TISSUE ENGINEERING: Part B
Volume 28, Number 3, 2022

DOI: 10.1089/ten.teb.2021.0012

termis
Tissue Engineering
& Regenerative Medicine
International Society

REVIEW ARTICLE

Highlights on Advancing Frontiers in Tissue Engineering

Nureddin Ashammakhi, MD, PhD,[1,2,*] Amin GhavamiNejad, PhD,[3,*] Rumeysa Tutar, PhD,[4] Annabelle Fricker, MSc,[5] Ipsita Roy, PhD,[5,6] Xanthippi Chatzistavrou, PhD,[7] Ehsanul Hoque Apu, DDS, PhD,[1] Kim-Lien Nguyen, MD,[8,9] Taby Ahsan, PhD,[10] Ippokratis Pountos, MD, PhD,[11] and Edward J. Caterson, MD, PhD[12]

The field of tissue engineering continues to advance, sometimes in exponential leaps forward, but also sometimes at a rate that does not fulfill the promise that the field imagined a few decades ago. This review is in part a catalog of success in an effort to inform the process of innovation. Tissue engineering has recruited new technologies and developed new methods for engineering tissue constructs that can be used to mitigate or model disease states for study. Key to this antecedent statement is that the scientific effort must be anchored in the needs of a disease state and be working toward a functional product in regenerative medicine. It is this focus on the wildly important ideas coupled with partnered research efforts within both academia and industry that have shown most translational potential. The field continues to thrive and among the most important recent developments are the use of three-dimensional bioprinting, organ-on-a-chip, and induced pluripotent stem cell technologies that warrant special attention. Developments in the aforementioned areas as well as future directions are highlighted in this article. Although several early efforts have not come to fruition, there are good examples of commercial profitability that merit continued investment in tissue engineering.

Keywords: 3D bioprinting, organ-on-a-chip, regenerative medicine, stem cells, tissue engineering

Impact Statement

Tissue engineering led to the development of new methods for regenerative medicine and disease models. Among the most important recent developments in tissue engineering are the use of three-dimensional bioprinting, organ-on-a-chip, and induced pluripotent stem cell technologies. These technologies and an understanding of them will have impact on the success of tissue engineering and its translation to regenerative medicine. Continued investment in tissue engineering will yield products and therapeutics, with both commercial importance and simultaneous disease mitigation.

[1]Department of Bioengineering, Henry Samueli School of Engineering, University of California, Los Angeles, California, USA.
[2]Department of Biomedical Engineering, College of Engineering, Michigan State University, Michigan, USA.
[3]Advanced Pharmaceutics and Drug Delivery Laboratory, Leslie L. Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada.
[4]Department of Chemistry, Faculty of Engineering, Istanbul University-Cerrahpasa, Istanbul, Turkey.
[5]Department of Materials Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, United Kingdom.
[6]Faculty of Medicine, National Heart and Lung Institute, Imperial College London, London, United Kingdom.
[7]Department of Chemical Engineering and Material Science, College of Engineering, Michigan State University, East Lansing, Michigan, USA.
[8]Department of Radiological Sciences, David Geffen School of Medicine, University of California, Los Angeles, California, USA.
[9]Division of Cardiology, David Geffen School of Medicine, University of California, Los Angeles, and VA Greater Los Angeles Healthcare System, Los Angeles, California, USA.
[10]RoosterBio, Inc., Frederick, Maryland, USA.
[11]Academic Department of Trauma and Orthopaedics, University of Leeds, Leeds, United Kingdom.
[12]Division of Plastic Surgery, Department of Surgery, Nemours/Alfred I. du Pont Hospital for Children, Wilmington, Delaware, USA.
*These authors contributed equally to this work.

Introduction

Tissue defects and organ loss can result from congenital problems, disease, damage, or surgical removal[1,2] and thus, tissue defects need to be regenerated and repaired. In addition, organ function needs to be regained. This is the promise of tissue engineering. Because of the shortages in organ and tissue supply,[3] many patients die every day while waiting for a transplant.[4,5] Tissue engineering and its popular and governmental support in concept was driven by this organ and tissue deficiency. Therefore, methods to develop autograft-like replacement tissues have been explored and demanded by the funding agencies. The major advance has been the development of the tissue engineering concept in the late 1980s and early 1990s.[6]

Tissue engineering aims at producing functional tissue constructs for use in reconstruction or regeneration of damaged or lost tissues and organs,[7,8] such as skin,[9] spinal cord,[10] and other organs.[11] In addition, secondary gain has been the development of models to study function,[12] disease[13] and test and develop drugs.[14] Tissue engineering can be achieved either *ex vivo*[15] or *in situ*[16] by using various molecules, materials, or cells to stimulate local tissue regenerative capacity. It is important to note that this effort of tissue engineering was a fundamental shift in the approach to the treatment of tissue loss. With the end goal being functional organs with a complex interplay of different cell signals and scaffolds, the effort mandated a ''system'' approach with engineering principles instead of the traditional reductionist methodology of experimentation. The ultimate end goal was beyond the knowledge of a fundamental mechanism, but rather a product to mitigate or cure a disease state.

The field of tissue engineering; however, continues to advance, taking advantage of recent developments in areas such as smart biomaterials,[16–18] induced pluripotent stem cell (iPSC),[19] three-dimensional (3D) bioprinting[20,21] technologies, and dynamic culture methods.[22,23] It is also renewed by new technologies such as genetic engineering, extracellular vesicles (EVs), and artificial intelligence. Literature in the field is vast, and there are excellent reviews of different aspects of tissue engineering.[24–26] Therefore, the purpose of the current article is to highlight only major and recent advances in the field (Fig. 1).

Advancing Frontiers in Tissue Engineering

Advances in biomaterials and their application in tissue engineering

In tissue engineering, biomaterials are used to provide micro- and nanostructural characteristics, morphology, and surface properties that support cells and can be loaded with appropriate growth factors. Biomaterials used, in the form of matrices or scaffolds for tissue engineering, can be engineered in a way that helps to direct cell growth through specific designs.[27] Although biomaterials have been used in tissue engineering since the early 1990s,[6,28] how they have been used and the applications they are used for are constantly evolving. Outlined here are recent advances in the use of biomaterials for tissue engineering (Table 1). It is important to note that the field often has an eye on commercialization, such that the Food and Drug Administration (FDA) approval for a new biomaterial may be a hurdle that shapes the evolution of tissue engineering constructs. It is important to note that this barrier can and has in part limited the imagination of the field, because it is often more expeditious to adapt a natural biomaterial or harness one that has an FDA track record instead of designing *de novo* a material that will require significant vetting before its clinical use.

Advances in polymeric biomaterials

Natural polymers. Natural polymers that are most commonly used in tissue engineering are collagen,[29] gelatin,[30] chitosan,[31] alginate,[32] hyaluronic acid (HA),[33] and polyhydroxyalkanoates (PHAs),[34] because of their availability and biocompatibility.[35,36] Peptides present on some of them, such as collagen, help cell attachment, migration, and function.[29] Silk, a natural polymer,[37] has been increasingly popular in many tissue engineering applications due to its high processability, strength, and elasticity.[38,39] The PHAs comprise another group of natural polymers with special interest, as they are characterized by degradation through surface erosion that helps to maintain their general structure.[40]

Guided growth of neuronal cells was observed *in vitro* following the use of highly aligned electrospun fibers of a blend of the poly(3-hydroxybutyrate) [p(3HB)], and poly(3-hydroxyoctanoate) [p(3HO)].[41] In another application, p(3HO) was used to produce cardiac patches, which exhibited favorable mechanical properties closely matching those of native cardiac muscle, and surface topography that enabled efficient cell adhesion and proliferation.[42] To produce new constructs with improved properties, polymers can also be used in combination, for example, electrospun fibers made from a combination of chitosan and gelatin were found to enhance bone regeneration capability.[43] When PHAs were blended with the synthetic polymer polycaprolactone (PCL) to produce a scaffold that delivers seeded cardiac progenitor cells and implanted in the postmortem murine heart, the implants enabled the adhesion of cardiac progenitor cells, stem cell proliferation, and retention.[44]

Natural extracellular matrixes. Natural extracellular matrixes (ECM) have been used in a wide range of tissue engineering applications.[45,46] The ECM provides a natural structure that maintains some of the biological cues of the native tissues. The ECM chemical cues also help with cell attachment, differentiation, and function. There are various methods that have been used for the preparation of mammalian-tissue-based decellularized matrices, including chemical, biological, and physical methods and their combinations.[47] The majority of research, though, has been focused on the decellularization of tissues or organs.[48] It was shown that seeding decellularized hearts can result in obtaining contractile hearts by day 4 after keeping them in a bioreactor.[49] Using electrical stimulation and physiological load, constructs pump function was achieved by day 8. This represents an interesting area for the application of decellularized ECM in the tissue engineering of various organs.

Kusuma *et al.* made a major advance by demonstrating that immortalized cell lines can produce high-quality ECM from a single cell source.[50] Moreover, processing steps such as homogenization, pepsin digestion, or urea extraction have been used to create solutions that can be used to create

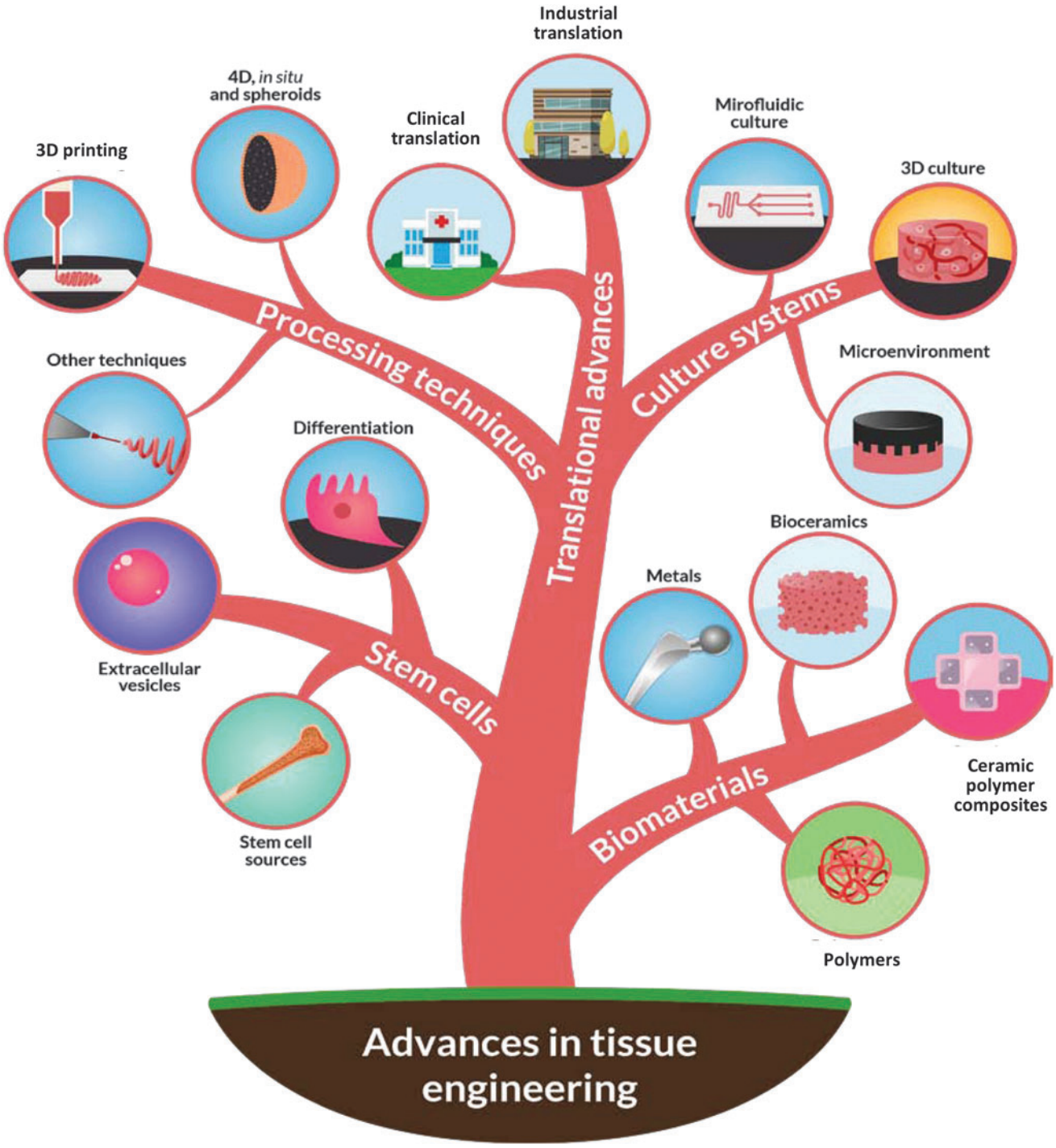

FIG. 1. Schematic illustration showing overview of major and recent advances in tissue engineering. 3D, three-dimensional; 4D, four-dimensional. Color images are available online.

surface coatings that retain some of the key properties of the native ECM. The ECM is proposed for numerous applications, due also to its versatile processing characteristics that have already allowed its use in 3D printing[51] and electrospinning.[52] For example, Kim *et al.*[53] used skin-derived ECM bioink for the 3D printing of skin tissue, with some success. Further, Carvalho *et al.*[54] combined cell-derived ECM with PCL and electrospun the solution to create microfibrous scaffolds for bone tissue engineering. The incorporation of ECM in the fibers enhanced cell proliferation and osteogenic differentiation, maintaining similar mechanical properties to PCL alone. The regulatory requirements of the field allow the strategy for efficient decellularization to appear to be one of the most viable pathways toward a product in short order.[49]

Despite the many positive attributes of the decellularized matrix for use in the field of tissue engineering, it does also come with limitations. One such limitation is its degradation

Table 1. Advantages, Disadvantages, or Limitations of Different Biomaterials Used for Tissue Engineering

No.	Biomaterial	Advantages	Disadvantages/ limitations	Types of tissue engineering products	Refs.
A	Polymers				
1	Natural polymers	• Biocompatibility • Cell adhesion motifs • High processability • Elasticity • Degradability	• Limited mechanical properties	• Various tissues such as heart, bone, liver, and cartilage	27–31
2	ECM	• Mimicking native tissue		• Tissues such as bone, skin, meniscus, and kidney	32–36
3	Synthetic polymers	• Can be bioresorbable and processed in a controlled way • High mechanical properties	• Inflammation • No cell adhesion molecules	• Tissues such as bone, cartilage, nerve, and brain	37–40
4	Hydrogels	• Cells, drugs, and biomolecule delivery • Minimally invasive techniques	• Mechanical properties • Adhesive strength • Cell adhesion	• 3D bioprinting • Injectable materials and drug delivery vehicles for regeneration • Minimally invasive regenerative therapeutics • Cartilage regeneration	41–43
5	Smart and functional polymers—composites	• Biological properties • Antibacterial activity • Physical properties, e.g., self-healing, shape-memory, stimuli-responsiveness	• Controllability of responsiveness may be affected by environment	• Injectable regenerative therapeutics for treating bone defects	8,44,45
B	Bioceramics	• Bioactive • Biocompatible • High compression strength • 3D printed scaffolds with mechanical characteristics comparable to human cortical bone	• Low tensile strength • Brittleness • Weak under cyclic or high loads	• Hard tissue engineering such as bone, cartilage, and tooth	46–51
C	Ceramic-polymer composites	• Cell incorporation • Enhanced tissue infiltration	• Brtittleness	• Injectable or 3D-printed composites for dental and cartilage tissue engineering	52–55
D	Metals	• Biocompatibility • Degradable metal alloys • Improved mechanical properties	• Uncontrolled corrosion	• Absorbable implants for bone repair • 3D porous scaffolds	56,57

3D, three-dimensional; ECM, extracellular matrixes.

rate, and this is a property that often needs consideration when using biomaterials for tissue engineering. For optimal regeneration, the degradation rate of decellularized matrix should be closely matched with the regeneration rate of the target tissue, and in many of applications this means that the degradation rate needs to be reduced.[55] Current decellularization methods and processes achieve both a thorough removal of all cells and retention of other nonantigenic parts of the original tissue composition that can aid/guide in tissue regeneration.[56]

Decellularization is also not a ''one-size-fits-all'' approach, and the protocols must be adapted for different tissue types while integrating factors such as their density and the matrix components. Decellularized matrix produced from tissues, which have specific mechanical properties, must maintain structural matrix components such as collagen fibers and many proteins that are necessary as endoskeleton and thus decellularization protocols need to be tuned to preserving these components. Increased preservation of active factors and structural components would also increase the bioactivity of decellularized matrix, making it an even better natural guidance material for tissue engineering.[57]

It is important to note why this strategy is imperative and that it is linked to the ''systems approach'' already mentioned in contrast to precedent scientific reductionist work. In a system approach, the ''principle'' is that the ECM or the scaffold is imperative to drive and maintain differentiation. One can ask the fundamental question as to whether an osteoblast is an osteoblast when it is not surrounded by its ECM. Many in the stem cell field would argue that the cells and the ECM are intrinsic to one another and that molecular flexibility in differentiation and dedifferentiation occur without the union of the cell and the ECM. With this principle in mind, the strategies of decellularized matrices are

rational because we do not have all the cues that are both physical and chronologic to the complex interplay between the cell and its ECM. Certainly, with further study and insight, smart or rational designs will incorporate the natural cues found in the natural ECM and allow synthetic polymers to support cell differentiation with similar efficiency to natural polymers.

Synthetic polymers. Synthetic polymers have been widely used in tissue engineering because they are widely available and inexpensive; can be bioresorbable; and can be processed in a controlled and multitude of ways to make them suitable for different applications. Commonly used synthetic polymers include polylactide (PLA),[58–60] polyglycolide (PGA),[58] poly(lactide-*co*-glycolide) (PLGA),[59,61,62] PCL,[60,63,64] poly(glycerol sebacate) (PGS),[64] and polydimethylsiloxane (PDMS). The PDMS has unique applications in tissue engineering among synthetic polymers due to high oxygen (O_2) diffusivity, ease of fabrication, biocompatibility, and flexibility.[65] It has been explored for engineering of cell sheets,[66] and it is widely used for the development of 3D organ-on-a-chip (OoC) cultures[67] that helped advancing the field of engineering tissue models tremendously (see the Advances in Microfluidic Culture Systems section). The PDMS is a nonbiodegradable polymer, and it was therefore used more commonly in *ex vivo* rather than implantable tissue engineering constructs.[67]

Blends of synthetic polymers were also explored to combine the properties of different materials.[68] Synthetic polymers have been also used in combination to build different phases in the resulting structure. For example, Fang *et al.* used PLA to produce shell and PGA to produce core in electrospun nanofibres.[58] The materials were found to accelerate wound healing *in vivo*. Synthetic polymers can also be combined with natural biomaterials to form semi-synthetic polymers. For example, Jiao *et al.*[63] melt-blended PCL and HA to 3D print scaffolds for bone tissue engineering. Constructs had improved mechanical characteristics as compared with those that were made from PCL alone.

The bioresorbability of many synthetic polymers poses advantages in many tissue engineering applications. However, controlling the rate at which degradation occurs can be a clinical challenge. Xu *et al.*[62] experimented with adding magnesium to PLGA to make composite films with low ranges of magnesium weight percentages, and they found that magnesium extended the duration of degradation and also improved the tensile strength of the films.

Hydrogels. Another advancing recent frontier has been in the area of hydrogels. Hydrogels have been extensively used for 3D bioprinting, which has been a very active area of research in the past few years.[69] Hydrogels can be made from various natural or synthetic polymers and have been used for the engineering of different tissues, because of their ability to encapsulate cells,[70,71] while having the permeability required for the diffusion of O_2 and nutrients across the material. An aspect in which previously they have fallen short is their mechanical properties[72] and the lack of adhesiveness.[73] Recently, however, these problems were largely addressed. For example, Shirzaei Sani *et al.*[74] produced an adhesive HA/elastin-like polypeptide hybrid hydrogel, which is characterized by remarkable adhesive, antimicrobial activity, and tunable physical properties. This enhances the translation of the hydrogels to the clinical practice as it was limited due to their poor mechanical characteristics, low adhesive strength, and their weakness to inhibit bacterial colonization.

Reinforcement of hydrogels can be achieved through interpenetrating secondary networks.[75] The addition of a second network enables conventional hydrogels to be used in many emerging biofabrication techniques toward achieving hierarchical architectures and developing personalized medicine. These interpenetrating hydrogels can find applications in tissue engineering and drug delivery systems as well as in developing *in vitro* disease models for drug discovery and screening. Hybrid hydrogels were found to have greater adhesive strength to the tissue being engineered, as compared with commercially available tissue adhesives. A great potential of hydrogels is their use as injectable materials to deliver cells, drugs, and biomolecules[16] for regenerative purposes that can often be achieved by using minimally invasive techniques.[76,77]

A recent study[78] looking into cartilage repair found that HA hydrogels could be used to encapsulate chondrocytes and support cell survival and the regeneration of cartilaginous tissue. Aside from HA, alginate, and collagen, ECM hydrogels have been used in tissue engineering, and also for cell encapsulation. In addition, microencapsulation of cells to produce microgels was also explored.[79–81] Hydrogels such as these can be blended and processed through 3D bioprinting, where cells are encapsulated and printed into designed structures and then crosslinked to provide appropriate mechanical properties. For example, 3D-printed scaffolds of collagen/alginate hydrogel have been used for cartilage tissue engineering[82] and ECM hydrogel for cardiac patches.[83]

The regeneration of damaged tissue can be achieved either via *ex vivo* or *in situ* methods. In *ex vivo* tissue engineering, scaffolds are combined with cells and biomolecules outside the body to obtain cell-laden tissue constructs for implantation (Fig. 2A).[16] However, the *ex vivo* tissue regeneration has limitations, such as tissue morbidity and the lack of reliable cell sources. On the other hand, *in situ* tissue engineering requires precise control of the biochemical and biophysical cues to stimulate resident host cells and attract cells to the site of injury requiring regeneration (Fig. 2B). On the other hand, *in situ* tissue regeneration can be achieved by stimulating endogenous cells using either extracellular signals or cell reprogramming. In the first approach, cells are primed via extracellular factors, such as through modulating the biophysical and biochemical characteristics of the biomaterial (Fig. 2B).[16] In the second approach, direct manipulation of the cellular gene- expression program is accomplished through cellular reprogramming (Fig. 2B).[16] Because of its relation to biomaterials, we review the first approach in this section.

