EBioMedicine 74 (2021) 103717

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.elsevier.com/locate/ebiom

Engineering mammalian living materials towards clinically relevant therapeutics

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ARTICLE INFO

Article History:
Received 13 July 2021
Revised 28 October 2021
Accepted 11 November 2021
Available online xxx

Keywords:
Biohybrid constructs
Biomaterials
Biomedicine
Cell engineering
Living materials
Tissue Engineering

ABSTRACT

Engineered living materials represent a new generation of human-made biotherapeutics that are highly attractive for a myriad of medical applications. In essence, such cell-rich platforms provide encodable bioactivities with extended lifetimes and environmental multi-adaptability currently unattainable in conventional biomaterial platforms. Emerging cell bioengineering tools are herein discussed from the perspective of materializing living cells as cooperative building blocks that drive the assembly of multiscale living materials. Owing to their living character, pristine cellular units can also be imparted with additional therapeutically-relevant biofunctionalities. On this focus, the most recent advances on the engineering of mammalian living materials and their biomedical applications are herein outlined, alongside with a critical perspective on major roadblocks hindering their realistic clinical translation. All in all, transposing the concept of leveraging living materials as autologous tissue-building entities and/or self-regulated biotherapeutics opens new realms for improving precision and personalized medicine strategies in the foreseeable future.

1. Introduction

Living tissues can be perceived as dynamic materials that have been extensively optimized by Nature throughout time, essentially yielding complex and highly evolved biofunctional modules designed to carry out specific activities within the body while maintaining homeostasis [1]. Traditionally, organ transplantation relies on replacing such irreversibly damaged or diseased modules for restoring lost functions that are vital for guaranteeing patient survival and improving quality of life. In this sense, it has been increasingly established that recreating functional tissue analogues via tissue engineering technologies will be critical for overcoming the inconsistent supply and shortage of donor organs in the clinical setting [2]. In addition, the successful generation of tissue-specific modules is highly attractive for advancing current drug screening and disease modelling platforms, namely by allowing to replace the use of animal models with tissue-like platforms that are potentially more predictive of clinical outcomes in humans [1].

Conventional biomaterial-based platforms (i.e., hydrogels, sponges, nano/microparticles, fibre meshes, etc.) envisioned for clinical applications are often designed to convey biophysical support and/or integrate bioactive cues for instructing or potentiating cellular bioactivity. Such strategies often rely on the inherent role of cells physically-embedded within scaffolds or the response of patients' tissue resident cells following administration. In addition, these platforms typically present cell densities several orders of magnitude below those found in the majority of soft native tissues (i.e., 10^7 – 10^9 cells/cm^3) [3,4]. In fact, similar to how the functional modules of the human body are the organs, the functional units of our tissues are the living cells and cell-secreted extracellular matrices (ECMs) that compose them. As a result, such conventional platforms exhibit underwhelming biofunctionalities in pre-clinical studies and are often limited in their ability to respond and adapt in biological scenarios [5].

These limitations have fuelled the pursuit of living materials, an emerging conceptualization that seeks to exploit the unique attributes of living cells, namely as: (i) continuous/programmable biofactories that generate different biologics (e.g., *de novo* synthesized extracellular matrix, extracellular vesicles, growth factors, cytokines, etc.), and as (ii) microtissue precursors [6]. In addition, such constructs should inherit cells' ability to interpret/adapt to their microenvironment, yielding bioresponsive constructs with autonomous and dynamic behaviour [7]. Following this rationale, living materials are herein referred to as macro-scale platforms comprising cells as the fundamental building blocks, or in which cells are the primary actuators responsible for driving the response mode of these constructs. As broadly recognized throughout different reports, living materials can be assembled via bottom-up processes and are fully comprised by cells or cells-biomaterials combinations where

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https://doi.org/10.1016/j.ebiom.2021.103717

biomaterials generally serve as linkers or are present in relatively low contents to ensure the assembly of bioarchitectures exhibiting "living character", which is mainly conveyed by cells. On the other hand, platforms that are assembled in a more traditional top-down approach (i.e., seeding on top of biomaterial scaffolds) and partially contain living cells could also be included in the broad definition of "living materials", but present vastly inferior cellular contents and fail to recapitulate the native tissue formation that is bottom-up and multi-scaled by design.

