PERSPECTIVE

A decade of progress in tissue engineering

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Tremendous progress has been achieved in the field of tissue engineering in the past decade. Several major challenges laid down 10 years ago, have been studied, including renewable cell sources, biomaterials with tunable properties, mitigation of host responses, and vascularization. Here we review advancements in these areas and envision directions of further development.

The aim of tissue engineering is to develop tissue and organ substitutes for maintaining, restoring or augmenting functions of their injured or diseased counterparts *in vivo*[1,2]. We have previously described a number of challenges that have hindered clinical applications of tissue engineering technology[2,3]. These limitations included a paucity of renewable sources of functional cells that are immunologically compatible; a lack of appropriate biomaterials with desired mechanical, chemical and biological properties; and an inability to generate large, vascularized tissues that can easily integrate into the host's circulatory system with the architectural complexity of native tissues. Over the past decade, the field of tissue engineering has witnessed tremendous progress toward overcoming these challenges as a result of our improved understanding of biology, materials science, chemistry and engineering strategies, and the convergence of these disciplines (**Fig. 1** and **Box 1**).

In this Perspective we focus on improved methodology for tissue engineering that has been achieved as a result of progress in the following areas (**Fig. 2**).

- The discovery of methods to generate induced pluripotent stem cells (iPSCs), which has paved the way for personalized medicine[4,5].
- The finding that substrate stiffness can modulate stem cell differentiation, enabling new ways of controlling cell phenotypes using physical cues[6].
- Advanced chemistries that have enabled more efficient and versatile biomaterial conjugations, to achieve precise patterning of biomolecules and biomaterials in the presence of biological entities[7,8].
- Refined delivery mechanisms that enable biochemical cues such as growth factors and cytokines to be presented with improved bioavailability and bioactivity[9,10].
- Increased understanding of the interaction between foreign bodies and the body's immune surveillance system, which has promoted rational design of biomaterials to achieve mitigated inflammatory responses[11,12].
- The development of new biomaterials and scaffolds that has led to fabrication of better biomimetic tissues[1,13,14].
- Advances in biofabrication technologies including programmed self-assembly[15,16] and three-dimensional (3D) bioprinting[17–24], which have allowed generation of complex biological structures with integrated vasculature and multiple cell or extracellular matrix (ECM) types at high spatial resolution.

Advances in cell engineering

Our understanding of how cells can be reprogrammed has advanced considerably over the past decade and thus increased the available methods to reprogram cells. The landscape of the stem cell field has substantially changed since the discovery of iPSCs. Adult cells initially were reprogrammed into iPSCs by introducing a set of four specific genes encoding critical reprogramming factors (Oct4 and Sox2 with either c-Myc and Klf4 or Nanog and Lin28)[4,5]. Inducible pluripotency from many types of somatic cells has made autologous cell sources a likely solution to many tissue-engineering applications. As iPSCs can

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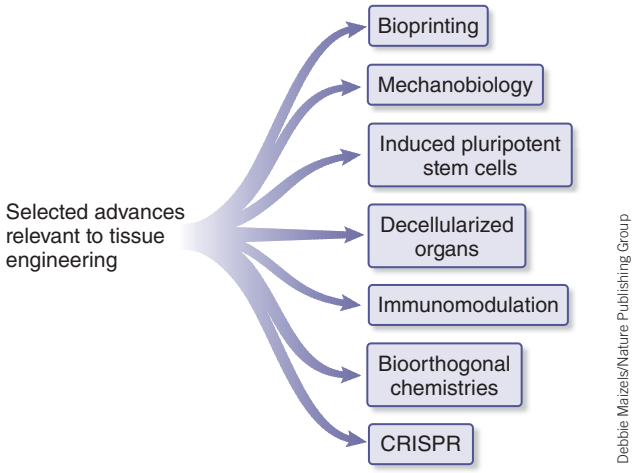

Figure 1 | Important advances in tissue engineering.

