retrospective review of all patients treated with the most common bioartificial DRT used in our practice (Integra DRT, Integra Lifesciences Corporation, Plainsboro, NJ) for combat-related traumatic wounds from November 2009 through July 2013 was completed. Our computerized surgical scheduling system was searched for all patients during the study period treated with this specific bioartificial DRT, and medical records were screened. The primary outcome of the study was healing of the wound following DRT placement, as measured by successful take or stable definitive coverage with second stage skin grafting, flap procedure, or in the cases of delayed primary closure, healing of the closed wound. Secondary outcomes measured included number of irrigation and debridement procedures prior to DRT placement, time from arrival to DRT placement, time from DRT placement to definitive closure, and overall time from injury to definitive closure.

Results: A total of 190 patients with 280 wounds met inclusion criteria, of which 251 (90%) had complete records. Patients underwent a median of 3 irrigation and debridement procedures (range 0-19 procedures) prior to DRT placement over a median of 8 days (range 1-66 days) (see Figures 1 & 2). Overall, the median time from DRT placement to definitive closure (split and full thickness skin grafting, delayed primary closure and flap coverage) was 15 days (range 0-57 days). The median time from injury to definitive closure was 35 days (range 9-105 days). In patients with complete records, overall healing rate after first attempt at definitive closure or autologous skin grafting was 86%.

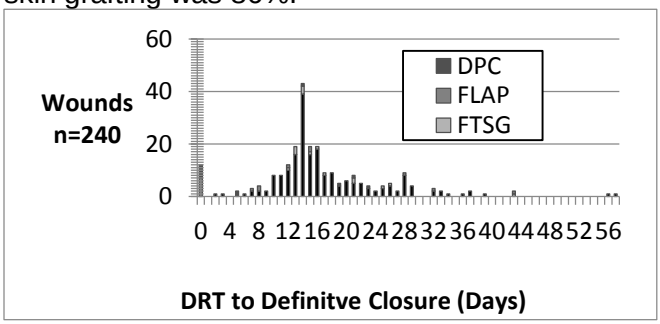

Figure 1: Days from DRT to definitive closure with STSG, FTSG, Flap or DPC. N=240 (198 STSG, 15 FTSG, 16, FLAP, 11, DPC)

	Injury to arrival Days (Range)	Arrival to DRT Days (Range)	I&D Procedures Procedures (Range)	DRTto DC Days (Range)	Injury to DC Days (Range)
Upper Extremities	5 (2-38)	8 (1-66)	3 (0-14)	14 (0-57)	34 (11-82)
Lower Extremities	5 (2-47)	8 (1-53)	3 (0-19)	15 (0-37)	35 (9-101)
Non-Extremity	5 (3-9)	13 (3-59)	8 (1-18)	16 (14-56)	64 (24-105)

Figure 2: Chronology of wound treatment. Days from injury to arrival at our facility, days from arrival to artificial dermal grafting (ADG), number of irrigation and debridement (I&D) procedures after arrival and prior to ADG, days from ADG to definitive closure (DC), days from injury to DC. All values are given as median (range).

Conclusion:

Bioartificial DRTs have played an increasing role in the treatment of traumatic war wounds. Utilizing the beneficial biologic scaffold and structural aspects of these materials, our clinical group has been able to successfully achieve definitive closure of wounds complicated by significant infectious burden and hypo- or avascular tissue while maintaining adequate function. However, long-term functional outcomes are still pending and further investigation is necessary to truly evaluate the utility of these products beyond combat casualty care and correlate with civilian traumatic and oncologic reconstructions. This study reports the largest consecutive case series of DRTs employed for traumatic combat injuries within the clinical literature.

Biomembrane from porcine cartilage extracellular matrix contributes enhancement of efficacy of Microfracture for cartilage repair- Clinical results followed up 1 year postoperatively

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It is well known that cartilage defects in the knee rarely heal. The microfracture remains the most cost effective, first-line treatment for cartilage defects. The technique, however, is limited by a number of factors such as repair with fibrocartilage rather than hyaline cartilage, variable results depending on defect size, and functional deterioration over time. Newer surgical techniques for microfracture aim to enhance tissue repair by the addition of a biomembranes which would stabilize the blood clot after microfracture.