Smart and functional polymers. Smart polymers used in tissue engineering include those with self-healing,[84–86] shape memory,[76,87–89] or stimuli-responsiveness[18,90–92] properties. The ability to change the shape of 3D-printed objects via environmental stimuli, such as heat, moisture, water, pH, or light as a function of time, is known as four-dimensional (4D) printing, and it also has recently gained

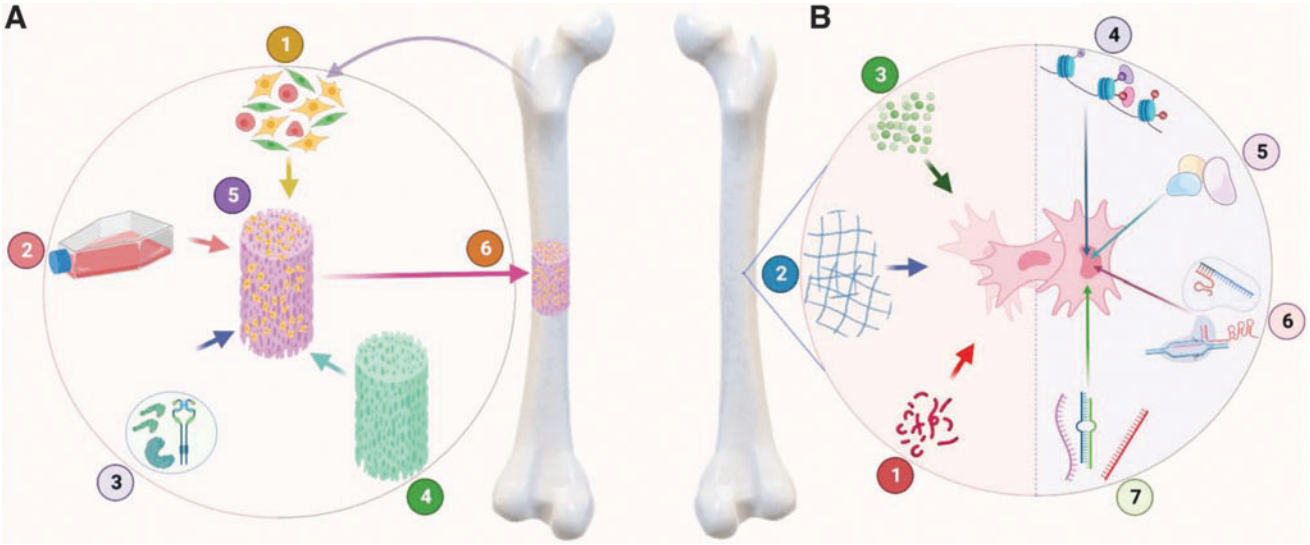

FIG. 2. Schematic illustration showing **(A)** Use of traditional *ex vivo* tissue engineering approach, which is based on the use of cells (1) cultured (2) with growth factors (3) and scaffolds (4) to develop pre-seeded constructs (5) outside the body before their implantation (6). **(B)** Use of *in situ* tissue regeneration to harness the innate regenerative capacity in the body either through extracellular signal manipulation by using bioactive (1) or immunomodulatory (2) biomaterials or bioactive molecules (3), or through an intracellular reprograming approach, which employs epigenetic transformation (4), transcription factors (5), gene editing (6), or an RNA-based approach (7). Created with Biorender.com. Color images are available online.

considerable interest.[93] For example, Invernizzi *et al.* have developed a novel 4D-printable smart material, using PCL and 2-ureido-4[1H]-pyrimidinone (UPy), which is a thermally activated shape memory polymer with self-repairing abilities.[94] The incorporation of methacrylates bearing UPy (UPyMA) monomers had provided self-healing properties to the 4D-printed structures, and the possibility to print actuators for soft robotics had been shown for the first time in this work. Synthesizing smart hydrogels that provide both self-healing and shape memory properties at the same time is expected to be further investigated in the following years.[95–97]

Although there are many clear reasons why smart and functional polymers have gathered attention, they do have drawbacks that should be considered. For example, in the body environment, triggering thresholds of changes, for example, temperature or pH, may affect the responsiveness of these polymers, which, in turn, will affect controllability of the construct and the included cells.[69] A shape memory material implanted in the body may lead to injury of the neighboring tissues or loss of function when it returns to its original shape.[98] These challenges have to be mitigated before full benefits can be gained from utilizing smart polymers for tissue engineering.

Stimuli-responsive and self-healing hydrogels have also emerged as pharmaceutical carriers for tissue engineering.[84,94–105] One such example application of these self-healing hydrogels that is being explored is bone regeneration that can be achieved by providing an optimal microenvironment for new bone formation and for therapeutic drug delivery. Unlike conventional hydrogels, these constructs can resist mechanical stress, while protecting their therapeutic cargos from degradation and maintaining their sustained release for the long-term performance required for bone tissue healing.[84,99] To this end, a hydrogel made of chondroitin sulfate (ChS), known for its regenerative capacities, was developed for bone tissue repair with the material being cross-linked to mimic cranial bone characteristics.[100]

With excellent self-healing, injectability, and *in vivo* tissue adhesion abilities, ChS-based hydrogel exhibited good cytocompatibility when it was used to encapsulate rat-derived mesenchymal stem cells (MSCs). Most importantly and compared with phosphate-buffered saline-loaded hydrogel, the injection of bone morphogenetic protein (BMP)-4 loaded hydrogel into a murine bone defect model led to defect repair through the formation of new cranial bone tissue, with a significant decrease in the defect size after 12 weeks.

Over the past few years, significant progress in the development of advanced functional polymers with tunable chemical, physical, and biological properties has been achieved.[101,102] This resulted in novel applications in 3D and 4D bioprinting[103] and drug delivery.[104–106] For example, Zhang *et al.* recently developed a biocompatible hydrogel ink, which contains self-healing precrosslinked hydrogel microparticles of chitosan methacrylate and polyvinyl alcohol hybrid hydrogels. Their results showed advanced structures with a high aspect ratio, and excellent shape accuracy at organ-proper scales could be quickly produced.[107]

Several drug delivery systems have been based on the use of advanced polymers. Consequently, functional hydrogels that can provide the required dosage in both proper chronicity and location can mitigate complications and enhance success with clinical application.[108–110]

Advances in bioceramic biomaterials. Ceramics are attractive materials for tissue engineering, because of their highly bioactive and biocompatible characteristics.[111] Ceramics, including bioactive glass ceramics, have been used in bone tissue engineering applications for many years

due to their well-matched mineral characteristics.[112–114] They are strong and osteoconductive, which makes them ideal for application in hard tissue engineering.[66–69] They are strong in compression, however, weak in tension, and very brittle. Bioactive ceramic and glass-ceramic scaffolds were also produced by 3D printing processes such as ''robocasting''[115] and the ''freeze extrusion fabrication'' that combines extrusion printing with freeze-drying.[116] The high strength values of scaffolds fabricated by additive manufacturing result from their ability to maintain highly interconnected channels with high alignment, at a porosity of 50–60%. These scaffolds presented an elastic response under compression, with an average compressive strength of 140 MPa and an elastic modulus of 5–6 GPa, which are comparable to those of the human cortical bone.

Although bioactive ceramic and glass-ceramic scaffolds can effectively mimic porous bone, provide required compressive strength,[117] and contain channels in their 3D structure for tissue ingrowth,[115] they are brittle and not suitable for applications in locations exposed to cyclic or high loads. Thus, scaffolds made of pure ceramics were not very successful when they were used in load-bearing regions of the body.[118] It is also imperative to note that these scaffolds, in particular once seeded with cells and remodeled by biologic ingrowth and calcified ECM production, will change their structural capacity and thus often can be used as a bridge technology. Therefore, the development of advanced scaffolds that can maintain bioactivity properties is required. To achieve this, the obvious engineering solution that has been implemented was the development of composite materials.

Advances in ceramic polymer composite biomaterials. Bioactive glass was used in the form of particles,[119] fibers,[120] or scaffolds,[121] and it was combined with polymers to develop various composites for tissue engineering. For example, bioactive glass nanoparticles have been incorporated into freeze-cast gelatin-chitosan foams with a pore size range between 150 and 300 μm.[122] The low strength of the composite was improved by a decrease in its porosity. Bioactive glass–collagen–phosphatidylserine scaffolds (65 wt.% 58S sol–gel bioactive glass) were developed with 75% porosity, a pore size of 300 μm, and a compressive strength of 1.5 MPa.[123] However, connectivity between pores was poor, limiting scaffold application in tissue engineering.

In addition, Nikpour *et al.*[124] developed a composite with bioactive glass-ceramic and dextran hydrogel because of its biocompatibility and hydrophilicity, which enable the incorporation of cells and nanoparticles in the structure. Chatzistavrou *et al.* also looked at the combination of bioactive glass-ceramic particles with appropriate matrixes (e.g., ECM, collagen–fibrin microspheres) and stem cells to enhance odontogenic differentiation and trigger new dentine formation in dental tissue engineering approaches.[125,126] Another study also looked at producing nano-bioactive glass-ceramic particles that were incorporated with Calcarea phosphorica aiming at assessing the effect of these nanoparticles on osteoblast differentiation.[127] It was found that these particles had osteogenic potential, as they promoted mouse mesenchymal cell proliferation.

Ceramics are also being coated with polymers, which can help in achieving surface functionalization, controlled delivery of growth factors and drugs, and enhanced bioactivity.[128] In one example, Luginina *et al.*[64] combined bioactive glass particles with electrospun PGS/PCL for the engineering of cartilage. This combination helped to maintain smaller projected cell areas as well as rounded cell phenotype. Scaffolds made of 13–93 bioactive glass were seeded with rat-bone-marrow-derived MSCs and implanted in the subcutis of rats for 4 weeks, which resulted in tissue infiltration of the scaffolds.[121] Moreover, vessels can form inside scaffolds in *in vitro* cultures and when the construct is implanted *in vivo* newly formed vessels may connect to the host blood vessels.[129] Further, scaffolds made from bioactive glass fibers and PLGA mesh were also developed and investigated for bone tissue engineering using osteoprogenitor cells representing craniosynostotic osteoprogenitors, with the view of using this approach for the reconstruction of the crania of these patients using autologous cells derived from removed tissues.[130]

Advances in metal biomaterials. Metals are a group of interesting materials that can also be used for developing scaffolds for tissue engineering. At a historic level, gold and other materials with malleable properties have been used before the time of Hippocrates. Because most of metals are not biodegradable, they cannot be replaced by tissues. Therefore, the use of this group of materials for tissue engineering has been very limited except for the recent activity in biodegradable metal alloys, which represents an expanding research frontier. These materials combine both the properties of metals and biodegradability sought in polymers. In addition, their use helps to avoid many problems associated with the use of biodegradable polymers, such as inflammation[131,132] and osteolysis.[133] In this group of biodegradable metals, magnesium-based alloys have been explored and various implants have been developed especially for application in the treatment of bone tissue.[134] A combination of both biodegradable polymers and metals has also been investigated,[135] for example, a biodegradable magnesium-reinforced biodegradable PLA membrane was developed for application in guided bone regeneration.[136] In future, it is expected to see more studies on the combination of metals with polymers.

Metals, in general, exhibit improved mechanical properties (i.e., yield strength, ultimate tensile strength, hardness, etc.), and they are considered the best alternative for structural support. In addition to mechanical performance, absorbable metals should be compatible and nontoxic with controlled corrosion behavior. A metal that can be considered absorbable should corrode in the body's environment without generating toxic corrosion products. Thus, they should meet an appropriate balance between maintaining the required mechanical performance and corroding within a required period while the native tissue is regenerating.

Iron (Fe), magnesium (Mg), and their alloys have been investigated as absorbable metals, for biomedical applications in cardiovascular and orthopedic surgery. Mg is biocompatible, reduces thrombogenicity, and is critical for several cellular functions, such as intracellular transport, signal transduction, and energy metabolism.[137] Absorbable stents[138] and bone screws[139] made of Mg-based alloys are already commercially available.[140,141]

However, the uncontrolled and fast corrosion of Mg in biological environments remains a challenge.[142] Mg-based alloys are still being optimized toward meeting the expectations of absorbable metallic implants.[132] Zinc (Zn) was

incorporated as an alternative to Mg, because of its moderate corrosion rate in simulated body fluid.[143] In one study, Bowen *et al.*[144] presented an outstanding corrosion behavior and biocompatibility of Zn vascular stents in rat aorta. Current research proposes that Zn alloys could potentially overcome the challenges of Mg alloys used as absorbable implants. Current research work also includes advances in other biomedical applications such as 3D porous Zn scaffolds.[145]

Through previous research it became clear that each biomaterial brings with it certain advantages and disadvantages, and to create specialized scaffolds and other tissue engineering constructs we must be able to utilize multiple materials in combination, so that their varied advantages can be exploited. In some sense, this requires that the engineering process begins with a clinical problem that dictates the design and needs of the bioresorbable material properties. Early tissue engineering began with a polymer or a construct and looked to find an application. Therefore, as the field has advanced, our approach to design and fabrication should also evolve.

Advances in stem cells and their application in tissue engineering

Although primary cells can be used for tissue engineering,[146] the use of stem cells offers the advantage of access to cells that can be directed to differentiate to the desired cell type.[147] The use of autologous cells, in particular, can help to avoid the problems associated with allo-transplantation. Therefore, stem cells represent a very important and almost inexhaustible source for tissue engineering[148,149] and regenerative medicine.[150,151] Stem cells can also be used for engineering tissues either with or without a biomaterial as a matrix[152] (Fig. 3). In addition to engineering tissues for regenerative purposes, stem cells were recently used for the engineering of cancer spheroids to develop models for cancer studies.[153]

Advances in stem cell sources. Stem cells are divided according to their differentiation potency into various lineages as totipotent, pluripotent, and multipotent. Totipotent stem cells can give rise to the three primary germ cell layers of the embryo and also give rise to extra-embryonic tissues.[154] Pluripotent stem cells can give rise to all tissues in the body, except the placenta and umbilical cord. Embryonic stem cells (ESCs) represent an important type of pluripotent stem cells that were explored for cell therapy and tissue regeneration.[155,156] Because of the associated ethical issues and regulatory restrictions, research continued to explore other possibilities.[157]

In 2006, scientists succeeded in developing pluripotent stem cells from adult somatic cells,[19] that is, iPSCs, by using clustered regularly interspaced short palindromic repeats (CRISPR) technology, which can be used for either the activation (CRISPRa) or interference (CRISPRi) with the expression of certain genes.[158] The iPSCs are currently being intensively studied because of their pluripotency but without having many of the issues associated with ESCs.[159] Today, they represent an advancing frontier in tissue engineering, because of their potential to differentiate into many cell types.[158] For example, iPSCs that were generated from human anterior cruciate ligament were used in the repair of ligaments and tendons. Further, iPSCs will be an invaluable tool for the development of personalized therapeutics.[160,161]

Compared with pluripotent cells, multipotent stem cells such as MSCs can produce only certain cell types.[162] MSCs are derived from mesodermal embryonic tissues, have high regenerative ability, and are precursors of different mesenchymal tissues such as bone and cartilage.[163] MSCs can be isolated from different tissues such as bone marrow,[164] adipose tissue,[165–167] amniotic membrane,[168] umbilical cord,[168–170] placenta,[171] dental pulp,[172] and other sources that are being continuously explored.[173]

Recently, MSCs that were isolated from the synovial fluid and synovial membrane were investigated for cartilage tissue engineering.[174] Among these, adipose tissue represents an attractive source[175] because of its abundance, easiness of accessibility, and the possibility of retrieval of stem cells that were proved to differentiate to different lineages such as fat, bone, and cartilage.[176] Generally, MSCs have been the most widely investigated stem cells[177,178] for various tissue engineering applications.[179] Their versatile behavior *in vivo* and *in vitro* made stem cells favorable for research and clinical applications.[180,181] Stem cells can also be used for immunomodulation,[182,183] which can be explored for application in tissue engineering and regenerative therapy.[184]

Recent advances in stem cell-derived extracellular vesicles. In addition to iPSCs, advancing frontiers in stem cell technology and its application in tissue engineering include stem cell-derived EVs. EVs are produced by cells in the form of exosomes, microvesicles, or apoptotic bodies; they

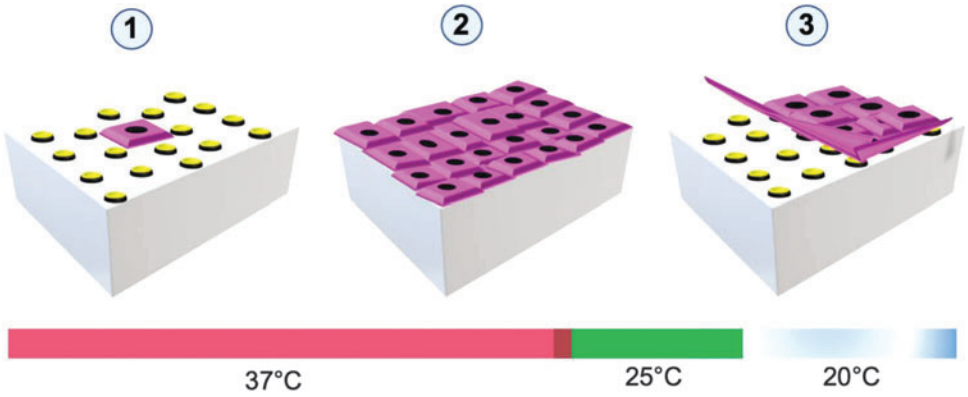

FIG. 3. Engineering of cell sheets composed of cells only using a modified poly(N-isopropylacrylamide) (PNIPAAm) surface.[430] Color images are available online.

carry peptides, lipids, or nucleotides such as RNA and DNA, and they have been increasingly recognized as an important means of molecular communication between cells and organs.[185] In particular, EVs secreted by MSCs have been investigated for tissue regeneration since they can produce important effects without the need to use cells. They have been investigated for skin, bone, cartilage, and neuronal regeneration.[178,186]

To increase the efficacy of EVs, MSCs were preconditioned by using hypoxia to produce primed MSCs.[187] This paves a new way of devising regenerative strategies based on the use of stem cell-derived EVs, which will help to eliminate many of the problems associated with the use of cell-based products. It is expected that research in this area will expand and extend to clinical translation in the future. It also underscores the importance of closing the knowledge and understanding gap that we still have in relation to the stem cell microenvironment.

Advances in stem cell differentiation. The most important challenge in the development of stem cell-based treatments in tissue engineering applications is the identification of biophysically and biochemically different tissue-specific environments.[188] In this process, besides defining differentiation and growth factors that mimic the stem cell environment, it has been reported that determining the physical properties and mechanical forces of stem cell matrix such as morphology and stiffness are also important.[189] Studying the effects of ECM biological, physical, and chemical effects on stem cells will help to develop methods that can influence cell differentiation.[190] It was also found that making the surface architecture and the stiffness properties of the biomaterials similar to those of certain native tissues favors the differentiation of the stem cells to cells specific to these tissues.[191]

Stem cell fate control is a crucial issue for stem cell research and applications. In a recent study, magnetic nanoparticles were used to guide stem cell differentiation,[192] with the help of an externally applied magnetic field that was used to pull iron oxide particle-laden ESCs together and form spheroids. Then, opposing magnetic fields were used to stretch them and lead to cardiac lineage differentiation. When both physical and chemical factors were combined and applied to ESC, significantly higher myogenic differentiation was observed.[193,194]

Differentiation of stem cells into desired cell type is possible by identifying factors such as matrix microenvironment and epigenetic mechanisms[195] that regulate the fate of stem cells.[190,194,196] For instance, an injectable hydrogel was developed by using HA, horseradish peroxidase, galactose oxidase, and tyramine; it was used as a crosslinker. Experiments in mice demonstrated the biocompatibility of the material, which makes it a good candidate for use in biomedical applications such as tissue engineering applications.[197]

In a recent study, the porosity of hydrogel biomaterial was shown to influence MSCs and their response to insulin-like growth factor-1 (IGF-1).[198] Unlike nanoporous alginate hydrogel, microporous ones could sensitize MSCs to the growth factor. Adding cell–cell adhesion mediating molecule (N-cadherin) mimicking peptide to nanoporous alginate added the effect that macroporous had in eliciting MSCs paracrine activity in response to IGF-1. This demonstrated the role of physical properties of the biomaterials further, and also the possibility to influence this by using chemical ways. Combined, these methods will help us to control the behavior of stem cells further in the future and tailoring it toward desired activity and fate, for regenerating desired tissues.

Advances in cell maturation strategies. There are several technologies that have been developed to increase cell maturation by using electrical and physical cues. For influencing cell maturation, physical cues such as surface patterning[199] or mechanical stretching[200] have been investigated. For example, physical conditioning of cardiomyocytes (both primary myocytes and human pluripotent stem cell-derived cardiomyocytes) embedded in a collagen hydrogel was achieved by using an automated stretch device.[200] More recently, the effect of electrical stimulation on cell maturation and the differentiation of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) was investigated.[201] It was found that the application of electrical stimulation during cell differentiation makes hiPSC-CMs behave similar to cardiac cells. It was demonstrated that hiPSC-CMs derived by using electromechanical stimulation can be used to engineer heart tissue.[202] In fact, the maturation of early stage cardiomyocytes was achieved by using electrical stimulation for two weeks in the presence of various growth factors. This area represents an advancing frontier in tissue engineering, and it is worth investigating further to also look at the outcome of *in vivo* applications of cells matured using electrical stimulation.

In another recent study, electrical stimulation of neonatal rat cardiomyocyte-embedded in gelatin hydrogel led to their maturation.[203] It was also shown that the organization of the cells within the gelatin hydrogels was improved by employing this strategy. For skeletal muscle tissue engineering, a gelatin-carboxymethyl cellulose biomaterial was combined with carbon nanotubes to increase electrical properties of the biomaterial.[204] Electric pulse stimulation was applied and led to enhanced myogenic differentiation and maturation of C2C12 myoblasts to form a skeletal muscle tissue.

Advances in culture systems and their application in tissue engineering

Cell culture is an important and integral part of *ex vivo* tissue engineering. Because of the rarity of certain cell types in the human body and the potential donor site morbidity associated with the retrieval of cells in large numbers,[22,205] cells are propagated outside the body in an environment that can provide nutrients and possible stimulation of cells to proliferate, differentiate, and function.[22] This is carried out using static or dynamic culture methods. In recent years, there have been advances in cell culture methods such as 3D, 4D, and microfluidic OoC culture systems. Significant progress has been made in these areas, and therefore, they will be highlighted in this section.

Advances in cell culture microenvironment. The most common drawback of any of cell culture techniques is the need to use media, which may contain animal serum. There is evidence that fetal serum could be the source of endotoxins, mycoplasma, or viral contaminants.[206] Also, the serum itself has ECM components that may alter the cell

expression of proteins. Human autologous serum can alternatively be used, and it has been shown to be equivalent to fetal animal sera. However, it is often difficult to isolate human autologous serum in sufficient quantities, especially for use in prolonged and large-scale applications.[207] Serum-free media tend to substitute individual key components found in serum-containing media, which can be a safer approach.[208]

Among the key components, are the growth factors specific for the stem cell type and tissue culture application. For instance, Hasegawa and colleagues have created a medium for stem cell culture containing a replacement of wnt with a GSK3β and NFAT inhibitor.[209] Despite significant progress made with serum-free media, the use of new approaches for the elimination of protein from the media will make this technology more cost-effective and possible to scale up.

Other parameters of cell culture such as temperature can also be used to influence cells, for example, improving adipogenic differentiation.[210] Another area of interest is the co-cultivation induction, where the concomitant culture of stem cells and committed cells is carried out. This technique has been shown to upregulate the properties of the stem cells and to induce a ''physiologic'' differentiation process without the need for the use of morphogens and other differentiation induction media. In a recent study, cardiomyocytes were cultured together with iPSCs, and it was found that older cardiomyocytes serve as an adequate inducer for stem cell differentiation, recapitulating the environment necessary for cardiac cell differentiation.[211]

Imprinted micropatterns on the surface of plates allow cell-to-cell adhesion and determine the formation and characteristics of the culture. This approach has seen several potential applications. For example, the use of micropatterned surfaces allowed homogenous stem cell differentiation to chondrocytes.[212] In another study, a 3D micropatterned plate was used to culture hepatic endoderm iPSCs.[213] The cells quickly reaggregated and formed hundreds of round-shaped spheroids while they efficiently differentiated into hepatocyte-like cells expressing hepatic gene makers. In addition, growth factors can be printed in micropatterned surfaces. For example, a micropattern-immobilized nerve growth factor nanolayer was found to induce neurite growth and regulate neurite formation.[214]

Advances in 3D culture. Conventional two-dimensional (2D) systems are classically used for stem cell culture. Such culture uses a feeder layer of cells complemented with tissue culture media supplemented with growth factors or cytokines containing cues that support cells and drive them to proliferate or differentiate.[215] Two-dimensional cultures have several limitations, including: (1) the deformation of the cells during culture (flattening and elongation), (2) poor differentiation and cell junction formation, (3) unnatural high proliferation rates, and (4) significant differences in gene expression and phenotypes.[216] Recent advances in 2D cultures have tried to overcome some of these drawbacks. Adaptations of the tissue culture biomaterial properties have been shown to modify cell fate.

On the other hand, 3D culture systems are better at recapitulating *in vivo* conditions. Several studies showed the effect of 3D culture systems on improving cell morphology, proliferation, differentiation, and response to stimuli.[217] Three-dimensional culture could be divided into either anchorage-dependent (scaffold-based) or anchorage-independent ones using specialized 3D platforms.[218] The former can benefit from recent advances in processing techniques mentioned earlier, such as 3D bioprinting[20,219,220] and electrospinning,[221,222] to create complex structures.[223] Such cell culture models should mimic cells' natural environment, providing interactions between the cells and the microenvironment, nutrients, O_2, and waste product removal.