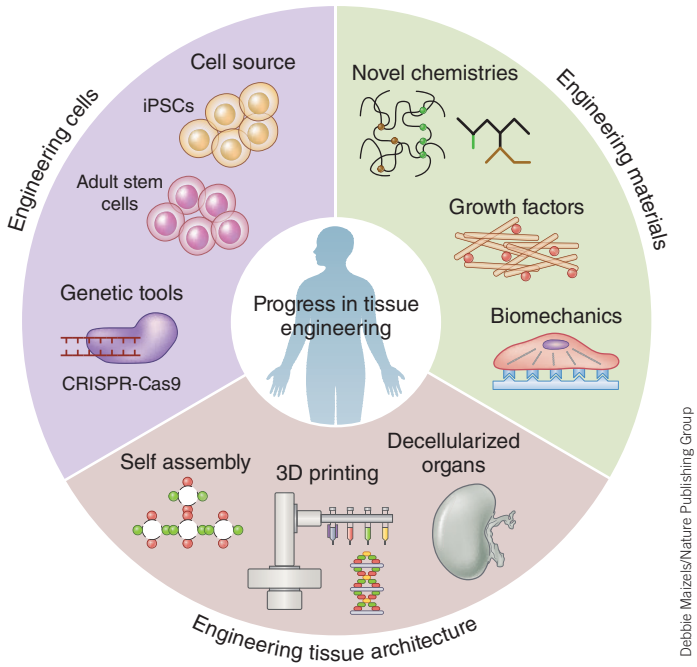

Figure 2 | Summary of tissue engineering progress in the past decade. Additional cell sources have become available, including iPSCs and adult stem cells, as well as genetic editing tools that enable greater cell manipulation. Improved chemistries and growth factor delivery mechanisms, as well as advances in understanding biophysical cues on cellular behaviors and tissue architecture technologies have contributed to engineering tissues of considerably improved structural, compositional and functional resemblance to their native counterparts.

be derived from patients easily, they potentially enable new approaches for personalized medicine, where an individual's own cells may be used to engineer and repair tissues. Furthermore, allogeneic iPSCs combined with immunoisolation capsules are also promising for use as a versatile source for treating diseases such as diabetes (for example, Viacyte, http://viacyte.com/).

Adult stem cell research has yielded several major breakthroughs, for example, the homing capability of mesenchymal stem cells (MSCs) has been identified as a powerful method of inducing tissue regeneration. These cells can be engineered to produce a pool of desired growth factors and cytokines beneficial for local wound healing or disease treatment[25–28]. Although such phenomenon has been confined to preclinical trials, we envision its future clinical translation in treating internal wounds and diseases that are not easily accessible by conventional strategies in a minimally invasive fashion. New adult stem cell sources such as adipose-derived stromal cells[29] and amniotic-fluid-derived stem cells[30] have been established as other renewable adult stem cells sources that can be differentiated into multiple lineages in a similar manner to MSCs.

Innovative methods of genetic manipulation of cells have also been developed, most notable being the clustered regularly interspaced short palindromic repeats (CRISPR) technology[31–34]. CRISPR technology allows specific targeting of DNA followed by cutting at a precise location to achieve genomic editing of mammalian cells with unprecedented ease and accuracy. We envision CRISPR technology and its variations to potentially change the landscape of personalized tissue engineering in the future to promote versatility of cell engineering and tissue modulation. Examples include efforts exerted on genetic editing of pig organs for potential human transplantation[35–37].

Active modulation of cell growth using biomaterials

Evolution of cell sources has demanded the development of advanced biomaterials to actively modulate cellular behaviors in terms of adhesion, proliferation, migration, differentiation and maturation. Over the past decade, advanced chemistries using strategies for conjugation of bioactive molecules have been proposed to improve the compatibility and activity of the biomaterials. For example, bioorthogonal click chemistry has contributed to substantial improvements in diversity and complexity of biomaterial formulations because of its extremely high selectivity, versatility, simplicity and yield[7,8,38]. These organic reactions can be conducted in biologically and physiologically relevant environments, allowing dynamic patterning of growth factors and manipulation of their availability and release kinetics in the presence of cells. Early protein delivery systems were based on reversible binding to heparin[9,39,40]. Application of rigorous *in vitro* selection processes and directed evolution[10] have facilitated the engineering of affinity-mediated release systems using a greater diversity of noncovalent forces, such as ionic, hydrophobic, van der Waals interactions and hydrogen bonding. The increasingly versatile and sophisticated biomolecule delivery systems could allow for orchestrated presentation of multiple proteins and growth factors in a manner resembling *in vivo* dynamics.

