Skeletal Muscle Tissue Engineering

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Skeletal muscle (SKM) represents nowadays a complex and arguing tissue to be generated *in vitro* for tissue engineering purposes. Several attempts have been pursued to develop hydrogels with different formulations resembling *in vitro* the characteristics of SKM tissue *in vivo*. Topographical cues have been applied on the hydrogels to guide cellular orientation and facilitate myogenic differentiation of blended myocites and maturation of the constructs. 3D bioprinting technique allows controlled spatial deposition of cells into ECM based hydrogels and provide the proper SKM native-like tissue microenvironment.

Keywords: extracellular matrix ; hydrogel ; skeletal muscle ; tissue engineering

1. Topographical Cues

To successfully reproduce a 3D scaffold able to mimic SKM tissue in vitro, the structural guidance for muscle cells should be provided to induce efficient differentiation. Engineering the topography of biomaterial substrates to determine cell fate takes advantage of the natural contact-mediated signaling events that occur between cells and ECM [1]. Various strategies have been developed to guide muscle cells such as ECM mimetic topographical structure and mechanical or electrical stimulation methods [2][3]. Example of topographic cues that influence cell morphology and organization include microscale topographical features presented by micropatterned substrates, aligned polymeric fibrous matrices mimicking native ECM proteins, and 3D scaffolds with anisotropic porosity within which myoblasts can organize into wide and long myotubes.

For fabricating aligned micro/nanostructures, numerous methods have been employed to obtain appropriate topographical cues, which are an easy way to achieve the formation of myotubes owing to the efficient interactions among cells. The advantage of this approach is the readiness and reproducibility of the supports, and the possibility to generate different shapes and geometries. Recently, Kim et al. described an innovative 3D printing method with a SKM-specific dECM to bioengineer biochemically and topographically mimicked SKM constructs. They combined a dECM methacrylate (MA) derived from porcine SKM (as a bioink) with fibrillated PVA to fabricate a uniaxially oriented dECM-MA patterned structure. They demonstrated that the printed and topographically predetermined constructs accelerated the myogenic differentiation of murine myoblasts in comparison to a simpler gelatin methacrylate (GelMa)-based cell-laden structure [4].

In another study, Jiwiawat et al. demonstrated the efficacy of 2D micropatterned structures in inducing human iPSC-derived myogenic progenitors to form highly aligned and contracting myotubes. Clear nuclear alignment of differentiated cells was observed and, depending on the width of micropatterned lines, different elongated myotubes were formed. Thus, topographical cues from micropatterning and physiological substrate stiffness improved the formation of well-aligned and multi-nucleated myotubes similar to myofibers, with spontaneous contractile behavior across the long axis of the pattern [5]. Recently, a new and simple method has been developed by Yang et al. to induce myoblasts alignment. They set up a modified plasma treatment on a hybrid polycaprolactone (PCL) scaffold consisting of melt-printed perpendicular PCL struts and an electrospun PCL fibrous mat. For the hybrid scaffold production, the surface of the electrospun mat was selectively roughened with a plasma process supplemented with a template. This innovative type of plasma-treated hybrid scaffold demonstrated strong potential as a biomaterial for muscle tissue regeneration because of the significant enhanced cell alignment in comparison with the use of a hybrid scaffold with a non-roughened electrospun fiber surface or a hybrid scaffold with the whole surface roughened[6].

Another appealing approach to characterize the contribution of different tissue-specific topographies in driving instructive cell niches (made of ECM) is represented by Silica BIoReplication (SBR). SBR is a process that converts biological samples into silica, faithfully preserving the original topography at the nanoscale. Tang et al. exploited this method to firstly demonstrate that the precisely replicated tissue topography harbored sufficient information to direct the fate of human MSCs without the need of exogenous factors such as soluble growth factors or immobilized ECM molecules. They suggested as the tissue microenvironment captured by SBR profoundly affected MSC biology, and that the topographical cues were sufficient in initiating and directing differentiation of MSC, despite the absence of any biochemical cues [7].

