Biofabrication of tissue-specific extracellular matrix proteins to enhance the expansion and differentiation of skeletal muscle progenitor cells

Cite as: Appl. Phys. Rev. **6**, 021309 (2019); https://doi.org/10.1063/1.5088726
Submitted: 14 January 2019 . Accepted: 10 April 2019 . Published Online: 26 April 2019

Longkun Li, Guihua Liu, Peter Timashev, Xiuzhi Susan Sun, Tracy Criswell, Anthony Atala, and Yuanyuan Zhang

Applied Physics Reviews REVIEW scitation.org/journal/are

Biofabrication of tissue-specific extracellular matrix proteins to enhance the expansion and differentiation of skeletal muscle progenitor cells

Cite as: Appl. Phys. Rev. **6**, 021309 (2019); doi: 10.1063/1.5088726
Submitted: 14 January 2019 · Accepted: 10 April 2019 ·
Published Online: 26 April 2019

Longkun Li,[1,a)] Guihua Liu,[2] Peter Timashev,[3] Xiuzhi Susan Sun,[4] Tracy Criswell,[5] Anthony Atala,[5] and Yuanyuan Zhang[5,a)]

AFFILIATIONS

[1]Department of Urology, Xinqiao Hospital, Amy Medical University, Chongqing, China
[2]Reproductive Medicine Research Center, the Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China
[3]Institute for Regenerative Medicine, Sechenov University, Moscow, Russia
[4]Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, USA and Biological and Agricultural Engineering, Kansas State University, Manhattan, Kansas 66506, USA
[5]Wake Forest Institute for Regenerative Medicine, Winston-Salem, North Carolina 27109, USA

Note: This paper is part of the Special Topic on 3D Bioprinting: Physical and Chemical Processes.
[a)]**Authors to whom correspondence should be addressed:** lilongk@hotmail.com and yzhang@wakehealth.edu

ABSTRACT

Skeletal muscle precursor cells (MPCs) are considered key candidates for cell therapy in the treatment of skeletal muscle dysfunction due to injury, disease, or aging. However, expansion of a sufficient number of functional skeletal muscle cells *in vitro* from a small tissue biopsy has been challenging due to changes in the phenotypic expression of these cells under nonnatural microenvironmental or traditional culture conditions. This review provides an overview of recent progress in the design and biofabrication of advanced tissue-specific extracellular matrix (ECM) proteins for use in the enhancement of expansion and differentiation of MPCs for cell therapy and 3D bioprinting. We start with a brief introduction about the existing progress, drawbacks, and emerging challenges in the culture and maintenance of long term primary human MPCs for cell therapy. With regard to MPC proliferation, elongation, fusion, and differentiation into mature myofibers, we systematically summarize the benefits and limitations of recent progress. The importance of tissue-specific ECM in skeletal muscle regeneration is discussed, in particular, the mechanisms, rationale, strategy, and methodologies for using tissue-specific ECM proteins for myogenesis in 2D and 3D culture environments. Furthermore, perspectives on the challenges in developing tissue-specific ECM proteins for cell therapy using human MPCs are described. Finally, we propose potential strategies for overcoming the challenges in the development of advanced tissue-specific ECM proteins for promoting cell therapy with human skeletal muscle cells.

TABLE OF CONTENTS

INTRODUCTION

There are three types of muscles in the body: Skeletal muscle, cardiac muscle, and smooth muscle. Each type of muscle has a different capacity for regeneration after insult. Smooth muscle cells have the greatest ability to regenerate, whereas cardiac muscle cells do not regenerate well,[1] if at all. Skeletal muscle stem/progenitor cells [muscle precursor cells (MPCs) or satellite cells] possess a moderate regenerative capacity and can form new muscle tissue after injury.[2] Trauma-associated skeletal muscle injuries or genetic muscle defects are common clinical problems. Despite significant progress in the field, there are many remaining challenges in the treatment of severe skeletal muscle defects. Cell-based therapy has the potential to provide effective treatments for injured or diseased muscle (e.g., muscular dystrophy). The ideal methodology behind cell therapy would be to obtain a

small biopsy of healthy tissue from the patient and expand the cells multiple times *in vitro*. However, the challenge is to maintain their stem cells and self-renew the potential, potentially induce them to differentiate into a specific target cell type *in vitro*, and finally implant the cells back into the patient. Most stem cells lose their "stemness," or ability to differentiate into a desired phenotype, in the *in vitro* cell culture environment used for cell expansion. Although skeletal MPCs have been frequently used in cell therapies for multiple indications, these cells either lose their skeletal muscle characteristics or stop growing and become senescent when cultured *in vitro*, indicating that traditional 2D cell culture lacks key components provided in the natural muscle microenvironment for the maintenance of their proliferation and myogenic differentiation potential. The extracellular matrix (ECM) of a tissue provides structural and biochemical support to the surrounding cells.[3] Importantly, it also releases a broad portfolio of cellular growth factors/cytokines required for the promotion of tissue repair after injury.