We developed a novel cartilage extracellular matrix (CECM) membrane to protect blood clots after microfracture. The CECM membrane was made of ECM fabricated naturally by cultured porcine chondrocytes, and then decellularized and multi-layered to confer optimal mechanical strength. Previously we reported that the CECM membrane which is mainly composed of collagen and proteoglycan was very thin (30–60 μm thick) and bendable, but had good tensile strength (85.64 N), suitable for protecting blood clots from leakage in cartilage defect. Moreover, the CECM membrane showed low but enough diffusion coefficient to allow delivery of small proteins in synovial fluid into the repaired tissue. In a beagle model, covering the cartilage defect with the CECM membrane after microfracture generated more hyaline cartilage-like tissues than the BST alone in histology and chemical analyses. Additionally CECM, an integral cartilage ECM composite, shows an inhibitory effect on vessel invasion both in vitro and in vivo, resultantly preventing neovascularization during the cartilage repair. These characteristics may attribute enhancement of cartilage repair of microfracture. The purpose of the study was to evaluate whether the CECM made of cartilage extracellular matrix, designed to provide cartilage-like favourable environments as well as to prevent against washout of blood clot after microfracture, would enhance cartilage repair compared with the conventional microfracture technique.

Methods: A prospective trial was designed to compare the CECM cover after microfracture with conventional microfracture among patients with grade III–IV symptomatic cartilage defect in the knee joint. Seventy four patients aged 18–60 years were assigned to either the microfracture/ CECM or microfracture groups (n = 30). Among them, 69 knees in the microfracture/ CECM or in the microfracture were followed up for 1 year. Cartilage repair was assessed with magnetic resonance images taken 6 months, 1 year postoperatively, and the clinical outcomes were also recorded.

Results Compared with conventional microfracture, microfracture/ CECM resulted in greater degree of cartilage repair ($p = 0.043$). In the intra-group analysis, while microfracture showed moderate to good degree of cartilage repair in nearly 50 % of the patients (47 % at 6 months to 50 % at 1 years; n.s.), microfracture/ CECM maintained an equivalent degree of repair up to 1 years (88 % at 6 months to 75 % at 1 years; n.s.). The clinical outcome at 1 year also showed improved knee score and satisfaction and decreased pain in each group, but the difference between the two groups was not statistically significant.

Conclusions: Compared with conventional microfracture, CECM cover after microfracture yielded superior outcome in terms of the degree of cartilage repair during 1 year of follow-up. This implies that initial protection of blood clot and immature repair tissue at the microfractured defect is important for the promotion of enhanced cartilage repair, which may be obtained by the application of a CECM.

Collagen Matrix: Structure & Function - Translating to New Opportunities in Regenerative Medicine

Dr. Kenneth Burhop, Chief Scientific Officer, Integra LifeSciences, Plainsboro, NJ, United States

Over the past 25 years biomaterials research has focused on understanding, designing and manufacturing collagen matrix materials for the treatment of burns, wounds, dural repair, and a host of other regenerative medicine applications.

Considerations for tissue engineering of products to treat wounds include promoting regeneration, while preventing fibrosis and infection. Research has demonstrated that factors such as “macro- and micro-architecture, biochemistry, pore structure and the resorption rate of the scaffold are critical factors controlling proper biocompatibility, material function and healing of collagen based regenerative biomaterials.

Integra® Dermal Regeneration Template (IDRT) is currently approved as indicated for the post-excisional treatment of life-threatening full-thickness or deep partial-thickness thermal injuries where sufficient autograft is not available at the time of excision or not desirable due to the physiological condition of the patient and has been on the market since 1996. As a result of the extensive research, use, and history in the treatment of burn patients (going back to the pioneering work of Yannas and Burke), as well as the extensive data collected in a variety of other clinical indications (ie, over 12 million implants world-wide) this collagen matrix provides an ideal “model” with which to study new biomaterial design.

Research has shown that cell behavior and cell fate can be extensively manipulated through biomaterial design. In recent years, there has been an increased focus on the interaction of synthetic and naturally derived engineered extracellular matrix constructs with other cells such as progenitor stem cells. Research has also shown that not all matrices behave in the same manner, and the composition of the matrix significantly influences cell interactions and physiologic variables such as the regulation of the inflammatory response, fibrosis, vasculogenesis and tissue regeneration.

This presentation will review examples of the research experience gained with various matrices as examples of regenerative medicine scaffolds in a variety of different clinical indications and discuss the basic structure-function aspects of this class of products and illustrate how this understanding might translate into new products that might provide better care for patients around the world.