2. Stiffness and Elastic Modulus

Although topographical cues represent a fundamental element to be considered, mechanical properties of materials utilized for SKM tissue engineering approaches exert a significant influence on cellular behavior and consequently on tissue functionality. Mechanical properties relate to forces exerted on the material and the resulting changes in shape. Among them, stiffness plays a fundamental role when considering mechanotransduction processes, and it should match that of native SKM tissue allowing for cell exposure to relevant mechanical forces thereby influencing cell fate [8]. Mechanotransduction occurs via intracellular signaling, influencing cellular responses such as proliferation, differentiation, metabolic activities, and maintaining the tissue identity with regard to functionality [9]. In SKM tissue, substrate stiffness orchestrates the formation of functional myotubes and a finely engineered control over it can be achieved by changing variables such as the composition of multi-polymer composites, the molecular weights of polymer constituents, crosslinking agents and crosslinking times [8]. Gilbert et al. demonstrated that biophysical properties such as matrix stiffness and elastic modulus play crucial roles in muscle stem cells (MuSCs) self-renewal and function in muscle regeneration. They obtained a tunable PEG hydrogel platform by altering the percentage of PEG polymer in solution and produced hydrogels with a range of stiffness including a formulation that mimicked the elastic modulus of adult murine SKM. They demonstrated as MuSCs cultured on the tissue-specific stiffness hydrogel were able to self-renew and to generate stem cell progeny that could potently repair damaged muscle. These results laid a milestone for the future SKM tissue engineering approaches by providing insight into the potency of tissue stiffness on stem cell fate regulation [10].

The natural response of all materials to stress is not purely elastic but also has a viscous component such as occurs with living tissues. The viscoelastic response of a material is sometimes described through the dynamic or complex modulus, which is represented by storage (E') and loss (E'') of moduli. Similarly, for deformations resulting from shear forces, the shear storage modulus (G') and the shear loss modulus (G'') are frequently evaluated by rheology and oscillatory experiments [11].

3. 3D Printing in Skeletal Muscle Engineering

The recently developed 3D printing technology is an additive manufacturing method that promises to bridge the difference between artificially engineered and native tissues. Such 3D printing is emerging as a scaffold fabrication approach finely mimicking native tissue complexity [12]. Indeed, 3D printing is a tool to assemble scaffolds with a high precision and accuracy, creating intricately detailed biomimetic 3D structures [13]. Scaffolds generated by 3D printing can have complex micro-geometries and, in practice, a layer-by-layer stratification can precisely deliver different cells or mechanical cues in the designed 3D architecture resembling the tissue of interest. Printability is a fundamental element to be considered exploiting a biomaterial for scaffold production and parameters to evaluate the resolution of the 3D bioprinted components of the scaffolds become crucial [14].

SKM complexity can be dissected using this innovative technology and, nowadays, lot of efforts have been pursued to develop optimal biomaterials suitable for 3D printing.

4. Bioinks

The most important element in designing a successful 3D bioprinting approach is the bioink, which is defined by Groll et al. as a formulation of cells suitable for processing by an automated biofabrication technology that may also contain biologically active components and biomaterials [15]. The bioink represents the building block of bioprinted constructs with a crucial role to support and provide an appropriate environment for incorporated cells [16]. Different characteristics should be considered when developing an optimal bioink for 3D bioprinting application such as printability including viscosity, gelation kinetics, filament stability and biocompatibility, mechanical properties, and nutrient diffusion capacity. An accurate process is required for adjusting these multiple parameters in order to design a bioink satisfying the biofabrication window requirements, in order to obtain bioinks with suboptimal, yet passable, print fidelity, while maintaining cell viability. Since the native ECM varies from tissue to tissue, bioinks should properly mimic the ECM of the target tissue, to support proliferation and differentiation of the specific cell populations homing that tissue [13][17]. A bioactive bioink should properly facilitate cell-matrix interactions to allow remodeling processes and new ECM synthesis [18].

Additionally, bioinks should have the cells homogeneously distributed in suspension to avoid cell aggregation and deposition and to extend the bioprinting time for making larger constructs [19][20].

5. dECM-Derived Bioinks in SKM

As above mentioned, dECM scaffolds are tissue specific and, since they are extracted from the tissue itself, they are biomimetic and contain a variety of proteins, proteoglycans, and cytokines that can aid cells in precise differentiation, maturation and tissue formation [21]. dECM-based bioinks have been produced from different tissues, including SKM, and in most of the cases have been used to regenerate the same tissue of origin. It should be noted that, in addition to the choice of a specific tissue, it is possible to obtain dECM bioinks from subjects of different ages or diseases, greatly expanding the spectrum of applications. Indeed, not only the tissue specific composition, but also age, injuries, diseases or degeneration, such as fibrosis or chronic inflammation, are important conditions that change dECM features and influences on target cells. Although literature is scarce about dECM-based bioinks for SKM, some examples highlight the potentiality of such approach.