Currently, several matrices have been used in MPC culture including collagen, laminin, fibronectin,[4] and gelatin.[5] In addition, certain tissue extracts enriched in the matrix (e.g., Matrigel®, extracts from amnion)[5] have well-documented, effects on the growth and survival of cells in culture. However, these extracts are not tissue-specific, therefore promoting cell expansion but not retention of specific myogenic phenotypes through expansion over time. Moreover, commonly used nonhuman tissue extracts enriched in the matrix, such as Matrigel, which is derived from a murine sarcoma, cannot be used for clinical purposes. Thus, in this review, we focus on the beneficial impact of porcine-derived tissue specific ECM (pECM) on the expansion and retention of the regenerative potential of human MPCs *in vitro*.

CHARACTERISTICS OF MPCs

The physiological functions of the skeletal muscle are varied including locomotion and maintenance of posture, as well as the generation of body heat, control of vascular tone, and maintenance of glucose metabolism.[6] Skeletal muscle is the largest organ in the human body, making up 42% of the body mass of an adult male and 36% of the body mass of an adult female.[7] The skeletal muscle consists of elongated multinucleated myofibers, local skeletal muscle stem cells (MPCs, also called satellite cells), peripheral motor nerves, neuromuscular junctions, blood vessels, and the supportive ECM structure that is mechanically able to support all of the functions of skeletal muscle in the human body. Healthy skeletal muscle is considered as a rather static tissue. Two types of cells exist in the skeletal muscle tissue: MPCs and the differentiated multinucleated myofibers. The myofibers are terminally differentiated and nonproliferative in postnatal life.[8] However, the pool of MPCs, located below the basal lamina of the tissue undergo asymmetric divisions after injury in order to produce the progenitor cells required for tissue repair and to replenish the pool of quiescent stem cells needed for future injuries and repair.[9]

Despite usually inactive, skeletal MPCs initiate proliferation and differentiate into daughter myogenic precursor cells following injury and can be identified by their expression of Pax3/Pax7. After multiple cell divisions, the MPCs exit the cell cycle and fuse to form myofibers. MPC proliferation, differentiation, and fusion is a complex process that is tightly associated with the interaction of the cell surface markers on the MPCs with ECM molecules, such as proteoglycans (PGs). Proteoglycans (PGs) are macromolecules containing a central core protein with attached carbohydrates, called glycosaminoglycans (GAGs) that are located at both the cell surface of MPCs and on the local ECM. Recent evidence has emerged demonstrating the role of PGs in regulating myogenesis, myogenic differentiation, and potential neuromuscular junction organization.[10] *In vitro*, both biological factors and physical manipulation can promote MPC proliferation and myogenic differentiation. Biophysical interactions with the ECM (collagen), adhesion proteins (gelatin, fibronectin, and laminin), and/or stiff substrates (polystyrene or gels with a Young's modulus of 12 kPa) have been demonstrated to modulate the growth and differentiation MPCs *in vitro*.[11] Clearly, the presence of collagens, growth factors, cytokines, and enzymatic proteins in the skeletal muscle extracellular matrix (mECM) plays an important role in the muscle tissue development and regeneration processes.

Several types of stem cells have been investigated for use in stem cell therapy for muscle tissue repair, including mesoangioblasts or pericytes,[12] embryonic stem cells (ESCs),[13] induced pluripotent stem cells (iPSCs),[14] and mesenchymal stem cells (MSCs) isolated from the bone marrow[15] and adipose tissue.[16] However, perhaps most obviously, the most commonly used cells for skeletal muscle repair are mesenchymal cells/mesangioblasts.[17–19] For MPC therapy to be successful, there is a need to investigate the appropriate matrix for the retention and promotion of cell growth and myogenic differentiation *in vitro*.[11]

THE EXTRACELLULAR MATRIX

The native ECM consists of a highly complex, tissue-specific network of proteins and polysaccharides, which help regulate tissue regeneration. The cell microenvironment is extraordinarily active, with communal regulatory pathways between cells and the ECM guiding many physiological processes, such as cell migration, stem cell differentiation, and tissue regeneration. The main components of the ECM are represented by collagens, proteoglycans, cell-cell and cell-ECM adhesion complexes, matrix metalloproteinases (MMPs), signaling molecules, and nonstructural matricellular proteins. The ECM is comprised of about 300 proteins as the core "matrixome," which consists of 43 collagen subunits, 36 proteoglycans (PGs), and ~200 complex glycoproteins.[20] Two types of ECM are present in the mammalian tissue, the interstitial connective tissue that supports most stromal cells and the basement membrane which primarily supports the epithelium. The interstitial matrix of the skeletal muscle includes collagen I, fibronectin, PGs, GAGs, tenascine C, and elastin.[21]

