Emerging Fabrication Techniques for Engineering Extracellular Matrix Biomimetic Materials

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Abstract

There is need to address the challenges of organ shortage, through development of tissues and organs with alternatives to those of the allograft-kind. This illustrates the quest behind novel biofabrication strategies such as 3D bio-printing, which is necessary to create artificial multi-cellular tissues/organs. Several findings have been reported in this review. First, the role of ECM components in tissue regenerative medicine is presented. Different ECM components such as collagen, gelatin, elastin, fibronectin, laminins and glycosaminoglycans are concisely examined for their tissue regenerative medicine applications. Next, current state of research on extrusion-based 3D bio-printing techniques and their limitations are reviewed. For example, we show that cell viability is still a challenge with extrusion, while the use of natural polymers such as collagen in improving composites' mechanical properties is limited. Lastly, we examine unresolved research questions necessary to advance the present state of research in the field.

Keywords: 3D Bioprinting; Extracellular Matrix; Extrusion; Biomaterials; Tissue Engineering.

1.0 Introduction

With an ever increasing world population of over 7 billion people, hundreds of billions of dollars are annually committed on hard tissue repairs and regeneration. These costs are usually spent on bone and tooth structures containing calcium phosphate minerals [1], as current research shows that over 85% of the world's population require repair or replacement of tissues [4]. Several

diseases affecting majority of the population without appropriate medical therapies include congenital aortic valve stenosis [2], rheumatoid arthritis [3] and others. More so, these health challenges are mostly associated with ageing in the growing population due to an increasing fraction of the elderly as well as traumatic bone fracture cases. All of these indicate the potential of bone tissue engineering [5] in addressing these and other challenges in tissue engineering and regenerative medicine [11]. Hence, the need for biomaterials that could regulate cell phenotype and function in development, hemostasis and in response to injury [6].

Detailed research has reported that the extracellular matrix (ECM) provides biochemical and biophysical signals that aid cell survival [7], amid different functions. Since the mammalian ECM contains these intrinsic features, they constitute an acceptable ideal microenvironment for functional tissue engineering. Also, the using the ECM regulates stem cell differentiation [9, 10]. While research has previously investigated the design of ECM- mimick biomaterials using conventional micro-fabrication strategies of micromolding, photolithography [8], and others, there is an increasing interest on the use of new techniques.

Unfortunately, none of these techniques can be used to build ECM-mimicking scaffolds with designed architecture, because research has shown that limitations in conventional processing techniques hinders the development of new materials having multidimensional architecture [12]. So, the use of 3D bioprinting is an attractive alternative to other options. This is because, several studies including reviews have shown the role of 3D printed scaffolds in enhancing tissue regeneration, as well as other regenerative medicine applications [13, 14, 15]. For example, efforts have been made to examine the extrusion bioprinting of soft materials for biological model fabrication [16], three-dimensional bioprinting and decellularized ECM-based biomaterials for in vitro tissue engineering [17]. Moreover, other recent studies have reviewed several decellularized hydrogels in bone tissue engineering [18], and a more specific use of coaxial bioprinting for engineering tissue constructs [19, 20].

This review is focused on the different ECM components such as collagen, gelatin, elastin, fibronectin, laminins and glycosaminoglycans for tissue regeneration. Also, current state of research on extrusion-based 3D bio-printing techniques and their limitations are reviewed. For example, we show that cell viability is still a challenge with extrusion, while the use of natural polymers such as collagen in improving composites' mechanical properties is limited. More so,

unresolved research questions necessary to advance the present state of research in the field were presented.

2.0 ECM Biopolymer Components for Tissue Regeneration

Tissues and organs are not composed of solely cellular components; instead, they converge with an extracellular matrix (ECM). The structure and role of the ECM differ depending on tissue natures. The ECM provides a microenvironment that is essential for cellular functionality and regulation[21].

The ECM offers biophysical, biochemical, and biomechanical cues for cellular components. Furthermore, it provides structural support, functions as an adhesive substrate, presents growth factors to its receptors, sequesters, stores growth factors, senses, and transduces mechanical signals. Also, the ECM is involved in regulating numerous cellular purposes for instance survival, adhesion, migration, proliferation, differentiation, and supporting cells for binding[22, 23]. The ECM organizes large fibrillar 3-D nets composed of glycosaminoglycans (GAGs) and various types of proteins such as collagen, fibronectin, elastin, and laminin. Among the numerous constituents of the ECM fibrous proteins (i.e., collagen and elastin), adhesive glycoproteins (fibronectin and laminin and glycosaminoglycans (i.e., hyaluronic acid) are recognized as the main components[24] as shown in Figure 1.
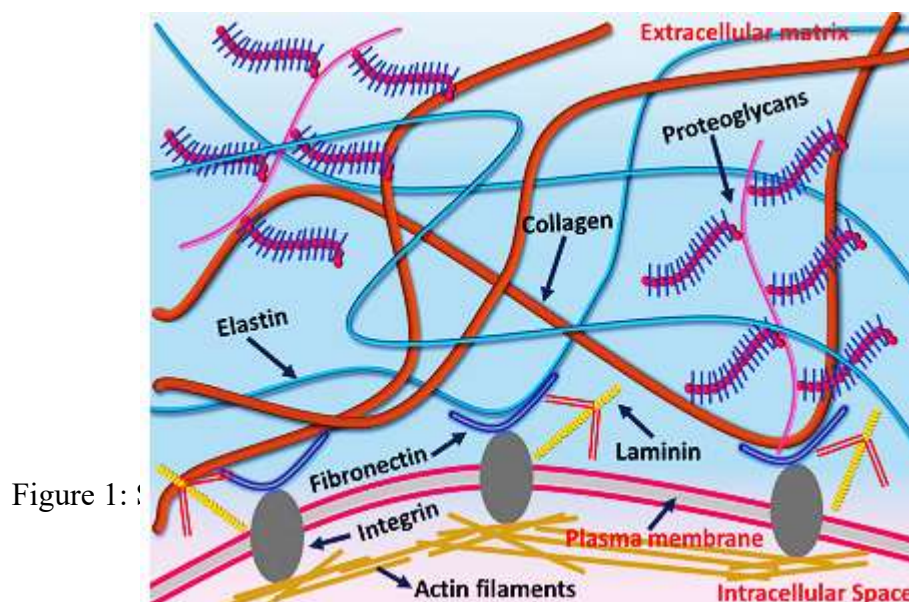

Figure 1: S

2.1. Collagen for tissue regeneration:

Collagen is the most abundant structural protein in the mammalian ECM that imparts tensile strength to prevent deformation of **vascular tissue**[25]. Collagen provides structural properties and resilience to the injured tissue in tissue regeneration manner[26]. Collagen delivers mechanical integrity to the vasculature and other biological tissues during tissue regeneration [27]. Some ECM molecules including type I collagen and fibronectin are known to be over expressed in renal tissue regeneration, promoting fibrosis and tissue dysfunction[28]. A good balance between inflammation and ECM remodeling is essential to maintain homeostasis and to restore essential functions after tissue damage[26]. Collagens provide tensile strength, regulate cell adhesion, support chemotaxis and migration, and direct tissue development in tissue engineering process[29].