Physical forces have been used on many occasions in the past decade to regulate cell responses. Although early discoveries had been based on planar substrates with various stiffness[6,41], the field has rapidly progressed to the use of 3D matrices to more accurately direct lineage specification of stem cells[42]. Dynamic modulation of the matrices through cell-mediated degradation revealed that the differentiation of embedded stem cells is directed by the generation of localized cellular traction, and that this is independent of the overall matrix mechanics[43]. As with biochemical cues, it has been found that stem cells remember

BOX 1: GLOSSARY

Biofabrication. Any fabrication process that includes cells and/or bioactive molecules.
Bioprinting. A technique that relies on a motorized dispensing system to achieve fabrication of well-organized biological constructs typically involving live cells in three dimensions.
Sacrificial bioprinting. Bioprinting technique in which one biomaterial serves as a template embedded in a secondary material and is removed thereafter to construct hollow structures.
Embedded bioprinting. Bioprinting technique in which one biomaterial is directly deposited in a self-healing matrix for fabrication of freeform structures.
Bioorthogonal chemistry. Chemical conjugation that can occur in the presence of biological entities.
Mechanobiology. A field of science focusing on how physical forces and mechanics influence biological systems including the cells and tissues.
Immunomodulation. Set of approaches to tailor and modify the response of the immune system.
Clustered regularly interspaced short palindromic repeats (CRISPR). A targeted genome-editing tool with extremely high precision and efficiency.
Induced pluripotent stem cells (iPSCs). Stem cells generated directly from adult somatic cells by introducing a set of pluripotency-associated genes into cells, or through chemical reprogramming or protein delivery.
Hydrogel. A network of macromolecular polymers containing a large amount of water.
Decellularized organ. An organ processed to selectively remove its inhabiting cells, leaving only the extracellular matrix scaffold of the original organ.

past mechanical doses and can undergo either reversible (below the threshold) or irreversible (above the threshold) activation as a consequence of subsequent mechanical stimulus[44]. The accumulation of mechanobiology knowledge has led to a fundamentally different approach to modulate cell behavior compared to traditional strategies based on biochemistry.

Another major trend in the past decade has been the increased use of immunomodulation of biomaterial-host interactions, which is critical to achieve enhanced performance of implanted biomaterials. If left unmodulated, inflammatory responses of the innate host microenvironment usually initiate a cascade of cellular events leading to foreign body reactions that manifest as inflammation, formation of giant cells, fibrosis, and eventually damage to the implant and the surrounding host tissues[45,46]. Large libraries of formulations have been screened for the effect of size and chemistry on host immunity, in an attempt to mitigate the response to these formulations as a foreign substance. For example, the use of implanted spheres larger than 1.5 mm in diameter significantly reduced foreign body reactions and fibrosis compared to smaller spheres, and this was the case for a broad spectrum of biomaterials, including hydrogels, ceramics, metals and plastics, potentially owing to a lack of macrophage accumulation on large-sized spheres[11]. Furthermore, several triazole-containing analogs have been identified for modification of alginate, which substantially abrogated foreign body reactions in rodents and nonhuman primates by inhibiting the recognition of such analogs by macrophages[12]. Monocytes and macrophages have an essential role during integration of implanted tissues. In inflammation, macrophages can be distinguished into two subtypes, the M1 immune effecter cells that mainly produce proinflammatory cytokines and M2 macrophages that are commonly associated with an anti-inflammatory response[47,48]. As the roles of the M2 macrophages have become better understood, it has become clear that foreign body responses of implanted tissues may also be controlled by directing the phenotype of locally residing or recruited macrophages[49–51]. Therefore, harnessing the plasticity of macrophage subtypes has been proposed as one of the most promising methods to reduce proinflammatory response upon the induction of specific signals[52,53].

Engineering the architecture of tissue scaffolds

The ability to create tightly controlled 3D architectures for tissue engineering has progressed considerably owing to two major technologies; programmed modular self-assembly and 3D bioprinting. Programmed modular self-assembly provides a convenient way to construct sophisticated synthetic architectures[54,55]. Self-organizing structures can be achieved by using DNA strands with sequence complementarity that pair under appropriate physical conditions[56,57]. Such a concept has been translated to the macroscale, where biomaterial and tissue building blocks attached with specially designed programmable DNA glues can be induced to assemble across multiple length scales spanning from a few hundred micrometers up to centimeters[15]. Similarly, degradable DNA glues were conjugated to single cells to achieve programmed tissue assembly, after which the DNA linkers can be degraded using DNase to release the assembled tissue[16]. Although the nondegradable DNAs facilitate long-term assembly of the biomaterial building blocks into desired tissue architectures, the use of degradable DNAs is better suited for cases in which the living cells can actively fuse into an integral piece after assembly without further need for DNA to stabilize the structures.