The combination of materials science with synthetic biology and directed evolution in this interdisciplinary field has recently led to several pioneering works exploiting the programmability and autonomous behaviour of living systems (i.e., prokaryotic or eukaryotic cells) to design cellular devices and materials encoded with unique functions or exhibiting improved performances over conventional biomaterials [8–12]. Recent research endeavours showcasing the resiliency, programmable behaviours and versatile biomolecule-producing nature of prokaryotic living materials are extensively overviewed elsewhere in other seminal reports [13–15]. Herein, we particularly focus on the progress underlining the development of eukaryotic living materials owing to their human relevance and translatability to clinical applications.

On this focus, this review outlines recent advances in harnessing mammalian cells to produce dynamic and responsive living materials that are highly attractive for biomedical applications. Moreover, we highlight emerging cell engineering strategies (i.e., encompassing chemical and biotechnological tools) that can enable the programmed or autonomous assembly of multi-scale living materials, and how their modular combination can further expand the range of biological functionalities that can be encoded in these platforms. Collectively, innovations in this nascent field are also critically discussed concerning the translational roadblocks that must be overcome for commercialization and clinical use of living materials intended for tissue engineering and regenerative medicine purposes. Due to their dynamic nature, living materials can ultimately provide continuous and self-regulated therapeutic activities imparted with bioactive lifetimes and bioresponsiveness that are unattainable by non-living biomaterial platforms, therefore being expected to provide substantial biomedical breakthroughs in the foreseeable future.

2. Design Considerations for Programming Living Materials Assembly and Behaviours

To materialize the development of living materials, researchers have at their disposal a vast toolbox of cell engineering technologies and several manufacturing strategies that have been employed for driving the cooperative assembly of unitary living cells into cell-rich biological constructs [16]. Building on this, the design of living materials according to the nature of the driving forces responsible for their assembly, in particular whether arising from cell surface components that are naturally-present (i.e., cell adhesion proteins) or artificially incorporated (i.e., via cell surface engineering technologies) will be discussed in the following sections (**Scheme 1**). In addition, the assembly mode of these constructs is herein distinguished in two different scenarios, namely if it occurs autonomously or in a guided/programmed manner. Finally, this subsection will also illustrate that the cellular building blocks selected - in native or engineered forms - can determine the behaviour and functionalities of the living materials produced.