Collagen acts as scaffolds providing ECMs and tissues with their structural organization and mechanical properties in tissue regeneration. In addition to their role as tissue designers, collagens interact with cell surface receptors, and regulate numerous biological processes either as full-length proteins or via their bioactive fragments, called matricryptins or matrikines, released by limited proteolysis.

2.2. Gelatin for tissue regeneration:

Gelatin (Gel) is one of the major components of the ECM of various tissues[30]. Gelatin is a polypeptide partially hydrolysis or denatured product of collagen biocompatible, biodegradable and lower immunogenic[31, 32].

Gautam *et al*[33]., stated that Human osteoblasts cells exhibited effective cell adhesion and significant proliferation over the gelatin nanofibers. The *in vivo* analysis by Samadian *et al*.,[34] presented that gelatin can induce the neo-bone formation, osteocyte in lacuna woven bone formation, and angiogenesis in the defect location[35].

2.3. Elastin for tissue regeneration:

Elastin is an elastic protein of ECM and usually found in skin, lungs and blood vessels. Elastin provides mechanical strength, elasticity, firmness, and suppleness to the damaged tissues in the tissue engineering procedure[36, 37]. A different important ECM component that delivers elasticity to the damaged tissue is elastin, which is involved in cellular functions such as cellular attachment, proliferation, differentiation, and migration[38]. Elastin is responsible for resilience and elasticity for cell expansion, differentiation and migration in tissue regeneration[39]. It is a highly cross-linked protein that self-assembles in the ECM to form robust elastic fibers which are essential for maintaining the structural integrity of injured tissues and organs [38].Also, Elastin provides the tissues with long-range elasticity, especially with participation of elastin when it is deposited on a scaffold in the tissue regeneration.

2.4.Fibronectin for tissue regeneration:

Fibronectin is a complex ECM glycoprotein that binds to integrin and other ECM proteins such as collagen. The importance of ECM proteins in mediating **bone** repair in tissue engineering is evident, and fibronectin has emerged as a pivotal regulator of this process[40, 41]. Fibronectin functions in several stages of tissue regeneration[42]. Fibronectin acts as a 3-D scaffold directly following trauma, guiding the assembly of additional ECM components [43]. Also, fibronectin regulates cellular behavior via integrin-binding and growth factor-binding domains, promoting downstream responses including cell recruitment, proliferation and differentiation in tissue engineering method [44]. Fibronectin mediates cellular activities by binding with receptors in the tissue regeneration procedure[45].

In various functions of Fibronectin in the tissue regeneration process includes embryonic development, fibrosis and blood clotting, providing binding sites for mammalian cells[46]. Fibronectin can bind various ECM molecules such as collagen I and III, gelatin, thrombospondin, and heparin [47]. Fibronectin typically functions through interactions with the ECM network in tissue engineering[48]. Fibronectin also plays an essential role in guiding cell behavior, guiding and polarizing the formation and the progression from rostro to caudal of these cells and tissue segments in the tissue regeneration[49]. Fibronectin interacts with many other ECM proteins as well as small molecules, glycosaminoglycans (GAGs), cell surface receptors and other FN molecules in tissue regeneration process[50].[29]

Fibronectin displays a highly specific enrichment in hair follicle stem cells (HFSC) at the onset of regeneration[51].

2.5. *Laminins for tissue regeneration*:

Laminins are a structurally minor component of the ECM [52], that have been shown to play important roles in facilitating cell attachment and mechano-transduction by interacting with cells through cell—membrane receptors including integrins and syndecans[53]. Laminins aid mediating processes based on receptor- and matrix-binding properties in the tissue regeneration process[31]. Laminin mediates a number of cellular functions such as cell adhesion, migration, differentiation and survival in tissue engieering[27]. Laminin usually incorporated into matrix for cell expansion, differentiation and migration[54].

2.6. *Glycosaminoglycans for tissue regeneration:*

GAGs are multifaceted anionic un-branched hetero-polysaccharides which represent major structural and functional ECM components of connective tissues[55]. Glycosaminoglycan chains regulate several signaling pathways in normal and pathological processes through their interactions with different classes of matrix proteins in tissue engineering process.

GAGs participate in a number of vascular events such as the regulation of vascular permeability, lipid metabolism, hemostasis, and thrombosis, but also interact with vascular cells, growth factors, and cytokines to modulate cell adhesion, migration, and proliferation[56].

Glycosaminoglycans are long, un-branched polysaccharide chains made up primarily of repeating disaccharide units. These disaccharide subunits are composed of one hexuronic acid and one amino sugar linked by glycosidic bonds and these variations in disaccharide composition are used to distinguish the major classes of GAGs: Hyaluronic Acid (HA), Chondroitin Sulfate (CS), Dermatan Sulfate (DS), Keratan Sulfate and Heparan Sulfate (HS). Glycosaminoglycans are six types including chondroitin sulfate , dermatan sulfate , heparan sulfate , keratan sulfate (KS), heparin and hyaluronic acid (hyaluronan) . Glycosaminoglycans established interactions with proteins and growth factors for tissue organizations .

Proteoglycans are composed of GAG chains that are covalently linked to the core protein and considered to have a significant role in chemical signaling among cells.

2.7.Decellularized extracellular matrix (dECM) for tissue regeneration:

Decellularization is the process of removing cells and cellular debris from a tissue or organ and isolating the extracellular matrix (ECM). Decellularized ECM is a complex configuration of natural ECM components. dECM isolated from native tissues or organs after decellularization. The dECM contains proteins, proteoglycans, and glycosaminoglycans responsible for cell adhesion, cell remodeling, and mechano-transduction in tissue engineering[57]. Significantly, dECMs do not contain many immunogenic components that are found in the native tissue, making them attractive for cardiovascular tissue engineering and regenerative medicine applications. In addition, tissue-specific dECMs also retain the biochemical and structural properties necessary for tissue function in the regeneration process [58]. dECM provides the unique structural and functional components to support cell growth in the tissue engineering[36]. dECM broadly used for various tissue engineering and regeneration applications owing to providing ECM biological signals to cells[21].

