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Human Perinatal-Derived Biomaterials

*Marc C. Moore, Aurore Van De Walle, Jerry Chang, Cassandra Juran, and Peter S. McFetridge**

Human perinatal tissues have been used for over a century as allogeneic biomaterials. Due to their advantageous properties including angiogenecity, anti-inflammation, anti-microbial, and immune privilege, these tissues are being utilized for novel applications across wide-ranging medical disciplines. Given continued clinical success, increased adoption of perinatal tissues as a disruptive technology platform has allowed for significant penetration into the multi-billion dollar biologics market. Here, we review current progress and future applications of perinatal biomaterials, as well as associated regulatory issues.

1. Introduction

The first reported clinical use of perinatal-derived biomaterials was at Johns Hopkins over a century ago in 1910 as an aid for dermal wound healing.[1] In recent years, both clinical and research applications of these biomaterials have increased in scope to include roles in tissue engineering, regenerative medicine, and cell-based therapies, especially in the field of orthobiologics.[2,3] Perinatal tissues are an abundant source of extracellular matrix components, discrete biomolecules, and growth factors with potential use in wide ranging surgical procedures.[4] Clinical success with current products have driven broader applications and novel materials that span most clinical specialities, see **Table 1**.[5] These biomaterials have translated well to regenerative medicine applications by improving wound site integration due to their low immunogenicity, positive wound healing characteristics and minimal tissue inflammation.[6,7] The two anatomical components that have seen the most clinical use are the umbilical vein and amniotic membrane (AM), although numerous preclinical investigations have assessed the potential of the chorion, villous placenta and umbilical artery (**Figure 1**).

In excess of $212M of placental derived biomaterials are sold per annum in the USA, not including placenta derived cord blood and cell therapies (see **Table 2**). These materials have significant potential to benefit a variety of therapeutic requirements and thus have room for extensive commercial growth. Lyophilized and dried versions of these biomaterials have further clinical and commercial benefit due to ease of transportation and storage requirements (no refrigeration) that simplifies logistics, particulary for surgical suites in remote locations.

In contrast to cadaveric and fetal tissue donation, placental tissues are donated and recovered from healthy individuals. These tissues are normally considered medical waste and can be recovered without harm to the donor or fetus, thus having minimal ethical and or legal issues. Obtaining the appropriate authorization for collection of perinatal tissues is also generally easier than cadaveric (musculoskeletal tissue or organ) and fetal demise tissue recoveries. Perinatal tissues are normally incinerated with other biohazard waste, and thus most patients donate their placentas knowing that it could go to help someone in need. Companies and researchers must primarily negotiate with doctors, hospital administrators and the parent to obtain the appropriate authorization for collection. Unlike cadaveric tissue, perinatal tissues do not have the same anatomic and demographic limitations, where tissue banks ideally require tendons from young donors and bone with thick cortical walls for sports medicine grafts. Cadaveric donor tissue necessitates serological testing after the complex donor recovery process has been completed, adding time and expense if the tissue fails to meet standards. By comparison, with potential for large numbers of donor contributions, the ability to prescreen perinatal tissue prior to recovery reduces costs and speeds processing time frames.

Prof. M. C. Moore
Stephenson School of Biomedical Engineering
University of Oklahoma
202 W. Boyd Street, Carson Engineering Center
Norman, OK 73019, USA
Dr. A. Van De Walle
Laboratoire Matière et Systèmes
Complexes MSC
UMR 7057
CNRS & University Paris Diderot
75205 Paris Cedex 13, France
J. Chang
BioD Logics, LLC
7740A Trinity Road, Cordova, TN 38018, USA
Dr. C. Juran
NASA Ames Research Center
Space Bioscience BLDG 236 MS: 236-7
Moffett Field, CA 94035, USA
Prof. P. S. McFetridge
J. Crayton Pruitt Family Department of Biomedical Engineering
University of Florida
JG-56 Biomedical Sciences Building
P.O. Box 116131, Gainesville, FL 32611-6131, USA
E-mail: pmcfetridge@bme.ufl.edu

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2. Advantageous Properties for Regenerative Medicine

Research has shown perinatal tissues to have a range of properties, including being angiogenic, antimicrobial, anti-inflammatory, osteogenic, and neurogenic, as well as having unique structural and mechanical properties (**Figure 2**). These diverse inherent properties may be attributed to their derivation and role during pregnancy. For example, the epithelium of the amniotic membrane (amniotic ectoderm) is derived from the epiblast (embryonic ectoderm) approximately 8 days after fertilization, whereas gastrulation, which is the tipping point at which cell fate and ultimately organ and tissue function begins to become specified, does not occur until days 15–17.[8] Other components of the placenta such as the chorion differentiate from the extraembryonic trophectorderm, while the mesenchymal cells are from extraembryonic mesoderm of the primitive streak.[8] Some of the basic functions of the amniotic membrane are to provide the developing embryo with protection from desiccation, to regulate the pH of amniotic fluid to keep it at 7.10,[9] metabolic functions such as the transport of water and soluble materials, and the production of bioactive factors, growth factors and cytokines.[10] The chorion does not make direct contact with the amniotic fluid, but it adds mechanical strength to the fetal membranes and also serves as the point from which the chorionic villi emerge, which carry nutrients from the maternal circulation system.[11,12]

Marc Moore is an assistant professor in the Stephenson School of Biomedical Engineering at the University of Oklahoma. His research interests are in the field of naturally derived biomaterials for use in wound healing, tissue engineering, and regenerative medicine applications.

Peter McFetridge is an associate professor in the J. Crayton Pruitt Family Department of Biomedical Engineering at the University of Florida. His primary research focuses on engineering viable 'living' tissue and organs for the repair and regeneration of diseased tissues. His groups research encompasses angiogenesis, arterial regeneration, articular cartilage as well as effects on primary and stem cell phenotype driven by mechanical and nutrient variation in the ECM microenvironment.