Despite several advantages of 3D over 2D culture, 3D culture still have some drawbacks such as uneven distribution of nutrients, growth factors, and O_2, which often results in making cells residing far away from the surface of the matrix inactive.[224] In addition, many tissue-engineered constructs are looking for regenerative models of culture as opposed to mature quiescent ECM–cell relationships. Increased costs, differences in experiment replication, and data interpretation are additional drawbacks of this type of culture[225] that remain to be addressed in future development activities.

Scaffold-based anchorage-dependent culture techniques utilize a scaffold of variable architecture ranging from a simple extracellular-like matrix to complex multilayer structures. Scaffold selection is largely dependent on the target tissue to be engineered, advantaging physical factors providing structural stability and the cellular composition of the target tissue. Three-dimensional bioprinting has revolutionized the construction of such complex structures. However, because the development of functional vasculature in transplantable devices has not been achieved, successful *in vivo* applications and clinical translation are largely affected.

Special 3D anchorage-independent techniques include the use of a low attachment vessel,[226] magnetic levitation,[227] or hand-drop technique,[228] including the use of magnetic forces.[192] The low attachment plate technique uses a culture vessel with an ultra-low attachment coating. Anchorage-independent techniques force cells to aggregate, form spheres, and subsequently create their own ECM. The most common form of these techniques is the spheroid culture, which is used in the engineering of cartilage.[229]

Magnetic levitation utilizes a magnetic force to levitate cultured cells mixed with magnetic nanoparticles. This technique is shown to have reproducible results and to reduce necrosis in the spheroid core. Stem cells cultured in these conditions maintain their properties and remain quiescent for subsequent clinical use.[230] One area of interest in anchorage-independent culture is the development of organoids. Organoid formation involves the utilization of a tissue culture technique that allows self-organizing and self-renewing of 3D cultures. Organoid cultures have been described for several organs, including the kidney, eye, brain, gut, and lungs.[231]

More recently, Tseng *et al.* demonstrated the capacity of assembling adipospheres from multiple cell types, including adipose tissue-derived stem cells, endothelial cells, and leukocytes, that recreate tissue organization.[232] This technique enabled the formation of vessel-like endothelial structures with lumens and differentiation of unilocular adipocytes. The hand-drop technique utilizes the self-aggregation properties of cells when no attachment wall is found. The cells aggregate to form spheroids, and the

control of the volume of the cell suspension enables the control of the spheroid size. The outcome of this type of cell culture is better as compared with that of static cultures.

Recently, investigators explored the conversion of adipocytes to cardiomyocytes for application in cardiovascular tissue engineering.[233] In applications for retinal degeneration, the hand-drop technique was utilized to convert adipocytes to retinal precursors and showed improved differentiation yield, with these precursor-like cells responding to glutamate neurotransmitters.[234] This technique has been used in many other preclinical studies, including cartilage repair, bone healing, and cardiac tissue regeneration.[235,236]

Four-dimensional culture platforms utilize a complex 3D-bioprinted or imprinted structure with a predetermined time-dependent dynamic morphological change. This is achieved by the control and manipulation of the behaviors of stem cells responding to cues that aim at replicating the topographical and mechano-biological environment of the target tissue. These systems could find applications in studying tissue biology and pathophysiology, preclinical testing, and tissue biofabrication.[237–239] As far as tissue engineering is concerned, the use of 4D culture systems is in its infancy. However, some promising studies were published. For example, Miao *et al.* have utilized this technique to create neural tissue with a time-dependent self-morphing regulation of neural stem cells that enhances neural differentiation of cells along with significant axonal alignment.[238] Further studies will be of interest in this area of research, as it is structurally most replicative of the regenerative process of healing.

Advances in microfluidic culture systems. Microfluidic systems are designed for cultures under perfusion, allowing a continuous supply of O_2 and nutrients (Fig. 4). This enables the long-term maintenance of constructs at physiologically relevant nutrient supply rates. The use of a microfluidic-based approach in cartilage regeneration allowed enhanced conjugation of the key growth factor, transforming growth factor-beta 3 and its sustained release.[240] In another study, biomimetic neural tissue fibers having hierarchically ordered nerve fibers were created by using a microfluidic system, which contained a coaxial triple-channel chip and a stretching loading device.[241] Authors reported good performance of the resulting nerve fibers.

The microfluidic system was also used for the production of a gene delivery system composed of nanocomplexes of plasmids encoding for BMP-2 and chitosan.[242] The results demonstrated the potential of using this system for *in situ* bone tissue regeneration. Another application of microfluidic systems is the development of OoC platforms, which aim at reproducing the function of organs or tissues.[243,244] Applications of OoC are currently limited to the development of basic tissue functions and of certain disease models,[245–247] and it points to new avenues for the study of novel tissue engineering strategies.

Advances in processing techniques and their application in tissue engineering

There are several techniques that have been used to develop scaffolds, matrices, or tissue constructs, such as salt leaching, molding, spinning, freeze-drying,[248] solvent casting and particulate leaching,[249] electrospinning,[221,222] selective laser sintering and 3D printing,[250] and 4D bioprinting.[69] However, we will highlight in this section only the recent developments in the most advancing frontiers of fabrication techniques[251] (Fig. 5).

Advances in 3D printing. Tissue engineering has adopted the 3D printing technique[252] for the fabrication of scaffolds and later to create cell-laden multi-cellular[253] and complex[254] tissue constructs, and the technique was termed ''3D bioprinting.'' Three-dimensional bioprinting is gaining increasing popularity, with more companies innovating to produce 3D bioprinters. The method employs most commonly extrusion, inkjet, laser, or stereolithography, with each of these methods having its own advantages and limitations.[25,255] Therefore, new approaches include combining 3D bioprinting with conventional manufacturing methods. Different combinations of various fabrication techniques can be used, for example, combining electrospinning with 3D bioprinting[256] or 3D printing with 3D bioprinting[257,258] to produce advanced scaffolds.

Three-dimensional bioprinting has several advantages over other tissue engineering techniques.[259] It allows the creation of well-defined, customized structures that mimic native tissues. These tissues have functional cellular components; therefore, cellular migration from the host is not essential. Further, cellular interaction and key signaling molecules can be incorporated into the design of the printed constructs. The overall cost of 3D bioprinting is lower when compared with currently used graft materials with no donor site morbidity.[260] Host tissue regeneration that occurs in pace with the degradation of the implanted 3D-bioprinted construct can hopefully be achieved in future by controlling material properties of the construct bioink. The field is still in its infancy, and therefore we expect to still see shortcomings of current 3D bioprinted constructs.

One problem is the choice of the material that can address both the biology and the anatomy of the tissue to be treated. A lot of our current understanding of these issues is based on our experiments on animals, which may make the design of structures with appropriate properties that fit human tissue structure and function challenging. Further, several aspects of the 3D bioprinting process, such as the isolation of cells, culture conditions, and identification of the signals and growth factors, need to be considered. Our current inability to incorporate vasculature and the potential degradation of the structures limits the success of implanted constructs, and it requires the attention and development of innovative solutions.[259,261,262] There are two areas of 3D bioprinting that deserve special discussion, the 4D[263] and the *in situ*[264,265] bioprinting.

Despite the numerous challenges, in the past decade, an increasing number of studies were published regarding the creation of biomimetic constructs for future clinical applications. The most important applications so far include skin,[266] musculoskeletal[267] cardiovascular,[268] neural,[269] and other tissues.[270] The studies present developments made in bioinks that are composed of different biomaterials, cell types, and additives such as growth factors, drugs, or osteoconductive elements, which were tested either *in vitro* or *in vivo*.[271] In addition, 3D-bioprinted products often have

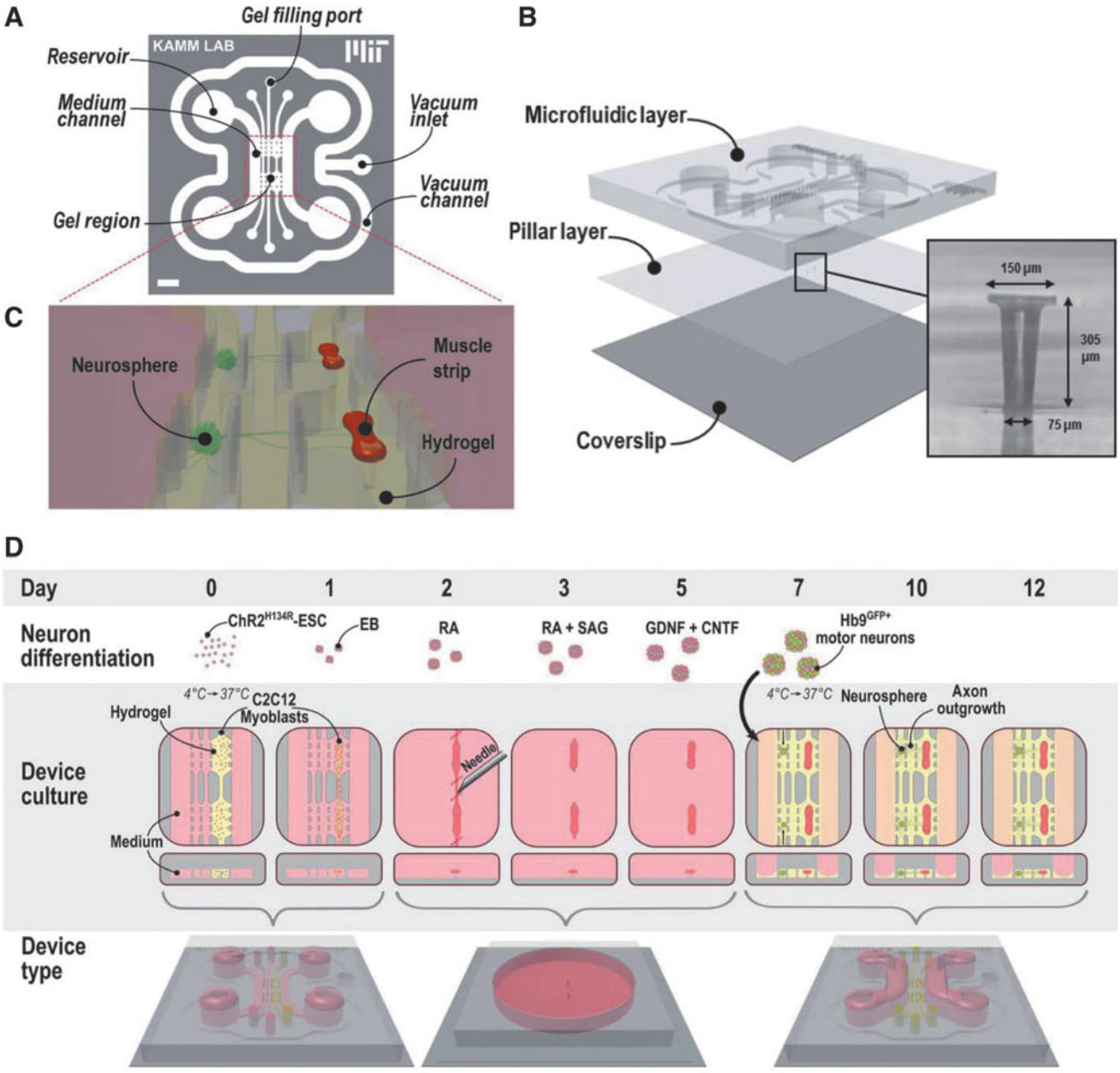

FIG. 4. **(A)** Schematic illustration of the design of microfluidic chip that has three parallel gel regions, six gel filling ports, and two medium channels connected to four medium reservoirs. The device also contains a surrounding vacuum channel. Scale bar, 2 mm. **(B)** The device comprises a microfluidic layer on a polydimethylsiloxane membrane featuring two sets of two capped pillars (*inset*). The membrane is itself bonded to a coverslip. (**C**) Schematic illustration showing the final coculture arrangement: embedded in a hydrogel, muscle bundles that are wrapped around and exerted force to the pillars. They are innervated by neurospheres, which are placed in the opposite gel chamber separated by a 1-mm-wide gel region. **(D)** Schematic illustration showing the differentiation process of the ESCs into motor neurons (MNs). *Row 2:* Schematic illustration displaying the *top* and *front* views of the tissue in the microfluidic device. *Row 3:* Three-dimensional illustrations showing the version of the device used at the corresponding days. ChR2, and Channelrhodopsin-2; CNTF, ciliary neurotrophic factor; EBs, embryoid bodies; ESCs, embryonic stem cells; GDNF, glia-derived neurotrophic factors; HS, horse serum; RA, retinoic acid; SAG, smoothened agonist. Reproduced from Uzel *et al.*,[431] which is an open-access article distributed under the terms of the Creative Commons Attribution license. Color images are available online.

significant contributions from established FDA-approved component parts, including cells, signals, and scaffolds, and thus the fabrication technique can often produce and amalgamate products that incorporate existing technology with a novel effect. Available evidence is encouraging; however, we are far from achieving the full complex organ engineering that tissue engineering has promised.

Advances in 4D, *in situ*, and spheroid printing. Four-dimensional bioprinting uses smart stimuli-responsive

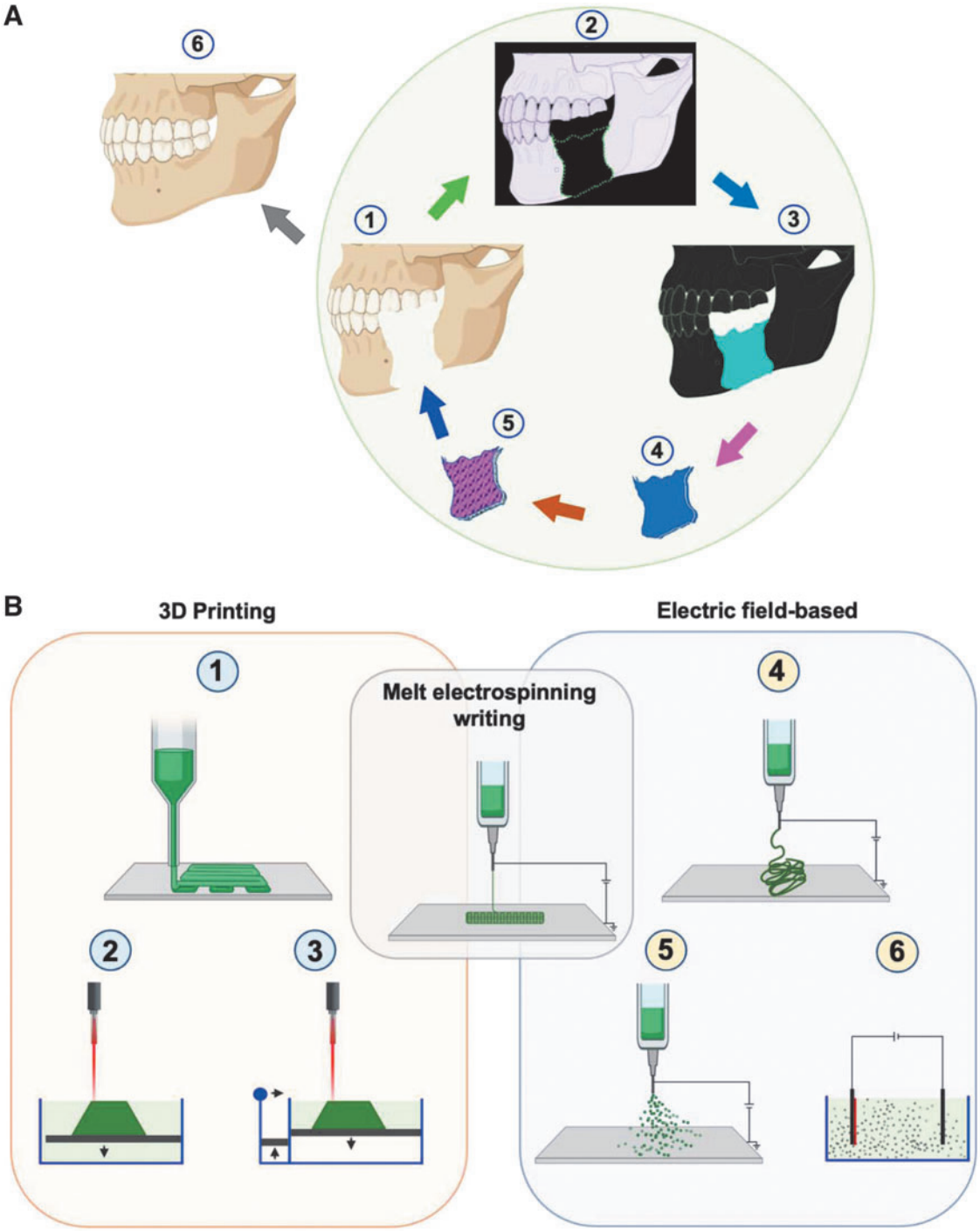

FIG. 5. Advances in fabrication techniques for tissue engineering. **(A)** Three-dimensional printing and electric-field-assisted techniques can be very useful for 3D construction of tissue defects (1) based on data-derived from imaging such as computed tomography (2), which is transferred to a design template for 3D bioprinting (4), to produce living constructs (5) that are transplanted to bridge defects and heal damaged tissue (6). **(B)** In 3D printing (1–3), layer-by-layer deposition of polymeric gel results in the formation of predesigned 3D constructs. In the electric-field-assisted technique (5–6), an electric field is used to control for directing and depositing polymeric fibers. In melt-electrospinning writing (4), both 3D printing and electric-field-assisted methods are combined. Created with Biorender.com. Color images are available online.

materials,[18,90] which are programmed to change their properties and bioactivity over time in response to local or external stimuli.[272] An example of the application of 4D bioprinting can be in guided nerve generation, using materials such as graphene hybrid in a 4D construct, which can provide physical guidance, chemical cues, dynamic self-entubulation, and seamless integration.[273]

In addition, *in situ* bioprinting[265] is of great interest. It employs special hardware and it completely eliminates the need for *ex vivo* manipulation of the grafts.[264] This approach

can find applications in cases where the exact dimensions of the tissue are not known preoperatively, such as, for example, after debridement of tissues following trauma, infection, or cancer resection. Several hand-held[265] or scanner-controlled 3D printing devices have been developed and the available studies show that such structures retain the high resolution of the 3D bioprinting technique and can match the exact needs of tissues to be constructed.[274]

Spheroids were recently used as building blocks of constructs that were produced by 3D bioprinting. In this method, spheroids are sucked in, and then released in a controlled fashion. This approach tries to lend developmental biology approaches. This technique will allow the development of 3D constructs using biomaterial-free bioinks and precise deposition of spheroids into the resulting construct.[275] It was also possible to use spheroid-based 3D bioprinting in combination with the freeform 3D bioprinting method that enabled patterning of the printed spheroids into the desired shape of constructs (Fig. 6A).[276]

Advances in other processing techniques. Electrospinning is a versatile technique relying on the use of an electric field to produce thin micro- and nanofibers[277] that can also be combined with drugs.[222,278,279] This technique has also been used for some time to produce nanofiber-based scaffolds that mimic ECM in several aspects and explored for the engineering of various tissue constructs[277,280] such as bone,[281] cartilage,[282] nerve conduits,[283] blood vessels,[284] skin,[24] and other tissues. In addition to its use for *ex vivo* tissue engineering, electrospinning was also experimented for *in situ* applications, for example, for the treatment of skin wounds[24] and for ocular drug delivery.[285]

Different materials have been utilized for electrospinning, and various modifications of the technique of the spinning process have been developed to allow for combining the benefits and properties of more than one material.[286] For example, in coaxial electrospinning, it is possible to use a material in the core and a different one in the shell that can have different degradation profiles and can be loaded with different molecules or drugs.[287] Examples of the successful use of co-axial[287] and triaxial[288] electrospinning techniques include the engineering of osteochondral tissues.[289] Among the interesting recent advances in electrospinning is cell-laden electrospinning (Fig. 6B), in which muscle cells were encapsulated in fibrin.[290] It has been found that incorporating these cells and modifying electrospinning conditions significantly enhanced cell viability under a 4.5 kV electric field.

Another advancing frontier involves the use of electrospinning in combination with 3D printing and bioprinting to bring in various properties, such as reinforcement, to improve the mechanical properties of the resulting 3D-bioprinted constructs.[291] In addition, combining electrospinning with 3D printing provides a microporous structure that can enhance cell proliferation and infiltration of the structure[292] (Fig. 6C). Further, more control over the process of fiber laying of electrospinning, which classically randomly falls on the collecting surface, enabled the use of the techniques in a similar way as 3D printing.[293] Once this is well controlled, it can be one form of 3D printing and bioprinting in future, used on its own.

In addition, melt-spinning has been used for tissue engineering.[294] Melt electrospinning-based printing is an emerging printing technique that can print fibers with diameter in the range of nanometers, providing a high degree of resolution, porosity, and pore interconnectivity.[295–297] For example, Brown *et al.* combined melt electrospinning with a digitally controlled collector and developed a new class of 3D printer called melt-electrospinning writing (MEW), which enabled the deposition of well-defined filaments.[298] The MEW has the advantage of avoiding problems related to solvents that are used in conventional electrospinning.[299]

Recently, and for the first time it was possible to have automated coupled melt-electrospinning and melt-electrowriting, by using a modified elongated nozzle to direct-write melt-electrospun polymeric thin fibers onto a collection surface.[300] In one interesting development, multilayers were developed by electrospinning, and layers have complementary moieties that lead to the formation of covalent bonds (such as hydrazide and aldehyde groups) between electrospun fibers when they are brought together under mechanical loading.[301] The technique can be useful in tissue engineering of advanced structures in future, which can become stronger on exposure to stress, for example, blood vessel engineering,[284] guided nerve regeneration,[283] or tendon[282] and ligament engineering.[302]

All these advances open new avenues and application territories of the techniques and provide us with more options and versatility toward mimicking the complexity and heterogeneity of the native tissues to be engineered, by combining various processing techniques. With these novel approaches, we come one step closer toward developing successful engineered constructs, *ex vivo* or *in situ*.

Translational advances in tissue engineering

Successful transfer of technology from bench to industrial production of engineered tissue products has been progressive, but slow, in part because the clinicians often do not have embedded design and architecture input to the early stage tissue engineered constructs. Although there was a bolus of products, primarily focused on engineered skin tissue, that was approved in the late 1990s and early 2000s,[303] only a few products have subsequently emerged.[303] Although a few individuals have gained expertise in translating basic tissue engineering research to commercial products, researchers in the field of tissue engineering and regenerative medicine (TERM), overall, lack experience in translational sciences. One factor that may highlight the impact and enable faster commercial translation is academic–industry partnerships among tissue engineers, clinical investigators, clinicians, and industry partners.