3.0.   3D ECM Bio-printing

There is need to address the challenges of organ shortage, through development of tissues and organs alternative to those of allograft-kind [59]. This illustrates the quest behind novel biofabrication strategies necessary to create artificial multi-cellular tissues/organs. To this end, research has presented 3D – printing as an alternative method of developing custom-made equipment/devices for specific and personalized treatment and diagnostics [60] in tissue engineering [61, 62, 63], pharmaceutical screening [63, 65], and drug manufacturing [66, 67]. Increasing interest on the use of 3D bio-printing technique is due to the failure of conventional techniques to fabricate constructs requiring structural, mechanical and biological complexity [Ji and Guvendiren, 2017].

3.1. Extrusion Bio-printing

Extrusion bio-printing is fundamentally the squeezing out of bioink through a nozzle by mechanical or pneumatic forces to form filaments which are systematically assembled on a surface to form 3D architectures.

It is unarguably the most widely used technique amongst other bioprinting techniques such as photo-curing-based bio-printing and droplet-based bioprinting [92]. This is because of its numerous advantages. For instance, extrusion bio-printing is used to print a wide range of biocompatible materials. It also has a wide range of viscosity in the range of 30mPa/s to 6 x 10^7mPa/s [93], hence can be used to provide structural support due to high viscosity and for maintaining cell viability due to low viscosity. Furthermore, the setup of extrusion printing is quite simple. However, its limitation compared to droplet-based printing is that cell viability is lower. According to Smith et al, Chang et al [94, 95] pressure and diameter of nozzle affect the cell viability of extrusion bio-printed materials.

3.1.1. Coaxial 3D Extrusion

While current reviews are presenting in-depth discussion on coaxial wet – spinning extrusion technique for printing [68], there is need for more review studies since 3D bioprinting can be used to fabricate cell-laden constructs for applications as in-vitro models or for therapeutic applications [69]. Self-assembled multicellular heterogeneous brain tumor fibers can be produced using co-axial extrusion 3D bioprinting system with alginate/gelatin as external shell [70].

To illustrate, the coaxial bioprinting provides cell-favorable gelatin methacryloyl (GelMA) microenvironments for tissue engineering and pharmaceutical screening application. This is observed when the coaxial extrusion is used to bioprint gelatin methacryloyl/alginate core/sheath microfibers to obtain 3D microfibrous constructs. Liu et al [79] suggests that it is possible to construct cell-laden GelMA materials at low concentrations of less than 2%, to support proliferation, as well as spreading for different cell types, implies a minimization and reduction of bioprinting requirement occur. Also, Hong et al [81] suggests that coaxial bioprinting of cell-laden

vascular constructs using a gelatin-tyramine bioink can be used to develop 3D tissue architecture vascularization, demonstrating a blood vessel – like structure.

Building on the work of Liu et al, Pi et al [80] confirm that tubular tissue constructs possess cell viability and undergoes proliferation and differentiation, when they examined the digitally tunable microfluidic bioprinting of multilayered cannular tissues using blend bioinks consisting GelMA, alginate and Polyethylene glycol (PEG). So, it is possible to promote cell growth and their proliferation, when the bioprinted cannular tissues were perfused with fluids. Similarly, Colosi et al [81] demonstrates the versatility of 3D fabricated coaxial bioink with the incorporation of low viscosity bioink. This bioprinting technique could control the bioink arrangement deposition, besides enhancing cell migration and arranging within each fiber. Furthermore, coaxial bioprint-based bioink can be used to develop

Using this bioprinting technique extrudes shell structures, without compromising their nature and structural integrity. Dai et al [70] reports that it is possible to study tumor microenvironment in vitro for tumor – stromal interactions using these self-assembled fiber-based 3D models. From this study, the coaxial bioprinted tumor fibers possess high expression of the used glioma stem/progenitor cell biomarker reported. Tumor stromal cells interacts with each other, which can result to an observable fusion. Moreover, studies have shown that coaxial bioprinting provides opportunity for cell-cell interactions in 3D space [71]. Besides, the possibility of fabricating 'core-shell' cell fiber structures demonstrate their scaffolding and soft nature, which is essential for tissue engineering and drug delivery applications [72, 73, 74]. The importance of these structures is in their ability to aid initial cell viability, vascularization, differentiations ad tissue heterogeneity [75, 76, 77].

The coaxial 3D extrusion-based bioprinting is versatile [78], as the tissue constructs obtained are usually viable, and could undergo proliferation and differentiation. All of these features indicate the wide applications in biomedical engineering.

3.1.2. 3D-Printed Electrospun Materials, and their Mechanical Properties

Electrospinning and 3D bioprinting techniques can be used to fabricate novel 3D asymmetric construct (SAC) for skin tissue regeneration [82].
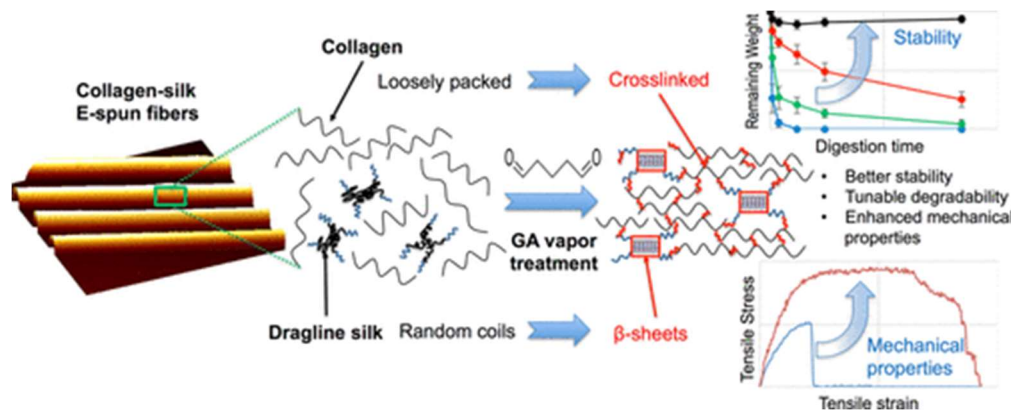
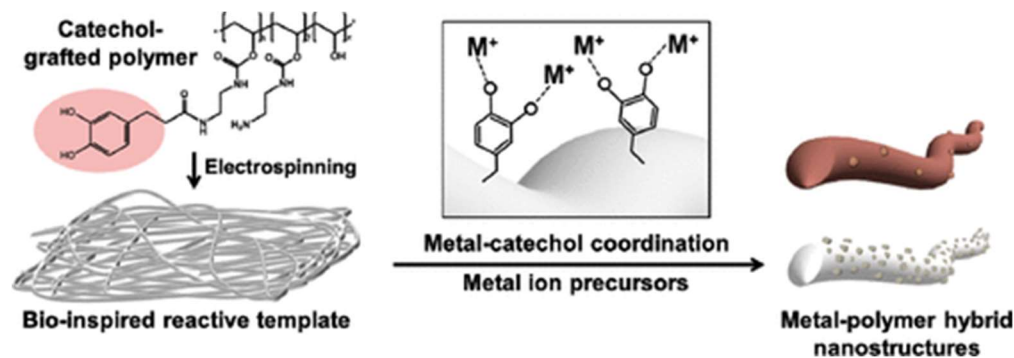Figure 2: Improving Mechanical Properties of Collagen-based Materials [99]