2.1. Angiogenicity

While the amnion is avascular, the main body of the placenta contains extensive vascular beds that are rich in basement membrane components and contain high concentrations of pro-angiogenic molecules.[13] ELISA assays have identified over 25 cytokines within the vascular bed shown to be directly related to angiogenesis and vascular regulation, including ANG, TGF-ß, TNF-α, IGF-1, SDF-1, VEGF, and vWF.[14,15] Liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis has also identified high concentrations of pro-angiogenic basement membrane components within the placenta including type IV collagen, heparin sulfate, desmosomes, fibrillin, laminin, and fibronectin.[15]

2.2. Antimicrobial

Amniotic fluid (AF) and vernix, the wax-like substance coating the fetus in the third trimester, have been shown to reduce bacterial counts and promote healing of infected wounds.[16] Antibody activity and antibacterial factors, including lysozyme, transferrin, and many immunoglobulins, are present in amniotic fluid,[17] which have been shown to inhibit growth of E. Coli and other anaerobic bacteria.[5] Current research has also shown that the chorion and placental trophoblast layers prevent the progression of infection in the pregnant uterus by serving as both physical and biomolecular barriers. These tissues displayed broad up-regulation of antimicrobial proteins HBD1-3 and elafin when exposed to TNFα and IL-1.[18]

While the precise nature of antimicrobial action is not well understood, it is clear that more than one mechanism contributes to this activity. A study by Kim et al. (2002) suggested that histones H2A and H2B may play an antimicrobial role as a endotoxin-neutralizing barrier, having shown dose-dependent inhibition of bacterial lypopolysaccharides (LPS) and endotoxin activity.[19] Investigations by Leipke et al. reported that hemoglobin-derived peptides purified from a human placenta peptide library exhibited antimicrobial activity in humans. These peptides inhibited the growth of gram-positive and gram-negative bacteria and yeasts in micromolar concentrations, as well as reduced endotoxin activity by binding to LPS.[20] Analysis has also shown the presence of neutrophil defensin (DEFA1), an antimicrobial peptide, within placental tissue.[21] The antimicrobial properties of the placenta are likely the result of multiple molecular interactions that work simultaneously to inhibit microbe growth and endotoxin activity. Further development of novel antimicrobial therapies derived from placental tissues are likely as more in-depth mechanistic studies are completed.

2.3. Anti-Inflammatory and Immune-Mediators

Anti-inflammatory cytokines in the placenta include a range of annexins,[22] defensins,[23] and interleukins,[24] all of which have been shown to be immunomodulatory. For example, placental annexin 1 (ANXA1) modulates components of inflammatory reactions such as metabolism of arachidonic

Table 1. Ongoing clinical studies of human amniotic tissues.

Clinical Trial ID[a)]	Study title[a)]	Product	Number of patients	Condition
NCT02632929	Prevention of Amputation in a High Risk Population With Comprehensive Care and Amniotic Tissue	AmnioExcel®	20	DFU at Amputation Risk
NCT02344329	A Comparison of AmnioExcel® and Total Contact Casting (TCC-EZ) Versus Standard Wound Care and TCC-EZ in Treating Diabetic Foot Ulcers	AmnioExcel®	20	DFU
NCT02679209	Clinical and Radiographic Evaluation of Amniotic Chorion Membrane and Demineralized Freeze-dried Bone Allograft in Periodontal Intrabony Defects: A Randomized Controlled Clinical Trial	Human Amniotic Membrane	60	Periodontal Intrabony Defects
NCT02132104	Amniotic Graft for Preventing Intrauterine Adhesions Following Hysteroscopic Surgery	Human Amniotic Membrane	208	Prevention of Intrauterine Adhesions
NCT02102776	Intraoperative Mitomycin C Application, Amniotic Membrane Transplantation and Conjunctival Autograft After Primary Pterygium Excision: A Multi-center Randomized Clinical Trial	Human Amniotic Membrane	750	Conjunctival Autograft for Primary Pterygium
NCT01693133	A Multicenter, Prospective, Randomized, Controlled, Comparative Parallel Study of Dehydrated Human Amnion/Chorion Membrane (dHACM) Wound Graft in the Management of Diabetic Foot Ulcers	EpiFix®	84	DFU
NCT02399826	A Prospective, Randomized, Comparative Parallel Study of Amniotic Membrane Graft in the Management of Diabetic Foot Ulcers	AmnioBand™	40	DFU
NCT02361814	The Effect of Human Amniotic Membrane Allograft on Functional Recovery After Flexor Tendon Repair: a Pilot Study	Human Amniotic Membrane	10	Recovery after Flexor Tendon Repair
NCT02609594	A Multi-center Randomized Controlled Clinical Trial Evaluating Two Application Regimens of Amnioband Dehydrated Human Amniotic Membrane and Standard of Care vs. Standard of Care Alone in the Treatment of Venous Leg Ulcers	AmnioBand™	240	VLU
NCT02461641	The NuTech NuShield And Affinity Membrane Product Evaluation for the Treatment Neuropathic Diabetic Ulcers (DFU)	NuShield™, Affinity®	90	DFU
NCT02011503	A Randomized Controlled Clinical Trial Evaluating The Application Of Dehydrated Human Amnion/Chorion Membrane (dHACM) Plus Standard Of Care Vs. Standard Of Care Alone In The Treatment Of Venous Leg Ulcers	EpiFix®	120	VLU
NCT02587104	A single-center, non-randomized study with subjects each receiving EpiFix mesh.	EpiFix® Mesh	20	DFU
NCT02166294	A Multi-center, Randomized, Parallel, Crossover Design Study of Non-healing Diabetic Foot Ulcers, Treated With Cryopreserved, Umbilical Cord Allograft (NEOX® CORD 1K) Versus Standard of Care That Are Followed for 12 Weeks	NEOX® CORD 1K	30	DFU
NCT02702406	A Multi-center, Randomized, Controlled Study of Non-healing Diabetic Foot Ulcers (DFU) Treated With Standard of Care With or Without Cryopreserved Umbilical Cord Allograft	NEOX® CORD 1K	114	DFU
NCT02571738	A Multicenter, Randomized, Single-Blind Study with an Open-Label Extension Option to Further Evaluate the Safety and Efficacy of Cryopreserved Human Amniotic Membrane for the Treatment of Chronic Diabetic Foot Ulcers	Cryopreserved Human Amniotic Membrane	224	Chronic DFU
NCT02506452	Evaluation of Biovance, a Dehydrated Decellularized Human Amniotic Membrane Allograft, in Diabetic Foot Ulcers	Biovance®	60	DFU

DFU = diabetic foot ulcer; pt = patient; pts = patients; VLU = venous leg ulcer; dHACM = dehydrated human amnion/chorion membrane.; [a)]Ref. [76].

acid, inhibition of iNOS, inhibition of COX-2, and inhibition of neutrophil and monocyte migration.[25] Other investigations have shown the complexity of the inflammatory response where isolated placental trophoblasts secrete both pro-inflammatory (IL-1β, IL-6, IL-8, TNF-α) and anti-inflammatory cytokines (IL-4, IL-10) when subjected to pathogenic strains of bacteria (including *Escherichia coli* and *Staphylococcus aureus*).[26] This is important as cell therapies derived from these tissues may have similar anti-inflammatory properties.