Biofabrication approaches such as 3D bioprinting, an extension from existing 3D printing (i.e., deposition of noncellular materials[58–60]), offer unprecedented versatility to manipulate cells and biomolecules (e.g., proteins and ECMs) with precise control over composition and spatial distribution to

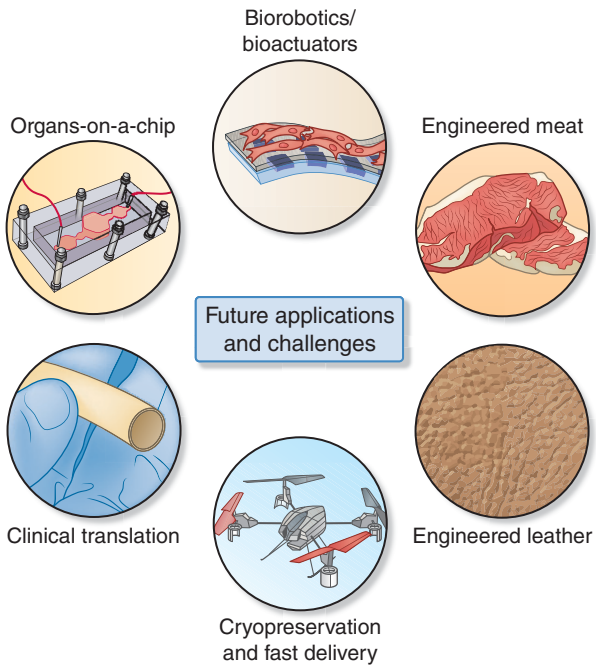

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Figure 3 | Future and challenges of tissue engineering: clinical translation of tissue engineering products, organs-on-a-chip and disease modeling, biorobotics/bioactuators, engineered meat and leather, and cryopreservation and fast delivery.

recapitulate the fine shape, structure and architecture of native tissues[17,18,21–24]. Since the debut of biofabrication technology in the form of cell-laden inkjet printing[61,62], development of this technology over the past decade has led to its widespread use in tissue engineering[21,63]. A wide variety of biomaterials can be used for bioprinting, enabling broad applicability to a myriad of tissue types. In particular, sacrificial bioprinting has made it possible to produce interconnected vascular networks in hydrogel matrices[19,20,64–66]. Embedded bioprinting supported direct fabrication of freeform shapes by preventing them from collapsing during the bioink deposition process due to gravity[67–69]. Bioprinters equipped with multiple nozzles extruding different biomaterials boosted the capacity to build complex tissues featuring spatial heterogeneity of cells and matrix compositions[18,24,70]. More recently, smart biomaterials that can evolve their shapes as a function of time in a prescribed manner upon externally applied stimuli such as humidity, pH and temperature, have been integrated to establish a new strategy termed four-dimensional (4D) bioprinting[71,72]. The unique extra dimension of time conferred by 4D bioprinting promises to bring dynamic temporal control in addition to the spatial hierarchy into fabricated tissues.

Natural tissue structures have also gained popularity as a source of scaffolds—for example, the use of decellularized tissues[73]. However, use of decellularized whole organs and their applications in whole-organ engineering were only developed about a decade ago[74–77]. In this approach, isolated donor organs are perfused with detergents to remove all cellular and immunogenic species while preserving the underlying ECM and potentially embedded vascular network. Desired cell types and/or stem cells (for example, iPSCs from patients) can be subsequently infused to repopulate these decellularized organs and render them functional. Using such a strategy, a variety of organs have been developed, including the blood vessels[78–80], heart[74,81], lung[82,83], liver[84,85], kidney[86,87], bladder[88] and pancreas[89]. Although the use of decellularized organs can maximally recapitulate the structural complexity of pristine organs and potentially their functionality, the limit of donor sources posts intrinsic limitations to the widespread application of the technology to organ transplant surgery.