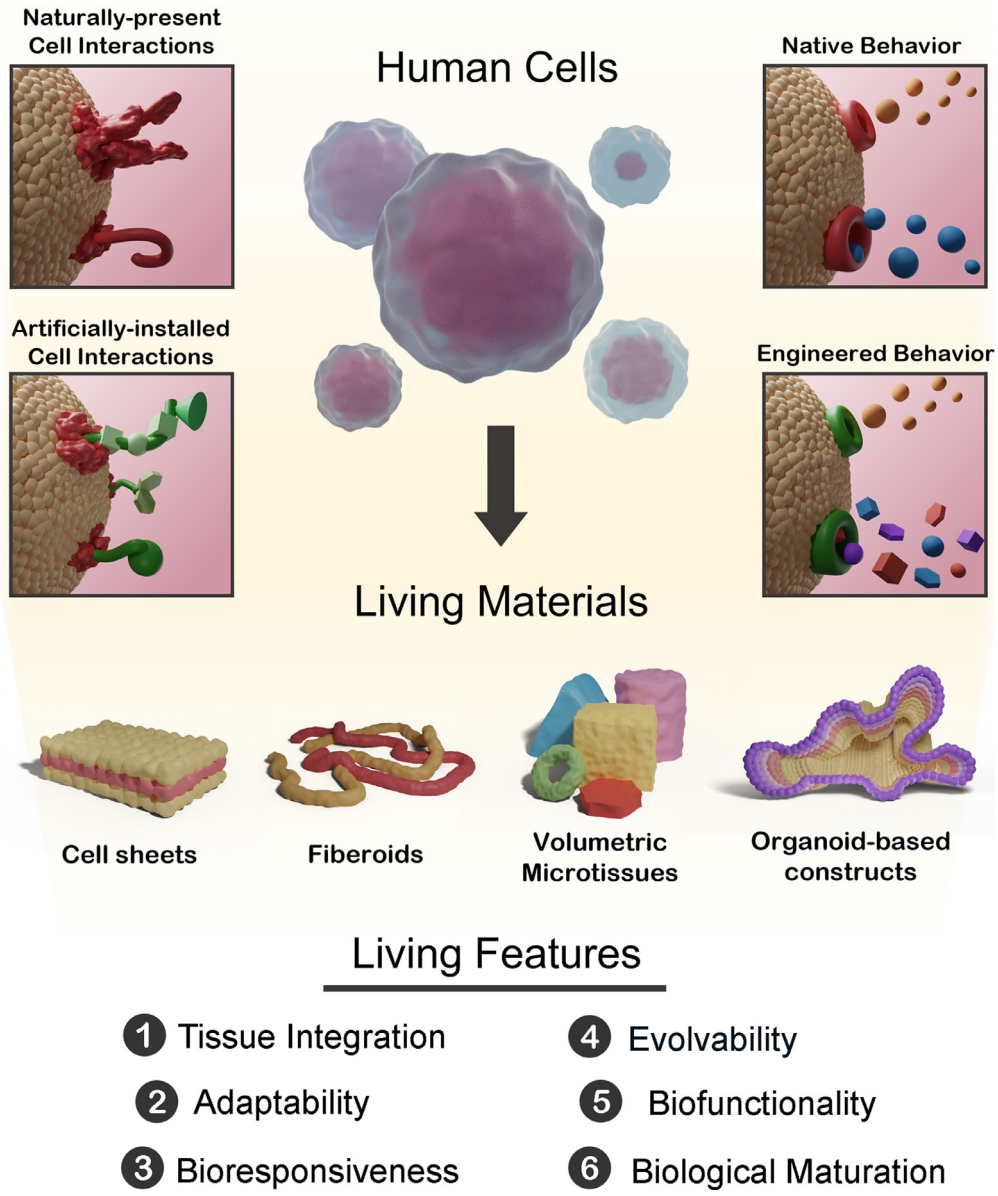
2.1. Assembly Driven by Naturally-occurring Motifs

By exploring naturally occurring processes, living materials can be assembled solely from native cell-cell or cell-ECM linkages, and therefore the formation of these constructs does not require specialized cell engineering methodologies. This approach relies on the presence of cell adhesion transmembrane proteins, namely calcium-dependent cadherins and selectins, as well as calcium-independent integrins and immunoglobulin superfamily, which are responsible for endowing cells with recognition, binding and adhesion capabilities [17]. The vast majority of mammalian living materials are generated via cadherin-based crosslinking of cytoskeletons among adjacent cells and have been continuously developed for scaffold-free tissue engineering in the form of cell sheets [18], fiberoids/other complex shapes [19,20], and spheroid-fused constructs [4]. Analogous to the use of cell-cell interactions, cell-ECM interactions (i.e., via integrins) can be alternatively exploited for producing cell-rich constructs. In these approaches, cells are combined with interfacial fibronectin/gelatin films to drive the sequential formation of multilayered tissues via integrin binding [21,22]. Due to its highly modular nature, multiple layers of different cell types can be combined together into a seamless hierarchic living material.