Given the composition of anti-inflammatory proteins within the placenta, complex biomolecules and biomaterials derived from these tissues may ultimately aid in the development of immunosuppressive therapies, and lead to the creation of advanced functional materials that modulate the immune response while enhancing wound healing.

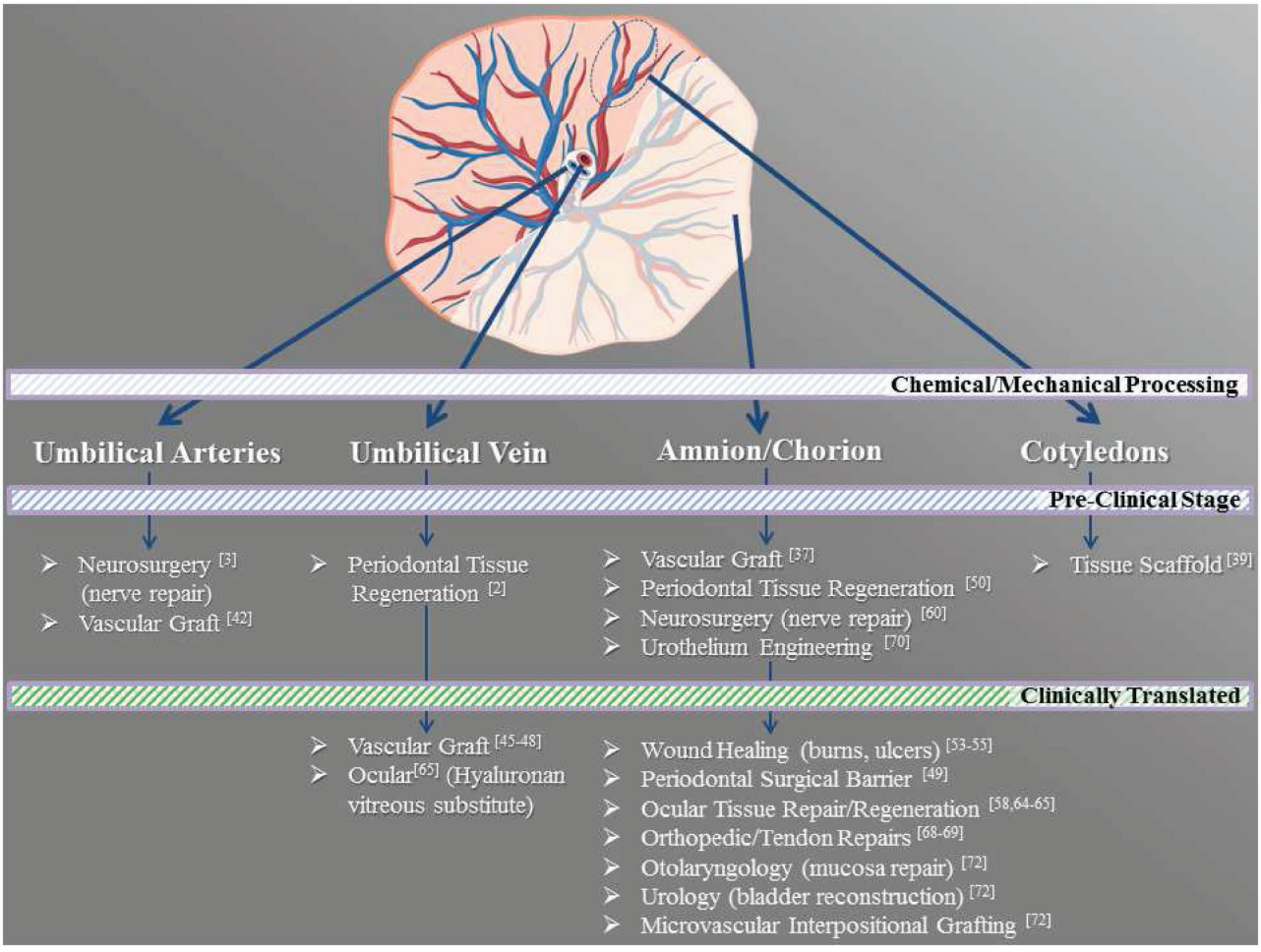

Figure 1. Placenta as a biomaterial source. Among components used clinically, the placenta can be dissected into four main groups including the umbilical arteries, the umbilical vein, the fetal membranes, and the villous placenta (cotyledons). Processing these components is fundamental to their use as biomaterials, with the most common methods including both chemical and mechanical based purification, extraction, and isolation techniques. Many preclinical studies are in progress to evaluate the use of the placental biomaterials as part of novel tissue engineering and regenerative medicine approaches. Medical disciplines in which these materials have been successfully clinically applied include cardiology, dentistry, dermatology, neurology, opthamology, orthopedics, and general surgery.

2.4. Low Immunogenicity and Immunoregulation

Perinatal cells and biomaterials often lack or exhibit a reduced expression of surface antigens.[6] While cells derived from human umbilical cord tissue are still under investigation, comparative studies of cells from miniature swine cord tissue have been shown to have low MHC class I (MHC1) expression and no MHC class II (MHC2) expression with IFN-γ simulation.[27] Over a century of anecdotal evidence, in ocular and burn applications and more recently with extensive use in the USA without any FDA reported adverse events, supports the hypothesis that these materials, including maternal derived tissues in the placenta, exhibit some degree of immune privilege.