Figure 3: Role of Electrospinning in Metal-Composite Nanostructures [100]

Miguel et al [82] suggests that the 3D printed skin construct can be used for tissue regeneration in skin wounds healing. Producing the top layer of the skin construct is possible through electrospinning, as a result of easy cell penetration and nutrient exchange [83, 84]. Then, the 3D bioprinting has capability to replace broken dermis structure through an improved sequential layer-by-layer deposition of hydrogel materials. The 3D SAC possess morphology, adequate porosity, mechanical properties, wettability, antimicrobial activity, and a cytotoxic profile, which ensures their usefulness as skin substitutes during the healing process. Examining the mechanical properties of the top layer of the construct, Miguel et al reports a similarity to native human skin.

They fabricated a mechanical resistant nanofibrous top layer to enhance the cell-cell and cell-ECM interactions at nano-scale level. For this top layer, the mechanical properties of tensile strength, Young's modulus and elongation at break were examined to be 34.92 ± 7.39MPa, 27.92 ± 8.09MPa and $155.37\pm5.71\%$, respectively, though these values were presented as excellent at wet state.

Other studies have shown that native skin presents same mechanical properties at 5-30MPa (tensile strength), 4.6-20MPa (Young's Modulus), and elongation at break of 35-115% [85, 86]. The differences between the mechanical properties of the study and those of native skin can be attributed to the use of poly (caprolactone) PCL [87]. Though this study also reports that the hydrogel display a lower mechanical performance due to the higher porosity of the top layer, it is actually difficult to relate porosity to mechanical properties [88]. While Venugopal et al [89] evaluates the influence of porosity on production of PCL/nanohdroxyapatite/collagen nanofibers and reports that an increase in the pore size (from 2-15µm to 5-50 µm) decreased the tensile strength (2.72MPa to 1.28MPa), only a negative influence is nonetheless reported.

Moving forward, Chen et al [90] also claims that the 3D electrospinning hybrid technique is innovative for development of electrospun fiber-reinforced cartilage decellularized matrix (CDM) with customizable shape and controlled inner structure scaffold for cartilage regeneration. Collagen fibers can improve the mechanical properties of the 3D-printed scaffolds, as they display an elastic nature in wet condition. This is however outdated because current studies show that natural polymers including collagen possess low mechanical strength [91]. Adding the fibers to scaffold materials can only improve their stiffness and toughness, not necessarily the main mechanical properties. Therefore, other composite materials such as iron should be added to the composite scaffold to enhance their mechanical properties, and optimal condition for more applications. Iron ions in composite materials have been severally reported to aid mechanical stabilization of biomaterials.

3.1.3. **Microfludics: A new –layer of control for extrusion –based 3D printing**.

Serex et al. [96] was able to demonstrate multiple smart printing heads that allow the use of new materials, enhance the print resolution and printing of multi-material parts by the application of extrusion 3D bio- printing. In this study, multi-material printing was achieved by careful design of the print head composed of micro channel merging into one just before the extrusion point. Additionally, multiplexing and switching between materials during printing was achieved by using values made from polydimethysiloxane (PDMS). The advantage of static mixer over meander-based mixer in the formation of new materials was also demonstrated.

Further in the study, enhanced resolution was achieved by dispersing the material using sheath flow and varying the core to laminar flow. The problem of residual fluid was resolved by using highly volatile materials such as perfluoro (methydecabin) (PFD) as sheath flow.

Through a cross flow design, the authors demonstrated a print head allowing the concentration of particles in solution which can be used to increase the filler concentration of a composition just before it is printed.

Mousavi et al., [97] fabricated a bio hybrid oxidized alginate (OA)/ myocardial extra cellular matrix (ECM) injectable hydrogels with improved electromechanical properties for tissue engineering. The potential of the synergistic effect of the oxidized alginate/ myocardial ECM incorporated with amine reduced graphene oxide to enhance the electromechanical properties of hydrogel for cardiac tissue engineering. The presence of the fabricated hydrogel exhibited an excellent biological and electromechanical properties which was achieved with 4% (W/W) OA/mycocardial ECM incorporated with 25ppm of amine reduced graphene oxide.

Liu et al. [98] fabricated cell- loaded with gelatin methacryloyl (GelMA)/alginate core/sheath microfibers composite with 3D-micro environment. This process was adopted to reduce core bio ink printing requirements and facilitates the fabrication of the call at low concentration. Importantly, the biopolymer composite was printed using a co-axial nozzle –assisted 3D bioprinting. A favourable fabrication of was made by co-axial extrusion bioprinting of GelMA/alginate core/ sheath microfibers. Interestingly, the GelMA hydrogel was used as a core, while the alginate and sheath serves a template support. The novel technique provides extremely low concentrations of the cell microenvironment against the conventional bioprinting strategies. More so, the method afforded a high degree of the encapsulated cell.

4.0. Perspectives and Future Research

1. How can neurite extension test beds be further adapted to understand the role of chemical molecules for stimulating cellular interaction and regeneration in nerve tissue engineering? [101].

2. How can dorsal root ganglia (DRG) test beds be modified to ensure they utilize controlled release of growth factors?

3. What are the dose-dependent and gradient effects associated with the use of glial cell line-derived neurotrophic factor (GDNF) platform? [102]

4. What is the potential mechanism of genes in regulating signaling pathways?

5. How do external stimuli affect the interaction of specific genes in a tightly controlled microenvironment? [103]

6. How is endothelial cell viability related to jet velocity and cell penetration in research involving cell biology? [104]

7. How can modular hyaluronic acid and gelatin-based hydrogel platform be utilized for numerous applications in biomedicine? [105]

8. What is the effect of PCL scaffold pore sizes on the mechanical strength and cell retaining capacity of chitosan thermo-gel incorporated with 3D-printed poly (ε-caprolactone) scaffold?