Providing the correct sequence of biochemical cues to cells, both *in vivo* and *in vitro*, is critical for triggering specific biological outcomes. Physical and biochemical ECM properties promote tissue healing primarily via two methods: (1) stimulating the cells adjacent to the injured tissue to restore the histological structure and function; and (2) inhibiting chronic inflammation and fibroblast overgrowth due to over-reaction of the immune system initiated after tissue damage.[22] The ideal niche for *in vitro* primary cell culture and maintenance of cell function is the substrate derived from the homologous tissue or organ. Tissue-specific ECM coatings on culture dishes offer a promising platform for cell culture that more closely mimics the mature *in vivo* ECM microenvironment.[23] The necessity or preference for a tissue specific ECM has recently attracted attention for the culture of primary stem cells. The initial premise for the use of tissue specific

ECM for stem cell culture is based on its potential to mimic the histological structure, composition, and function of the native matrix. Recently, tissue-specific ECM has been used in primary culture for human cells such as human hepatocytes,[24–26] skin cells, and skeletal MPCs.[23,24,27,28]

Our group has developed tissue-specific ECM-based coating substrates for skin, skeletal muscle, and liver cell cultures.[24] Adult skin, skeletal muscle, and liver tissues were decellularized and the resulting acellular matrices were homogenized and reconstituted.[24] The ECM suspensions were used to coat culture dishes and tissue matched and nontissue matched cell types were grown on these coatings to assess adhesion, proliferation, and maintenance of phenotype and cell function at several time points. Each cell type showed enhanced proliferation and differentiation in cultures containing ECM from their tissue of origin. Although subtle compositional differences in the three ECM types were not investigated in this study, these results suggest that tissue-specific ECMs provide a culture microenvironment that is similar to the *in vivo* environment when used as coating substrates, and this new culture technique has the potential for use in drug development and the development of cell-based therapies. In addition, we demonstrated that a 3D liver-specific ECM improved the culture of hepatocytes and resulted in considerably increased cell growth and maintenance of cell function; therefore, this system could potentially be used in liver tissue regeneration, drug discovery, or toxicology studies.[25]

In preclinical experiments, site-specific or homologous extracellular matrix facilitates the remodeling of bladder,[29] urethra,[30,31] penis,[32] myocardial tissue,[33] and cartilage.[34] Interestingly, tissue specific ECM prompted remolding of esophageal mucosa with a greater extent than heterologous ECM when used *in vivo*.[35] Our previous study demonstrated that implantation of a heart tissue specific decellularized matrix thickened the left ventricular infarcted wall, prevented paradoxical left ventricular systolic bulging, and improved left ventricular ejection fraction after myocardial infarction in rats.[33]

Thus, tissue specific ECM offers the biophysical and biochemical features that regulate cell and tissue function—the "niche in a dish" for cell biology research, regenerative medicine, and cell-based therapies. Tissue specific ECM acts as a ligand "reservoir" by binding abundant growth factors and bioactive molecules. The key growth factors maintained within the ECM are proteolytically released when used in cell culture, resulting in enhanced cell proliferation and differentiation.[36]

SKELETAL MUSCLE ECM (mECM)

The rationale behind the myogenic effect of skeletal muscle ECM (mECM) is based on previous studies from our group and others describing the benefits of ECM derived from tissue specific organs vs nontissue specific organs when used in selected anatomic locations.[24,27,28,37] Our studies showed that an optimal substrate for MPC survival, proliferation, myogenic differentiation, and fusion is the native skeletal muscle ECM with tissue-specific bioactivity, as compared to the same cells grown on other ECMs.[24,37] In addition, the fragments of mECM formed a structural scaffold that might maintain the physical structure of motor units and provide the framework for force transmission of muscle tissue *in vivo*.

ECM molecules heavily influence the differentiation process of MPCs during tissue repair.[38] Excessive fibronectin, a glycoprotein component of the ECM, can bind to other ECM components such as collagen, fibrin, and heparin and inhibit myotube formation.[39] Obviously, each ECM component plays a different role during MPC differentiation. A better understanding of the signals provided by the mECM niche will allow for targeting specific individual ECM components to regulate MPC behavior and might provide therapeutic potential for enhancing skeletal muscle regeneration and functional recovery. Moreover, for *in vitro* culture, the effects of particular ECM components must be considered, depending on the desired differentiation stage of the cells.

DECELLULARIZATION OF THE mECM

Decellularization refers to the process used to remove cellular components from a tissue while leaving behind an ECM scaffold of an allogeneic or xenogeneic host tissue,[40] which can provide a cell-free microenvironment for cell growth *in vitro* and potential *in vivo* implantation. The ideal decellularization method is to remove the maximum number of immunogenic cellular components, while preserving the tissue architecture (ECM) and associated bioactive compounds (cytokines and growth factors). The use of a cell-free ECM with bioactive compounds can bypass potential autoimmune rejection from the host due to the removal of the immune-reactive cellular components. Moreover, a cell-free ECM can promote cell growth and differentiation of grafted cells and improve tissue development.