In addition to the lack of immunogenic molecules within placental biomaterials, they also contain immunomodulatory molecules including members of the B7 family of immunomodulatory cell-associated proteins, which have been shown to exist near the maternal-fetal interface.[28] Other immune focused proteins found in the human placenta, amnion, and chorion include inhibin, ativin, and follistatin which may also function as growth factors or immune modulators.[29]

2.5. Extracellular Matrix (ECM) Structure

Structural properties of the placenta's ECM vary widely and are consistent with functions of a complex organ. Components such as the amniotic and chorionic membranes have complex mechanical properties complementary to their physiological function. A variety of methods have been used to test the mechanical properties of the membranes making comparative data difficult. It appears that the fetal membranes are mechanically heterogeneous, with the force required to rupture under uniaxial tension being much greater than the forced to rupture the membrane using biaxial puncture.[30] Fetal membranes from twin and cesarian deliveries have been shown to have different mechanical properties relative to those from full-term normal vaginal births. With the maximum tensile strength

Table 2. Perinatal-derived biologics and biomaterial industry: Companies, revenue, and products.

Company name	2014 Revenue (MM $'s)	Product names and descriptions
Alliqua Biomedical, Inc.	Unknown	Biovance®: Decellularized, dehydrated human amniotic membrane allograft (chorion removed).
AmnioGenix	$5.00[c)]	AmnioDryFlex®: Dehydrated tissue allograft derived from human amnion.
		AmnioMTM: Flowable human placental tissue allografts.
Amniotic Therapies, Inc.	Unknown	Amniotic AlphaPATCH™: Dehydrated tissue allograft derived from human amnion.
Amniox Medical, Inc.	Unknown	NEOX CORD 1K™: Cryopreserved umbilical cord/amniotic membrane wound allograft.
Applied Biologics	$1.00[b)]	Flograft®: Cryopreserved, injectable amniotic fluid-derived allograft.
BioD Logics, Inc.	$14.00	AmnioExcel®: Dehydrated amniotic membrane allograft provided in prescribed multiple geometric configurations.
Bone Bank Allografts	$2.50[b)]	SteriShieldII™ Dual Layer: Two dehydrated, laminated amnion layers.
Human Regenerative Technologies, LLC	Unknown	Flowable Hydratek®: Cryopreserved amnion and chorion based flowable products.
		Membrane Hydratek®: Single and Laminated-Multilayer dehydrated amnion products.
Medline Industries	Unknown	Revitalon®: Dehydrated human amnion/chorion membrane allograft.
MiMedx Group, Inc.	$117.00[a)]	EpiFix®: Dehydrated human amnion/chorion membrane allograft.
		AmnioFix®: Dehydrated composite amniotic tissue membrane.
MTF Woundcare	Unknown	AmnioBand™: Dehydrated human placental membrane comprise of amnion and chorion.
		VersaShield®: Decellularized, dehydrated human amniotic membrane allograft.
NuTech Medical	$2.50[b)]	Affinity®: Fresh amniotic allograft.
		NuShield™: Sterilized, dehydrated human placental allograft.
Osiris Therapeutics, Inc.	$59.9[d)]	Grafix®: Cryopreserved placental membrane; human viable wound matrix.
Skye Biologics	[B]$0.95[b)]	Woundex® Flow: Flowable sterile, human placental tissue allografts.
		Woundex® Membrane: Sterile, dehydrated human amniotic membrane product sheets.
Tri-State Biologics	$10.00[b)]	Amniovo™: Sterilized, dehydrated composite human amniotic tissue membrane.
TOTAL REVENUE (ESTIMATED)	**$212.85**	

[a)]Ref. [77]; [b)]Estimate from Manta Co. (Columbus, OH); [c)]Estimate from Zoominfo. (Waltham, MA); [d)]Ref. [78].

of fetal membranes derived from normal vaginal births, twin births, and cesarean-section births being 0.21, 0.45 and 0.22 kg cm^{-2}, respectively;[11] likely resulting from changing hormones concentrations during labour and mechanical stress of the birthing process. As detailed by Kanamaya et al., mechanical differences between pathological and normal membrane mechanics are also the result of compositional variation (eg. Type III collagen).[31]

In addition to variation in the membranes ECM composition that have been noted to influence membrane integrity, dysfunction of several biochemical pathways has also been implicated in the tissues mechanical rupture. As the precise mechanism of failure is unknown, it has become the focus of numerous recent investigations. Up-regulation of extracellular matrix metalloproteinase inducer (EMMPRIN) and the subsequent expression of matrix metalloproteinases (MMPs) results in excessive collagen degradation and amnion weakening.[32] More recently, MMP activation has been shown to cause detachment of amnion epithelial cells from surrounding tissue, a biochemical event known as the amniotic cell epithelial to mesenchymal transition (EMT). This results in a decrease in the tensile strength of the amnion and may ultimately play a role in membrane rupture.[33]

While the materials inherent mechanical properties complement a variety of clinical applications, modifications to the materials have been made to suit broarder clincial use. A variety of processing techniques have been used to modify the tissues mechanical properties, including chemical treatments, decellularization, drying, cryopreservation, lyophilization, sterilization, and layering. Crosslinking agents such as glutaraldehyde have been used to prolong mechanical stability, prevent degradation, and increase biomaterial mechanical strength.[34] These are often used in barrier applications with the amnion to inhibit cellular infiltration and have shown evidence of reduced fibrosis.[35] Sterilization and decellularization agents have also been shown to alter mechanical strength,[36] and as such, care should be taken during processing to ensure the ECM maintains its desirable mechanical attributes. Lamination of multiple amniotic sheets has been used to create biomaterials with improved tensile strength and handling characteristics. In addition to uses as a more robust sheet, these