9. What is the effect of chitosan thermogel on in-vivo application of chitosan-based 3D printed PCL scaffolds in load bearing bone defects?

5.0. Conclusion

Current research on biomedical application of several ECM components in tissue regenerative medicine, and emerging extrusion-based fabrication techniques have been reviewed in this work. New insights on improving the mechanical stability involving the use of iron ions have also been discussed. Having reviewed current advances, we propose that the coaxial-based extrusion method is versatile and of more potential significance in tissue regenerative medicine. The field is expected to evolve more with

Conflict of Interest

The authors have no known conflict to declare

6.0. References

1. Zhang K, Wang S, Zhou C, Cheng L, Gao X, Xie X, Sun J, Wang H, Weir MD, Reynolds MA, Zhang N, Bai Y, Xu HHK. Advanced smart biomaterials and constructs for hard tissue engineering and regeneration. Bone Res., 2018. 6 (1), 1-15

2. Clift CL, Su YR, Bichell D, Smith HCJ, Bethard JR, Norris-Caneda K, Comte-Walters S, Ball LE, Hollingsworth MA, Mehta AS, Drake RR, Angel PM. Collagen fiber regulation in human pediatric aortic valve development and disease. Sci. Rep., 2021. 11 (1), 1-17

3. Kuchler-Bopp S, Mariotte A, Strub M, Po C, De Cauwer A, Schulz G, Bellinghen XV, Fioretti F, Clauss F, Georgel P, Benkirane-Jessel N, Bornert F. Temporomandibular joint damage in K/BxN arthritic mice. Int. J. Oral Sci., 2020. 12 (1), 1-9

4. Kohn DH, Lemons JE. Dental applications. Biomater. Sci., 2020. 1119-33

5. Bhattacharjee P, Gupta P, Christakiran MJ, Nandi SK, Mandal BB. Silk-based matrices for bone tissue engineering applications. Nanostructure Eng. Cells Tiss Org., 2018. 439-472

6. Hussey GS, Dziki JL, Badylak SF. Extracellular matrix-based materials for regenerative medicine. Nat. Rev. Mater., 2018.

7. Mecham RP. Overview of extracellular matrix. Curr. Proc Cell Biol. Chapter 57, 2012. 10.1.1-10.1.16

8. Murphy SV, et al. Prog. Polym. Sci., 2012. 37, 1079.

9. Jang J, Kim TG, Kim BS, Kim SW, Kwon SM, Cho DW. Tailoring mechanical properties of decellularized extracellular matrix bioink by vitamin B2-induced photo-crosslinking. Act. Bio., 2016. 33, 88-95

10. Smith LR, Cho S, Discher DE. Stem Cell Differentiation is Regulated by Extracellular Matrix Mechanics. Physiology (Bethesda). 2018. 33(1):16-25

11. Samuel Ogbeide Ebhodaghe. Natural Polymer Scaffolds for tissue engineering applications. Journal of Biomaterials Science, Polymer Edition, 2021. 32 (16).

12. Chahal S, Kumar A, Hussian FSJ. Development of biomimetic electrospun polymeric biomaterials for bone tissue engineering: A review. J. Biomater. Sci Polym Ed., 2019.

13. Pati F, Jang J, Ha DH, Won KS, Rhie JW, Shim JH, Kim DH, Cho DW. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat. Commun., 2014. 5, 3935

14. Kim YS, Majid M, Melchiorri AJ, Mikos AG. Applications of decellularized extracellular matrix in bone and cartilage tissue engineering. Bioeng. Transl. Med., 2019. 1, 83-95

15. Samuel Ogbeide Ebhodaghe. Hydrogel-based biopolymers for regenerative medicine applications: a critical review. Int. J. Polym. Mater. Polym. Biomater., 2020. 1-18.

16. Jiang T, Munguia-Lopez JG, Kinsella JM. Extrusion bioprinting of soft materials: An emerging technique for biological model fabrication. Appl. Phys. Rev., 2019

17. Das S, Jang J. 3D bioprinting and decellularized ECM-based biomaterials for in vitro CV tissue engineering.

18. Pacifici A, Laino L, Pacifici L, etal. Decellularized hydrogels in bone tissue engineering: A topical review. Int. J. Mater. Sci., 2018

19. Kjar A, McFarland B, Huang Y, et al. Engineering of tissue constructs using coaxial bioprinting. Bioactive Mater., 2021.

20. Dai, X., Liu, L., Ouyang, J. *et al.* Coaxial 3D bioprinting of self-assembled multicellular heterogeneous tumor fibers. *Sci Rep, 2017.* **7,** 1457.

[21]   Y. Jo, S. H. Hwang, and J. Jang, Employing Extracellular Matrix-Based Tissue Engineering Strategies for Age-Dependent Tissue Degenerations, *International Journal of Molecular Sciences,* vol. 22, 2021.

[22]   K. Pfisterer, L. E. Shaw, D. Symmank, and W. Weninger, The Extracellular Matrix in Skin Inflammation and Infection, *Frontiers in Cell and Developmental Biology,* vol. 9, 2021-July-06 2021.

[23]   F.-S. Fattahi, Nanoscience and nanotechnology in fabrication of scaffolds for tissue regeneration, *International Nano Letters,* vol. 11, pp. 1-23, 2021/03/01 2021.

[24]   J. Jin, Q. Saiding, X. Wang, M. Qin, Y. Xiang, R. Cheng*, et al.*, Rapid Extracellular Matrix Remodeling via Gene-Electrospun Fibers as a "Patch" for Tissue Regeneration, *Advanced Functional Materials,* vol. 31, p. 2009879, 2021/04/01 2021.

[25]   A. Khanna, M. Zamani, and N. F. Huang, Extracellular Matrix-Based Biomaterials for Cardiovascular Tissue Engineering, *Journal of Cardiovascular Development and Disease,* vol. 8, 2021.

[26]   R. Sobreiro-Almeida, R. Quinteira, and N. M. Neves, Renal Regeneration: The Role of Extracellular Matrix and Current ECM-Based Tissue Engineered Strategies, *Advanced Healthcare Materials,* vol. 10, p. 2100160, 2021/07/01 2021.