Advances in clinical translation

Influencing factors. Clinical translation is affected by several factors that are related to the technology, approval, and acceptance by doctors[304,305] and patients.[305] Although there have been advances in the field, clinical translation has been limited, not because of science or technology, but largely due to factors including scalability, cost, regulatory issues, and uptake.[305] There are engineered tissue products that are in clinical use or are moving toward clinical translation such as skin, cartilage, bone, vascular grafts, cardiac tissues, and bladder.[305,306] More complex structures such as heart, lung, liver, and kidney have been recreated

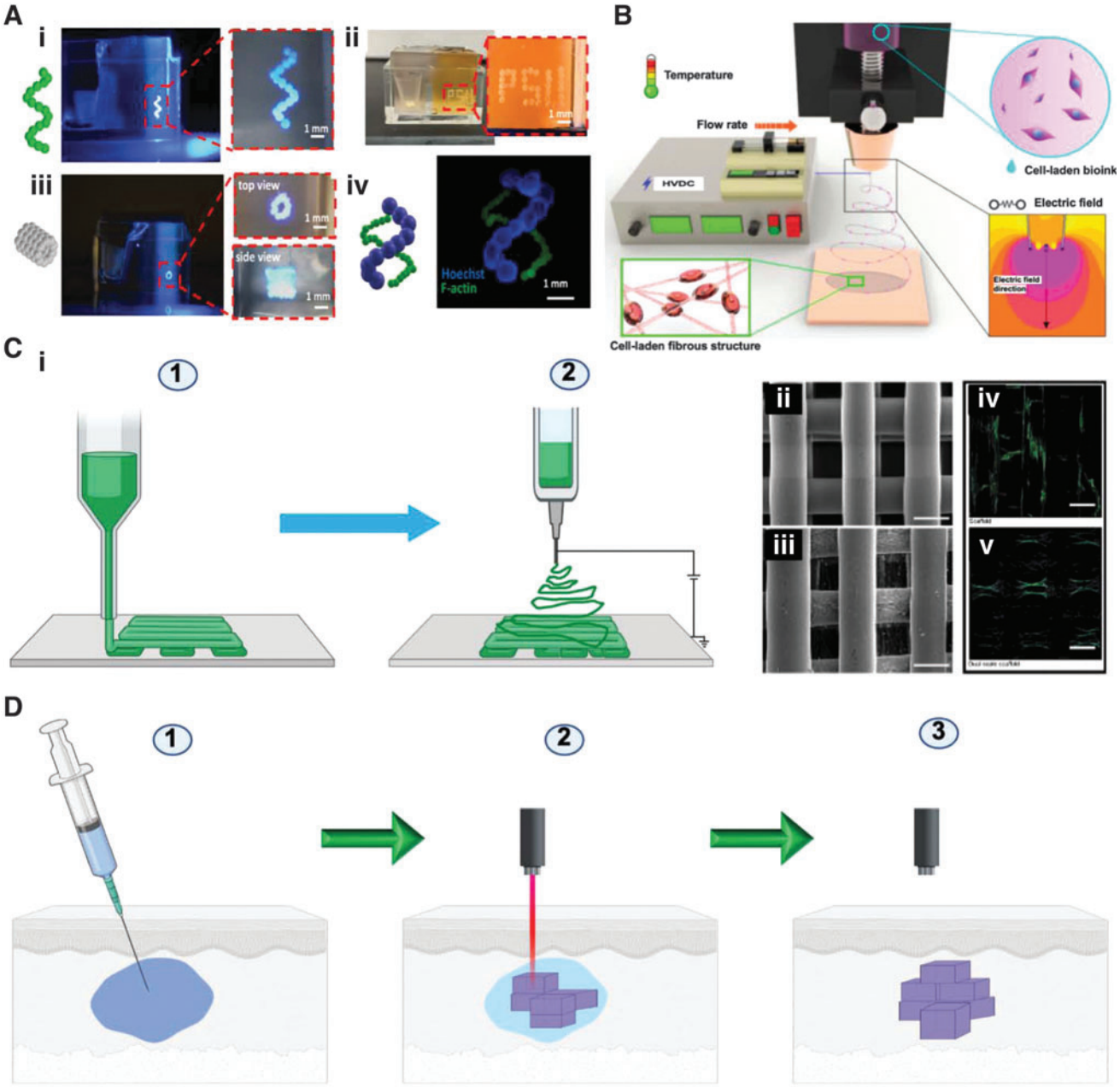

FIG. 6. Various fabrication techniques for tissue engineering constructs. **(A)** Use of mesenchymal stem cell spheroids to 3D bioprint a helix shape **(i)**, Penn State University initials **(ii)**, 5-layer tubular structures **(iii)**, and double helix-shape constructs **(iv)**. One hundred fifty micrometers (F-actin) and 450 μm (Hoechst) in radius in 1.2% Carbopol yield-stress gel. Magnified zone is indicated by *dashed red line*. Reproduced from Ayan *et al.*,[276] which is licensed under a Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). **(B)** Cell-electrospinning process with the processing parameters. Reproduced from Hong *et al.*,[432] which is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). **(C)** Combined electrospinning and 3D printing. **(i)** Schematic illustration of the composite scaffold with electrospinning and 3D printing. Step (1) polymer polycaprolactone was used to 3D print the constructs and electrospinning to produce nanofibers, resulting in the formation of dual-scale scaffolds. Created with Biorender.com. Scanning electron microscope images of the scaffolds that were produced by using 3D printing **(ii)** and dual-scale scaffolds that were produced by using electrospinning and 3D printing **(iii)** (scale bar = 300 μm). Confocal laser scanning microscopy images of the scaffolds that were produced by using 3D printing **(iv)** and dual-scale scaffolds that were produced by using electrospinning and 3D printing **(v)** (scale bar = 300 μm). Reproduced from Vyas *et al.*,[433] which is an open access article distributed under the terms of the Creative Commons CC BY license **(ii–v)**. **(D)** Intravital 3D bioprinting, which is carried out by injecting a solution of the polymer into a certain tissue site to be treated in a living body. In this example, a two-photon excitation is used for the construction of a 3D object by gelating the polymer solution, and object intravital imaging is used for identification. Created with Biorender.com. Color images are available online.

and are still in preclinical animal studies. Clinical translation of complex structures and whole organs face a completely different set of challenges.[305]

Acellular products. Successful clinical applications of engineered tissue products have flourished in the past couple of decades,[304] and they were approved by the United States FDA, for example, Integra for skin and INFUSE for bone regeneration.[76,307,308] The former is composed of collagen, glycosaminoglycans, and polysiloxane, and it was approved by FDA in 2002 for use in the treatment of burns[307] and then for the treatment of diabetic foot ulcers in 2016.[309] INFUSE is a BMP2-containing collagen sponge that was also approved by the FDA in 2002 for use in lumbar fusion.[310] In addition to these biomaterials, the FDA has also approved native-tissue derived ECM for application in the treatment of complex wounds[311] and nerve regeneration.[312]

Currently, there are several clinical trials exploring the use of materials in achieving *in situ* regeneration of nervous,[10,313] cardiac,[314] and musculoskeletal[315] tissues. The Humacyte acellular vascular graft, which is made by laying smooth ECM on PGA with cultured smooth muscle cells that are subsequently removed from the graft,[316] is now in an open-label, nonrandomized, phase II clinical trial[317] for patients with life-threatening limb or torso vascular trauma. The primary outcome measures include primary graft patency along with frequency and severity of adverse outcomes.

For cartilage and osteochondral repair, TruFit and MaioRegen acellular devices have been developed. TruFit is composed of a PLGA, 10% calcium sulfate, PGA fibers, and surfactant and is used for cartilage repair.[318] Unfortunately, a 2-year clinical study showed no significant improvement in knee scores.[319] Compared with autologous osteochondral transplantation, knee scores were worse in the group that received TruFit.[320] Other clinical studies with TruFit showed improvement in symptoms and radiologic outcomes but lack direct comparison with conventional surgical cartilage repair techniques.[321,322] Although there was excitement with MaioRegen (a three-layer scaffold composed of collagen I and hydroxyapatite) for the treatment of osteochondral lesions when medium-term results showed significant improvement of knee scores,[323] the 5-year results showed failure of repair.[324]

Cellular products. Several notable cellular products have been approved by the FDA. In 1997, TransCyte, which is composed of fibroblasts and nylon mesh,[325] was approved for the treatment of burns.[326] In 1998, Apligraf, which is composed of fibroblasts, keratinocytes, and collagen matrix,[327] and another product for the treatment of skin venous ulcers[328] were approved. For the treatment of nonhealing diabetic foot ulcers,[329] in 2001, the FDA approved Dermagraft, a construct made from a synthetic polymer, PGA with fibroblasts. OrCel is a collagen sponge-based scaffold seeded with keratinocytes and fibroblasts, which was used in the treatment of burns.[330] Laserskin and Hyalograft are hyaluronan-based matrices seeded with fibroblasts and keratinocytes, which were used in the treatment of diabetic foot ulcers[331] and chronic wounds of the lower extremity.[332] Matriderm® is an acellular matrix composed of coupled collagen and elastin,[333] which can be seeded with fibroblasts and keratinocytes and used for the treatment of full-thickness skin loss.[333]

Most of the identified cellularized products are for skin regeneration.[334] Although these products are very valuable, it is also important to develop and test cellular products for the treatment of tissues with much less intrinsic regenerative capacity such as the heart.[335] Even though many of these products have not yet been approved or are not widely utilized in the field, it is important to mention that an iPSC-derived cell sheet based product (TERUMO BCT) for the treatment of heart failure was developed, but it has not yet been approved by the FDA.[336] This cellular therapy may have a major impact on the treatment of heart failure, which constitutes one of the major causes of mortality in the world.[337]

Autologous cellular products. The FDA-approved autologous cell-based products include matrix-assisted autologous chondrocyte implantation (MACI), which is composed of matrix and chondrocytes and used for the treatment of full-thickness cartilage defects, and they were approved in 2001.[76,307] Since 2001, several MACI products have been commercialized,[318] including BioSeed®-C[338] and Hyalograft® C.[339] Fibrin glue is used in BioSeed as a cell carrier, and polyglactin 910/poly-p-dioxanone fleece is used as a scaffold. Significant benefits were demonstrated in clinical studies.[338,340] Hyalograft C, which uses HA as a matrix, has been investigated in 28 trials. Relative to microfracture therapy, Hyalograft C showed improved patient scores.[318] However, Hyalograft C did not undergo a phase III clinical trial and was withdrawn from the market[318] due to problems with manufacturing practices and comparative studies.[341]

Other MACI products available outside of the United States include CaReS®, which uses collagen type I hydrogel seeded with autologous chondrocytes. The results of a prospective multicenter clinical trial in 116 patients (49 women and 67 men; mean age, 32.5 ± 8.9 years) demonstrated significant improvement in the knee scores at 12–60 months after treatment with CaReS; there was a significant reduction in global pain scores and an improvement in the health-related quality of life (SF-36) scores.[342]

In 2017, phase III clinical trial of another MACI product, NeoCart®, was completed. NeoCart relies on the use of type I collagen scaffold.[343] At the 5-year follow-up, although magnetic resonance imaging showed significant improvements from earlier follow-up time points, subchondral bone lesions were seen in 80% of patients. NOVOCART® 3D, a third-generation ACI, employs type I/III collagen biphasic scaffolds[344]; clinical trials have been performed.[345] and phase III clinical trials are in progress.[346–349] One report showed significantly improved knee scores from the preoperative state.[350] NOVOCART 3D may serve as the treatment of choice for children and adolescents.[351]

Several other engineered tissues reached the clinical testing stage but faced several challenges. In 2010, tissue-engineered trachea that employed a decellularized allograft[352] along with autologous MSCs was implanted into a 12-year-old boy.[353] Though successful, full restoration of the biomechanical properties of the trachea took a long time (18 months). Bladder tissue reconstruction was another success.

In 2005, Atala *et al.* reported implantation of engineered bladder in patients needing cystoplasty for end-stage bladder disease.[354] Collagen or PGA-collagen scaffolds were seeded with the patient's own bladder cells. Unfortunately, a phase II study consisting of children with neurogenic bladder resulting from spina bifida showed no functional improvement in bladder compliance or capacity and the prevalence of serious adverse events prevented further development.[355] The performance of the engineered bladder is still far from replacing that conventional use of gastrointestinal tissue for augmentation cystoplasty.[306] As a result, a combination of techniques with neurovascular muscle transfer and tissue engineering was proposed.[356] The common thread is that neovascularization had to occur *in vivo* on the construct, because the vascular component of tissue engineering is not optimized.

However, some clinical success was seen in large vessel tissue engineering related to congenital heart disease. In one patient, tissue-engineered pulmonary artery using PCL–PLA copolymer (weight ratio, 1:1) reinforced with woven PGA tubular scaffolds and seeded with autologous peripheral vein-derived cells was found to be successful, and follow-up at seven months showed no evidence of graft occlusion or aneurysms.[357]

Later, the same group showed both safety and absence of adverse events at four years in a cohort of 25 patients with congenital heart disease who had extracardiac total cavopulmonary surgeries with tissue-engineered vascular grafts serving as conduits.[358] One major challenge hindering clinical application of engineered vessel grafts is related to standardizing the engineered parameters such as scaffold structure and materials, for which 3D printing may offer solutions in terms of reproducibility.[306] Another interesting product is Holoclar®, which employs autologous stem cells that are cultured on a fibrin matrix and transplanted to treat damaged outer layer of the cornea; Holoclar was approved by the European Commission in 2015.[306,359,360]

Biomaterial-free cellular products. Biomaterial-free cellular grafts have also been tested in patients. In one case report, iPSC-derived retinal pigment epithelial (RPE) sheets were used in the treatment of age-related macular degeneration (AMD) of a 70-year-old woman.[361] Two phase I/II clinical studies have been conducted to assess the safety and tolerability of ESC-derived RPEs in the treatment of Stargardt's disease ($n=9$ patients) and patients with AMD ($n=9$) and provided first evidence of medium- to long-term safety, graft survival, and possible biological activity of these cells. No evidence of adverse proliferation or rejection was seen in these patients who were followed up for a median duration of 22 months.[362]

Three-dimensional bioprinted products. There are opportunities for translating 3D bioprinting technologies into the clinic. However, there remain significant challenges and limitations that need to be addressed. Some challenges include the production of tissue constructs that have a clinically relevant size, function, and vascularization.[363] Tissues such as cartilage, bone, and skin are more feasible than complex tissues such as the myocardium. The physiologic components and functional requirements necessary for mimicking native tissues are significantly more challenging to engineer. Successful engineering of complex tissues requires time, development of multicomponent bioinks,[271] and improvements in materials, cell sourcing, and fabrication techniques.[363] Scalability and costs are added barriers. To the best of our knowledge, there are no 3D-bioprinted products currently undergoing clinical testing. To advance translational aspects of 3D printing and bioprinting, we have organized sessions in the Annual meeting of the Society for Biomaterials, 2019 and World Biomaterials Congress 2020.

Industrial translational advances. Product concepts in the TERM field are challenging. Despite promising clinical outcomes,[364] many therapeutics have limited insight into the target mechanism of action. This, in turn, leads to a poor understanding of the critical quality attributes that function, in part, to gauge acceptable levels of variability, either inherent to the biology or due to the process.[365] Further, a critical eye needs to be kept on the efficacy and approach to developing therapies given the cost of resources for development and translation to the clinic.[364]

Many initial product concepts emerge from basic science research, largely supported by federal grants from the National Institutes of Health (NIH) and National Science Foundation (NSF). These funding mechanisms explicitly favor innovation, which may come at the cost of advancing simpler effective approaches. In addition, for some areas, the patent landscape is crowded and complicated requiring some product concepts to needlessly contort to remain unconflicted.[366] The ultimate goal, however, is to develop a therapeutic that has a clear increase in efficacy over the standard of care,[367] but if not careful the long path of translation may induce drift away from that goal.

As these products move from concept to realization, there are a host of business-related challenges that emerge.[368] Even an efficacious approach needs to have a tenable business model to be ubiquitously and consistently available to patients. If the process to manufacture the product is not scalable, only a limited number of patients can benefit from the product. Further, if the financial models for generating the product, looking at both cost of goods and reimbursement levels, are not favorable, then eventually no corporate entity can support production. These considerations are often taken too late, leading to false starts as we try to develop therapies for aiding patients.

Limitations in supporting production infrastructure also comprise a concern for the nascent TERM industry. Although some larger corporations will develop full manufacturing and testing facilities internally, widespread translation will require contract organizations to support smaller businesses. However, there is a paucity of Contract Manufacturing Organizations (CMOs) and Contract Testing Organizations (CTOs) that are experienced in the technical aspects unique to the TERM field. In particular, the robust workforce for manufacturing and testing for this field is lacking. Fortunately, efforts by academic programs have recently emerged to address the specialized workforce required production.[369]

Another production concern is the availability of proper raw materials. For example, the lack of specialized cytokines and biomaterials that are cGMP grade will remain an issue until the demand for enough is established.[370] Even for raw materials shared with other more established industries, such as Pharma, the TERM industry does not currently require the scale of material to provide the leverage needed to

implement common supply chain strategies, in turn affecting the cost of goods. Unfortunately, only as more TERM products are translated toward the commercial scale will these resource issues be more fully addressed.

The challenges for the translation of TERM products include issues from tenable product concepts to manufacturing issues to regulatory hurdles.[371] One of the major hurdles in getting TERM products to the clinic has been the time for regulatory review and approval in the United States.[304] The FDA has developed programs to accelerate the process, including the Regenerative Medicine Advanced Therapy (RMAT) designation, which was enacted in the 21st Century Cures Act in December 2016.[372] The RMAT designation applies to those regenerative medicine therapies that target serious or life-threatening conditions and has the potential to address unmet medical needs. Although this relatively new regulatory pathway will help ease one obstacle, many practical challenges still exist in commercializing TERM products. Only as the field continues to forge forward will some of these issues be overcome. Continued discussions among the community, such as ones at the TERMIS meetings, are critical for identifying the problems and sharing the solutions.

Challenges and Future Directions

There are already more than 100,000 publications and 9000 patents in the field of tissue engineering,[371] but many bottlenecks still exist at the translational interface. At this time, the pipeline for academic–industry collaborations with active participation by clinical investigators and clinicians is underdeveloped. Additional efforts in tissue engineering need to address both the scientific challenges and translational potential to achieve synergistic success that will have an impactful benefit on patients in the clinic.

One of the most important challenges in tissue engineering has been the death of cells in the scaffolds after their implantation in the body.[365] Cells can survive on diffusion only at a distance of $\sim$100–200 μm away from the source of nutrient supply.[373] Because angiogenesis takes time,[374] various strategies have been explored to provide cells in the engineered tissue with essential nutrients and O_2 while awaiting new vessel formation.[375–377] One strategy that has been developed recently is to deliver O_2 into the engineered tissues by using O_2-generating biomaterials,[378] which have been shown to be also effective when they are used as a part of 3D-bioprinted tissue constructs.[379]

To enhance angiogenesis, accurate cell positioning in printed constructs[380] and angiogenic growth factors[381] can play important roles to avoid failure of engineered tissues[382] or implanted constructs.[383,384] To ensure continued blood supply to engineered constructs, strategies for vascularization,[375,385] or prevascularization of scaffolds through the use of microsurgery were investigated.[377] The need for a functional vascular network increases with the complexity and size of the target tissue or organ. These vascular networks could be used to support the grafts during the immediate postfabrication period.

Although many of the early tissue engineering experiments were proof-of-concept and demonstrated function,[386] they were mostly carried out in immune-deficient animals[387] where normal immune reactions are not functional. After implantation in immunocompetent animals, immune response to the construct is a challenging problem.[388] This response includes nonspecific inflammatory reactions to matrix materials and possible reactions to allogeneic cells.[389]

Various strategies to address these issues have been developed, such as the use of autologous cells and the use of biocompatible materials. In addition, the exploration of autologous native ECM derived materials has been pursued.[390] The use of immune reaction modulating agents such as anti-inflammatory agents[391–393] embedded or integrated with the biomaterial has been explored for optimizing reactions toward implanted biodegradable materials. Further, cellular constructs that have no foreign materials added represent an interesting approach.[394] Recent developments in the use of *in situ* tissue regeneration can be used as an alternative to *ex vivo* engineering, and it will help to avoid many of the current problems associated with the *ex vivo* engineering approach.[395]

Successful results of tissue engineering were demonstrated in early short- to medium-term animal experiments. Later, it was shown that function cannot be always sustained, such as it was seen in experiments with pancreatic endocrine[396] and liver tissue engineering.[397] Therefore, strategies to enhance the survival and function of implanted engineered tissue constructs were investigated.[398] Durability of the implanted engineered tissue constructs is an important aspect, and long-term studies are required to demonstrate this. *In vivo* imaging[399] and cell tracking[400] have recently evolved and they enable better evaluation and monitoring during the post-implantation period. Sensor technology is an emerging area that can be taken as an enabling tool to advance our capabilities in monitoring our implants further and pursue timely intervention if needed.[401,402]

Many of the engineered tissues are small in size because of limitation, partly imposed by difficulty in providing nourishment to deeper parts of the engineered tissue constructs and required vascularization for larger-sized constructs. Even with the most recent developments in the use of 3D bioprinting for engineering tissues, the production of clinically relevant sizes of constructs remains a challenge.[258] Some strategies have been developed to address this, such as the printing of supportive structures[258] or printing into a supportive sacrificial material.[403] The latter is hoped to help produce larger constructs. It remains, however, to get mechanically stable constructs that can preserve their physical characteristics and mechanical properties for a time enough to support tissues while they are in the healing stage. Most challenges in this sense are related to constructs intended for use in hard tissue such as the bone. Important strategies can be sought by combining acellular frames and scaffolds with cellular constructs, as has been suggested earlier.[20]

The use of engineered tissue products may be associated with safety issues related to cell, material, and molecule sources, during retrieval, processing, storage, transport, and application phases.[255,404] Ethical issues are especially related to the source of cells, for example, xenogeneic grafts, chimeric constructs, or ESCs.[405] Also, aspects related to the use of stem cell therapies that are risky, untested, and unproven scientifically by unregulated clinics need to be addressed.[406] Further, ethical aspects related to applications and making the therapy available when needed and for patients who need it are important, given the lack of

availability of sufficient organs and tissues needed to provide vital functions and reduce the death of patients on the waiting list.[407] Ethical aspects related to clinical trials should be properly analyzed and addressed. In clinical trials, it is sometimes difficult to design appropriate control groups because of ethical reasons, and therefore, results should be evaluated accordingly, and so also when applications are submitted for approval by regulatory bodies.

Because of financial reasons or the availability of other therapeutic alternatives, health service providers and insurance companies may not provide or approve engineered tissue products, which imposes another challenge facing wider application of engineered tissues. In addition, acceptance by doctors[408] and patients is also an important factor that will influence the future of the use of engineered tissue therapeutics. Wider clinical application will, thus, be influenced by patient education, marketing, safety, and efficacy proof. Influencing factors will also include safety, efficacy, and price as well as coverage of the products by insurance companies for defined indications, where alternatives are not available, inefficient, or more expensive. It is clear that there is a niche for engineered tissue products in certain clinical indications, for example, skin for the treatment of face lesions, younger patients, and large burn wounds.[409]

In the future, we expect to see more of *in situ* tissue engineering that can be accomplished by creating an environment *in vivo* that stimulates an individual's own resident cells to achieve regeneration. This can be achieved via various approaches that may employ bioresponsive materials to influence immune cells, progenitors, or stem cells, or utilize transcription factors and RNA-based strategies to reprogram cells.[16] Further, the use of *in situ*[264,265] and intravital[410] (Fig. 6D) 3D bioprinting will advance our capabilities further toward achieving less or minimally invasive delivery of regenerative therapeutics.[76]

Using iPSCs[411] and stem cell-derived EVs,[412] as well as customized implants using 3D printing[413,414] allows for the development of more customized and personalized treatment modalities in the future. There is a significant need for the development of autologous endocrine tissues, for example, for the treatment of diabetes,[396] and for musculoskeletal tissues that are important for craniomaxillofacial reconstruction,[397] which can be addressed by using these new tissue and cellular engineering approaches.

One advancing frontier is related to the use of electroconductive materials for tissue engineering, which can be useful in many applications such as neural tissue engineering.[415,416] Care of the patients will be advanced by integrating capabilities of sensors and actuators; communication and remote control, which will enable real-time monitoring of implanted constructs and timely intervention by providing the right treatment at the right time.[417] Diagnosis, design of treatment, installation of implants, follow-up, and optimization will benefit from advances made through the Internet of Things.[418]

Advances in microfluidic OoC systems[245] have led to the publication of several experiments on studying tissues, developing disease models, and testing drugs.[419] However, their use for advancing tissue engineering for the purpose of regenerative medicine needs still to be harnessed and there are many untapped opportunities to be explored. In addition, OoC systems will enhance our ability to perform cell culture studies in a 3D dynamic environment, which makes it possible to mimic the *in vivo* microenvironment.[243] Growth and spread of cells can be monitored in a controlled manner on microstructures created with 3D printers, and it can be possible to mimic the real-time events.[420] Further, the use of such advanced *in vitro* systems will allow us to also test and decide on the ideal cell type for use in the engineering of certain tissues and defined clinical applications.

Next-generation studies in tissue engineering applications will be especially focused on the use of smart biomaterials,[421–423] stem cell studies,[424] development of nanotechnology, new biofabrication techniques, and the integration of advances made in synthetic biology.[425,426] Especially with increasing studies and published results on stem cells, it will become easier to imitate target tissues and organs.[427] Further studies in the area would allow overcoming the safety and efficacy concerns encountered with many stem cell types, such as the iPSCs and MSCs. Methodologies to isolate, *ex vivo* manipulate, and culture these cells need further evaluation.[428]

In tissue engineering applications, the selection and design of biomaterials that are suitable for target tissue and organ is one of the most important issues. In addition, the harmony and integrity of the cells with biomaterials have made the use of biological materials as scaffolds useful for the integration of organs with 3D systems.[429] In this context, the most important issue will be to increase the use of technological applications such as computer modeling, artificial intelligence, OoC platforms, and 3D printing for understanding the interactions of cells and tissues with biomaterials *in vivo*. Thus, it will be possible to use the new-generation biocompatible smart materials in tissue engineering applications and to meet patient requirements in real time.[305]

Conclusions

Overall, it can be concluded that our current armamentarium in tissue engineering has made different advances at different levels, including biomaterials, stem cell technologies, fabrication techniques, industrial production innovation, and clinical applications. Through the integration of these facets by multidisciplinary teams with sustained funding, future developments should lead to optimized tissue constructs, successful products, and wider adoption for clinical application.