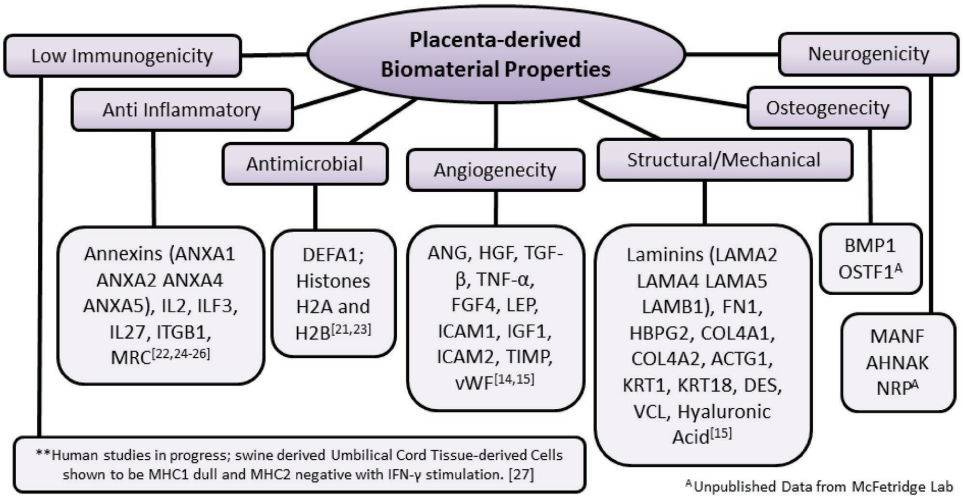

Figure 2. Placental-derived biomaterial properties and proteins. Placental biomaterials have been shown to be angiogenic, antimicrobial, anti-inflammatory and antimicrobial, in addition to having low immunogenicity and wound healing characteristics. This wide array of properties is the result of material derivation from a highly vascular organ that is associated with fetal development and facilitates the exchange of nutrients and waste between two separate blood supplies, while also acting as a barrier to prevent reactivity between two immune systems.

modifications have shown potential in vascular and neuronal applications.[34,37,38] With the positive characteristics associated with these materials, it is likely that novel approaches will be taken to modify the base structure and function, either on their own or in combination with other materials, to bring innovative therapies to the clinic.

3. Placental-Derived Biomaterials by Medical Specialty

Perinatal tissues provide a unique platform for biomaterial development with applications in a wide range of medical disciplines, including cardiology, dentistry, dermatology, neurology, opthamology, orthopedics, and general surgery. The most common (published) application of these materials has been use of the amnion as a wound cover and the umbilical vein as a vascular graft. More recently, modified placental tissues have become commerically available as mechanically micronized, injectable amnion tissue scaffolds that are widely available as a treatment option. These clinically available materials have been used as cell-viable tissues (containing placental stem cells) as well as chemically processed forms, such as glutaraldehyde treated.[7]

In contrast to most clinically available synthetic or metallic materials, these biomaterials are rich in functional biologics that require delicate processing conditions in order to maintain function.[39] Collection of placental tissues typically includes donor consent, an initial screening for communicable diseases (prebirth), followed by a second screening and microbial testing of the tissue (post birth). Materials are collected from the hospital using approved procedures, and are then transported to a dedicated processing facility where components of the tissue are isolated and prepared for construction toward a specific indication. The final steps include packaging, labeling and terminal sterilization, unless they are in a cell viable form where aseptic techiniques are required and the terminal sterilization treatment is ommited. In the case of extracting specific placental biomolecules, processing methods include chemical and mechanical based purification, extraction, and isolation techniques.[40] These methodologies, in particular any aggressive treatment such as terminal sterilization, must be optimized to maintain the materials natural characteristics and minimize tissue or molecular degradation.

3.1. Cardiovascular

Until recently, vascular applications have focused on chemically stabilized umbilical veins and arteries for vascular reconstruction.[41,42] Typically these vessels have been treated with glutaraldehyde to cross-link their protein structure as an approach to 'mask' foreign epitopes and prevent subsequent immune-based degradation. These manually dissected vessels have translated well as peripheral bypass grafts because of their improved patency over synthetic alternatives and availability in long sections (average 50 to 60 cm) without bifurcations.[43]

The earliest reports of glutaraldehyde cross-linked human umbilical veins (gHUV) had shown improvements in graft patency compared to synthetic materials, with no reports of infection, rejection, or aneurysm formation for a periods up to 9 months post-surgery.[42,44,45] Patency rates approach the efficacy of autogenous saphenous veins, but with decreased operative time and morbidity.[45] While most studies have shown postive results, Dardik et al. hypothesized that there was a potential for aneurysmal degeneration in these grafts due to the tanning process preventing biological remodeling, which may lead to material fatigue rather than occlusive failure.[46] To limit the potential of degradation and aneurysm, processing treatments have been modified to include external wrapping of polyester Dacron meshes.[42,47] In addition to current clinical applications, modified umbilical veins have been assessed as vascular substitutes for arteriovenous shunts,[44] popliteal, tibial, and peroneal arteries.[45] Although these processed grafts

out perform synthetic materials, the current gold standard (transplanted autologous arteries) remains the grafting material of choice due to extended patency rates. This has been hypothesised to be due to chemical stabilization which inhibits cell migration and graft remodeling.

In an effort to improve patency rates, research has been oriented toward omitting chemical cross-linking to promote biological integration with host cell populations via dynamic reciprocity. As with most other tissue engineering approaches where ex vivo tissues are used, these vessels have been processed using procedural decellularization to remove soluble allogeneic biomolecules with the aim to minimize negative immune responses. In 2005, an automated technique was developed to rapidly and uniformly dissect the umbilical vein and arteries.[36] This approach made it possible to dissect the umbilical vessels while preserving their mechanical properties and maintaining a uniform wall thickness; shown to be a significant improvement over manual dissection methods. Importantly, burst pressure, mechanical compliance and structural anatomy were preserved. Decellularized umbilical vessels processed this way and reseeded with vascular cells using controlled culture conditions have been shown to positively influence remodeling characteristics leading to viable tissue.[48]

A recently developed cardiovascular application of the amnion is the formation of laminated multi-layer tubes from rolled sheets for vascular reconstruction (**Figure 3**).[34] Still in early development, these amniotic tubes have been shown to have positive endothelial cell (EC) and smooth muscle cell (SMC) adhesion and repopulation characteristics, while also upregulating SMC contractile remodeling markers (ACTN1, SM22) and matrix remodeling markers (COL1A1, MMP2) indicating biological adaptation when cultured in perfusion bioreactors.[34] In comparison to the HUV and HUA, where vessel diameter is constrained, this technique allows for the creation of a variety of vessel diameters, wall thicknesses, shapes, lengths, and material compliance for distinct anatomical locations. In vivo applications using this approach have shown positive biological integration and patency when used as a xenograft in a rabbit interpositional carotid model.[37]

3.2. Dentistry

The HUV and the AM have also shown potential as surgical barriers for periodontal tissue regeneration. HUV sections have been longitudinally dissected to create biomaterial sheets to protect dental wound environments where the vascular basement membrane comprised of compact collagen acts as a microbial barrier at the oral interface while the more porous ablumenal surface (adjacent to the wound site) promotes cellular migration and remodeling.[2] In vitro analysis has shown the scaffold to support human gingival fibroblast adhesion and ECM remodeling, indicating the vascular bioscaffold may serve well in clinical periodontal applications as both a surgical barrier and also as a resorbable guide for tissue regeneration.