[27]   H. Yuan, X. Li, M.-S. Lee, Z. Zhang, B. Li, H. Xuan*, et al.*, Collagen and chondroitin sulfate functionalized bioinspired fibers for tendon tissue engineering application, *International Journal of Biological Macromolecules,* vol. 170, pp. 248-260, 2021/02/15/ 2021.

[28]   S. Ullah and X. Chen, Fabrication, applications and challenges of natural biomaterials in tissue engineering, *Applied Materials Today,* vol. 20, p. 100656, 2020/09/01/ 2020.

[29]   C. Ma, H. Wang, Y. Chi, Y. Wang, L. Jiang, N. Xu*, et al.*, Preparation of oriented collagen fiber scaffolds and its application in bone tissue engineering, *Applied Materials Today,* vol. 22, p. 100902, 2021/03/01/ 2021.

[30]   B. Velasco-Rodriguez, T. Diaz-Vidal, L. C. Rosales-Rivera, C. A. García-González, C. Alvarez-Lorenzo, A. Al-Modlej*, et al.*, Hybrid Methacrylated Gelatin and Hyaluronic Acid Hydrogel Scaffolds. Preparation and Systematic Characterization for Prospective Tissue Engineering Applications, *International Journal of Molecular Sciences,* vol. 22, 2021.

[31]   M. Keshvardoostchokami, S. S. Majidi, P. Huo, R. Ramachandran, M. Chen, and B. Liu, Electrospun Nanofibers of Natural and Synthetic Polymers as Artificial Extracellular Matrix for Tissue Engineering, *Nanomaterials,* vol. 11, 2021.

[32]   S. Sharifi, M. M. Islam, H. Sharifi, R. Islam, D. Koza, F. Reyes-Ortega*, et al.*, Tuning gelatin-based hydrogel towards bioadhesive ocular tissue engineering applications, *Bioactive Materials,* vol. 6, pp. 3947-3961, 2021/11/01/ 2021.

[33]   S. Gautam, C. Sharma, S. D. Purohit, H. Singh, A. K. Dinda, P. D. Potdar*, et al.*, Gelatin-polycaprolactone-nanohydroxyapatite electrospun nanocomposite scaffold for bone tissue engineering, *Materials Science and Engineering: C,* vol. 119, p. 111588, 2021/02/01/ 2021.

[34]   B. Massoumi, M. Abbasian, B. Khalilzadeh, R. Jahanban-Esfahlan, A. Rezaei, H. Samadian*, et al.*, Gelatin-based nanofibrous electrically conductive scaffolds for tissue engineering applications, *International Journal of Polymeric Materials and Polymeric Biomaterials,* vol. 70, pp. 693-702, 2021/07/03 2021.

[35]   H. Samadian, H. Khastar, A. Ehterami, and M. Salehi, Bioengineered 3D nanocomposite based on gold nanoparticles and gelatin nanofibers for bone regeneration: in vitro and in vivo study, *Scientific Reports,* vol. 11, p. 13877, 2021/07/06 2021.

[36]   J. Xu, H. Fang, S. Zheng, L. Li, Z. Jiao, H. Wang*, et al.*, A biological functional hybrid scaffold based on decellularized extracellular matrix/gelatin/chitosan with high biocompatibility and antibacterial activity for skin tissue engineering, *International Journal of Biological Macromolecules,* vol. 187, pp. 840-849, 2021/09/30/ 2021.

[37]   N. K. Karamanos, A. D. Theocharis, Z. Piperigkou, D. Manou, A. Passi, S. S. Skandalis*, et al.*, A guide to the composition and functions of the extracellular matrix, *The FEBS Journal,* vol. n/a, 2021/02/19 2021.

[38]   A. Sharma, P. Sharma, and S. Roy, Elastin-inspired supramolecular hydrogels: a multifaceted extracellular matrix protein in biomedical engineering, *Soft Matter,* vol. 17, pp. 3266-3290, 2021.

[39]   M. Stojic, J. Ródenas-Rochina, M. L. López-Donaire, I. González de Torre, M. González Pérez, J. C. Rodríguez-Cabello*, et al.*, Elastin-Plasma Hybrid Hydrogels for Skin Tissue Engineering, *Polymers,* vol. 13, 2021.

[40]   C. J. Dalton and C. A. Lemmon, Fibronectin: Molecular Structure, Fibrillar Structure and Mechanochemical Signaling, *Cells,* vol. 10, 2021.

[41]   W. Zhang, Y. Liu, and H. Zhang, Extracellular matrix: an important regulator of cell functions and skeletal muscle development, *Cell & Bioscience,* vol. 11, p. 65, 2021/03/31 2021.

[42]   H.-S. Hung, K.-B. Chang, C.-M. Tang, T.-R. Ku, M.-L. Kung, A. Y. Yu*, et al.*, Anti-Inflammatory Fibronectin-AgNP for Regulation of Biological Performance and Endothelial Differentiation Ability of Mesenchymal Stem Cells, *International Journal of Molecular Sciences,* vol. 22, 2021.

[43]   J. Klavert and B. C. J. van der Eerden, Fibronectin in Fracture Healing: Biological Mechanisms and Regenerative Avenues, *Frontiers in Bioengineering and Biotechnology,* vol. 9, 2021-April-16 2021.

[44]   J. V. Korpershoek, M. Rikkers, T. S. de Windt, M. A. Tryfonidou, D. B. F. Saris, and L. A. Vonk, Selection of Highly Proliferative and Multipotent Meniscus Progenitors through Differential Adhesion to Fibronectin: A Novel Approach in Meniscus Tissue Engineering, *International Journal of Molecular Sciences,* vol. 22, 2021.

[45]   M. Assunção, D. Dehghan-Baniani, C. H. K. Yiu, T. Später, S. Beyer, and A. Blocki, Cell-Derived Extracellular Matrix for Tissue Engineering and Regenerative Medicine, *Frontiers in Bioengineering and Biotechnology,* vol. 8, 2020-December-03 2020.

[46]   J. Wang, J. Li, J. Liu, M. Lin, S. Mao, Y. Wang*, et al.*, Adsorption Force of Fibronectin: A Balance Regulator to Transmission of Cell Traction Force and Fluid Shear Stress, *Biomacromolecules,* vol. 22, pp. 3264-3273, 2021/08/09 2021.

[47]   M. L. Leite, D. G. Soares, G. Anovazzi, I. P. Mendes Soares, J. Hebling, and C. A. de Souza Costa, Development of fibronectin-loaded nanofiber scaffolds for guided pulp tissue regeneration, *Journal of Biomedical Materials Research Part B: Applied Biomaterials,* vol. 109, pp. 1244-1258, 2021/09/01 2021.