Several decellularization approaches currently being utilized use either a single or a combination of approaches such as (1) physical methods (temperature, force, pressure, and electrical disruption), (2) chemical methods (acids, alkaline treatments, ionic detergents such as sodium dodecyl sulfate (SDS) and nonionic detergents such as Triton X-100 and zwitterionic detergents) and/or (3) enzymatic treatments (lipases, thermolysin, galactosidase, nucleases, and trypsin).[40,41] Despite effective removal of cellular components, these harsh methods also abolish most bioactive factors.

The optimal decellularization method would be to remove all the xenogeneic immuno-reactive cellular components while retaining the bioactive proteins within the ECM. Fetal bovine serum (FBS) has been successfully used in decellularization processes due to the nucleases in the serum that plays a key role in the degradation of potential immuno-reactive DNA/RNA remaining after cell lysis.[27,28,42] In addition, the use of serum maximally retains the proteins within the ECM compared to other decellularization reagents such as detergents (i.e., SDS, Triton X-100).[42] Skeletal muscle is a thick tissue with both a high cell and matrix density, which often necessitates a combination of decellularization methods. Based on our previous studies,[27,28] we developed a decellularization approach that includes homogenization of the tissue into small particles (5–100 μm) for increased decellularization efficiency, repeated rinsing with FBS for efficiently and gently washing away cellular components while retaining the bioactive proteins. Finally, the ECM is lyophilized to preserve the growth factors and cytokines retained within the ECM long-term.

Our studies demonstrated that a simple method using a combination of serum and water washes (Fig. 1) shortens the process of muscle ECM decellularization to further protect native bioactive proteins, as compared to other methods that commonly use DNase as a degradation method.[27,28] In the clinical setting, the patient's own serum could be used in these processes, in replacement of the

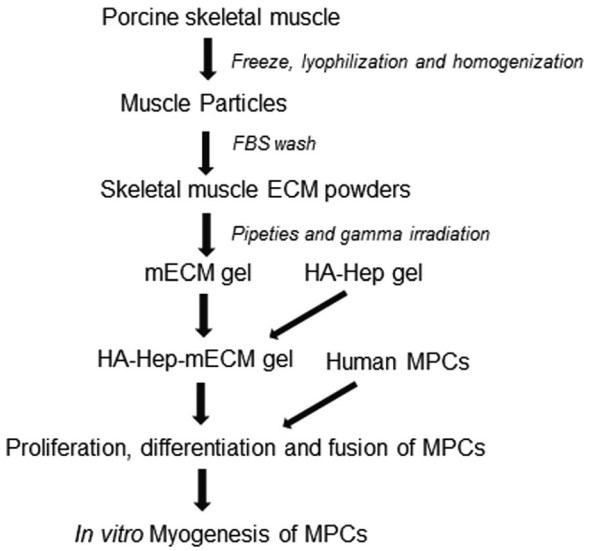

FIG. 1. Outline of the fabrication process of the synthetic mECM hydrogel and its effects on myogenesis.

animal-derived serum, to prevent the potential risk of xenogeneic contamination.

As already mentioned, bioactive growth factors, cytokines, and ECM proteins (collagen, fibronectin, and laminin) regulate cellular behavior and function. Our previous study demonstrated that decellularized substrates from the mECM retained a wide range of bioactive growth factors and cytokines, as well as macromolecules for its basal structure.[24] Our recent data examined the protein and ECM components of our decellularized mECM. These data demonstrated that the total collagen (62 mg/ml), elastin (56.1 mg/ml), and GAG (0.24 mg/ml) contents within the mECM were much higher than other tissue ECMs. Collagen type I was the predominant collagen type present, while collagen types III and IV were present in relatively low percentages. In addition, bone morphogenetic protein-5 (BMP-5, 1139.9 pg/ml), Fibroblast Growth Factor 4 (FGF4) (1022.3 pg/ml), insulin-like growth factor-binding protein-3 (IGFBP-3, 1756.5 pg/ml), and IGFBP-4 (1135.1 pg/ml) were present in relatively high levels, compared to other tissue ECMs. Other important cytokines were also present, including the basal fibroblast growth factor (bFGF, 21.5 pg/ml), hepatocyte growth factor (HGF, 88.3 pg/ml), stem cell factor-receptor (SCF receptor, 103.3 pg/ml), transforming growth factor beta-3 (TGFβ-3, 33.5 pg/ml) as regulating cell adhesion and ECM formation,[43] and vascular endothelial growth factor (VEGF, 146.8 pg/ml), but at relatively low concentrations. These bioactive molecules play a critical role in cell proliferation, differentiation, and myogenic fusion of MPCs during skeletal muscle regeneration and demonstrate the importance of using an ECM for *in vitro* cell culture.