Regardless of the native driving force employed, cell assembly occurs randomly across the unitary thick constructs (i.e., fiberoids and others) and within the same layer (i.e., cell sheets), resulting in limited spatial control within the established assemblies. Strategies aiming to improve the selectivity of cell assemblies in living materials could draw inspiration from the natural embryonic development, in which cells from one tissue bind specifically to cells of the same type rather than cells from other tissues [17]. For instance, cell adhesion proteins have been demonstrated to establish either homophilic (i.e., bind to identical motifs in another cell) or heterophilic interactions (i.e., recognize different type of motifs in another cell), and have been implicated in the selective cell-cell binding among different cell types (i.e., epithelial, placental and neural cadherin members) that are fundamental for organ formation [23]. Ultimately, a deeper understanding of these native mechanisms combined with advanced manipulation technologies (e.g., optogenetics or magnetogenetics) for on-demand presentation of cell adhesion proteins could enable a precise spatiotemporal modelling of the intrinsic organization of cell assemblies during the manufacturing of living materials. On another level, recent innovations in biofabrication technologies through the use of microgel-based supporting baths have enabled the assembly of macro-scale cell-only constructs leveraging the expanded spatial control offered by 3D bioprinting [24]. Also, advances in polymer-based viscoelastic supporting baths may provide alternative solutions for further improving the spatial resolution of the living constructs [25]. Collectively, a combination of precise intra-tissue modelling of cell-cell interactions/segregations allied to macro-scale constructs architectural control represents an attractive roadmap for engineering native-like tissue constructs exhibiting human morphometric features.

2.2. Assembly Driven by Artificially-incorporated Motifs

Alternatively, specific functional groups can be artificially-incorporated in cell surfaces in order to drive the assembly of living materials. To this end, several cell surface engineering technologies have been pursued, namely via: (i) chemical functionalization, (ii) cell membrane coatings (iii) lipophilic insertion, (iv) metabolic glycoengineering, and (v) genetic engineering [1,16]. The chemical route is particularly valuable since cell membranes inherently display a broad range of functional groups (i.e., amine, carboxyl, hydroxyl, phosphate and thiol) that can serve as cell-crosslinking nodes with complementary biomaterials or be chemically-functionalized with crosslinking moieties of interest through standard bioconjugation techniques. However, it is important to consider that such groups are ubiquitous in glycoproteins backbones, hence unspecific modification can also occur at regions that are critical for physiological functions. The latter may negatively affect engineered cells viability and/or bioactivity downstream, ultimately influencing living materials performance and biological maturation. To tackle these bottlenecks, researchers



Scheme 1. Advanced engineering technologies for leveraging human cells as building blocks of living materials. Such biological constructs can be generated by exploiting endogenous cell adhesion transmembrane proteins or artificially-engineered surface motifs, while their collective behaviour can be further customized to encode distinct response modes under biological or external stimuli. Under these strategies. a broad range of living materials (i.e., cell sheets, fiberoids, volumetric microtissues and organoid-based constructs) can be assembled across multiple length scales and displaying different degrees of organizational complexity. These cell-rich platforms present attractive living features for biomedicine, such as autonomous tissue integration, bioresponsiveness/adaptiveness to surrounding microenvironments, biological maturation, evolvability, resilience, self-powering/self-maintenance and biofunctionality.