[48]   L. Chen, M. Carlton, X. Chen, N. Kaur, H. Ryan, T. J. Parker*, et al.*, Effect of fibronectin, FGF-2, and BMP4 in the stemness maintenance of BMSCs and the metabolic and proteomic cues involved, *Stem Cell Research & Therapy,* vol. 12, p. 165, 2021/03/06 2021.

[49]   J. Patten and K. Wang, Fibronectin in development and wound healing, *Advanced Drug Delivery Reviews,* vol. 170, pp. 353-368, 2021/03/01/ 2021.

[50]   G. Schierano, R. A. Canuto, M. Mauthe von Degerfeld, R. Navone, B. Peirone, G. Preti*, et al.*, Role of rhBMP-7, Fibronectin, And Type I Collagen in Dental Implant Osseointegration Process: An Initial Pilot Study on Minipig Animals, *Materials,* vol. 14, 2021.

[51]   S. Estrach, L. Tosello, F. S. Tissot, L. Cailleteau, L. Cervera, K. B. Jensen*, et al.*, Fibronectin meshwork controls epithelial stem cell fate, *bioRxiv,* p. 2021.05.26.445735, 2021.

[52]   N. Ziemkiewicz, G. Hilliard, A. J. Dunn, J. Madsen, G. Haas, J. Au*, et al.*, Laminin-111 Enriched Fibrin Hydrogels Enhance Functional Muscle Regeneration Following Trauma, *Tissue Engineering Part A,* 2021.

[53]   N. Nazeri, R. Karimi, and H. Ghanbari, The effect of surface modification of poly-lactide-co-glycolide/carbon nanotube nanofibrous scaffolds by laminin protein on nerve tissue engineering, *Journal of Biomedical Materials Research Part A,* vol. 109, pp. 159-169, 2021/02/01 2021.

[54]   J.-H. Lee, P. Parthiban, G.-Z. Jin, J. C. Knowles, and H.-W. Kim, Materials roles for promoting angiogenesis in tissue regeneration, *Progress in Materials Science,* vol. 117, p. 100732, 2021/04/01/ 2021.

[55]   A. J. Lepedda, G. Nieddu, M. Formato, M. B. Baker, J. Fernández-Pérez, and L. Moroni, Glycosaminoglycans: From Vascular Physiology to Tissue Engineering Applications, *Frontiers in chemistry,* vol. 9, pp. 680836-680836, 2021.

[56]   H. Sodhi and A. Panitch, Glycosaminoglycans in Tissue Engineering: A Review, *Biomolecules,* vol. 11, 2021.

[57]   D. Olvera, B. N. Sathy, and D. J. Kelly, Spatial Presentation of Tissue-Specific Extracellular Matrix Components along Electrospun Scaffolds for Tissue Engineering the Bone–Ligament Interface, *ACS Biomaterials Science & Engineering,* vol. 6, pp. 5145-5161, 2020/09/14 2020.

[58]   X. Zhang, X. Chen, H. Hong, R. Hu, J. Liu, and C. Liu, Decellularized extracellular matrix scaffolds: Recent trends and emerging strategies in tissue engineering, *Bioactive Materials,* 2021/09/23/ 2021.

59. Matai I, Kaur G, Laurencin CT, et al. Progress in 3D bioprinting technology for tissue/organ regenerative engineering. Biomater., 2020.

60. Ghilan A, Chiriac AP, Nita LE, Rusu AG, Neamtu I, Chiriac VM. Trends in 3D printing processes for biomedical field: Opportunities and challenges. J. Polym. Env., 2020. 28, 1345-67

61. Williams D, Thayer P, Martinez H, Gatenholm E, Khademhosseini A. A perspective on the physical, mechanical and biological specifications of bioinks and the development of functional tissues in 3D bioprinting. Bioprint., 2018. 9, 19-36

62. You F, Eames BF, Chen X. Application of extrusion-based hydrogel bioprinting for cartilage tissue engineering. Int. J. Mol. Sci., 2017. 18, 1597

63. Holzl K, Lin S, Tytgat L, Van Vlierberghe S, Gu L, Ovsianikov A. Bioink properties before, during, and after 3D bioprinting. Biofab., 2016. 8, 032002

64. Chang R, Emami K, Wu H, Sun W. Biofabrication of a three-dimensional liver micro-organ as an in vitro drug metabolism model. Biofab., 2010. 2, 045004

65. Vanderburgh J, Sterling JA, Guelcher SA. 3D printing of tissue engineered constructs for in vitro modelling of disease progression and drug screening. Ann. Biomed. Eng., 2017. 45, 164-179

66. Norman J, Madurawe RD, Moore CMV, Khan MA, Khairuzzaman A. A new chapter in pharmaceutical manufacturing: 3D - printed drug products. Adv. Drug Deliv. Rev., 2017. 108, 39-50

67. Lepowsky E, Tasoglu S. 3D printing for drug manufacturing: a perspective on the future of pharmaceuticals. Int. J. Bioprint., 2018. 4(1)

68. Costantini M, Colosi C, Swieszkowski W, Barbetta A. Co-axial wet-spinning in 3D bioprinting; state of the art and future perspective of microfluidic integration. Biofab., 2019. 11, 012001

69. Shin M, Galarraga JH, Kwon MY, Lee H, Burdick JA. Gallol-derived ECM-mimetic adhesive bioinks exhibiting temporal shear-thinnning and stabilization behavior. Act. Biomater., 2019. 95, 165-175

70. Dai X, Liu L, Ouyang J, Li X, Zhang X, Lan Q, Xu T. Coaxial 3D bioprinting of self-assembled multicellular heterogeneous tumor fibers. Sci. Rep., 2017. 7: 1457

71. Onoe H, et al. Metre-long cell-laden microfibres exhibit tissue morphologies and functions. Nat. Mater., 2013. 12, 584-90

72. Yu Y, et al. Three-dimensional bioprinting using self-assembling scalable scaffold - free "tissue strands" as a new bioink. Sci. Rep., 2016. 6, 28714

73. de Obaldia EE, et al. Analysis of the mechanical response of biomimetic materials with highly oriented microstructures through 3D printing, mechanical testing and modeling. J. Mech. Behav. Biomed. Mater., 2015. 48, 70-85.

74. Cornock R, Beirne S, Thompson B, Wallace GG. Coaxial additive manufacture of biomaterial composite scaffolds for tissue engineering. Biofab., 2014. 6, 025002

75. Ahn SH, et al. A novel cell-printing method and its application to hepatogenic differentiation of human adipose stem cell-embedded mesh structures. Sci. Rep., 2015. 5, 13427.