SYNTHETIC MECM HYDROGEL

Although a variety of substrates have been used in the engineering of the skeletal muscle, the main component is typically a collagen gel including collagen, glycoproteins, and proteoglycans.[27,28] The mECM milieu also contains several key myogenic growth factors (including HGF, FGF, and IGFBP) and angiogenic growth factors (including VEGF and bFGF) that are significantly different from the milieu of other tissue ECMs.[27,28] Other potent cytokines are also present within the mECM, including the bone morphogenetic protein-5 (BMP-5), stem cell factor (SCF) receptors, and transforming growth factor (TGFβ-3). Furthermore, solutions derived from the mECM include different types and amounts of ECM proteins, such as collagen (types I, III, and IV), elastin, and GAGs[28] in contrast to solutions derived from the ECM of other tissues.

Different modification strategies have been developed to improve the growth and proliferation of cells on the mECM. Supplementation of bioactive compounds to the mECM offers the potential for greater control over the proliferation and differentiation potential of cultured MPCs over time. Direct implantation of growth factors *in vivo* generally does not achieve expected outcomes since most growth factors are unstable and have a short effective half-life due to inactivation by enzymes within the tissue. To amplify the efficacy and the biological activity of growth factors, several systems have been developed for tissue repair and cellular regeneration that can control their dose, time-frame, and site of release.[44] Protein delivery techniques using hydrogels formulated to include heparin have shown promise in imitating the natural tissue environment during regeneration.[27,28]

We combined mECM with hyaluronan-based hydrogel and heparin (HA-Hep) to generate a synthetic mECM hydrogel that significantly improved the proliferation and differentiation of MPCs (Fig. 1), compared to kidney ECM and HA-Hep gel alone.[27] Binding bioactive proteins within the mECM with heparin in a HA gel appear to prolong the half-life of these factors and alter their interaction with cell surface receptors.[27] Uniform mECM-HA-Hep substrate-based coatings or surface modifications provided an extracellular microenvironment to guide the differentiation of MPCs *in vitro*.[27] Porcine-derived skeletal muscle ECM (pECM) was generated as described and combined with a hyaluronic acid-based hydrogel decorated with heparin (pECM-HA-Hep). The cell proliferation and myogenic differentiation capacity of human MPCs was assessed when grown on gel-alone, ECM or each ECM-HA-Hep substrate. Human MPC proliferation was significantly enhanced when cultured on the ECM-HA-Hep substrates as compared to the other substrates tested, with the greatest proliferation on the muscle ECM-HA-Hep (mECM-HA-Hep) substrate. The number of differentiated myotubes was significantly increased on the mECM-HA-Hep substrate as compared to the other gel-ECM substrates as well as an increase in the numbers of MPCs expressing specific myogenic cell markers (i.e., Myosin, Desmin, MyoD, and Myf5). In conclusion, skeletal muscle ECM-HA-Hep as a culture substrate provided an optimal culture microenvironment potentially due to its similarity to the *in vivo* environment.

Another approach to promote cell proliferation and myogenic differentiation of human MPC *in vitro* with the mECM in combination with a modified alginate hydrogel conjugated with gelatin and heparin (Alg-G-Hep) as a substrate was recently developed.[28] This Alg-G-Hep substrate, together with the mECM, significantly enhanced cell expansion, differentiation, and maturation of MPCs compared to cells grown on the individual substrates (i.e., gelatin, Matrigel®, or mECM alone). In Western-blot and immunocytochemical analyses, the Alg-G-Hep-ECM enhanced expression of myogenic markers (MyoD, Myf5, Myogenin, Desmin, and Myosin) and myotube formation. This study demonstrated that it was the signaling molecules and growth factors released from the mECM that modulated cell proliferation, differentiation, and maturation of the MPCs. This technique

could be a cost-effective tool for *in vitro* skeletal muscle cell differentiation and maturation, with potential applications in tissue engineering and drug development.

A novel synthetic peptide hydrogel (PepGel) system that uses native protein domains has recently been discovered by our group. One of the PepGel hybrids, named h9e, is a triblocked amphiphilic peptide-based hydrogel that was rationally designed using the native domain proteins from human muscle and spider silk with selective amino acids that are commonly found in the human body upon degradation.[45] PepGel hybrids are highly compatible with biological systems, which have been demonstrated both *in vitro* using cell-based culture models[46] and *in vivo* subcutaneous injection.[47] PepGel hybrids are well-defined scaffolds with advanced shear-thinning and sol-gel self-recoverable features induced by either pipetting or syringing under neutral pH at room or body temperature. With backbone structure modifications, a variety of PepGels can be created with desirable hydrogel properties to meet specific applications including tissue-specific mECM scaffolds. PepGel hybrids have been successfully used for *in vitro* cell culture or *in vivo* cell delivery. For instance, PepGel PGmatrix, a commercial product developed by PepGel LLC in Manhattan, KS, has been used for (1) 3D cultures of various cancer cells[48,49] and (2) safe *in vivo* delivery of drugs, antigens, and live viruses.[50,51]

Several key questions that still need to be addressed:

1. The bioactive compounds present in the mECM that act on MPCs still need to be identified by proteomics.
2. Well defined synthetic hydrogel systems derived from native human muscle protein domains and mECM are needed to advance the function of the reservoir of mECM bioactive compounds, which might be superior for biocompatible and sustained release of the bioactive compounds for MPC therapeutic applications.
3. It is unclear how excessive accumulation of ECM impedes muscle regeneration. How can the over accumulation of ECM in pathological conditions which results in tissue fibrosis after injury be prevented?
4. There are challenges to the development of tissue-specific ECM proteins for cell therapy that can be used for bioprinting of the human skeletal muscle.
5. How can physical and mechanical elements, such as exercise and neural signaling, be incorporated into the *in vitro* environment to maintain the physical function of MPC during culture?
6. *In vitro* experiments showed that the mECM can act to provide a niche for MPCs to improve survival and differentiation potential. More investigations on the myogenic effect of the mECM on muscle injury and regeneration *in vivo* are needed.

In conclusion, progenitor cells demonstrate enhanced proliferation and differentiation in cultures containing ECM from their specific tissue of origin.[24] Tissue-specific ECM provides a natural biological microenvironment that is able to mediate normal cellular behavior and tissue organization. A proper decellularization process is necessary to create a natural scaffold and niche for cell growth, differentiation, and tissue development. The skeletal muscle ECM plays a role in early muscle development and promotes cell proliferation and myogenic differentiation of myogenic progenitors *in vitro*. Therefore, cell-based therapies with a tissue specific ECM may also stimulate regeneration of skeletal muscle tissues *in vivo*. Implanted myogenic progenitors within the ECM are most likely to participate in host muscle ECM remodeling, which provokes functional and histological structural repair. The real impact of the combination of myogenic progenitors and the ECM on *in vivo* muscle repair needs further investigation, and a well-defined skeletal muscle derived synthetic hydrogel featuring the skeletal muscle ECM still needs to be developed.

ACKNOWLEDGMENTS

This is to disclose that Dr. Xiuzhi Susan Sun is the primary inventor of the peptide hydrogel named as PepGel, and she is a founder and technical consultant of PepGel LLC.

REFERENCES

[1]D. C. Zebrowski, S. Vergarajauregui, C.-C. Wu, T. Piatkowski, R. Becker, M. Leone *et al.*, "Developmental alterations in centrosome integrity contribute to the post-mitotic state of mammalian cardiomyocytes," Elife **4**, e05563 (2015).
[2]F. S. Tedesco, A. Dellavalle, J. Diaz-Manera, G. Messina, and G. Cossu, "Repairing skeletal muscle: Regenerative potential of skeletal muscle stem cells," J. Clin. Invest. **120**(1), 11–19 (2010).
[3]A. D. Theocharis, S. S. Skandalis, C. Gialeli, and N. K. Karamanos, "Extracellular matrix structure," Adv. Drug Delivery Rev. **97**, 4–27 (2016).
[4]U. Kuhl, M. Ocalan, R. Timpl, and K. von der Mark, "Role of laminin and fibronectin in selecting myogenic versus fibrogenic cells from skeletal muscle cells in vitro," Dev. Biol. **117**(2), 628–635 (1986).
[5]M. E. Danoviz and Z. Yablonka-Reuveni, "Skeletal muscle satellite cells: Background and methods for isolation and analysis in a primary culture system," Methods Mol. Biol. **798**, 21–52 (2012).
[6]C. Hoffmann and C. Weigert, "Skeletal muscle as an endocrine organ: The role of myokines in exercise adaptations," Cold Spring Harbor Perspect. Med. **7**(11), a029793 (2017).
[7]E. H. Marieb and K. S. Francisco, *Human Anatomy & Physiology*, 8th ed. (Benjamin Cummings, 2010), p. 312, ISBN 978-0-8053-9569-3.
[8]D. Schumacher, B. K. Tischer, J. P. Teifke, K. Wink, and N. Osterrieder, "Generation of a permanent cell line that supports efficient growth of Marek's disease virus (MDV) by constitutive expression of MDV glycoprotein E," J. Gen. Virol. **83**(Pt 8), 1987–1992 (2002).
[9]F. Relaix, D. Rocancourt, A. Mansouri, and M. Buckingham, "A Pax3/Pax7-dependent population of skeletal muscle progenitor cells," Nature **435**(7044), 948–953 (2005).
[10]E. Brandan and J. Gutierrez, "Role of skeletal muscle proteoglycans during myogenesis," Matrix Biol. **32**(6), 289–297 (2013).
[11]B. C. Syverud, J. D. Lee, K. W. VanDusen, and L. M. Larkin, "Isolation and purification of satellite cells for skeletal muscle tissue engineering," J. Regener. Med. **3**(2), 117 (2014).
[12]M. Sampaolesi, S. Blot, G. D'Antona, N. Granger, R. Tonlorenzi, A. Innocenzi *et al.*, "Corrigendum: Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs," Nature **494**(7438), 506 (2013).
[13]H. Chang, M. Yoshimoto, K. Umeda, T. Iwasa, Y. Mizuno, S. Fukada *et al.*, "Generation of transplantable, functional satellite-like cells from mouse embryonic stem cells," FASEB J. **23**(6), 1907–1919 (2009).
[14]F. S. Tedesco, M. F. Gerli, L. Perani, S. Benedetti, F. Ungaro, M. Cassano *et al.*, "Transplantation of genetically corrected human iPSC-derived progenitors in mice with limb-girdle muscular dystrophy," Sci. Transl. Med. **4**(140), 140ra89 (2012).
[15]J. H. Lee, P. A. Kosinski, and D. M. Kemp, "Contribution of human bone marrow stem cells to individual skeletal myotubes followed by myogenic gene activation," Exp. Cell Res. **307**(1), 174–182 (2005).
[16]S. V. Forcales, "Potential of adipose-derived stem cells in muscular regenerative therapies," Front. Aging Neurosci. **7**, 123 (2015).
[17]C. A. Collins, I. Olsen, P. S. Zammit, L. Heslop, A. Petrie, T. A. Partridge *et al.*, "Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche," Cell **122**(2), 289–301 (2005).
[18]D. Montarras, J. Morgan, C. Collins, F. Relaix, S. Zaffran, A. Cumano *et al.*, "Direct isolation of satellite cells for skeletal muscle regeneration," Science **309**(5743), 2064–2067 (2005).
[19]Z. Qu-Petersen, B. Deasy, R. Jankowski, M. Ikezawa, J. Cummins, R. Pruchnic *et al.*, "Identification of a novel population of muscle stem cells in mice: Potential for muscle regeneration," J. Cell Biol. **157**(5), 851–864 (2002).