Acknowledgments

Thanks are due to Dr. Mohammed Xohdy and to Mr. Adam Marsh for drawing Figure 1.

Disclosure Statement

No competing financial interests exist.

Funding Information

The authors acknowledge funding from the National Institutes of Health (1UG3TR003148-01) and the American Heart Association (18TPA34230036 and 442611-NU-80922). Dr. Nguyen receives grant funding from the American Heart Association (18TPA34170049), the National Institutes of Health (R01HL148182), and the Veterans Health Administration (I01-CX001901).

References

1. Mulzer, J., Schutte, B., Lindner, J., Okpanyi, S.N., and Eurich, R. Studies on DNA content of tissues and organs. 1. Changes in cell content of some mesenchymal and parenchymatous rat organs during development, maturation, and in part aging (determinations of DNA). Arzneimittelforschung **29,** 207, 1979.
2. Chong, A.S., and Alegre, M.L. The impact of infection and tissue damage in solid-organ transplantation. Nat Rev Immunol **12,** 459, 2012.
3. Wagener, G. Multiple listings: good for a few, but no solution for the organ shortage. Transplantation **104,** 671, 2020.
4. Colvin, M., Smith, J.M., Hadley, N., *et al*. OPTN/SRTR 2016 annual data report: heart. Am J Transplant **18(S1),** 291, 2018.
5. Giwa, S., Lewis, J.K., Alvarez, L., *et al*. The promise of organ and tissue preservation to transform medicine. Nat Biotechnol **35,** 530, 2017.
6. Langer, R., and Vacanti, J.P. Tissue engineering. Science **260,** 920, 1993.
7. Vacanti, J.P., and Langer, R. Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. Lancet **354 Suppl 1,** Si32, 1999.
8. Langer, R. Tissue engineering: perspectives, challenges, and future directions. Tissue Eng **13,** 1, 2007.
9. Natan, B., Hanjun, K., Marcus, J.G., *et al*. Biofabrication of endothelial cell, dermal fibroblast, and multilayered keratinocyte layers for skin tissue engineering. Biofabrication 2020 [Epub ahead of print]; DOI: 10.1088/1758-5090/aba503.
10. Ashammakhi, N., Kim, H.-J., Ehsanipour, A., *et al*. Regenerative therapies for spinal cord injury. Tissue Eng Part B Rev **25,** 471, 2019.
11. Saveleva, M.S., Ivanov, A.N., Prikhozhdenko, E.S., *et al*. Hybrid functional materials for tissue engineering: synthesis, in vivo drug release and SERS effect. J Phys Conf Ser **1461,** 012150, 2020.
12. Gonzalez-Andrades, M., Garzon, I., Alaminos, M., *et al*. Advances in the field of tissue engineering and regenerative medicine: state of the art and regulatory issues. J Biomater Tiss Eng **3,** 245, 2013.
13. Mohammadinejad, R., Kumar, A., Ranjbar-Mohammadi, M., *et al*. Recent advances in natural gum-based biomaterials for tissue engineering and regenerative medicine: a review. Polymers (Basel) **12,** 176, 2020.
14. Jensen, G., Morrill, C., and Huang, Y. 3D tissue engineering, an emerging technique for pharmaceutical research. Acta Pharm Sin B **8,** 756, 2018.
15. Singh, A. Biomaterials innovation for next generation ex vivo immune tissue engineering. Biomaterials **130,** 104, 2017.
16. Gaharwar, A.K., Singh, I., and Khademhosseini, A. Engineered biomaterials for in situ tissue regeneration. Nat Rev Mater **5,** 686, 2020.
17. Koons, G.L., Diba, M., and Mikos, A.G. Materials design for bone-tissue engineering. Nat Rev Mater **5,** 584, 2020.
18. Ashammakhi, N., and Kaarela, O. Stimuli-responsive biomaterials: next wave. J Craniofac Surg **28,** 1647, 2017.
19. Takahashi, K., and Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell **126,** 663, 2006.
20. Ashammakhi, N., Hasan, A., Kaarela, O., *et al*. Advancing frontiers in bone bioprinting. Adv Healthc Mater **8,** 1801048, 2019.
21. Jorgensen, A.M., Yoo, J.J., and Atala, A. Solid organ bioprinting: strategies to achieve organ function. Chem Rev **120,** 11093, 2020.
22. Hacking, S.A., Ashammakhi, N., and Khademhosseini, A. 2.1.3—Cells and surfaces in vitro. In: Wagner, W.R., Sakiyama-Elbert, S.E., Zhang, G., and Yaszemski, M.J., eds. Biomaterials Science (Fourth Edition). Academic Press, 2020, pp. 661–681.
23. Nguyen, L., Bang, S., and Noh, I. Tissue regeneration of human mesenchymal stem cells on porous gelatin microcarriers by long-term dynamic in vitro culture. Tissue Eng Regen Med **16,** 19, 2019.
24. Kamel, R.A., Ong, J.F., Eriksson, E., Junker, J.P.E., and Caterson, E.J. Tissue engineering of skin. J Am Coll Surg **217,** 533, 2013.
25. Murphy, S.V., and Atala, A. 3D bioprinting of tissues and organs. Nat Biotechnol **32,** 773, 2014.
26. Ashammakhi, N., Ndreu, A., Nikkola, L., Wimpenny, I., and Yang, Y. Advancing tissue engineering by using electrospun nanofibers. Regen Med **3,** 547, 2008.
27. Chan, B.P., and Leong, K.W. Scaffolding in tissue engineering: general approaches and tissue-specific considerations. Eur Spine J **17(S4),** 467, 2008.
28. Dolcimascolo, A., Calabrese, G., Conoci, S., and Parenti, R. Innovative Biomaterials for Tissue Engineering. IntechOpen, 2019.
29. Sell, S.A., Wolfe, P.S., Garg, K., McCool, J.M., Rodriguez, I.A., and Bowlin, G.L. The use of natural polymers in tissue engineering: a focus on electrospun extracellular matrix analogues. Polymers (Basel) **2,** 522, 2010.
30. Massoumi, B., Abbasian, M., Khalilzadeh, B., *et al*. Gelatin-based nanofibrous electrically conductive scaffolds for tissue engineering applications. Int J Polym Mater Polym Biomater **70,** 693, 2020.
31. Nezhad-Mokhtari, P., Akrami-Hasan-Kohal, M., and Ghorbani, M. An injectable chitosan-based hydrogel scaffold containing gold nanoparticles for tissue engineering applications. Int J Biol Macromol **154,** 198, 2020.
32. Chawla, D., Kaur, T., Joshi, A., and Singh, N. 3D bioprinted alginate-gelatin based scaffolds for soft tissue engineering. Int J Biol Macromol **144,** 560, 2020.
33. Nazir, R., Bruyneel, A., Carr, C., and Czernuszka, J. Collagen type I and hyaluronic acid based hybrid scaffolds for heart valve tissue engineering. Biopolymers **110,** e23278, 2019.
34. Pramanik, N., Dutta, K., Basu, R.K., and Kundu, P.P. Aromatic pi-conjugated curcumin on surface modified polyaniline/polyhydroxyalkanoate based 3D porous scaffolds for tissue engineering applications. ACS Biomater Sci Eng **2,** 2365, 2016.
35. Wang, X.Y., Wang, G., Liu, L., and Zhang, D.Y. The mechanism of a chitosan-collagen composite film used as biomaterial support for MC3T3-E1 cell differentiation. Sci Rep **6,** 39322, 2016.
36. O'Brien, F.J. Biomaterials & scaffolds for tissue engineering. Mater Today **14,** 88, 2011.
37. Kundu, B., Kurland, N.E., Bano, S., *et al*. Silk proteins for biomedical applications: bioengineering perspectives. Prog Polym Sci **39,** 251, 2014.

38. Najberg, M., Haji Mansor, M., *et al*. Aerogel sponges of silk fibroin, hyaluronic acid and heparin for soft tissue engineering: composition-properties relationship. Carbohydr Polym **237,** 116107, 2020.
39. Brito-Pereira, R., Correia, D.M., Ribeiro, C., *et al*. Silk fibroin-magnetic hybrid composite electrospun fibers for tissue engineering applications. Compos Part B Eng **141,** 70, 2018.
40. Jendrossek, D., and Handrick, R. Microbial degradation of polyhydroxyalkanoates. Annu Rev Microbiol **56,** 403, 2002.
41. Lizarraga-Valderrama, L.R., Taylor, C.S., Claeyssens, F., Haycock, J.W., Knowles, J.C., and Roy, I. Unidirectional neuronal cell growth and differentiation on aligned polyhydroxyalkanoate blend microfibres with varying diameters. J Tissue Eng Regen Med **13,** 1581, 2019.
42. Bagdadi, A.V., Safari, M., Dubey, P., *et al*. Poly(3-hydroxyoctanoate), a promising new material for cardiac tissue engineering. J Tissue Eng Regen Med **12,** e495, 2018.
43. Ranganathan, S., Balagangadharan, K., and Selvamurugan, N. Chitosan and gelatin-based electrospun fibers for bone tissue engineering. Int J Biol Macromol **133,** 354, 2019.
44. Constantinides, C., Basnett, P., Lukasiewicz, B., *et al*. In vivo tracking and 1H/19F magnetic resonance imaging of biodegradable polyhydroxyalkanoate/polycaprolactone blend scaffolds seeded with labeled cardiac stem cells. ACS Appl Mater Interfaces **10,** 25056, 2018.
45. Dhandayuthapani, B., Yoshida, Y., Maekawa, T., and Kumar, D.S. Polymeric scaffolds in tissue engineering application: a review. Int J Polym Sci **2011,** 1, 2011.
46. Ashammakhi, A., Apu, E.H., and Caterson, E.J. Self-healing biomaterials to heal tissues. J Craniofac Surg 2020 [Epub ahead of print]; DOI: 10.1097/SCS.0000000000007267.
47. Ge, F., Lu, Y., Li, Q., and Zhang, X. Decellularized extracellular matrices for tissue engineering and regeneration. Adv Exp Med Biol **1250,** 15, 2020.
48. Fernandez-Perez, J., and Ahearne, M. The impact of decellularization methods on extracellular matrix derived hydrogels. Sci Rep **9,** 14933, 2019.
49. Ott, H.C., Matthiesen, T.S., Goh, S.K., *et al*. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med **14,** 213, 2008.
50. Kusuma, G.D., Brennecke, S.P., O'Connor, A.J., *et al*. Decellularized extracellular matrices produced from immortal cell lines derived from different parts of the placenta support primary mesenchymal stem cell expansion. PLoS ONE **12,** e0171488. DOI:10.1371/journal.pone.0171488.
51. Dzobo, K., Motaung, K.S.C.M., and Adesida, A. Recent trends in decellularized extracellular matrix bioinks for 3D printing: an updated review. Int J Mol Sci **20,** 4628, 2019.
52. Schoen, B., Avrahami, R., Baruch, L., *et al*. Electrospun extracellular matrix: paving the way to tailor-made natural scaffolds for cardiac tissue regeneration. Adv Funct Mater **27,** 1700427, 2017.
53. Kim, B.S., Kwon, Y.W., Kong, J.-S., *et al*. 3D cell printing of in vitro stabilized skin model and in vivo prevascularized skin patch using tissue-specific extracellular matrix bioink: a step towards advanced skin tissue engineering. Biomaterials **168,** 38, 2018.
54. Carvalho, M.S., Silva, J.C., Udangawa, R.N., *et al*. Co-culture cell-derived extracellular matrix loaded electrospun microfibrous scaffolds for bone tissue engineering. Mater Sci Eng C **99,** 479, 2019.
55. Wassenaar, J.W., Braden, R.L., Osborn, K.G., and Christman, K.L. Modulating in vivo degradation rate of injectable extracellular matrix hydrogels. J Mater Chem B **4,** 2794, 2016.
56. Obata, T., Tsuchiya, T., Akita, S., *et al*. Utilization of natural detergent potassium laurate for decellularization in lung bioengineering. Tissue Eng Part C Methods **25,** 459, 2019.
57. Liao, J., Xu, B., Zhang, R.H., Fan, Y.B., Xie, H.Q., and Li, X.M. Applications of decellularized materials in tissue engineering: advantages, drawbacks and current improvements, and future perspectives. J Mater Chem B **8,** 10023, 2020.
58. Fang, Y., Zhu, X., Wang, N., *et al*. Biodegradable core-shell electrospun nanofibers based on PLA and γ-PGA for wound healing. Eur Polym J **116,** 30, 2019.
59. Qi, F., Wu, J., Li, H., and Ma, G. Recent research and development of PLGA/PLA microspheres/nanoparticles: a review in scientific and industrial aspects. Front Chem Sci Eng **13,** 14, 2019.
60. Sartore, L., Inverardi, N., Pandini, S., Bignotti, F., and Chiellini, F. PLA/PCL-based foams as scaffolds for tissue engineering applications. Mater Today **7,** 410, 2019.
61. Sun, X., Wang, J., Wang, Y., and Zhang, Q. Collagen-based porous scaffolds containing PLGA microspheres for controlled kartogenin release in cartilage tissue engineering. Artif Cells Nanomed Biotechnol **46,** 1957, 2018.
62. Xu, T.O., Kim, H.S., Stahl, T., and Nukavarapu, S.P. Self-neutralizing PLGA/magnesium composites as novel biomaterials for tissue engineering. Biomed Mater **13,** 035013, 2018.
63. Jiao, Z., Luo, B., Xiang, S., Ma, H., Yu, Y., and Yang, W. 3D printing of HA/PCL composite tissue engineering scaffolds. Adv Ind Eng Polym Res **2,** 196, 2019.
64. Luginina, M., Schuhladen, K., Orru, R., Cao, G., Boccaccini, A.R., and Liverani, L. Electrospun PCL/PGS composite fibers incorporating bioactive glass particles for soft tissueengineering applications. Nanomaterials **10,** 978, 2020.
65. Liu, J., Zheng, H.Y., Dai, X.Y., Poh, P.S.P., Machens, H.G., and Schilling, A.F. Transparent PDMS bioreactors for the fabrication and analysis of multi-layer prevascularized hydrogels under continuous perfusion. Front Bioeng Biotechnol **8,** 568934, 2020.
66. Qian, Z.C., Ross, D., Jia, W.K., Xing, Q., and Zhao, F. Bioactive polydimethylsiloxane surface for optimal human mesenchymal stem cell sheet culture. Bioact Mater **3,** 167, 2018.
67. Wu, Q.R., Liu, J.F., Wang, X.H., *et al*. Organ-on-a-chip: recent breakthroughs and future prospects. Biomed Eng Online **19,** 9, 2020.
68. de Cassan, D., Becker, A., Glasmacher, B., *et al*. Blending chitosan-g-poly(caprolactone) with poly(caprolactone) by electrospinning to produce functional fiber mats for tissue engineering applications. J Appl Polym Sci **137,** 48650, 2020.
69. GhavamiNejad, A., Ashammakhi, N., Wu, X.Y., and Khademhosseini, A. Crosslinking strategies for 3D bioprinting of polymeric hydrogels. Small **16,** e2002931, 2020.
70. Bjørge, I.M., Costa, A.M.S., Silva, A.S., Vidal, J.P.O., Nóbrega, J.M., and Mano, J.F. Tuneable spheroidal hydrogel particles for cell and drug encapsulation. Soft Matter **14,** 5622, 2018.
71. Huang, Q., Zou, Y., Arno, M.C., *et al*. Hydrogel scaffolds for differentiation of adipose-derived stem cells. Chem Soc Rev **46,** 6255, 2017.

72. Al-Sabanh, A., Burnell, S.E.A., Simoes, I.N., *et al*. Structural and mechanical characterization of crosslinked and sterilised nanocellulose-based hydrogels for cartilage tissue engineering. Carbohyd Polym **212,** 242, 2019.
73. Roy, C.K., Guo, H.L., Sun, T.L., *et al*. Self-adjustable adhesion of polyampholyte hydrogels. Adv Mater **27,** 7344, 2015.
74. Shirzaei Sani, E., Portillo-Lara, R., Spencer, A., *et al*. Engineering adhesive and antimicrobial hyaluronic acid/elastin-like polypeptide hybrid hydrogels for tissue engineering applications. ACS Biomater Sci Eng **4,** 2528, 2018.
75. Dhand, A.P., Galarraga, J.H., and Burdick, J.A. Enhancing biopolymer hydrogel functionality through interpenetrating networks. Trends Biotechnol **39,** 519, 2021.
76. Ashammakhi, N., Ahadian, S., Darabi, M.A., *et al*. Minimally invasive and regenerative therapeutics. Adv Mater **31,** e1804041, 2019.
77. GhavamiNejad, A., Lu, B., and Wu, X.Y. Transdermal drug delivery via microneedle patches. In: Unnithan, A.R., Sasikala, A.R.K., Park, C.H., and Kim, C.S., eds. Biomimetic Nanoengineered Materials for Advanced Drug Delivery. Netherlands: Elsevier, 2019, pp. 37–52.
78. Han, S.-S., Yoon, H.Y., Yhee, J.Y., *et al*. In situ cross-linkable hyaluronic acid hydrogels using copper free click chemistry for cartilage tissue engineering. Polym Chem **9,** 20, 2018.
79. Kumar Meena, L., Rather, H., Kedaria, D., and Vasita, R. Polymeric microgels for bone tissue engineering applications—a review. Int J Polym Mater Polym Biomater **69,** 381, 2020.
80. Jiang, W., Li, M., Chen, Z., and Leong, K.W. Cell-laden microfluidic microgels for tissue regeneration. Lab Chip **16,** 4482, 2016.
81. Newsom, J.P., Payne, K.A., and Krebs, M.D. Microgels: modular, tunable constructs for tissue regeneration. Acta Biomater **88,** 32, 2019.
82. Yang, X., Lu, Z., Wu, H., Li, W., Zheng, L., and Zhao, J. Collagen-alginate as bioink for three-dimensional (3D) cell printing based cartilage tissue engineering. Mater Sci Eng C **83,** 195, 2018.
83. Noor, N., Shapira, A., Edri, R., Gal, I., Wertheim, L., and Dvir, T. 3D printing of personalized thick and perfusable cardiac patches and hearts. Adv Sci (Weinh) **2019,** 1900344. DOI: 10.1002/advs.201900344.
84. Talebian, S., Mehrali, M., Taebnia, N., *et al*. Self-healing hydrogels: the next paradigm shift in tissue engineering? Adv Sci (Weinh) **6,** 1801664, 2019.
85. Saunders, L., and Ma, P.X. Self-healing supramolecular hydrogels for tissue engineering applications. Macromol Biosci **19,** e1800313, 2019.
86. Rammal H GA, Erdem, A., Mbeleck, R., *et al*. Advances in biomedical applications of self-healing hydrogels. Mater Chem Front **5,** 4368, 2021.
87. Montgomery, M., Ahadian, S., Davenport Huyer, L., *et al*. Flexible shape-memory scaffold for minimally invasive delivery of functional tissues. Nat Mater **16,** 1038, 2017.
88. Xia, Y., He, Y., Zhang, F., Liu, Y., and Leng, J. A review of shape memory polymers and composites: mechanisms, materials, and applications. Adv Mater **33,** e2000713, 2020.
89. Darabi, M.A., Khosrozadeh, A., Wang, Y., *et al*. An alkaline based method for generating crystalline, strong, and shape memory polyvinyl alcohol biomaterials. Adv Sci **7,** 1902740, 2020.
90. Lu, Y., Aimetti, A.A., Langer, R., and Gu, Z. Bioresponsive materials. Nat Rev Mater **2,** 16075, 2016.
91. Tang, J.D., Mura, C., and Lampe, K.J. Stimuli-responsive, pentapeptide, nanofiber hydrogel for tissue engineering. J Am Chem Soc **141,** 4886, 2019.
92. Sood, N., Bhardwaj, A., Mehta, S., and Mehta, A. Stimuli-responsive hydrogels in drug delivery and tissue engineering. Drug Deliv **23,** 758, 2016.
93. Zhou, W.X., Qiao, Z., Zare, E.N., *et al*. 4D-printed dynamic materials in biomedical applications: chemistry, challenges, and their future perspectives in the clinical sector. J Med Chem **63,** 8003, 2020.
94. Invernizzi, M., Turri, S., Levi, M., and Suriano, R. 4D printed thermally activated self-healing and shape memory polycaprolactone-based polymers. Eur Polym J **101,** 169, 2018.
95. Gyarmati, B., Szilágyi, B.Á., and Szilágyi, A. Reversible interactions in self-healing and shape memory hydrogels. Eur Polym J **93,** 642, 2017.
96. Obiweluozor, F.O., GhavamiNejad, A., Park, C.H., and Kim, C.S. Mussel inspired locomotive: the moisture induced actuation in a poly(vinyl alcohol) film containing melanin-like dopamine nano spheres. RSC Adv **6,** 65089, 2016.
97. Obiweluozor, F.O., GhavamiNejad, A., Maharjan, B., Kim, J., Park, C.H., and Kim, C.S. A mussel inspired self-expandable tubular hydrogel with shape memory under NIR for potential biomedical applications. J Mater Chem B **5,** 5373, 2017.
98. Sun, J., Liu, Y.Y., and Leng, J.S. Mechanical properties of shape memory polymer composites enhanced by elastic fibers and their application in variable stiffness morphing skins. J Intell Mater Syst Struct **26,** 2020, 2015.
99. Liu, Y., and Hsu, S-h. Synthesis and biomedical applications of self-healing hydrogels. Front Chem **6,** 449, 2018.
100. Lü, S., Bai, X., Liu, H., *et al*. An injectable and self-healing hydrogel with covalent cross-linking in vivo for cranial bone repair. J Mater Chem B **5,** 3739, 2017.
101. Drury, J.L., and Mooney, D.J. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials **24,** 4337, 2003.
102. Zou, W., Dong, J., Luo, Y., Zhao, Q., and Xie, T. Dynamic covalent polymer networks: from old chemistry to modern day innovations. Adv Mater **29,** 1606100, 2017.
103. Unagolla, J.M., and Jayasuriya, A.C. Hydrogel-based 3D bioprinting: a comprehensive review on cell-laden hydrogels, bioink formulations, and future perspectives. Appl Mater Today **18,** 100479, 2020.
104. Li, J., and Mooney, D.J. Designing hydrogels for controlled drug delivery. Nat Rev Mater **1,** 1, 2016.
105. Vermonden, T., Censi, R., and Hennink, W.E. Hydrogels for protein delivery. Chem Rev **112,** 2853, 2012.
106. Fletcher, N.A., Babcock, L.R., Murray, E.A., and Krebs, M.D. Controlled delivery of antibodies from injectable hydrogels. Mater Sci Eng C **59,** 801, 2016.
107. Zhang, H., Cong, Y., Osi, A.R., *et al*. Direct 3D printed biomimetic scaffolds based on hydrogel microparticles for cell spheroid growth. Adv Funct Mater **30,** 1910573, 2020.
108. Ashammakhi, N. Drug release: proper control to help clinical application discussion. J Craniofac Surg **29,** 124, 2018.
109. GhavamiNejad, A., Park, C.H., and Kim, C.S. In situ synthesis of antimicrobial silver nanoparticles within an-