76. Colosi C, et al. Microfluidic bioprinting of heterogeneous 3D tissue constructs using low-viscosity bioink. Adv. Mater., 2016. 28, 677-684

77. Jia W, et al. Direct 3D bioprinting of perfusable vascular constructs using a blend bioink. Biomater., 2016. 106, 58-68

78. Colosi C, Shin SR, Khademhosseini A, et al. Microfluidic bioprinting of heterogeneous 3D tissue constructs using low-viscosity bioink. Adv. Mater, 2016.

79. Liu W, Zhang Z, Zhang YS, et al. Coaxial extrusion bioprinting of 3D microfibrous constructs with cell-favorable gelatin methacryloyl microenvironment. Biofab., 2018.

80. Pi Q, Maharjan S, Zhang YS, et al. Digitally tunable microfluidic bioprinting of multilayered cannular tissues. Adv. Mater., 2018.

81. Hong S, Kim JS, Jung B, Won C, Huang C. Coaxial bioprinting of cell-laden vascular construts using a gelatin - tyramine bioink. Biomater. Sci., 2019.

82. Miguel SP, Cabral CSD, Moreira AF, Correia IJ. Production and characterization of a novel asymmetric 3D printed construct aimed for skin tissue regeneration. Col. Surf. Biointer., 2019. 181, 994-1003

83. Miguel SP, et al. Electrospun polymeric nanofibres as wound dressings: A review. Col. Surf. B; Biointer., 2018. 169, 60-71

84. Miguel SP, et al. Electrospun Polycaprolactone/Aloe Vera_Chitosan

Nanofibrous Asymmetric Membranes Aimed for Wound Healing Applications.

Polymers, 2017. 9, 183.

85. Labet M, et al. Synthesis of polycaprolactone: a review.
Chem. Soc. Rev., 2009. 38, 3484-3504

86. Miguel SP, et al. Production and characterization of electrospun silk fibroin

based asymmetric membranes for wound dressing applications. Int. J. Biol.

Macromol., 2019. 121, 524-535

87. Morgado PI, et al. J. Poly(vinyl alcohol)/chitosan asymmetrical membranes:
Highly controlled morphology toward the ideal wound dressing
Membr. Sci., 2014. 469, 262-271

88. Hollister, SJ, et al. Porous scaffold design for tissue engineeringNat. Mater.,
2005. 4, 518

89. Venugopal J, et al. Biocomposite nanofibres and osteoblasts for bone tissue

engineering. Nanotech. 2007. 18, 055101

90. Chen W, Xu Y, Li Y, Jia L, Mo X, Jiang G, Zhou G. 3D printing electrospinning fiber-

reinforced decellularized extracellular matrix for cartilage regeneration. Chem. Eng. J,

2019.

91. Morgado PI, et al. Carb. Polym., 2017. 159, 136-145.

92. Zening GU, Jianzhong, FU, Hui Lin; Yong, HE. Development of 3D bioprinting: From

printing methods to biomedical applications. Assian Journal of Pharmaceutical Sciences

(2019)

93. Kogelenberg, S.V. Yue Z, Dinor JN, Bakers CS, Wallace GG. Advances in woundcare

(2017)

94. Smith CM, Stone AL, Parkill RL, Stewart RL, Simpkins MW, Kachurin AM, et al.

Three-dimensional bioassembly tool for generating viable tissue-engineered constructs.

Tisue Eng. 2014: 10(9-10). 156-76

95. Chang, R, Nan J, Sun W. Effects of dispensing pressure & nozzle diameter on cell survival from solid free form fabrication based direct cell writing. 2008 Tissue Eng. Part A- 14:(1): 41-8

96. Serex Ludovic, Arnaud Bertsch and Philippe Renaud. Microfluidics: A layer of control for the extrusion –based 3D printing. Micromachines 2018,9,86; doi: 10.3390/mi9020086

97. Mousavi Ali, Shohreh Mashayekhan, Nafiseh Baheiraei and Ali pourjavadi. Biohybrid oxidized aliginate/myocardial extra cellular matrix injectable hydrogels with improved electromechanical properties for cardiac tissue engineering. 2021,180, pp 692-708.

98. Liu Wanjun, Zhong Ning Hu, Yixiao Zhou, Lucia Maggio, Amir Miri, Alessio Fragasso, Xiangyu Jin, Ali Khade mhosseini and Yu shrike Zhang. Coaxial extrusion bioprinting of 3D microfibrous constructs with cell-favourable gelatin methacryoyl microenvironment. International Society for Biofabrication. 2017 https://doi.org/101088/1758-5090/aa9d44

99. Zhu B, Li W, Chi N, Lewis RV, Osamor J, Wang J. Optimization of Glutaraldehyde Vapor Treatment for Electrospun Collagen/Silk Tissue Engineering Scaffolds. *ACS Omega* 2017, 2, 6, 2439–2450

100. Son HY, Ryu JH, Lee H, Nam YS. Bioinspired Templating Synthesis of Metal–Polymer Hybrid Nanostructures within 3D Electrospun Nanofibers. *ACS Appl. Mater. Interfaces* 2013, 5, 13.

101. Ngo TB, Spearmen BS, Hlavac N, Schmidt CE. Three-dimensional bioprinted hyaluronic acid hydrogel test beds for assessing neural cell responses to competitive growth stimuli. ACS Biomater. Sci. Eng., 2020.

102. Bittner SM, Guo JL, Mikos AG. Spatiotemporal control of growth factors in three-dimensional printed scaffolds. Bioprinting, 2018. 12, e00032

103. Das S, Kim SW, Choi YJ, Lee SH, Kong JS, Park HJ, Cho DW, Jang J. Decellularized extracellular matrix bioinks and the external stimuli to enhance cardiac tissue development in vitro. Act. Bio., 2019. 95, 188-200

104. Catros S, Guillotin B, Bacakova M, Fricain JC, Guillemot F. Effect of laser energy, substrate film thickness and bioink viscosity on viability of endothelial cells printed by laser-assisted bioprinting. Appl. Surf. Sci., 2011. 257. 5142-47

105. Skardal A, Devarasetty M, Kang HW, Mead I, Bishop C, Shupe T, Lee SJ, Jackson J, Yoo J, Soker S, Atala A. A hydrogel bioink toolkit for mimicking native tissue biochemical and mechanical properties in bioprinted tissue constructs. Act. Bio., 2015.