Lyophilized AM have also been used as surgical barriers to aid reconstruction of the sublingual papilla following a total glossectomy,[49] as well as being used as a cell/AM transplant method for periodontal regeneration.[50] In investigations by Iwasaki et al., periodontal ligament stem cells (PDLSC) were

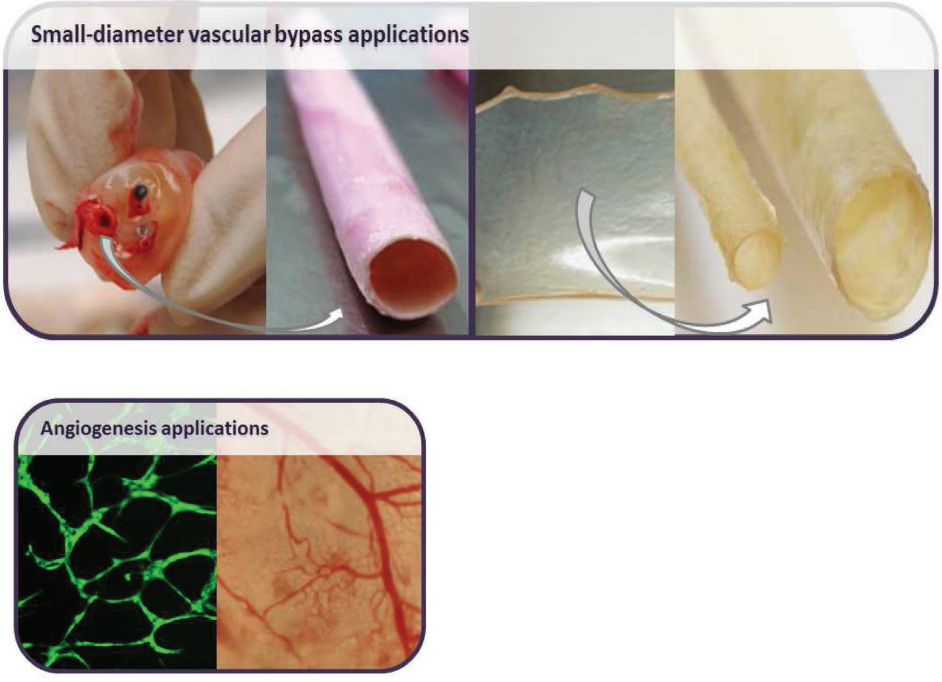

Figure 3. Placenta as a source for cardiovascular biomaterials. The human umbilical vein (top left) and rolled amniotic membrane (top right) have been proven effective as scaffolds for vascular reconstruction.[34,48] Placental derived matrix has been shown to induce angiogenesis.[15] Bottom left represents capillaries formed in vitro on human placental matrix coated TC plate. Bottom right image shows neo-angiogenesis of blood vessels after (rat) implantation of a biomaterial made using human placental matrix. Bottom right image reproduced with permission.[15] Copyright 2015, Elsevier.

seeded onto the surface of an AM (PDLSC-amnion), which was transplanted into (rat) periodontal defects. Results showed scaffolds to have therapeutic potential as a novel cell-based regenerative therapy that supports regeneration of tissues destroyed by periodontal diseases.[50]

3.3. Dermatology

The first reported uses of the AM were demal applications by Davis in 1910 to cover a granulation wound,[1] and Sabella in 1913 to treat a burned patient.[51] Since these first applications the amnion has been shown to impart a considerable reduction in pain and infection in patients, as well as increased epithelialization.[52] More recent dermal applications include surgical patches and injectable-micronized AM products for healing maladies ranging from skin burns to lower extremity ulcers,[53] as well as for relieving pain, promoting healing, and preventing loss of fluids.[54] The AM has also been found to be superior to human skin in reducing the microbial populations in infected rat burns.[55] In other clinical applications the AM is being used to treat severe and potentially life-threatening skin conditions such as Stevens-Johnsons syndrome and non-healing leg and foot ulcers.[53,56,57] Both traumatic and non-traumatic wounds that do not respond to current therapeutic treatments have been shown to respond positively to AM application.[53,57]

In comparison to other dermal wound healing therapies, the AM has a number of key properties that make it an effective wound repair membrane, these include the differentiation potential of its native *in situ* stem cells (if processed as a viable tissue) and the combination of its anti-inflammatory, anti-bacterial, anti-viral and minimal immunogenic characteristics. It is also an ideal patch for superficial wound healing because it acts as a physical barrier against bacterial infiltration, where it adheres to the wound surface, and prevents hematoma formation.[58]

3.4. Neurology

With research currently in the pre-clinical stages, perinatal tissues have received much attention by neurology researchers. Molecular analysis using mass spectroscopy has indicated these tissues contain neurogensis modulators including neural-related mesencephalic astrocyte derived neurotrophic factor (MANF), desmoyokin (AHNAK) and neuropilin-1 (NRP1) cytokines among others (unpublished data from McFetridge Lab). As such, it is not surprising that biomaterials derived from these tissues have significant potential in nerve related tissue regeneration environments.

Processed amnion and umbilical arteries have been assessed for their potential as tubular guides to bridge nerve defects by directing neuronal elongation, localizing growth factors, and inhibiting fibrotic cellular ingrowth.[3,59] Engineered bioscaffolds derived from decellularized umbilical arteries have comparable biomechanical properties relative to native nerves, and have been shown to effectively serve as a basis for the adherence and migration of neuronal-like PC12 cells.[3]

The AM has also been reported to be effective as a biodegradable conduit for peripheral nerve regeneration,[60] and has shown potential as an adhesion barrier to substitute the natural tissue of the dura mater and prevent cerebrospinal fluid (CSF) leakage; one of the most common and undesirable complications after craniotomy and craniectomy procedures.[61] Cells isolated from AM show potential to express neurosupportive factors;[62] amnion derived cells can be differentiated towards a Schwann cell-like lineage and their neurosupportive properties enhanced by the addition of neurotrophic factors.[63]

3.5. Ophthalmology

The AM has seen significant clinical use in ocular applications, due to its positive healing characteristics as well as its translucence, tissue matching mechanical properties, and biochemical composition.[64] These properties have made it ideal for the treatment of corneal injuries, conjunctival defects, caustic eye burns,[65] as well as ocular tissue regeneration.[66] The first documented ophthalmological use of the AM was in the 1940's when native AM sheets were used for the plastic repair of conjunctival defects.[67] Until the prevalent use of AM for conjunctival defect repair the typical treatment required transplantation of viable tissue from the patients opposite eye. This earlier approach was less than ideal due to difficulty obtaining sufficient tissue and the potential for donor site morbitity.