tifouling zwitterionic hydrogels by catecholic redox chemistry for wound healing application. Biomacromolecules **17,** 1213, 2016.
110. GhavamiNejad, A., SamariKhalaj, M., Aguilar, L.E., Park, C.H., and Kim, C.S. pH/NIR light-controlled multidrug release via a mussel-inspired nanocomposite hydrogel for chemo-photothermal cancer therapy. Sci Rep **6,** 33594, 2016.
111. Baino, F., Novajra, G., and Vitale-Brovarone, C. Bioceramics and scaffolds: a winning combination for tissue engineering. Front Bioeng Biotechnol **3,** 202, 2015.
112. Tellisi, N., and Ashammakhi, N. Comparison of meshes, gels and ceramic for cartilage tissue engineering in vitro. Eur J Plast Surg **35,** 159, 2012.
113. Rahaman, M.N., Day, D.E., Bal, B.S., *et al*. Bioactive glass in tissue engineering. Acta Biomater **7,** 2355, 2011.
114. El-Rashidy, A.A., Roether, J.A., Harhaus, L., Kneser, U., and Boccaccini, A.R. Regenerating bone with bioactive glass scaffolds: a review of in vivo studies in bone defect models. Acta Biomater **62,** 1, 2017.
115. Fu, Q., Saiz, E., and Tomsia, A.P. Direct ink writing of highly porous and strong glass scaffolds for load-bearing bone defects repair and regeneration. Acta Biomater **7,** 3547, 2011.
116. Huang, T.S., Rahaman, M.N., Doiphode, N.D., *et al*. Porous and strong bioactive glass (13–93) scaffolds fabricated by freeze extrusion technique. Mater Sci Eng C Mater **31,** 1482, 2011.
117. Wu, Z.Y., Hill, R.G., Yue, S., Nightingale, D., Lee, P.D., and Jones, J.R. Melt-derived bioactive glass scaffolds produced by a gel-cast foaming technique. Acta Biomater **7,** 1807, 2011.
118. Gao, C.D., Deng, Y.W., Feng, P., *et al*. Current progress in bioactive ceramic scaffolds for bone repair and regeneration. Int J Mol Sci **15,** 4714, 2014.
119. Du, X.Y., Fu, S.Y., and Zhu, Y.F. 3D printing of ceramic-based scaffolds for bone tissue engineering: an overview. J Mater Chem B **6,** 4397, 2018.
120. Esfahani, H., Jose, R., and Ramakrishna, S. Electrospun ceramic nanofiber mats today: synthesis, properties, and applications. Materials (Basle) **10,** 1238, 2017.
121. Fu, Q.A., Rahaman, M.N., Bal, B.S., Kuroki, K., and Brown, R.F. In vivo evaluation of 13–93 bioactive glass scaffolds with trabecular and oriented microstructures in a subcutaneous rat implantation model. J Biomed Mater Res A **95a,** 235, 2010.
122. Peter, M., Binulal, N.S., Nair, S.V., Selvamurugan, N., Tamura, H., and Jayakumar, R. Novel biodegradable chitosan-gelatin/nano-bioactive glass ceramic composite scaffolds for alveolar bone tissue engineering. Chem Eng J **158,** 353, 2010.
123. Xu, C.X., Su, P.Q., Chen, X.F., *et al*. Biocompatibility and osteogenesis of biomimetic bioglass-collagen-phosphatidylserine composite scaffolds for bone tissue engineering. Biomaterials **32,** 1051, 2011.
124. Nikpour, P., Salimi-Kenari, H., Fahimipour, F., *et al*. Dextran hydrogels incorporated with bioactive glass-ceramic: nanocomposite scaffolds for bone tissue engineering. Carbohydr Polym **190,** 281, 2018.
125. Chatzistavrou, X., Fenno, J.C., Faulk, D., *et al*. Fabrication and characterization of bioactive and antibacterial composites for dental applications. Acta Biomater **10,** 3723, 2014.
126. Chatzistavrou, X., Rao, R.R., Caldwell, D.J., *et al*. Collagen/fibrin microbeads as a delivery system for Ag-doped bioactive glass and DPSCs for potential applications in dentistry. J Non Cryst Solids **432,** 143, 2016.
127. Dinesh Kumar, S., Mohamed Abudhahir, K., Selvamurugan, N., *et al*. Formulation and biological actions of nano-bioglass ceramic particles doped with Calcarea phosphorica for bone tissue engineering. Mater Sci Eng C **83,** 202, 2018.
128. Subhapradha, N., Abudhahir, M., Aathira, A., Srinivasan, N., and Moorthi, A. Polymer coated mesoporous ceramic for drug delivery in bone tissue engineering. Int J Biol Macromol **110,** 65, 2018.
129. Tsigkou, O., Pomerantseva, I., Spencer, J.A., *et al*. Engineered vascularized bone grafts. Proc Natl Acad Sci U S A **107,** 3311, 2010.
130. Santos-Ruiz, L., Mowatt, D.J., Marguerie, A., *et al*. Potential use of craniosynostotic osteoprogenitors and bioactive scaffolds for bone engineering. J Tissue Eng Regen M **1,** 199, 2007.
131. Crupi, A., Costa, A., Tarnok, A., Melzer, S., and Teodori, L. Inflammation in tissue engineering: the Janus between engraftment and rejection. Eur J Immunol **45,** 3222, 2015.
132. Rezk, A.I., Sasikala, A.R.K., Nejad, A.G., *et al*. Strategic design of a mussel-inspired in situ reduced Ag/Au-nanoparticle coated magnesium alloy for enhanced viability, antibacterial property and decelerated corrosion rates for degradable implant Applications. Sci Rep **9,** 117, 2019.
133. Yusop, A.H., Bakir, A.A., Shaharom, N.A., Kadir, M.R.A., and Hermawan, H. Porous biodegradable metals for hard tissue scaffolds: a review. Int J Biomater **2012,** 641430, 2012.
134. Sheikh, Z., Najeeb, S., Khurshid, Z., Verma, V., Rashid, H., and Glogauer, M. Biodegradable materials for bone repair and tissue engineering applications. Materials **8,** 5744, 2015.
135. Ferrández-Montero, A., Lieblich, M., González-Carrasco, J.L., *et al*. Development of biocompatible and fully bioabsorbable PLA/Mg films for tissue regeneration applications. Acta Biomater **98,** 114, 2019.
136. Zhang, H.Y., Jiang, H.B., Kim, J.E., Zhang, S., Kim, K.M., and Kwon, J.S. Bioresorbable magnesium-reinforced PLA membrane for guided bone/tissue regeneration. J Mech Behav Biomed Mater **112,** 104061, 2020.
137. Saris, N.E.L., Mervaala, E., Karppanen, H., Khawaja, J.A., and Lewenstam, A. Magnesium—an update on physiological, clinical and analytical aspects. Clin Chim Acta **294,** 1, 2000.
138. Bosiers, M., Peeters, P., D'Archambeau, O., *et al.*; AMS INSIGHT Investigators. AMS INSIGHT-absorbable metal stent implantation for treatment of below-the-knee critical limb ischemia: 6-month analysis. Cardiovasc Intervent Radiol **32,** 424, 2009.
139. Windhagen, H., Radtke, K., Weizbauer, A., *et al*. Biodegradable magnesium-based screw clinically equivalent to titanium screw in hallux valgus surgery: short term results of the first prospective, randomized, controlled clinical pilot study. Biomed Eng Online **12,** 62, 2013.
140. Seitz, J.M., Lucas, A., and Kirschner, M. Magnesium-based compression screws: a novelty in the clinical use of implants. JOM **68,** 1177, 2016.
141. Cerrato, E., Barbero, U., Romero, J.A.G., *et al*. Magmaris (TM) resorbable magnesium scaffold: state-of-art review. Future Cardiol **15,** 267, 2019.
142. Gu, X.N., Zheng, Y.F., Cheng, Y., Zhong, S.P., and Xi, T.F. In vitro corrosion and biocompatibility of binary magnesium alloys. Biomaterials **30,** 484, 2009.

143. Vojtech, D., Kubasek, J., Serak, J., and Novak, P. Mechanical and corrosion properties of newly developed biodegradable Zn-based alloys for bone fixation. Acta Biomater **7,** 3515, 2011.
144. Kornev, V.A., Grebenik, E.A., Solovieva, A.B., Dmitriev, R.I., and Timashev, P.S. Hydrogel-assisted neuroregeneration approaches towards brain injury therapy: a state-of-the-art review. Comput Struct Biotecnol **16,** 488, 2018.
145. Capek, J., Jablonska, E., Lipov, J., Kubatik, T.F., and Vojtech, D. Preparation and characterization of porous zinc prepared by spark plasma sintering as a material for biodegradable scaffolds. Mater Chem Phys **203,** 249, 2018.
146. Howard, D., Buttery, L.D., Shakesheff, K.M., and Roberts, S.J. Tissue engineering: strategies, stem cells and scaffolds. J Anat **213,** 66, 2008.
147. Uz, U., Gunhan, K., Vatansever, S., Kivanc, M., and Yuceturk, A.V. Novel simple strategy for cartilage tissue engineering using stem cells and synthetic polymer scaffold. J Craniofac Surg **30,** 940, 2019.
148. Toma, J.G., Akhavan, M., Fernandes, K.J., *et al*. Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol **3,** 778, 2001.
149. Blau, H.M., Brazelton, T.R., and Weimann, J.M. The evolving concept of a stem cell: entity or function? Cell **105,** 829, 2001.
150. Bajada, S., Mazakova, I., Richardson, J.B., and Ashammakhi, N. Updates on stem cells and their applications in regenerative medicine. J Tissue Eng Regen Med **2,** 169, 2008.
151. Bajada, S., Mazakova, I., Ashton, B.A., Richardson, J., and Ashammakhi, N. Stem cells in regenerative medicine. In: Topics in Tissue Engineering. Oulu, Finland: Oulu, FinlandTopics in Tissue Engineering, 2008. Accessed September 29, 2020. Available from: https://www.oulu.fi/spareparts/ebook_topics_in_t_e_vol4/abstracts/bajada.pdf
152. Choi, A., Yoon, H., Han, S.J., Lee, J.H., Rhyou, I.H., and Kim, D.S. Rapid harvesting of stem cell sheets by thermoresponsive bulk poly(N-isopropylacrylamide) (PNIPAAm) nanotopography. Biomater Sci **8,** 5260, 2020.
153. Kundu, B., Bastos, A.R.F., Brancato, V., *et al*. Mechanical property of hydrogels and the presence of adipose stem cells in tumor stroma affect spheroid formation in the 3D osteosarcoma model. ACS Appl Mater Interfaces **11,** 14548, 2019.
154. Umehara, H., Kimura, T., Ohtsuka, S., *et al*. Efficient derivation of embryonic stem cells by inhibition of glycogen synthase kinase-3. Stem Cells **25,** 2705, 2007.
155. Xu, R.H., Chen, X., Li, D.S., *et al*. BMP4 initiates human embryonic stem cell differentiation to trophoblast. Nat Biotechnol **20,** 1261, 2002.
156. Khademhosseini, A., Ashammakhi, N., Karp, J.M., *et al*. Chapter 27—Embryonic stem cells as a cell source for tissue engineering. In: Lanza, R., Langer, R., Vacanti, J.P., and Atala, A., eds. Principles of Tissue Engineering (Fifth Edition). London, UK: Academic Press, 2020, pp. 467–490.
157. Zacharias, D.G., Nelson, T.J., Mueller, P.S., and Hook, C.C. The science and ethics of induced pluripotency: what will become of embryonic stem cells? Mayo Clin Proc **86,** 634, 2011.
158. Zhang, K., Chooi, W.H., Liu, S., *et al*. Localized delivery of CRISPR/dCas9 via layer-by-layer self-assembling peptide coating on nanofibers for neural tissue engineering. Biomaterials **256,** 120225, 2020.
159. Somoza, R.A., and Rubio, F.J. Cell therapy using induced pluripotent stem cells or somatic stem cells: this is the question. Curr Stem Cell Res Ther **7,** 191, 2012.
160. Kiskinis, E., and Eggan, K. Progress toward the clinical application of patient-specific pluripotent stem cells. J Clin Invest **120,** 51, 2010.
161. Paik, D.T., Chandy, M., and Wu, J.C. Patient and disease–specific induced pluripotent stem cells for discovery of personalized cardiovascular drugs and therapeutics. Pharmacol Rev **72,** 320, 2020.
162. Rosenbaum, A.J., Grande, D.A., and Dines, J.S. The use of mesenchymal stem cells in tissue engineering. Organogenesis **4,** 23, 2008.
163. Ding, D.C., Shyu, W.C., and Lin, S.Z. Mesenchymal stem cells. Cell Transplant **20,** 5, 2011.
164. Oryan, A., Kamali, A., Moshiri, A., and Baghaban Eslaminejad, M. Role of mesenchymal stem cells in bone regenerative medicine: what is the evidence? Cells Tissues Organs **204,** 59, 2017.
165. Strioga, M., Viswanathan, S., Darinskas, A., Slaby, O., and Michalek, J. Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. Stem Cells Dev **21,** 2724, 2012.
166. Niemelä, S., Miettinen, S., Sarkanen, J.R., and Ashammakhi, N. Adipose tissue and adipocyte differentiation: molecular and cellular aspects and tissue engineering applications. 2008. In: Topics in Tissue Engineering. Oulu, Finland: Oulu UniversityTopics in Tissue Engineering. Accessed September 29, 2020. Available from: https://www.oulu.fi/spareparts/ebook_topics_in_t_e_vol4/abstracts/niemela.pdf
167. Niemelä, S.M., Miettinen, S., Konttinen, Y., *et al*. Fat tissue: views on reconstruction and exploitation. J Craniofac Surg **18,** 325, 2007.
168. Hendrijantini, N., and Hartono, P. Phenotype characteristics and osteogenic differentiation potential of human mesenchymal stem cells derived from amnion membrane (HAMSCs) and umbilical cord (HUC-MSCs). Acta Inform Med **27,** 72, 2019.
169. Yaghoubi, Y., Movassaghpour, A., Zamani, M., Talebi, M., Mehdizadeh, A., and Yousefi, M. Human umbilical cord mesenchymal stem cells derived-exosomes in diseases treatment. Life Sci **233,** 116733, 2019.
170. Rajabzadeh, N., Fathi, E., and Farahzadi, R. Stem cell-based regenerative medicine. Stem Cell Investig **6,** 19, 2019.
171. Abumaree, M.H., Abomaray, F.M., Alshabibi, M.A., AlAskar, A.S., and Kalionis, B. Immunomodulatory properties of human placental mesenchymal stem/stromal cells. Placenta **59,** 87, 2017.
172. Amiryaghoubi, N., Pesyan, N.N., Fathi, M., and Omidi, Y. Injectable thermosensitive hybrid hydrogel containing graphene oxide and chitosan as dental pulp stem cells scaffold for bone tissue engineering. Int J Biol Macromol **162,** 1338, 2020.
173. Gargett, C.E., Schwab, K.E., and Deane, J.A. Endometrial stem/progenitor cells: the first 10 years. Hum Reprod Update **22,** 137, 2016.
174. Amemiya, M., Tsuji, K., Katagiri, H., *et al*. Synovial fluid-derived mesenchymal cells have non-inferior chondrogenic potential and can be utilized for regenerative therapy as substitute for synovium-derived cells. Biochem Biophys Res Commun **523,** 465, 2020.
175. Dai, R., Wang, Z., Samanipour, R., Koo, K.-I., and Kim, K. Adipose-derived stem cells for tissue engineering and regenerative medicine applications. Stem Cells Int **2016,** 6737345, 2016.

176. Zuk, P.A., Zhu, M., Mizuno, H., *et al*. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng **7,** 211, 2001.
177. Yong, K.W., Choi, J.R., Choi, J.Y., and Cowie, A.C. Recent advances in mechanically loaded human mesenchymal stem cells for bone tissue engineering. Int J Mol Sci **21,** 5816, 2020.
178. Maqsood, M., Kang, M., Wu, X., Chen, J., Teng, L., and Qiu, L. Adult mesenchymal stem cells and their exosomes: sources, characteristics, and application in regenerative medicine. Life Sci **256,** 118002, 2020.
179. Al-Moraissi, E.A., Oginni, F.O., Holkom, M.A.M., Mohamed, A.A.S., and Al-Sharani, H.M. Tissue-engineered bone using mesenchymal stem cells versus conventional bone grafts in the regeneration of maxillary alveolar bone: a systematic review and meta-analysis. Int J Oral Maxillofac Implants **35,** 79, 2020.
180. Docheva, D., Popov, C., Mutschler, W., and Schieker, M. Human mesenchymal stem cells in contact with their environment: surface characteristics and the integrin system. J Cell Mol Med **11,** 21, 2007.
181. Galanis, E., Atherton, P.J., Viker, K.B., *et al*. Phase I/II trial of adipose tissue derived mesenchymal stem cell delivery of a measles virus strain engineered to express the sodium iodide symporter in ovarian cancer patients. Mol Ther **28,** 589, 2020.
182. Aurora, A.B., and Olson, E.N. Immune modulation of stem cells and regeneration. Cell Stem Cell **15,** 15, 2014.
183. Nasef, A., Ashammakhi, N., and Fouillard, L. Immunomodulatory effect of mesenchymal stromal cells: possible mechanisms. Regen Med **3,** 531, 2008.
184. Li, H., Shen, S., Fu, H., *et al*. Immunomodulatory functions of mesenchymal stem cells in tissue engineering. Stem Cells Int **2019,** 9671206, 2019.
185. Bavisotto, C.C., Scalia, F., Gammazza, A.M., *et al*. Extracellular vesicle-mediated cell-cell communication in the nervous system: focus on neurological diseases. Int J Mol Sci **20,** 434, 2019.
186. Jiang, T., Wang, Z., and Sun, J. Human bone marrow mesenchymal stem cell-derived exosomes stimulate cutaneous wound healing mediates through TGF-β/Smad signaling pathway. Stem Cell Res Ther **11,** 198, 2020.
187. Pendse, S., Kale, V., and Vaidya, A. Extracellular vesicles isolated from mesenchymal stromal cells primed with hypoxia: novel strategy in regenerative medicine. Curr Stem Cell Res Ther **16,** 243, 2021.
188. Higuchi, A., Ling, Q.-D., Chang, Y., Hsu, S.-T., and Umezawa, A. Physical cues of biomaterials guide stem cell differentiation fate. Chem Rev **113,** 3297, 2013.
189. Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. Matrix elasticity directs stem cell lineage specification. Cell **126,** 677, 2006.
190. Padhi, A., and Nain, A.S. ECM in differentiation: a review of matrix structure, composition and mechanical properties. Ann Biomed Eng **48,** 1071, 2020.
191. Sung, T.C., Li, H.F., Higuchi, A., *et al*. Human pluripotent stem cell culture on polyvinyl alcohol-co-itaconic acid hydrogels with varying stiffness under xeno-free conditions. J Vis Exp 57314, 2018.
192. Du, V., Luciani, N., Richard, S., *et al*. A 3D magnetic tissue stretcher for remote mechanical control of embryonic stem cell differentiation. Nat Commun **8,** 400, 2017.
193. Tannaz, N.A., Ali, S.M., Nooshin, H., *et al*. Comparing the effect of uniaxial cyclic mechanical stimulation and chemical factors on myogenin and Myh2 expression in mouse embryonic and bone marrow derived mesenchymal stem cells. Mol Cell Biomech **11,** 19, 2014.
194. Even-Ram, S., Artym, V., and Yamada, K.M. Matrix control of stem cell fate. Cell **126,** 645, 2006.
195. Hsieh, J., and Gage, F.H. Epigenetic control of neural stem cell fate. Curr Opin Genet Dev **14,** 461, 2004.
196. Zhao, X., Cui, K., and Li, Z. The role of biomaterials in stem cell-based regenerative medicine. Future Med Chem **11,** 1777, 2019.
197. Wang, L., Li, J., Zhang, D., *et al*. Dual-enzymatically crosslinked and injectable hyaluronic acid hydrogels for potential application in tissue engineering. RSC Adv **10,** 2870, 2020.
198. Qazi, T.H., Mooney, D.J., Duda, G.N., and Geissler, S. Niche-mimicking interactions in peptide-functionalized 3D hydrogels amplify mesenchymal stromal cell paracrine effects. Biomaterials **230,** 119639, 2020.
199. Abdellatef, S.A., Ohi, A., Nabatame, T., and Taniguchi, A. The effect of physical and chemical cues on hepatocellular function and morphology. Int J Mol Sci **15,** 4299, 2014.
200. Zimmermann, W.H., Tiburcy, M., and Eschenhagen, T. Cardiac tissue engineering: a clinical perspective. Future Cardiol **3,** 435, 2007.
201. Crestani, T., Steichen, C., Neri, E., *et al*. Electrical stimulation applied during differentiation drives the hiPSC-CMs towards a mature cardiac conduction-like cells. Biochem Biophys Res Commun **533,** 376, 2020.
202. Dhahri, W., Romagnuolo, R., and Laflamme, M.A. Training heart tissue to mature. Nat Biomed Eng **2,** 351, 2018.
203. Zhang, F., Qu, K.Y., Li, X.P., *et al*. Gelatin-based hydrogels combined with electrical stimulation to modulate neonatal rat cardiomyocyte beating and promote maturation. Bio-Des Manuf **4,** 100, 2021.
204. Das, A., Ojha, M., Subramanyam, P., and Deepa, M. New volumetric CNT-doped gelatin-cellulose scaffolds for skeletal muscle tissue engineering. Nanoscale Adv **2,** 2885, 2020.
205. Giannoudis, P.V., and Pountos, I. Tissue regeneration. The past, the present and the future. Injury **36 Suppl 4,** S2, 2005.
206. Nims, R.W., and Price, P.J. Best practices for detecting and mitigating the risk of cell culture contaminants. In Vitro Cell Dev Biol Anim **53,** 872, 2017.
207. Pountos, I., Georgouli, T., and Giannoudis, P.V. The effect of autologous serum obtained after fracture on the proliferation and osteogenic differentiation of mesenchymal stem cells. Cell Mol Biol (Noisy-le-grand) **54,** 33, 2008.
208. Zhang, X., Wang, L., Zhang, X., *et al*. The use of KnockOut serum replacement (KSR) in three dimensional rat testicular cells co-culture model: an improved male reproductive toxicity testing system. Food Chem Toxicol **106,** 487, 2017.
209. Yasuda, S.Y., Ikeda, T., Shahsavarani, H., *et al*. Chemically defined and growth-factor-free culture system for the expansion and derivation of human pluripotent stem cells. Nat Biomed Eng **2,** 173, 2018.
210. Tirza, G., Solodeev, I., Sela, M., *et al*. Reduced culture temperature attenuates oxidative stress and inflammatory response facilitating expansion and differentiation of adipose-derived stem cells. Stem Cell Res Ther **11,** 35, 2020.