Choi et al. developed functional muscular constructs using a porcine SKM-derived dECM bioink, containing C2C12 cells and printed different patterns. They cultured the constructs for 7 days and observed high cell viability and increased cell proliferation compared with those prepared with collagen only. Moreover, high myogenic gene expression of C2C12 encapsulated into the dECM was observed at day 14 after differentiation induction, together with the preservation of ECM components and agrin, which allows the prepatterning of acetylcholine receptors [22]. More recently, the same group developed a novel VML treatment based on a 3D cell printing and SKM dECM-derived bioink that ensured both an organized structure and cell delivery with high viability [23]. Kim et al. employed a porcine SKM-derived dECM as biochemical component and a modified 3D cell-printing process to produce an in situ uniaxially aligned/micro-topographical structure. Myoblasts laden in the printed structure were aligned and differentiated with a high degree of myotube formation, owing to the synergistic effect of the SKM-specific biochemical and topographical cues [4].

References

1. Richard O. Hynes; The Extracellular Matrix: Not Just Pretty Fibrils. *Science* **2009**, *326*, 1216-1219, 10.1126/science.1176009 DOI:dx.doi.org.
2. Guoyou Huangab; Fei Li; Xin Zhao; Yufei Ma; Yuhui Li; Min Lin; Guorui Jin; Tian Jian Lu; Guy M. Genin; Feng Xu; et al. Functional and Biomimetic Materials for Engineering of the Three-Dimensional Cell Microenvironment. *Chemical Reviews* **2017**, *117*, 12764-12850, 10.1021/acs.chemrev.7b00094.
3. Yuhui Li; Guoyou Huangab; Xiaohui Zhang; Lin Wang; Yanan Du; Tian Jian Lu; Feng Xu; Engineering cell alignment in vitro. *Biotechnology Advances* **2014**, *32*, 347-365, 10.1016/j.biotechadv.2013.11.007.
4. Wonjin Kim; Hyeongjin Lee; Jiun Lee; Anthony Atala; James J. Yoo; Sang Jin Lee; GeunHyung Kim; Efficient myotube formation in 3D bioprinted tissue construct by biochemical and topographical cues. *Biomaterials* **2019**, *230*, 119632-119632, 10.1016/j.biomaterials.2019.119632.
5. Nunnapas Jiwlawat; Eileen Lynch; Brett N. Napiwocki; Alana Stempien; Randolph S. Ashton; Timothy J. Kamp; Wendy C. Crone; Masatoshi Suzuki; Micropatterned substrates with physiological stiffness promote cell maturation and Pompe disease phenotype in human induced pluripotent stem cell-derived skeletal myocytes. *Biotechnology and Bioengineering* **2019**, *116*, 2377-2392, 10.1002/bit.27075.
6. Gi Hoon Yang; Hojun Jeon; GeunHyung Kim; Alternately plasma-roughened nanosurface of a hybrid scaffold for aligning myoblasts. *Biofabrication* **2017**, *9*, 025035, 10.1088/1758-5090/aa77ba.
7. Sze Wing Tang; Wai Yuen; Ishdeep Kaur; Stella W. Pang; Nicolas H. Voelcker; Yun Wah Lam; Capturing Instructive Cues of Tissue Microenvironment by Silica Bioreplication. *SSRN Electronic Journal* **2019**, *102*, 114–126., 10.2139/ssrn.3454940.
8. Roberto Rizzi; Claudia Bearzi; Arianna Mauretti; Sergio Bernardini; Stefano Cannata; Cesare Gargioli; Tissue engineering for skeletal muscle regeneration. *Muscle Ligaments and Tendons Journal* **2012**, *2*, 230-234, .
9. Courtney A. Powell; Beth L. Smiley; John Mills; Herman H. VanDenburgh; Mechanical stimulation improves tissue-engineered human skeletal muscle. *American Journal of Physiology-Cell Physiology* **2002**, *283*, C1557-C1565, 10.1152/ajpcell.00595.2001.
10. Penney M. Gilbert; K. L. Havenstrite; K. E. G. Magnusson; A. Sacco; N. A. Leonardi; P. Kraft; N. K. Nguyen; S. Thrun; M. P. Lutolf; Helen M. Blau; et al. Substrate Elasticity Regulates Skeletal Muscle Stem Cell Self-Renewal in Culture. *Science* **2010**, *329*, 1078-1081, 10.1126/science.1191035.
11. 108. Meyers, M.; Chawla, K. Mechanical Behavior of Materials, ; Cambridge University Press: Cambridge, CA, USA, 2008; pp. ISBN 9780511810947..