[20]R. O. Hynes and A. Naba, "Overview of the matrisome—An inventory of extracellular matrix constituents and functions," Cold Spring Harbor Perspect. Biol. **4**(1), a004903 (2012).
[21]K. C. Clause and T. H. Barker, "Extracellular matrix signaling in morphogenesis and repair," Curr. Opin. Biotechnol. **24**(5), 830–833 (2013).
[22]T. L. Adair-Kirk and R. M. Senior, "Fragments of extracellular matrix as mediators of inflammation," Int. J. Biochem. Cell Biol. **40**(6–7), 1101–1110 (2008).
[23]J. A. DeQuach, V. Mezzano, A. Miglani, S. Lange, G. M. Keller, F. Sheikh *et al.*, "Simple and high yielding method for preparing tissue specific extracellular matrix coatings for cell culture," PLoS One **5**(9), e13039 (2010).
[24]Y. Zhang, Y. He, S. Bharadwaj, N. Hammam, K. Carnagey, R. Myers *et al.*, "Tissue-specific extracellular matrix coatings for the promotion of cell proliferation and maintenance of cell phenotype," Biomaterials **30**(23–24), 4021–4028 (2009).
[25]R. Lang, M. M. Stern, L. Smith, Y. Liu, S. Bharadwaj, G. Liu *et al.*, "Three-dimensional culture of hepatocytes on porcine liver tissue-derived extracellular matrix," Biomaterials **32**(29), 7042–7052 (2011).
[26]A. Skardal, L. Smith, S. Bharadwaj, A. Atala, S. Soker, and Y. Zhang, "Tissue specific synthetic ECM hydrogels for 3-D in vitro maintenance of hepatocyte function," Biomaterials **33**(18), 4565–4575 (2012).
[27]D. Zhang, Y. Zhang, Y. Zhang, H. Yi, Z. Wang, R. Wu *et al.*, "Tissue-specific extracellular matrix enhances skeletal muscle precursor cell expansion and differentiation for potential application in cell therapy," Tissue Eng. Part A **23**(15–16), 784–794 (2017).
[28]H. Yi, S. Forsythe, Y. He, Q. Liu, G. Xiong, S. Wei *et al.*, "Tissue-specific extracellular matrix promotes myogenic differentiation of human muscle progenitor cells on gelatin and heparin conjugated alginate hydrogels," Acta Biomater. **62**, 222–233 (2017).
[29]X. Hou, C. Shi, W. Chen, B. Chen, W. Jia, Y. Guo *et al.*, "Transplantation of human adipose-derived mesenchymal stem cells on a bladder acellular matrix for bladder regeneration in a canine model," Biomed. Mater. **11**(3), 031001 (2016).
[30]A. Shokeir, Y. Osman, M. El-Sherbiny, M. Gabr, T. Mohsen, and M. El-Baz, "Comparison of partial urethral replacement with acellular matrix versus spontaneous urethral regeneration in a canine model," Eur. Urol. **44**(5), 603–609 (2003).
[31]A. M. Kajbafzadeh, R. Abbasioun, S. Sabetkish, N. Sabetkish, P. Rahmani, K. Tavakkolitabassi *et al.*, "Future prospects for human tissue engineered urethra transplantation: Decellularization and recellularization-based urethra regeneration," Ann. Biomed. Eng. **45**(7), 1795–1806 (2017).
[32]A. M. Kajbafzadeh, R. Abbasioun, N. Sabetkish, S. Sabetkish, A. A. Habibi, and K. Tavakkolitabassi, "In vivo human corpus cavernosum regeneration: Fabrication of tissue-engineered corpus cavernosum in rat using the body as a natural bioreactor," Int. Urol. Nephrol. **49**(7), 1193–1199 (2017).
[33]W. Dai, P. Gerczuk, Y. Zhang, L. Smith, O. Kopyov, G. L. Kay *et al.*, "Intramyocardial injection of heart tissue-derived extracellular matrix improves postinfarction cardiac function in rats," J. Cardiovasc. Pharmacol. Ther. **18**(3), 270–279 (2013).