211. Chu, A.J., Zhao, E.J., Chiao, M., and Lim, C.J. Co-culture of induced pluripotent stem cells with cardiomyocytes is sufficient to promote their differentiation into cardiomyocytes. PLoS One **15,** e0230966, 2020.
212. Li, B., Gao, Y., Guo, L., *et al*. Synthesis of photo-reactive poly (vinyl alcohol) and construction of scaffold-free cartilage like pellets in vitro. Regen Biomater **5,** 159, 2018.
213. Zhang, R.R., Takebe, T., Miyazaki, L., *et al*. Efficient hepatic differentiation of human induced pluripotent stem cells in a three-dimensional microscale culture. Methods Mol Biol **1210,** 131, 2014.
214. Kim, S.M., Ueki, M., Ren, X., Akimoto, J., Sakai, Y., and Ito, Y. Micropatterned nanolayers immobilized with nerve growth factor for neurite formation of PC12 cells. Int J Nanomed **14,** 7683, 2019.
215. Pountos, I., Corscadden, D., Emery, P., and Giannoudis, P.V. Mesenchymal stem cell tissue engineering: techniques for isolation, expansion and application. Injury **38 Suppl 4,** S23, 2007.
216. Costa, E.C., Moreira, A.F., de Melo-Diogo, D., Gaspar, V.M., Carvalho, M.P., and Correia, I.J. 3D tumor spheroids: an overview on the tools and techniques used for their analysis. Biotechnol Adv **34,** 1427, 2016.
217. Antoni, D., Burckel, H., Josset, E., and Noel, G. Three-dimensional cell culture: a breakthrough in vivo. Int J Mol Sci **16,** 5517, 2015.
218. Ho, W.J., Pham, E.A., Kim, J.W., *et al*. Incorporation of multicellular spheroids into 3-D polymeric scaffolds provides an improved tumor model for screening anticancer drugs. Cancer Sci **101,** 2637, 2010.
219. Davoodi, E., Sarikhani, E., Montazerian, H., *et al*. Extrusion and microfluidic-based bioprinting to fabricate biomimetic tissues and organs. Adv Mater Technol **5,** 1901044, 2020.
220. Atala, A., and Forgacs, G. Three-dimensional bioprinting in regenerative medicine: reality, hype, and future. Stem Cells Transl Med **8,** 744, 2019.
221. Gao, X., Han, S., Zhang, R., Liu, G., and Wu, J. Progress in electrospun composite nanofibers: composition, performance and applications for tissue engineering. J Mater Chem B **7,** 7075, 2019.
222. Nikkola, L., Morton, T., Balmayor, E.R., *et al*. Fabrication of electrospun poly(d,l lactide-co-glycolide)80/20 scaffolds loaded with diclofenac sodium for tissue engineering. Eur J Med Res **20,** 54, 2015.
223. Jensen, C., and Teng, Y. Is it time to start transitioning from 2D to 3D cell culture? Front Mol Biosci **7,** 33, 2020.
224. Langhans, S.A. Three-dimensional in vitro cell culture models in drug discovery and drug repositioning. Front Pharmacol **9,** 6, 2018.
225. Kapałczyńska, M., Kolenda, T., Przybyła, W., *et al*. 2D and 3D cell cultures—a comparison of different types of cancer cell cultures. Arch Med Sci **14,** 910, 2018.
226. Merten, O.W. Advances in cell culture: anchorage dependence. Philos Trans R Soc B Lond B Biol Sci **370,** 20140040, 2015.
227. Souza, G.R., Molina, J.R., Raphael, R.M., *et al*. Three-dimensional tissue culture based on magnetic cell levitation. Nat Nanotechnol **5,** 291, 2010.
228. Abe-Fukasawa, N., Otsuka, K., Aihara, A., Itasaki, N., and Nishino, T. Novel 3D liquid cell culture method for anchorage-independent cell growth, cell imaging and automated drug screening. Sci Rep **8,** 3627, 2018.
229. Kronemberger, G.S., Dalmônico, G.M.L., Rossi, A.L., *et al*. Scaffold- and serum-free hypertrophic cartilage tissue engineering as an alternative approach for bone repair. Artif Organs **44,** E288, 2020.
230. Anil-Inevi, M., Yilmaz, E., Sarigil, O., Tekin, H.C., and Ozcivici, E. Single cell densitometry and weightlessness culture of mesenchymal stem cells using magnetic levitation. Methods Mol Biol **2125,** 15, 2020.
231. Dutta, D., Heo, I., and Clevers, H. Disease modeling in stem cell-derived 3D organoid systems. Trends Mol Med **23,** 393, 2017.
232. Tseng, H., Daquinag, A.C., Souza, G.R., and Kolonin, M.G. Three-dimensional magnetic levitation culture system simulating white adipose tissue. Methods Mol Biol **1773,** 147, 2018.
233. Bagheri-Hosseinabadi, Z., Seyedi, F., Mollaei, H.R., Moshrefi, M., and Seifalian, A. Combination of 5-azaytidine and hanging drop culture convert fat cell into cardiac cell. Biotechnol Appl Biochem **68,** 92, 2021.
234. Salehi, H., Razavi, S., Esfandiari, E., Kazemi, M., Amini, S., and Amirpour, N. Application of hanging drop culture for retinal precursor-like cells differentiation of human adipose-derived stem cells using small molecules. J Mol Neurosci **69,** 597, 2019.
235. Lawrence, L.M., Cottrill, A., Valluri, A., *et al*. Minimally manipulative method for the expansion of human bone marrow mesenchymal stem cells to treat osseous defects. Int J Mol Sci **20,** 612, 2019.
236. Kondo, S., Nakagawa, Y., Mizuno, M., *et al*. Transplantation of aggregates of autologous synovial mesenchymal stem cells for treatment of cartilage defects in the femoral condyle and the femoral groove in microminipigs. Am J Sports Med **47,** 2338, 2019.
237. Ji, K., Sameni, M., Osuala, K., Moin, K., Mattingly, R.R., and Sloane, B.F. Spatio-temporal modeling and live-cell imaging of proteolysis in the 4D microenvironment of breast cancer. Cancer Metastasis Rev **38,** 445, 2019.
238. Miao, S., Cui, H., Esworthy, T., *et al*. 4D self-morphing culture substrate for modulating cell differentiation. Adv Sci (Weinh) **7,** 1902403, 2020.
239. Nies, C., Rubner, T., Lorig, H., *et al*. A microcavity array-based 4D cell culture platform. Bioengineering (Basel) **6,** 50, 2019.
240. Qasim, M., Le, N.X.T., Nguyen, T.P.T., Chae, D.S., Park, S.J., and Lee, N.Y. Nanohybrid biodegradable scaffolds for TGF-β3 release for the chondrogenic differentiation of human mesenchymal stem cells. Int J Pharm **581,** 119248, 2020.
241. Chen, S., Wu, C., Liu, A., *et al*. Biofabrication of nerve fibers with mimetic myelin sheath-like structure and aligned fibrous niche. Biofabrication **3,** 035013, 2020.
242. Malek-Khatabi, A., Javar, H.A., Dashtimoghadam, E., Ansari, S., Hasani-Sadrabadi, M.M., and Moshaverinia, A. In situ bone tissue engineering using gene delivery nanocomplexes. Acta Biomater **108,** 326, 2020.
243. Ashammakhi, N., Darabi, M.A., Çelebi-Saltik, B., *et al*. Microphysiological systems: next generation systems for assessing toxicity and therapeutic effects of nanomaterials. Small Methods **4,** 1900589, 2020.
244. Bhatia, S.N., and Ingber, D.E. Microfluidic organs-on-chips. Nat Biotechnol **32,** 760, 2014.
245. Ashammakhi, N., Elkhammas, E., and Hasan, A. Translating advances in organ-on-a-chip technology for supporting organs. J Biomed Mater Res B Appl Biomater **107,** 2006, 2019.

246. Sontheimer-Phelps, A., Hassell, B.A., and Ingber, D.E. Modelling cancer in microfluidic human organs-on-chips. Nat Rev Cancer **19,** 65, 2019.
247. Zheng, F., Fu, F., Cheng, Y., Wang, C., Zhao, Y., and Gu, Z. Organ-on-a-chip systems: microengineering to biomimic living systems. Small **12,** 2253, 2016.
248. Brougham, C.M., Levingstone, T.J., Shen, N.A., *et al*. Freeze-drying as a novel biofabrication method for achieving a controlled microarchitecture within large, complex natural biomaterial scaffolds. Adv Healthc Mater **6,** 2017. DOI: 10.1002/adhm.201700598.
249. Prasad, A., Sankar, M.R., and Katiyar, V. State of art on solvent casting particulate leaching method for orthopedic scaffolds fabrication. Mater Today **4,** 898, 2017.
250. Eltom, A., Zhong, G.Y., and Muhammad, A. Scaffold techniques and designs in tissue engineering functions and purposes: a review. Adv Mater Sci Eng **2019,** 3429527, 2019.
251. Dutta, R.C., Dey, M., Dutta, A.K., and Basu, B. Competent processing techniques for scaffolds in tissue engineering. Biotechnol Adv **35,** 240, 2017.
252. Hull, C.W., inventor; UVP, Inc., assignee. Apparatus for production of three-dimensional objects by stereolithography. USA Patent 4,575,330 1986.
253. Vijayavenkataraman, S., Yan, W.C., Lu, W.F., Wang, C.H., and Fuh, J.Y.H. 3D bioprinting of tissues and organs for regenerative medicine. Adv Drug Deliv Rev **132,** 296, 2018.
254. Mandrycky, C., Wang, Z.J., Kim, K., and Kim, D.H. 3D bioprinting for engineering complex tissues. Biotechnol Adv **34,** 422, 2016.
255. Belk L TN, MacDonald, H., Erdem, A., Ashammakhi, N., and Pountos, I. Safety considerations in 3D bioprinting using Mesenchymal Stem Cells. Front Bioeng Biotechnol **8,** 924, 2020.
256. Paul, K., Darzi, S., McPhee, G., *et al*. 3D bioprinted endometrial stem cells on melt electrospun poly ε-caprolactone mesh for pelvic floor application promote anti-inflammatory responses in mice. Acta Biomater **97,** 162, 2019.
257. Merceron, T.K., Burt, M., Seol, Y.J., *et al*. A 3D bioprinted complex structure for engineering the muscle-tendon unit. Biofabrication **7,** 035003, 2015.
258. Kang, H.W., Lee, S.J., Ko, I.K., Kengla, C., Yoo, J.J., and Atala, A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. Nat Biotechnol **34,** 312, 2016.
259. Jessop, Z.M., Al-Sabah, A., Gardiner, M.D., Combellack, E., Hawkins, K., and Whitaker, I.S. 3D bioprinting for reconstructive surgery: principles, applications and challenges. J Plast Reconstr Aesthet Surg **70,** 1155, 2017.
260. Kahl, M., Gertig, M., Hoyer, P., Friedrich, O., and Gilbert, D.F. Ultra-low-cost 3D bioprinting: modification and application of an off-the-shelf desktop 3D-printer for biofabrication. Front Bioeng Biotechnol **7,** 184, 2019.
261. Fulco, I., Miot, S., Haug, M.D., *et al*. Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial. Lancet **384,** 337, 2014.
262. Nerem, R.M., and Seliktar, D. Vascular tissue engineering. Annu Rev Biomed Eng **3,** 225, 2001.
263. Ashammakhi, N., Ahadian, S., Fan, Z.J., *et al*. Advances and future perspectives in 4D bioprinting. Biotechnol J **13,** e1800148, 2018.
264. Ashammakhi, N., Ahadian, S., Pountos, I., *et al*. In situ three-dimensional printing for reparative and regenerative therapy. Biomed Microdevices **21,** 42, 2019.
265. Ying, G., Manríquez, J., Wu, D., *et al*. An open-source handheld extruder loaded with pore-forming bioink for in situ wound dressing. Mater Today Bio **8,** 100074, 2020.
266. Lei, Z.Y., and Wu, P.Y. A supramolecular biomimetic skin combining a wide spectrum of mechanical properties and multiple sensory capabilities. Nat Commun **9,** 1134, 2018.
267. Potyondy, T.U.J., Tebon, P.J., Byambaa, B., *et al*. Recent advances in 3D bioprinting of musculoskeletal tissues. Biofabrication 2020 [Epub ahead of print]; DOI: 10.1088/1758-5090/abc8de.
268. Liang, C.Y., Hu, Y.C., Wang, H.S., *et al*. Biomimetic cardiovascular stents for in vivo re-endothelialization. Biomaterials **103,** 170, 2016.
269. Khoyratee, F., Grassia, F., Saighi, S., and Levi, T. Optimized real-time biomimetic neural network on FPGA for bio-hybridization. Front Neurosci **13,** 377, 2019.
270. Jain, A., and Bansal, R. Applications of regenerative medicine in organ transplantation. J Pharm Bioallied Sci **7,** 188, 2015.
271. Ashammakhi, N., Ahadian, S., Xu, C., *et al*. Bioinks and bioprinting technologies to make heterogeneous and biomimetic tissue constructs. Mater Today Bio **1,** 100008, 2019.
272. Tamay, D.G., Dursun Usal, T., Alagoz, A.S., Yucel, D., Hasirci, N., and Hasirci, V. 3D and 4D printing of polymers for tissue engineering applications. Front Bioeng Biotechnol **7,** 164, 2019.
273. Miao, S., Cui, H., Nowicki, M., *et al*. Stereolithographic 4D bioprinting of multiresponsive architectures for neural engineering. Adv Biosyst **2,** 1800101, 2018.
274. Cui, H.T., Nowicki, M., Fisher, J.P., and Zhang, L.G. 3D bioprinting for organ regeneration. Adv Healthc Mater **6,** 10.1002/adhm.201601118, 2017.
275. Dong Nyoung, H., Bugra, A., Madhuri, D., *et al*. Aspiration-assisted bioprinting of co-cultured osteogenic spheroids for bone tissue engineering. Biofabrication 2020 [Epub ahead of print]; DOI: 10.1088/1758-5090/abc1bf.
276. Ayan, B., Celik, N., Zhang, Z., *et al*. Aspiration-assisted freeform bioprinting of pre-fabricated tissue spheroids in a yield-stress gel. Commun Phys **3,** 183, 2020.
277. Yang, Y., Nikkola, L., Ylikauppila, H., and Ashammakhi N, eds. Investigation of cell attachment on the scaffolds manufactured by electrospun PCL-hyaluronan blend. TNT2006 ''Trends in Nanotechnology'' Conference 2006; Grenoble, France, 2006.
278. Will, O.M., Purcz, N., Chalaris, A., *et al*. Increased survival rate by local release of diclofenac in a murine model of recurrent oral carcinoma. Int J Nanomedicine **11,** 5311, 2016.
279. GhavamiNejad, A., Unnithan, A.R., Ramachandra, A., *et al*. Mussel-inspired electrospun nanofibers functionalized with size-controlled silver nanoparticles for wound dressing application. ACS Appl Mater Interfaces **7,** 12176, 2015.
280. Bhardwaj, N., and Kundu, S.C. Electrospinning: a fascinating fiber fabrication technique. Biotechnol Adv **28,** 325, 2010.
281. Chee, B.S., de Lima, G.G., Devine, D., and Nugent, M.J.D. Electrospun hydrogels composites for bone tissue engineering. Applications of Nanocomposite Materials in Orthopedics. 2019, pp. 39–70. DOI: 10.1016/B978-0-12-813740-6.00003-X.

282. Chen, W.M., Xu, Y., Liu, Y.Q., *et al*. Three-dimensional printed electrospun fiber-based scaffold for cartilage regeneration. Mater Des **179,** 107886, 2019.
283. Frost, H.K., Andersson, T., Johansson, S., *et al*. Electrospun nerve guide conduits have the potential to bridge peripheral nerve injuries in vivo (vol 8, 16716, 2018). Sci Rep **9,** 10017, 2019.
284. Karkan, S.F., Davaran, S., Rahbarghazi, R., Salehi, R., and Akbarzadeh, A. Electrospun nanofibers for the fabrication of engineered vascular grafts. J Biol Eng **13,** 83, 2019.
285. Göttel, B., de Souza e Silva, J.M., Santos de Oliveira, C., *et al*. Electrospun nanofibers—a promising solid in-situ gelling alternative for ocular drug delivery. Eur J Pharm Biopharm **146,** 125, 2020.
286. GhavamiNejad, A., Sasikala, A.R.K., Unnithan, A.R., *et al*. Mussel-inspired electrospun smart magnetic nanofibers for hyperthermic chemotherapy. Adv Funct Mater **25,** 2867, 2015.
287. Aguilar, L.E., GhavamiNejad, A., Park, C.H., and Kim, C.S. On-demand drug release and hyperthermia therapy applications of thermoresponsive poly-(NIPAAm-co-HMAAm)/polyurethane core-shell nanofiber mat on non-vascular nitinol stents. Nanomed Nanotechnol **13,** 527, 2017.
288. Nagiah, N., Murdock, C.J., Bhattacharjee, M., Nair, L., and Laurencin, C.T. Development of tripolymeric triaxial electrospun fibrous matrices for dual drug delivery applications. Sci Rep **10,** 609, 2020.
289. Casanova, M.R., Reis, R.L., Martins, A., and Neves, N.M. The use of electrospinning technique on osteochondral tissue engineering. In: Oliveira, J.M., Reis, S.P.R.L., and Roman, J.S., eds. Osteochondral Tissue Engineering: nanotechnology, Scaffolding-Related Developments and Translation. vol. 1058, 2018. New York City: Springer International Publishing, pp. 247–263.
290. Guo, Y., Gilbert-Honick, J., Somers, S.M., Mao, H.-Q., and Grayson, W.L. Modified cell-electrospinning for 3D myogenesis of C2C12s in aligned fibrin microfiber bundles. Biochem Biophys Res Commun **516,** 558, 2019.
291. Maver, T., Smrke, D.M., Kurecic, M., Gradisnik, L., Maver, U., and Kleinschek, K.S. Combining 3D printing and electrospinning for preparation of pain-relieving wound-dressing materials. J Solgel Sci Technol **88,** 33, 2018.
292. Yu, Y.X., Hua, S., Yang, M.K., *et al*. Fabrication and characterization of electrospinning/3D printing bone tissue engineering scaffold. RSC Adv **6,** 110557, 2016.
293. Chen, Y.J., Shafiq, M., Liu, M.Y., Morsi, Y., and Mo, X.M. Advanced fabrication for electrospun three-dimensional nanofiber aerogels and scaffolds. Bioact Mater **5,** 963, 2020.
294. Vogel, R., Tandler, B., Haussler, L., Jehnichen, D., and Brunig, H. Melt spinning of poly(3-hydroxybutyrate) fibers for tissue engineering using alpha-cyclodextrin/polymer inclusion complexes as the nucleation agent. Macromol Biosci **6,** 730, 2006.
295. Park, J.-S. Electrospinning and its applications. Adv Nat Sci Nanosci Nanotechnol **1,** 043002, 2011.
296. Bisht, G.S., Canton, G., Mirsepassi, A., *et al*. Controlled continuous patterning of polymeric nanofibers on three-dimensional substrates using low-voltage near-field electrospinning. Nano Lett **11,** 1831, 2011.
297. Hochleitner, G., Jungst, T., Brown, T.D., *et al*. Additive manufacturing of scaffolds with sub-micron filaments via melt electrospinning writing. Biofabrication **7,** 035002, 2015.
298. Brown, T.D., Dalton, P.D., and Hutmacher, D.W. direct writing by way of melt electrospinning. Adv Mater **23,** 5651, 2011.
299. Costa, P.F., Vaquette, C., Zhang, Q., Reis, R.L., Ivanovski, S., and Hutmacher, D.W. Advanced tissue engineering scaffold design for regeneration of the complex hierarchical periodontal structure. J Clin Periodontol **41,** 283, 2014.
300. Großhaus, C., Bakirci, E., Berthel, M., *et al*. Melt electrospinning of nanofibers from medical-grade poly(ε-caprolactone) with a modified nozzle. Small **16,** 2003471, 2020.
301. Davidson, M.D., Ban, E., Schoonen, A.C.M., *et al*. Mechanochemical adhesion and plasticity in multifiber hydrogel networks. Adv Mater **32,** 1905719, 2020.
302. Saveh-Shemshaki, N., S.Nair, L., and Laurencin, C.T. Nanofiber-based matrices for rotator cuff regenerative engineering. Acta Biomater **94,** 64, 2019.
303. Yu, J.R., Navarro, J., Coburn, J.C., *et al*. Current and future perspectives on skin tissue engineering: key features of biomedical research, translational assessment, and clinical application. Adv Healthc Mater **8,** e1801471, 2019.
304. O'Donnell, B.T., Ives, C.J., Mohiuddin, O.A., and Bunnell, B.A. Beyond the present constraints that prevent a wide spread of tissue engineering and regenerative medicine approaches. Front Bioeng Biotechnol **7,** 95, 2019.
305. Chandra, P.K., Soker, S., and Atala, A. Chapter 1—Tissue engineering: current status and future perspectives. In: Lanza, R., Langer, R., Vacanti, J.P., and Atala, A., eds. Principles of Tissue Engineering (Fifth Edition). London, UK: Academic Press, 2020, pp. 1–35.
306. Colombo, F., Sampogna, G., Cocozza, G., Guraya, S., and Forgione, A. Regenerative medicine: clinical applications and future perspectives. J Microsc Ultrastruct **5,** 1, 2017.
307. Yano, K., Speidel, A.T., and Yamato, M. Four Food and Drug Administration draft guidance documents and the REGROW Act: a litmus test for future changes in human cell- and tissue-based products regulatory policy in the United States? J Tissue Eng Regen Med **12,** 1579, 2018.
308. Mao, A.S., and Mooney, D.J. Regenerative medicine: current therapies and future directions. Proc Natl Acad Sci U S A **112,** 14452, 2015.
309. Pahon, E. FDA Approves Integra Omnigraft Dermal Regeneration Matrix to Treat Diabetic Foot Ulcers. US Food and Drug Administration, 2016. Updated January 7, 2016; accessed November 1, 2020. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-integra-omnigraft-dermal-regeneration-matrix-treat-diabetic-foot-ulcers
310. Anonymous. Premarket Approval: INFUSE Bone Graft/LT-CAGE Lumbar Tapered Fusion Device: Food and Drud Adimistration; 2002. Accessed November 1, 2020. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P000058
311. Kimmel, H., and Gittleman, H. Retrospective observational analysis of the use of an architecturally unique dermal regeneration template (Derma Pure®) for the treatment of hard-to-heal wounds. Int Wound J **14,** 666, 2017.
312. Means, K.R., Jr., Rinker, B.D., Higgins, J.P., Payne, S.H., Jr., Merrell, G.A., and Wilgis, E.F. A multicenter, pro-