12. Natalja E. Fedorovich; Jacqueline Alblas; Wim E. Hennink; F. Öner; Wouter Dhert; Organ printing: the future of bone re generation?. *Trends in Biotechnology* **2011**, *29*, 601-606, 10.1016/j.tibtech.2011.07.001.
13. Thomas Billiet; Mieke Vandenhaute; Jorg Schelfhout; Sandra Van Vlierberghe; Peter Dubruel; A review of trends and li mitations in hydrogel-rapid prototyping for tissue engineering. *Biomaterials* **2012**, *33*, 6020-6041, 10.1016/j.biomaterial s.2012.04.050.
14. Liliang Ouyang; Rui Yao; Yu Zhao; Wei Sun; Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. *Biofabrication* **2016**, *8*, 035020, 10.1088/1758-5090/8/3/035020.
15. Jürgen Groll; Jason A. Burdick; Dong-Woo Cho; Brian Derby; Michael Gelinsky; Sarah C Heilshorn; T Jüngst; Jos Mald a; Vladimir A Mironov; Koichi Nakayama; et al. A definition of bioinks and their distinction from biomaterial inks. *Biofabri cation* **2018**, *11*, 013001, 10.1088/1758-5090/aaec52.
16. David Williams; Patrick Thayer; Hector Martinez; Erik Gatenholm; Ali Khademhosseini; A perspective on the physical, m echanical and biological specifications of bioinks and the development of functional tissues in 3D bioprinting. *Bioprintin g* **2018**, *9*, 19-36, 10.1016/j.bprint.2018.02.003.
17. David Chimene; Kimberly K. Lennox; Roland Kaunas; Akhilesh K. Gaharwar; Advanced Bioinks for 3D Printing: A Mater ials Science Perspective. *Annals of Biomedical Engineering* **2016**, *44*, 2090-2102, 10.1007/s10439-016-1638-y.
18. Farshid Guilak; Daniel Cohen; Bradley Estes; Jeffrey M. Gimble; Wolfgang Liedtke; Christopher S. Chen; Control of Ste m Cell Fate by Physical Interactions with the Extracellular Matrix. *Cell Stem Cell* **2009**, *5*, 17-26, 10.1016/j.stem.2009.0 6.016.
19. Eva Hoch; Thomas Hirth; Günter E. M. Tovar; Kirsten Borchers; Chemical tailoring of gelatin to adjust its chemical and physical properties for functional bioprinting.. *Journal of Materials Chemistry B* **2013**, *1*, 5675-5685, 10.1039/c3tb20745 e.
20. Cameron J. Ferris; Kerry J. Gilmore; Stephen Beirne; Donald McCallum; Gordon G Wallace; Marc In Het Panhuis; Bio-i nk for on-demand printing of living cells. *Biomaterials Science* **2013**, *1*, 224-230, 10.1039/c2bm00114d.
21. Falguni Pati; Jinah Jang; Dong-Heon Ha; Sung Won Kim; Jong-Won Rhie; Jin-Hyung Shim; Deok-Ho Kim; Dong-Woo Cho; Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nature Communication s* **2014**, *5*, 3935, 10.1038/ncomms4935.
22. Yeong-Jin Choi; Taek Gyoung Kim; Jonghyeon Jeong; Hee-Gyeong Yi; Ji Won Park; Woonbong Hwang; Dong-Woo Ch o; 3D Cell Printing of Functional Skeletal Muscle Constructs Using Skeletal Muscle-Derived Bioink.. *Advanced Healthc are Materials* **2016**, *5*, 2636-2645, 10.1002/adhm.201600483.
23. Yeong-Jin Choi; Young-Joon Jun; Dong Yeon Kim; Hee-Gyeong Yi; Su-Hun Chae; Junsu Kang; Juyong Lee; Ge Gao; J eong-Sik Kong; Jinah Jang; et al. A 3D cell printed muscle construct with tissue-derived bioink for the treatment of volu metric muscle loss. *Biomaterials* **2019**, *206*, 160-169, 10.1016/j.biomaterials.2019.03.036.