Currently, cryopreserved and dehydrated AM sheets represent the most commonly used ophthalmological biomaterials derived from placental tissues. In comparison to earlier AM uses that relied on the regenerative properties of viable cells within the ECM, these versions contain no viable perinatal cells and as such rely solely on the biomaterial's function and composition (including growth factors) as a tissue scaffold to guide regeneration.[64] Several research groups have concluded that AM is not simply a substitute of the cornea, but it represents an appropriate structure or scaffold capable of promoting regeneration by the patients own endogenous corneal limbal cells without the need for exogenous stem cells.[9]

3.6. Orthopedics

As in other clinical areas, there has been growing interest in the use of placental biomaterials in orthopedic applications. Placental tissues are an excellent source of stem cells and given the documented presence of osteogenic proteins, placental tissues have potential to be a platform for osteogenic and chondrogenic biomaterials.[68] The AM has been shown to be effective in preventing the formation of fibrous tendon adhesion without affecting healing, and when used as a graft it dramatically reduces post-operative adhesion formation.[35] Supporting these earlier studies, Willett et al. in 2014 detailed the application of micronized AM scaffolds injected via intra-articular delivery into a rat osteoarthritis model. Results from this approach show an attenuation of cartliage degradation and a positive therapeutic effect in modulating the progression of osteoarthritis.[69] Additionally, our lab has shown placental derived materials to prevent fibrosis,[21] and unpublished data

has shown the AM to contain cytokines involved in osteogenesis including bone-related BMP1 and OSTF1.

3.7. Other Surgical Applications

The AM is commonly used because of its ease of isolation, sheet-like geometry and immune privilege. These properties have made it an ideal material for a variety of surgical needs such as indirect organ reconstruction and as an aid to surgical recovery. Studies of the AM in urology have shown it to be a suitable scaffold that promotes urothelial cell proliferation and differentiation, which may enable the development of tissue-engineered urotheliums with molecular and ultrastructural properties comparable to that of native tissues.[70] The AM and amniotic fluid have also been used in gastroenterological surgery to prevent peritoneal adhesions.[71] Further clincial applications include treatments for congenital absence of the vagina, replacing nasal mucosa, bladder wall reconstruction, as well as microvascular interpositional grafting.[72]

4. Regulatory

Perinatal tissues have long been regulated by the US Food and Drug Administration (FDA) as a human cell, tissue, and cellular and tissue-based product (HCT/P). However, the increased success as a biomaterial in a variety of regenerative medicine applications has resulted in increased scrutiny and oversight by the FDA, which may ultimately impact the clinical utility of these materials.

HCT/Ps are regulated by the Center for Biologics Evaluation and Research (CBER) under the Code of Federal Regulations Parts 1270 and 1271. These regulations require those who are recovering, processing, and distributing allografts to follow procedures to prevent the transmission of relevant communicable disease agents or diseases (RCDADs) via the establishment of donor screening criteria and good tissue practices (GTPs). HCT/Ps are regulated solely under 21 CFR 1271.3(d)(1) and Section 361 of the Public Health Service (PHS) Act when they meet all of the criteria of 21 CFR 1271.10(a) which states that the materials must be minimally manipulated, intended only homologous use, and its manufacturing should not involve the combination of the cells or tissues with another article, except for water, crystalloids, or a sterilizing, preserving, or storage agent, provided that the addition does not raise new clinical safety concerns. Furthermore, the HCT/P cannot have a systemic effect and cannot be dependent upon the metabolic activity of living cells for its primary function; and if it does it must be for autologous use, for allogenic use in a first-degree or second-degree blood relative, or be for reproductive use.

If these criteria are not met, manufacturers of HCT/Ps not only have to comply with 21 CFR 1270 and 1271, but they must also gain regulatory approval via the traditional device, drug, and/or biologic pathways (premarket notification, premarket approval, investigational device exemption, investigational new drug application, biologic license application) before marketing their products. These alternate regulatory pathways increase both the time to market and the costs associated with these products and could thus also potentially stifle innovation.

Based upon a number of recent actions by the FDA's Center for Biologics Evaluation and Research (CBER), FDA regulators have been focusing their attention on these Section 361 HCT/Ps and have revised their opinion regarding the regulatory classification of perinatal tissues. The FDA recently published a number of Draft Guidance for Industry documents as a method to modify and promote increased regulation of specific HCT/Ps including but not limited to perinatal tissue derived products. The FDA reinforces that with regard to the minimal manipulation of HCT/Ps, the amniotic membrane must retain its original relevant characteristics to serve as a membranous barrier. Also, with regard to the homologous use of HCT/Ps, the FDA reinforces that the amnion serves to cover, protect, and act as a selective barrier for the movement of nutrients between the external and *in utero* environment; as such, an amniotic membrane used for wound healing of dermal ulcers and defects is not a homologous use, because wound healing of dermal lesions is not a basic function of amniotic membrane.

It is interesting to note that some of the information provided in these Draft Guidance documents is inconsistent with previous FDA positions that regard perinatal tissues as compliant with regulations. For example, even though dermal use of the AM has been cited by the FDA as not a basic function of the material, in a letter to Bio-Tissue dated November 26, 2001, the FDA stated "that both in utero and on the ocular surface, the amniotic membrane [...] acts as an anti-scarring agent, an anti-inflammatory agent, and a anti-angiogenic agent". The FDA further stated that "Bio-Tissue's amniotic membrane product... meets the criteria contained in 21 CFR 1271.10(a), including homologous use".[74] In the preamble to the final rule on Current Good Tissue Practice for HCT/P product establishments, the FDA noted that amniotic membrane for ocular repair (an eye wound) is homologous use. This was confirmed in the Federal Register that the FDA considered ocular repair as homologous use of amniotic membrane. In the FDA's guide,[75] it was stated that "Amniotic membrane when used alone or without added cells" is a product that meets the criteria in 21 CFR 1271.10(a). Additionally, the FDA has previously indicated that the "basic functions" of amniotic membrane *in utero* include anti-inflammatory, anti-scarring, and anti-angiogenic functions, and acting as a wound repair and wound healing agent.