Current and future potential applications of tissue engineering

Several tissue-engineering products have shown potential for clinical application over the past decade. A biomaterial-based scaffold termed Neuro-Spinal Scaffold developed by InVivo Therapeutics (http://www.invivotherapeutics.com/research-clinical-development/pipeline/bioengineered-neural-trails), could potentially facilitate new neuronal connections for use in spinal cord injury[90]. Humacyte (http://www.humacyte.com) has been testing, currently in clinical trials, vascular replacements fabricated by growing banked vascular smooth muscle cells on porous tubular scaffolds *in vitro* and decellularizing them[91]. L-C Ligament, a bioresorbable scaffold designed to facilitate regrowth of the anterior cruciate ligament (ACL) in the knee also entered the clinical trials in 2015 (http://softtissueregeneration.com/index.php/technology-overview/l-c-ligament). Although many of the current acellular products entering the market are inherited from earlier eras, successful clinical application of engineered tissues has been very limited largely because of the persisting challenges in achieving biological functions of cellularized constructs and their host compatibility. We anticipate that innovations in stem cells, genetic editing, biomaterial engineering, immunomodulation and biofabrication together will further boost the clinical translation of engineered tissues by tackling the critical challenges in the field (**Fig. 3**).

Aside from tissue substitutes for *in vivo* transplantation, technological advancements in tissue engineering have spurred new, unforeseen applications of engineering *in vitro* biomimetic tissue and organ models. These tissue and organ models are usually engineered at miniaturized scales that recapitulate the biology and physiology of their *in vivo* counterparts, featuring structural and architectural similarity, compositional resemblance in cell types and ECM moieties, ultimately producing a functional imitation. Such models have applications for improving the prediction of human drug responses and reducing the need for animal models in research. By taking advantage of stem cell technology, it has been shown that human iPSCs-derived cerebral organoids could be induced to form brain-mimicking structures[92] and familial Alzheimer's disease in which amyloid-β and phosphorylated tau proteins are expresssed[93]. Although these examples are highly biologically relevant, they alone do not necessarily recapitulate the dynamic physiological cues present in the human system.

An alternative approach is the use of organ-on-chip platforms that integrate biomimetic organ models with advanced

microfluidic technologies. This enables important physiological cues such as the vascular and interstitial fluid flows to be included in the model system, as well as an interconnected network among multiple organoids[94–101]. As such, a myriad of organ-on-chip models have been developed, including liver[102], lung[103,104], kidney[105], blood vessel[106], intestine[107] and bone marrow[108], among others. These different microfluidic organ models may be linked together to build human-body-like microphysiological systems to probe their interactions, collective drug responses and side effects of pharmaceutical compounds.

Unconventional applications of tissue engineering include the emergence of biological actuators and robotics. A free-swimming jellyfish-like biorobot was generated by populating a carefully designed elastomer substrate with cardiomyocytes, which exhibited spontaneous and predesigned synchronous beating patterns to propel the movement of the medusoids[109]. Conductive carbon nanomaterials may be incorporated into the substrate to improve the contraction of the biorobotics[110]. In addition, external controls such as those based on optogenetics can further realize remote actuation[111].

Engineered tissues are potentially edible as well. For example, Google spent €250,000 to support scientists growing cow stem-cell-derived muscles to make hamburgers. Meat engineering projects aim to reduce the environmental impact of greenhouse gases (i.e., methane) produced by farming and to improve animal welfare. Other examples include the company Modern Meadows, which produces food using cultured animal ingredients as well as leathers through biofabrication (http://www.modernmeadow.com).

In our opinion, improvement is needed in methods to preserve engineered tissues for efficient transfer from the site of fabrication to the site of transplantation. Although a combination of stepwise ultrafast vitrification protocols, optimized cryopreservant ingredients and metabolic preconditioning have indicated improved success[112–114], we envision the technological advancements achieved in peripheral fields may complement such improvements, for example, by expediting the shipment of tissues through the use of drones.

With convergence of multiple disciplines including biology, materials science, chemistry and engineering we have witnessed a decade's endeavor to advance tissue engineering. We are optimistic about the future: building functional tissues and organs at clinically relevant scales and construction of physiologically relevant *in vitro* tissue and disease models is the next major challenge. In addition, although the advances have enabled fabrication of tissues with structural and compositional accuracy, functional recapitulation of their native counterparts poses a major challenge toward tissue engineering. Emerging approaches that address this challenge include directing different cells toward desired lineages and improved efficiency potentially through the use of CRISPR technology (such as differentiation of iPSCs, genetic editing of MSCs to express growth factors and cytokines, and phenotype editing of macrophages for immunomodulation), devising common media for maintaining the functions and interactions of multiple cell types, as well as the optimization of cell-type-specific and disease- or wound-stage-specific biochemical and biomechanical cues that can achieve orchestrated dynamic presentation over the process of tissue maturation and regeneration. Through integration of complementary expertise using interdisciplinary approaches, we anticipate more developments in these areas to come in the next decade to advance this exciting field.

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