spective, randomized, pilot study of outcomes for digital nerve repair in the hand using hollow conduit compared with processed allograft nerve. Hand (N Y) **11,** 144, 2016.
313. Anonymous. The INSPIRE Study: Probable Benefit of the Neuro-Spinal Scaffold for Treatment of AIS A Thoracic Acute Spinal Cord Injury. US National Library of Medicine, 2014. Updated December 18, 2019; accessed November 1, 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT02138110
314. Anonymous. A Study of VentriGel in Post-MI Patients. US National Library of Medicine, 2014. Updated October 25, 2019; accessed November 1, 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT02305602
315. Anonymous. Musculotendinous Tissue Repair Unit and Reinforcement (MTURR). US National Library of Medicine, 2011. Updated August 14, 2017; accessed November 1, 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT01292876
316. Dahl, S.L., Kypson, A.P., Lawson, J.H., *et al*. Readily available tissue-engineered vascular grafts. Sci Transl Med **3,** 68ra9, 2011.
317. Anonymous. Humacyte Human Acellular Vessel (HAV) in Patients with Vascular Trauma. ClinicalTrials.gov, 2016. Updated October 12, 2020; accessed November 8, 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT03005418
318. Jiang, S., Guo, W., Tian, G., *et al*. Clinical application status of articular cartilage regeneration techniques: tissue-engineered cartilage brings new hope. Stem Cells Int **2020,** 5690252, 2020.
319. Azam, A., Forster, M., and Robertson, A. Clinical and radiological outcome for Trufit Plug in the treatment of chondral and osteochondral lesions at a minimum of 2years. J Orthop **15,** 47, 2018.
320. Hindle, P., Hendry, J.L., Keating, J.F., and Biant, L.C. Autologous osteochondral mosaicplasty or TruFit plugs for cartilage repair. Knee Surg Sports Traumatol Arthrosc **22,** 1235, 2014.
321. Bugelli, G., Ascione, F., Dell'Osso, G., Zampa, V., and Giannotti, S. Biphasic bioresorbable scaffold (TruFit®) in knee osteochondral defects: 3-T MRI evaluation of osteointegration in patients with a 5-year minimum follow-up. Musculoskelet Surg **102,** 191, 2018.
322. Di Cave, E., Versari, P., Sciarretta, F., Luzon, D., and Marcellini, L. Biphasic bioresorbable scaffold (TruFit Plug®) for the treatment of osteochondral lesions of talus: 6- to 8-year follow-up. Foot **33,** 48, 2017.
323. Berruto, M., Delcogliano, M., de Caro, F., *et al*. Treatment of large knee osteochondral lesions with a biomimetic scaffold: results of a multicenter study of 49 patients at 2-year follow-up. Am J Sports Med **42,** 1607, 2014.
324. D'Ambrosi, R., Valli, F., De Luca, P., Ursino, N., and Usuelli, F.G. MaioRegen osteochondral substitute for the treatment of knee defects: a systematic review of the literature. J Clin Med **8,** 783, 2019.
325. Anonymous. Premarket approval (PMA): transcyte human fibroblast-derived temporary skin substitute. Food and Drug Administration, 1998. Accessed November 1, 2020. Available from: www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P960007
326. Noordenbos, J., Doré, C., and Hansbrough, J.F. Safety and efficacy of TransCyte for the treatment of partial-thickness burns. J Burn Care Rehabil **20,** 275, 1999.
327. Wilkins, L.M., Watson, S.R., Prosky, S.J., Meunier, S.F., and Parenteau, N.L. Development of a bilayered living skin construct for clinical applications. Biotechnol Bioeng **43,** 747, 1994.
328. Zaulyanov, L., and Kirsner, R.S. A review of a bi-layered living cell treatment (Apligraf) in the treatment of venous leg ulcers and diabetic foot ulcers. Clin Interv Aging **2,** 93, 2007.
329. Marston, W.A. Dermagraft, a bioengineered human dermal equivalent for the treatment of chronic nonhealing diabetic foot ulcer. Expert Rev Med Devices **1,** 21, 2004.
330. Still, J., Glat, P., Silverstein, P., Griswold, J., and Mozingo, D. The use of a collagen sponge/living cell composite material to treat donor sites in burn patients. Burns **29,** 837, 2003.
331. Caravaggi, C., De Giglio, R., Pritelli, C., *et al*. HYAFF 11-based autologous dermal and epidermal grafts in the treatment of noninfected diabetic plantar and dorsal foot ulcers. A prospective, multicenter, controlled, randomized clinical trial. Diabetes Care **26,** 2853, 2003.
332. Uccioli, L. A clinical investigation on the characteristics and outcomes of treating chronic lower extremity wounds using the tissuetech autograft system. Int J Low Extrem Wounds **2,** 140, 2003.
333. Min, J.H., Yun, I.S., Lew, D.H., Roh, T.S., and Lee, W.J. The use of matriderm and autologous skin graft in the treatment of full thickness skin defects. Arch Plast Surg **41,** 330, 2014.
334. Vig, K., Chaudhari, A., Tripathi, S., *et al*. Advances in skin regeneration using tissue engineering. Int J Mol Sci **18,** 789, 2017.
335. Ghiroldi, A., Piccoli, M., Cirillo, F., *et al*. Cell-based therapies for cardiac regeneration: a comprehensive review of past and ongoing strategies. Int J Mol Sci **19,** 3194, 2018.
336. Therapeutic Apheresis. https://www.terumobct.com/Pages/Therapeutic%20Apheresis/Spectra%20Optia/Not-Approved-for-US.aspx (accessed August 4, 2021).
337. Davidson, P.M., Newton, P.J., Tankumpuan, T., Paull, G., and Dennison-Himmelfarb, C. Multidisciplinary management of chronic heart failure: principles and future trends. Clin Ther **37,** 2225, 2015.
338. Ossendorf, C., Kaps, C., Kreuz, P.C., Burmester, G.R., Sittinger, M., and Erggelet, C. Treatment of posttraumatic and focal osteoarthritic cartilage defects of the knee with autologous polymer-based three-dimensional chondrocyte grafts: 2-year clinical results. Arthritis Res Ther **9,** R41, 2007.
339. Brix, M.O., Stelzeneder, D., Trattnig, S., Windhager, R., and Domayer, S.E. Cartilage repair of the knee with Hyalograft C:(R) Magnetic Resonance Imaging assessment of the glycosaminoglycan content at midterm. Int Orthop **37,** 39, 2013.
340. Kreuz, P.C., Müller, S., Freymann, U., *et al*. Repair of focal cartilage defects with scaffold-assisted autologous chondrocyte grafts: clinical and biomechanical results 48months after transplantation. Am J Sports Med **39,** 1697, 2011.
341. Wylie, J.D., Hartley, M.K., Kapron, A.L., Aoki, S.K., and Maak, T.G. Failures and reoperations after matrix-assisted cartilage repair of the knee: a systematic review. Arthroscopy **32,** 386, 2016.
342. Schneider, U., Rackwitz, L., Andereya, S., *et al*. A prospective multicenter study on the outcome of type I collagen hydrogel-based autologous chondrocyte implantation

(CaReS) for the repair of articular cartilage defects in the knee. Am J Sports Med **39,** 2558, 2011.
343. Crawford, D.C., DeBerardino, T.M., and Williams, R.J., 3rd. NeoCart, an autologous cartilage tissue implant, compared with microfracture for treatment of distal femoral cartilage lesions: an FDA phase-II prospective, randomized clinical trial after two years. J Bone Joint Surg Am **94,** 979, 2012.
344. Gaissmaier, C.F.J., Schewe, B., Albrecht, D., *et al*. Development of NOVOCART® 3D, a novel system of scaffold augmented transplantation of autologous chondrocytes. In: Zanasi, S., Brittberg, M., and Marcacci, M., eds. Basic Science, Clinical Repair and Reconstruction of Articular Cartilage Defects: Current Status and Prospects. Bologna, Italy: Timeo Editore s.r.l., 2006, pp. 573–585.
345. Anonymous. Non-Interventional Study to Evaluate Safety and Efficacy of NOVOCART 3D in Patients with Cartilage Defects (NISANIK). ClinicalTrials.gov, 2015. Updated December 3, 2019; accessed November 8, 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT02348697
346. Anonymous. Non-Interventional Study with NOVOCART® 3D for the Treatment of Cartilage Defects of the Knee in Pediatric Patients (JUNOVO). ClinicalTrials.gov, 2017. Updated March 31, 2020; accessed November 8, 2020. Available from: https://clinicaltrials.gov/ct2/history/NCT04186208?V_2=View#StudyPageTop
347. Anonymous. Phase III Study to Evaluate Safety and Effectiveness of NOVOCART 3D Plus vs. Microfracture in Knee Cartilage Defects (N3D). ClinicalTrials.gov, 2012. Updated August 18, 2020; accessed November 8, 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT01656902
348. Anonymous. NOVOCART 3D Treatment Following Microfracture Failure. ClinicalTrials.gov, 2017. Updated January 31, 2019; accessed November 8, 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT03219307
349. Anonymous. NOVOCART®3D for Treatment of Articular Cartilage of the Knee (N3D). ClinicaTtrials.gov, 2013. Updated October 24, 2019; accessed November 8, 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT01957722
350. Müller, P.E., Gallik, D., Hammerschmid, F., *et al*. Third-generation autologous chondrocyte implantation after failed bone marrow stimulation leads to inferior clinical results. Knee Surg Sports Traumatol Arthrosc **28,** 470, 2020.
351. Niethammer, T.R., Holzgruber, M., Gülecyüz, M.F., Weber, P., Pietschmann, M.F., and Müller, P.E. Matrix based autologous chondrocyte implantation in children and adolescents: a match paired analysis in a follow-up over three years post-operation. Int Orthop **41,** 343, 2017.
352. Baiguera, S., Jungebluth, P., Burns, A., *et al*. Tissue engineered human tracheas for in vivo implantation. Biomaterials **31,** 8931, 2010.
353. Elliott, M.J., De Coppi, P., Speggiorin, S., *et al*. Stem-cell-based, tissue engineered tracheal replacement in a child: a 2-year follow-up study. Lancet **380,** 994, 2012.
354. Atala, A., Bauer, S.B., Soker, S., Yoo, J.J., and Retik, A.B. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet **367,** 1241, 2006.
355. Joseph, D.B., Borer, J.G., De Filippo, R.E., Hodges, S.J., and McLorie, G.A. Autologous cell seeded biodegradable scaffold for augmentation cystoplasty: phase II study in children and adolescents with spina bifida. J Urol **191,** 1389, 2014.
356. Stenzl, A., Ninkovic, M., Ashammakhi, N., Eder, I.E., and Bartsch, G. [Reconstruction of the lower urinary tract. Developments at the beginning of a new century]. Urologe A **40,** 368, 2001.
357. Shin'oka T, Imai, Y., and Ikada, Y. Transplantation of a tissue-engineered pulmonary artery. N Engl J Med **344,** 532, 2001.
358. Kurobe, H., Maxfield, M.W., Breuer, C.K., and Shinoka, T. Concise review: tissue-engineered vascular grafts for cardiac surgery: past, present, and future. Stem Cells Transl Med **1,** 566, 2012.
359. Pellegrini, G., Ardigò, D., Milazzo, G., *et al*. Navigating market authorization: the path holoclar took to become the first stem cell product approved in the european union. Stem Cells Transl Med **7,** 146, 2018.
360. Anonymous. Europe approves Holoclar®, the first stem cell-based medicinal product. EuroStemCell. Accessed November 1, 2020. Available from: https://www.eurostemcell.org/story/europe-approves-holoclar-first-stem-cell-based-medicinal-product
361. Anonymous. Transplantation of iPSC-derived RPE sheet into first AMD patient. Riken Center for Developmental Biology, 2014. Accessed November 1, 2020. Available from: http://www.cdb.riken.jp/en/news/2014/researches/0915_3047.html
362. Schwartz, S.D., Regillo, C.D., Lam, B.L., Eliott, D., Rosenfeld, P.J., Gregori, N.Z., Hubschman, J.P., Davis, J.L., Heilwell, G., Spirn, M., Maguire, J., Gay, R., Bateman, J., Ostrick, R.M., Morris, D., Vincent, M., Anglade, E., Del Priore, L.V., Lanza, R. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. Lancet **385,** 509, 2015.
363. Murphy, S.V., De Coppi, P., and Atala, A. Opportunities and challenges of translational 3D bioprinting. Nat Biomed Eng **4,** 370, 2020.
364. Hunsberger, J., Harrysson, O., Shirwaiker, R., *et al*. Manufacturing road map for tissue engineering and regenerative medicine technologies. Stem Cell Transl Med **4,** 130, 2015.
365. Evans, C.H. Barriers to the clinical translation of orthopedic tissue engineering. Tissue Eng Part B Rev **17,** 437, 2011.
366. Lu, L.C., Arbit, H.M., Herrick, J.L., Segovis, S.G., Maran, A., and Yaszemski, M.J. Tissue engineered constructs: perspectives on clinical translation. Ann Biomed Eng **43,** 796, 2015.
367. Webber, M.J., Khan, O.F., Sydlik, S.A., Tang, B.C., and Langer, R. A perspective on the clinical translation of scaffolds for tissue engineering. Ann Biomed Eng **43,** 641, 2015.
368. Chapekar, M.S. Tissue engineering: challenges and opportunities. J Biomed Mater Res **53,** 617, 2000.
369. Nerem, R.M. Cellular engineering. Ann Biomed Eng **19,** 529, 1991.
370. Chen, F.M., and Liu, X.H. Advancing biomaterials of human origin for tissue engineering. Prog Polym Sci **53,** 86, 2016.
371. Ghaemi, R.V., Siang, L.C., and Yadav, V.G. Improving the rate of translation of tissue engineering products. Adv Healthc Mater **8,** 1900538, 2019.

372. Barlas, S. The 21st Century Cures Act: FDA implementation one year later: some action, some results, some questions. P T **43,** 149, 2018.
373. Jain, R.K., Au, P., Tam, J., Duda, D.G., and Fukumura, D. Engineering vascularized tissue. Nat Biotechnol **23,** 821, 2005.
374. Schneider, M.K., Ioanas, H.-I., Xandry, J., and Rudin, M. An in vivo wound healing model for the characterization of the angiogenic process and its modulation by pharmacological interventions. Sci Rep **9,** 6004, 2019.
375. Shahabipour, F., Ashammakhi, N., Oskuee, R.K., *et al*. Key components of engineering vascularized 3-dimensional bioprinted bone constructs. Transl Res **216,** 57, 2020.
376. Rath, S.N., Pryymachuk, G., Bleiziffer, O.A., *et al*. Hyaluronan-based heparin-incorporated hydrogels for generation of axially vascularized bioartificial bone tissues: in vitro and in vivo evaluation in a PLDLLA-TCP-PCL-composite system. J Mater Sci Mater Med **22,** 1279, 2011.
377. Penttilä, H.T.R-M., Waris, T., Ellä, V., Kellomäki, M., Törmälä, P., and Ashammakhi, N., eds. Combining prefabricated microvascularied perichondrial flaps and bioabsorbable polylactide nonwoven scaffolds to tissue engineered cartilage. Joint Meeting of the Tissue Engineering Society International (TESI) and the European Tissue Engineering Society (ETES); 10–13.10.2004; Lausanne, Switzerland.
378. Ashammakhi, N., Darabi, M.A., Kehr, N.S., *et al*. Advances in controlled oxygen generating biomaterials for tissue engineering and regenerative therapy. Biomacromolecules **21,** 56, 2020.
379. Erdem, A., Darabi, M.A., Nasiri, R., *et al*. 3D bioprinting of oxygenated cell-laden gelatin methacryloyl constructs. Adv Healthc Mater **9,** 1901794, 2020.
380. Wust, S., Muller, R., and Hofmann, S. Controlled positioning of cells in biomaterials-approaches towards 3D tissue printing. J Funct Biomater **2,** 119, 2011.
381. Lee, J.W., Choi, Y.J., Yong, W.J., *et al*. Development of a 3D cell printed construct considering angiogenesis for liver tissue engineering. Biofabrication **8,** 015007, 2016.
382. Puumanen, K.A., Ruuskanen, M.M., Ashammakhi, N., *et al*. Tissue engineering of bone in muscle by using free periosteal grafts with a self-reinforced polyglycolide membrane scaffold. An experimental study in growing rabbits. Eur J Plast Surg **23,** 39, 2000.
383. Kawecki, F., Clafshenkel, W.P., Auger, F.A., *et al*. Self-assembled human osseous cell sheets as living biopapers for the laser-assisted bioprinting of human endothelial cells. Biofabrication **10,** 035006, 2018.
384. Cui, X., and Boland, T. Human microvasculature fabrication using thermal inkjet printing technology. Biomaterials **30,** 6221, 2009.
385. Tresoldi, C., Bianchi, E., Pellegata, A.F., Dubini, G., and Mantero, S. Estimation of the physiological mechanical conditioning in vascular tissue engineering by a predictive fluid-structure interaction approach. Comput Methods Biomech Biomed Engin **20,** 1077, 2017.
386. Guilak, F., Butler, D.L., Goldstein, S.A., and Baaijens, F.P.T. Biomechanics and mechanobiology in functional tissue engineering. J Biomech **47,** 1933, 2014.
387. McGovern, J.A., Griffin, M., and Hutmacher, D.W. Animal models for bone tissue engineering and modelling disease. Dis Model Mech **11,** dmm033084, 2018.
388. Boehler, R.M., Graham, J.G., and Shea, L.D. Tissue engineering tools for modulation of the immune response. Biotechniques **51,** 239, 2011.
389. Yuan, T., Li, K.F., Guo, L.K., Fan, H.S., and Zhang, X.D. Modulation of immunological properties of allogeneic mesenchymal stem cells by collagen scaffolds in cartilage tissue engineering. J Biomed Mater Res A **98a,** 332, 2011.
390. Sittinger, M., Bujia, J., Rotter, N., Reitzel, D., Minuth, W.W., and Burmester, G.R. Tissue engineering and autologous transplant formation: practical approaches with resorbable biomaterials and new cell culture techniques. Biomaterials **17,** 237, 1996.
391. Nikkola, L., Vapalahti, K., Huolman, R., Seppala, J., Harlin, A., and Ashammakhi, N. Multilayer implant with triple drug releasing properties. J Biomed Nanotechnol **4,** 331, 2008.
392. Piras AM CF, Chiellini, E., Nikkola, L., and Ashammakhi, N. New multicomponent bioerodible electrospun nanofibers for dual-controlled drug release. J Bioact Compat Polym **23,** 423, 2008.
393. Farah, S., Doloff, J.C., Müller, P., *et al*. Long-term implant fibrosis prevention in rodents and non-human primates using crystallized drug formulations. Nat Mater **18,** 892, 2019.
394. Washington, K.S., and Bashur, C.A. Delivery of antioxidant and anti-inflammatory agents for tissue engineered vascular grafts. Front Pharmacol **8,** 659, 2017.
395. Dias, J.R., Ribeiro, N., Baptista-Silva, S., *et al*. In situ enabling approaches for tissue regeneration: current challenges and new developments. Front Bioeng Biotechnol **8,** 85, 2020.
396. Sokolowska, P., Janikiewicz, J., Jastrzebska, E., Brzozka, Z., and Dobrzyn, A. Combinations of regenerative medicine and Lab-on-a-chip systems: new hope to restoring the proper function of pancreatic islets in diabetes. Biosens Bioelectron **167,** 112451, 2020.
397. Langer, R., and Vacanti, J. Advances in tissue engineering. J Pediatr Surg **51,** 8, 2016.
398. Luo, J.S., Qin, L.F., Zhao, L.P., *et al*. Tissue-engineered vascular grafts with advanced mechanical strength from human iPSCs. Cell Stem Cell **26,** 251, 2020.
399. Fragogeorgi, E.A., Rouchota, M., Georgiou, M., Velez, M., Bouziotis, P., and Loudos, G. In vivo imaging techniques for bone tissue engineering. J Tissue Eng **10,** 2041731419854586, 2019.
400. Kim, S.H., Park, J.H., Kwon, J.S., *et al*. NIR fluorescence for monitoring in vivo scaffold degradation along with stem cell tracking in bone tissue engineering. Biomaterials **258,** 120267, 2020.
401. Xu, Y.Y., Wu, X.Y., Guo, X., *et al*. The boom in 3D-printed sensor technology. Sensors (Basel) **17,** 1166, 2017.
402. Ashammakhi, N.H.A., Unluturk, B.D., Quintero, S.A., *et al*. Biodegradable implantable sensors: materials design, fabrication, and applications. Adv Funct Mater. 2021. DOI: 10.1002/adfm.202104149.
403. Lee, A., Hudson, A.R., Shiwarski, D.J., *et al*. 3D bioprinting of collagen to rebuild components of the human heart. Science **365,** 482, 2019.
404. Pountos, I., Tellisi, N., and Ashammakhi, N. Three-dimensional bioprinting: safety, ethical, and regulatory considerations. In: Guvendiren, M., ed. 3D Bioprinting in Medicine: Technologies, Bioinks, and Applications. Cham: Springer International Publishing, 2019, pp. 191–203.

405. Afshar, L. Ethical issues in perinatal tissue derivation and regenerative medicine. Stem Cells Biol Reg 229, 2016.
406. Mohammadi, D. The dangers of unregulated stem-cell marketing. Lancet **390,** 1823, 2017.
407. Seetapun, D., and Ross, J.J. Eliminating the organ transplant waiting list: the future with perfusion decellularized organs. Surgery **161,** 1474, 2017.
408. Hollister, S.J., and Murphy, W.L. Scaffold translation: barriers between concept and clinic. Tissue Eng Part B Rev **17,** 459, 2011.
409. Boyce, S.T., and Lalley, A.L. Tissue engineering of skin and regenerative medicine for wound care. Burns Trauma **6,** 4, 2018.
410. Urciuolo, A., Poli, I., Brandolino, L., *et al.* Intravital three-dimensional bioprinting. Nat Biomed Eng **4,** 901, 2020.
411. Maffioletti, S.M., Sarcar, S., Henderson, A.B.H., *et al.* Three-dimensional human iPSC-derived artificial skeletal muscles model muscular dystrophies and enable multilineage tissue engineering. Cell Rep **23,** 899, 2018.
412. Burrello, J., Monticone, S., Gai, C., Gomez, Y., Kholia, S., and Camussi, G. Stem cell-derived extracellular vesicles and immune-modulation. Front Cell Dev Biol **4,** 83, 2016.
413. Tellisi, N., Ashammakhi, N.A., Billi, F., and Kaarela, O. Three dimensional printed bone implants in the clinic. J Craniofac Surg **29,** 2363, 2018.
414. Abelseth, E., Abelseth, L., De la Vega, L., Beyer, S.T., Wadsworth, S.J., and Willerth, S.M. 3D printing of neural tissues derived from human induced pluripotent stem cells using a fibrin-based bioink. ACS Biomater Sci Eng **5,** 234, 2019.
415. Xu, C., Guan, S., Wang, S.P., *et al.* Biodegradable and electroconductive poly(3,4-ethylenedioxythiophene)/carboxymethyl chitosan hydrogels for neural tissue engineering. Mat Sci Eng C Mater **84,** 32, 2018.
416. Alizadeh, P.S.M., Tutar, R., Apu, E.H., Unluturk, B., Contag, C.H., and Ashammakhi, N. Use of electroconductive biomaterials for engineering tissues by 3D printing and 3D bioprinting. Essays Biochem **65,** 441, 2021.
417. Ashammakhi, N., Darabi, M.A., and Pountos, I. The dynamic cycle of future personalized and regenerative therapy. J Craniofac Surg **30,** 623, 2019.
418. Akyildiz, I.F., Chen, J., Ghovanloo, M., *et al.* Microbiome-gut-brain axis as a biomolecular communication network for the internet of bio-nanothings. IEEE Access **7,** 136161, 2019.
419. Tian, C., Tu, Q., Liu, W.M., and Wang, J.Y. Recent advances in microfluidic technologies for organ-on-a-chip. Trac-Trend Anal Chem **117,** 146, 2019.
420. Ashammakhi, N., Darabi, M.A., Çelebi-Saltik, B., *et al.* Therapeutic nanomaterials: microphysiological systems: next generation systems for assessing toxicity and therapeutic effects of nanomaterials (small methods 1/2020). Small Methods **4,** 2070001, 2020.
421. Zhang, K., Wang, S.P., Zhou, C.C., *et al.* Advanced smart biomaterials and constructs for hard tissue engineering and regeneration. Bone Res **6,** 31, 2018.
422. GhavamiNejad, A., Li, J., Lu, B., *et al.* Glucose-responsive composite microneedle patch for hypoglycemia-triggered delivery of native glucagon. Adv Mater **31,** e1901051, 2019.
423. GhavamiNejad, A., Lu, B., Giacca, A., and Wu, X.Y. Glucose regulation by modified boronic acid-sulfobetaine zwitterionic nanogels—a non-hormonal strategy for the potential treatment of hyperglycemia. Nanoscale **11,** 10167, 2019.
424. Nostro, M.C., and Keller, G. Generation of beta cells from human pluripotent stem cells: potential for regenerative medicine. Semin Cell Dev Biol **23,** 701, 2012.
425. Hoffman, T., Antovski, P., Tebon, P., *et al.* Synthetic biology and tissue engineering: toward fabrication of complex and smart cellular constructs. Adv Funct Mater **30,** 1909882, 2020.
426. Diltemiz, S.E.T.M., de Barros, N.R., Heinamaki, J., Seidlits, S., and Ashammakhi, N. Use of artificial cells as drug carriers. Mater Chem Front 2021, DOI: 10.1039/d1qm00717c.
427. Bradley, J.A., Bolton, E.M., and Pedersen, R.A. Stem cell medicine encounters the immune system. Nat Rev Immunol **2,** 859, 2002.
428. Ibarretxe, G., Alvarez, A., Cañavate, M.L., Hilario, E., Aurrekoetxea, M., and Unda, F. Cell reprogramming, IPS limitations, and overcoming strategies in dental bioengineering. Stem Cells Int **2012,** 365932, 2012.
429. Khademhosseini, A., Langer, R., Borenstein, J., and Vacanti, J.P. Microscale technologies for tissue engineering and biology. Proc Natl Acad Sci U S A **103,** 2480, 2006.
430. Tang, Z.L., Akiyama, Y., and Okano, T. Temperature-responsive polymer modified surface for cell sheet engineering. Polymers (Basel) **4,** 1478, 2012.
431. Uzel, S.G.M., Platt, R.J., Subramanian, V., *et al.* Microfluidic device for the formation of optically excitable, three-dimensional, compartmentalized motor units. Sci Adv **2,** e1501429, 2016.
432. Hong, J., Yeo, M., Yang, G.H., and Kim, G. Cell-electrospinning and its application for tissue engineering. Int J Mol Sci **20,** 6208, 2019.
433. Vyas, C., Ates, G., Aslan, E., Hart, J., Huang, B., and Barto, P. Three-dimensional printing and electrospinning dual-scale polycaprolactone scaffolds with low-density and oriented fibers to promote cell alignment. 3D Print Addit Manuf **7,** 105, 2020.

Address correspondence to:
Nureddin Ashammakhi, MD, PhD
Department of Bioengineering
Henry Samueli School of Engineering
University of California, Los Angeles
420 Westwood Plaza,
Engineering V
Los Angeles, CA 90095
USA

E-mail: n.ashammakhi@ucla.edu;
n.ashammakhi@gmail.com

Received: January 20, 2021
Accepted: July 15, 2021
Online Publication Date: October 26, 2021