Based on the available information, the FDA considers allogeneic amniotic membrane derived products, in a powder or flowable format, to be more than minimally manipulated and therefore not considered 361 HCT/P compliant. To that effect, it appears that FDA will require manufactures of these products to follow the regulatory approval process of a new drug or biologic. Furthermore, to date there are currently no FDA-approved placenta-derived stem cell treatments for orthopedic regenerative medicine applications. Those that are currently being investigated in clinical trials will need to undergo a regulatory pathway similar to that of most drug companies; thus, the regulatory hurdles will be significant and burdensome which will inhibit development of these materials, particularly novel designs and applications.

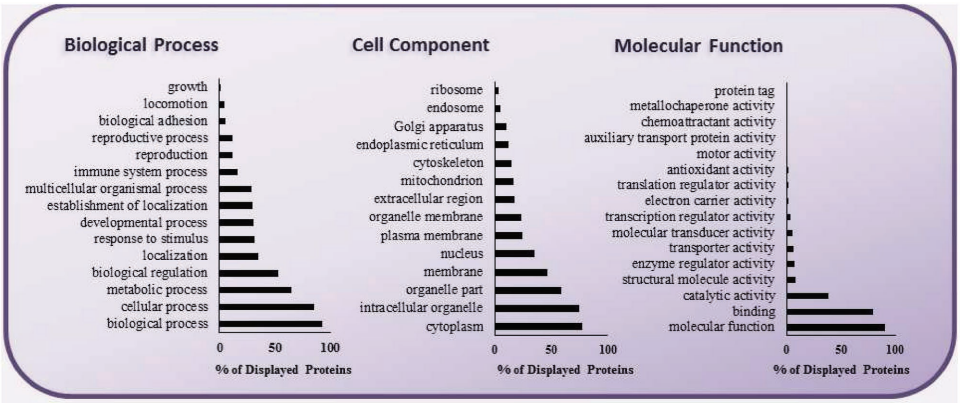

Figure 4. Compositional analysis of a placental protein complex by molecular function, role, and derivation. LC-MS/MS was used to analyzed a placenta protein complex (hPM) isolated from a whole placental villous bed with attached fetal membranes.[15] Detected proteins (data from McFetridge lab) were then grouped according to their associated biological process, cell component derivation, and molecular function.

5. Conclusion

Given the unique biological properties of perinatal derived-biomaterials, their potential applications are the basis for exciting developments in basic sciences, regenerative medicine research, as well as clinical applications. In contrast to the many bottom-up approaches to synthesize biologically functional materials (e.g. start with a polymer and add functional proteins one-by-one), the use of naturally derived materials allows researchers to use a top-down approach to create biomaterials. Functionality can be designed into these materials, if necessary, by selective removal of biomolecules in an already functional matrix. Future investigations may yield new commercial opportunities in the form of biomaterials which target multiple biological functions, including control of immunogenecity, inflammation, and angiogenesis.

The diverse biomolecular composition of perinatal tissues, which is the result of the placentas roles in facilitating a wide array of fetal developmental processes (**Figure 4**), allows the isolation of a considerable number of different functional molecules from a single starting material. Placental extracellular matrix extracts have been shown to contain in excess of 2600 proteins, with many more likely to exist (unpublished LC-MS/MS data, McFetridge Lab).[73] Thus, researchers may use these tissues as a starting point to derive biomaterials with compositions that target specific molecular processes such as angiogenesis, neurogenesis or osteogenesis.

Importantly, the placenta represents a "platform technology" that can be used clinically across a variety of regenerative medicine and biologic applications. For example, it can be used in multiple cardiovascular application to both create vascular grafts as well as induce angiogenesis for wound healing. While cadaveric tissues must primarily be used in application specific technologies as per the FDA's HCT/P regulations (eg. demineralized and decellularized cadaver bone must be used to regrow bone), placenta derived biomaterials can be used in wide-ranging commercial applications as a result of their derivation from an organ that facilitates multiple biological processes necessary for fetal growth and development.

While past studies have focused on the amniotic membrane and umbilical cord, the main placental bed and cotyledons have received only modest attention as potential 3D biomaterials. Whole placentas perfused with decellularization solutions have been shown to yield extensively bifurcated vascular trees rich in basement membrane and extracellular membrane components.[39] Sections of these materials have the potential for repair of small diameter blood vessels or could be used in conjunction with polymerizable biomaterials to engineer composite biomaterials and complex organs. For example, to form a vascular bed within a cell-dense tissue construct, a perfusable cotyledon bioscaffold and cells could be placed into an unpolymized scaffold, which upon gelation creates a cell dense scaffold with a perfusable vascular system.

Future applications are likely to explore the use of maternal-side perinatal tissues. Until recently, clinically marketed placental products have consisted entirely of components derived from the fetal side of the placenta such as the amniotic membrane and human umbilical cord, whose tissue and cellular components have naturally low antigenicity.[6] Future applications may include ABO and RH factors matched to the blood type of the intended recipient in order to make broader use of maternal cell and ECM components. Ultimately, future directions are expected to be diverse with novel materials, therapies and applications taking advantage of the tissues innate healing properties. As clinical use expands across a wide range of specializations, the growing published and anecdotal clinical evidence will continue to spur innovation provided that the regulatory pathway does not increase development costs past the current ecomomies. As our understanding of these complex perinatal biomaterials (and cells) improves by detailing modes of action, innovative discoveries in biomaterials and cell therapies will be facilitated.

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Conflict of Interest

The authors, Marc Moore, Aurore Van de Walle, Cassandra Juran, and Peter McFetridge, declare that they have no competing interests. Jerry Chang declares that he is employed by BioD Logics, LLC (Memphis, TN).

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