[34]S. Schwarz, L. Koerber, A. F. Elsaesser, E. Goldberg-Bockhorn, A. M. Seitz, L. Durselen *et al.*, "Decellularized cartilage matrix as a novel biomatrix for cartilage tissue-engineering applications," Tissue Eng. Part A **18**(21–22), 2195–2209 (2012).
[35]T. J. Keane, A. DeWard, R. Londono, L. T. Saldin, A. A. Castleton, L. Carey *et al.*, "Tissue-specific effects of esophageal extracellular matrix," Tissue Eng. Part A **21**(17–18), 2293–2300 (2015).
[36]R. O. Hynes, "The extracellular matrix: Not just pretty fibrils," Science **326**(5957), 1216–1219 (2009).
[37]A. Urciuolo, L. Urbani, S. Perin, P. Maghsoudlou, F. Scottoni, A. Gjinovci *et al.*, "Decellularised skeletal muscles allow functional muscle regeneration by promoting host cell migration," Sci. Rep. **8**(1), 8398 (2018).
[38]S. Grefte, M. J. W. Adjobo-Hermans, E. M. M. Versteeg, W. J. H. Koopman, and W. F. Daamen, "Impaired primary mouse myotube formation on cross-linked type I collagen films is enhanced by laminin and entactin," Acta Biomater. **30**, 265–276 (2016).
[39]Y. Ohtake, H. Tojo, and M. Seiki, "Multifunctional roles of MT1-MMP in myofiber formation and morphostatic maintenance of skeletal muscle," J. Cell Sci. **119**(Pt 18), 3822–3832 (2006).
[40]P. M. Crapo, T. W. Gilbert, and S. F. Badylak, "An overview of tissue and whole organ decellularization processes," Biomaterials **32**(12), 3233–3243 (2011).
[41]T. J. Keane, I. T. Swinehart, and S. F. Badylak, "Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance," Methods **84**, 25–34 (2015).
[42]L. Gui, S. A. Chan, C. K. Breuer, and L. E. Niklason, "Novel utilization of serum in tissue decellularization," Tissue Eng. Part C Methods **16**(2), 173–184 (2010).
[43]V. Kaartinen, J. W. Voncken, C. Shuler, D. Warburton, D. Bu, N. Heisterkamp *et al.*, "Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction," Nat. Genet. **11**(4), 415–421 (1995).
[44]S. H. Lee and H. Shin, "Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering," Adv. Drug Delivery Rev. **59**(4–5), 339–359 (2007).
[45]H. Huang, A. I. Herrera, Z. Luo, O. Prakash, and X. S. Sun, "Structural transformation and physical properties of a hydrogel-forming peptide studied by NMR, transmission electron microscopy, and dynamic rheometer," Biophys. J. **103**(5), 979–988 (2012).
[46]H. Huang, Y. Ding, X. S. Sun, and T. A. Nguyen, "Peptide hydrogelation and cell encapsulation for 3D culture of MCF-7 breast cancer cells," PloS One **8**(3), e59482 (2013).
[47]X. Li, A. Galliher-Beckley, H. Huang, X. Sun, and J. Shi, "Peptide nanofiber hydrogel adjuvanted live virus vaccine enhances cross-protective immunity to porcine reproductive and respiratory syndrome virus," Vaccine **31**(41), 4508–4515 (2013).
[48]P. G. Miller and M. L. Shuler, "Design and demonstration of a pumpless 14 compartment microphysiological system," Biotechnol. Bioeng. **113**(10), 2213–2227 (2016).
[49]J. Liang, X. S. Sun, Z. Yang, and S. Cao, "Anticancer drug camptothecin test in 3D hydrogel networks with HeLa cells," Sci. Rep. **7**, 37626 (2017).
[50]H. Huang, J. Shi, J. Laskin, Z. Liu, D. S. McVey, and X. S. Sun, "Design of a shear-thinning recoverable peptide hydrogel from native sequences and application for influenza H1N1 vaccine adjuvant," Soft Matter **7**(19), 8905–8912 (2011).
[51]J. Liang, G. Liu, J. Wang, and X. S. Sun, "Controlled release of BSA-linked cisplatin through a PepGel self-assembling peptide nanofiber hydrogel scaffold," Amino Acids **49**(12), 2015–2021 (2017).