Low-Immunogenic Matrix Suitable for Transplantation

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Transplantation is an effective treatment option for patients suffering from different end-stage diseases; however it is associated with a constant shortage of donor organs and lifelong immunosuppressive therapy. Using of pig organs is promising to replace human organs for transplantation. Matrix derived from porcine organs is a convenient substitute for the human matrix. As an initial step to create a new ex vivo tissue engineered model, we optimized protocols to obtain organ-specific acellular matrices and evaluated their potential as tissue engineered scaffolds for culture of normal cells and tumor cell lines. Our protocols include decellularization by perfusion in a bioreactor system and immersion-agitation on an orbital shaker with use of various detergents (SDS, Triton X-100) and freezing.

Completeness of decellularization in terms of residual DNA amount is important predictor of probability of immune rejection of materials of natural origin. However, according to our data and the data of the literature, the signs of cellular material still remain even after harsh decellularization protocols. In this regard, the matrices obtained from tissues of low-immunogenic pigs with α3Galactosyl-tranferase gene knock out (GalT-KO) may be a promising alternative to native animal sources. We studied induced effect of frozen and fresh fragments of GalT-KO skin on healing of full-thickness plane wounds in 80 rats. Commercially available wound dressings (Ksenoderm, Hyamatrix and Alloderm) as well as allogenic skin were used as a positive control and untreated wounds were analyzed as a negative control. The results were evaluated on the 4^{th} day after grafting, which corresponds to the time of start of normal wound epithelization. It has been shown that a non-specific immune response in models treated with GalT-Ko pig skin was milder than in all the control groups. These preliminary data may contribute to develop personalized transplantable organs from GalT-Ko pigs with significantly limited potential of immune rejection.

Ideally, a bioengineered organ must be biocompatible, non-immunogenic and support cell growth. Porcine organs are attractive for xenotransplantation, if severe immunologic concerns can be bypassed. Our results indicate that genetically modified pig tissues with knock-outed α3Galactosyl-tranferase gene may be used for production of low-immunogenic matrix suitable for transplantation.

A Macrophage Centric Approach to the Evaluation of ECM Scaffolds for Tissue Reconstruction

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The conventional approach to biomaterial design and development typically focuses upon mechanical and material properties with long-term objectives that include an inert host immune response and long-lasting mechanical and structural support. The emergence of, and interest in, tissue engineering and regenerative medicine have driven the development of novel cell-friendly biomaterials, materials with tailored degradation rates, materials with highly specific architectures and surfaces, and vehicles for delivery of bioactive molecules, among numerous other advancements. These advancements in biomaterial form and function, combined with new knowledge of innate and acquired immune system biology, have provided an impetus for re-examination of host-biomaterial interactions, including host-biomaterial interface events, spatial and temporal patterns of in vivo biomaterial remodeling, and related downstream functional outcomes. The present talk focuses upon the role of the host macrophage response in the remodeling of extracellular matrix based materials in regenerative medicine applications.

Biologic scaffolds composed of ECM have been shown to act as inductive templates for constructive remodeling across multiple tissue and organ systems. That is, ECM scaffold materials are capable of promoting the formation of new, functional, host derived tissue following placement. A number of key events have been identified in the remodeling process. These include early exposure to mechanical loading, rapid and complete degradation of the ECM scaffold with concurrent release of bioactive peptides, the recruitment of tissue resident and progenitor cell populations, and host-scaffold interactions resulting in shifting of the immune profile within the site of tissue remodeling. As is described above, host-scaffold interactions have now received considerable attention in the field of regenerative medicine. Multiple studies utilizing ECM scaffolds in pre-clinical applications have demonstrated that successful constructive remodeling is associated with a transition from an M1, proinflammatory macrophage phenotypic profile to an M2-like phenotype early in the remodeling process. This observation has been consistent across multiple tissue and organ systems, including skeletal muscle, the temporomandibular joint, and peripheral nervous system, among others. The present talk will highlight the preclinical evidence in each case, and human clinical evidence where available. Additionally, the present talk will highlight recent and ongoing work to define the macrophage phenotype associated with ECM scaffold remodeling, as it does not correspond to the canonical M1(IFN-γ, LPS) or M2 (IL-4, IL-13, IL-10) phenotypes commonly reported in the literature.

Developing a robust and mechanistic profile of host-ECM scaffold interactions has the potential to significantly advance the understanding of the manner in which ECM scaffolds promote constructive remodeling, provide guidance for improved scaffold design, and ultimately improve clinical success associated with their use.