have been devising elegant cell membrane engineering technologies based on non-covalent interactions with surface coatings (i.e., consisting of organic/inorganic polymers and/or nanomaterials) comprising crosslinking moieties, or functioning as supporting templates for subsequent (bio)conjugation approaches [26]. In addition, single cell coatings can present multilayered architectures incorporating multiple biomaterials deposited in a stepwise manner, which is particularly valuable for tuning cellular activities and further broadening the type of interactions and responsiveness that can be encoded in the assembly of living materials [27,28]. Still, despite presenting opportunities for reprograming cell surfaces, stable coatings are difficult to obtain in a consistent and uniform manner (particularly across large cell populations), which limits their scalability and applicability for assembling living materials across different length scales. On a different take, emerging technologies are attempting to leverage the spontaneous insertion of lipid-conjugated functional groups (i.e., as free molecules or in the form of fusogenic liposomes) into phospholipidic cellular membranes to install new functionalities in mammalian cells surfaces. This concept is routed in naturally occurring supramolecular membrane components and is hence highly adaptable to modify virtually any cell types with complementary groups (i.e., oxyamine, ketone, and others) or single stranded DNA molecules on their surfaces, enabling one to instruct their assembly in a cytocompatible and truly selective manner [29,30]. Despite these advances, taking into account that organ development is a highly time-orchestrated sequence of cell-cell recognition/actuation events, the unlocking of increasingly biofunctional living materials may thus require the encoding of user-defined temporal control into their assembly modes. If such fluctuations are essential for attaining microtissues with optimal biofunction, these tools would allow researchers to program cell-cell decision events and manipulate their timeframes to ultimately mirror those of developing tissues.

Adding to the library of technologies that provide direct outer cellular surface functionalization, other invaluable approaches dwelling on *"inside-out"* cell engineering concepts (i.e., metabolic glycoengineering and genetic engineering) have enabled researchers to exploit the endogenous intracellular biomachinery for precisely reprograming cell surfaces in an on-demand and spatiotemporally controlled mode.

Metabolic glycoengineering allows for live cells or entire tissues *in vivo* to be engineered with a wide variety of functional motifs (i.e., naturally-occurring or non-natural) upon incorporating synthetic monosaccharide analogues [31]. Such rationale has been exploited for directing the assembly of different cell types (i.e., T cells and Burkitt lymphoma cells) bearing complementary bioorthogonal groups (i.e., azide and bicyclo[6.1.0]nonyne) [32], or alternatively, for connecting cells to non-adhesive biomaterials [33]. However, although this is a highly versatile and biocompatible technology, some functional chemical motifs have limited incorporation efficiencies due to their large molecular sizes (i.e., bicyclo[6.1.0]nonyne or norbornene) or cross-reactivity with biological components (i.e., thiols, ketones or alkynes), thus requiring additional optimization.

Another interesting angle for giving rise to functionalized mammalian cells is the use of genetic engineering tools to completely customize surface motifs and driving highly complex self-organized cellular assemblies with near biological cell-to-cell selectivity [34]. In this context, one of the most elegant approaches for modulating the spatial organization in biological constructs is to exploit the spontaneous cadherin-driven differential sorting of multicellular populations. Such phenomenon occurs because cadherins exhibit different adhesive strengths according to their type (i.e., E-, N- and P-) and favour homotypic cellular interactions over heterotypic cross-associations [34,35]. Following this rationale, researchers have leveraged synthetic Notch (synNotch) receptors that can customize the cellular response (i.e., in terms of cadherin expression and other ligands) upon sensing cell-cell interactions [34]. For instance, A-type cells expressing CD19 ligands activate anti-CD19 synNotch in B-type receiver cells, inducing them to convert to C-type cells presenting high E-cadherin levels and surface GFP. Afterwards, owing to E-cadherin expression, C-type cells spontaneously self-adhere and are sorted into the centre of the construct. As a result, C-type cells reciprocally activate anti-GFP synNotch in spatially-adjacent A-type cells, which are converted to D-type cells expressing low E-cadherin levels and mCherry. In essence, these constructs presented two initially disordered cell genotypes that self-organized into three distinct phenotypes in spatially-segregated compartments. By tuning the combinations of adhesion molecules used and the timings of their expression, researchers disclosed a wide library of self-organizing multicellular structures that evolve similarly to developing tissues in the human body, a remarkable achievement considering the biological complexity involved in such processes. Most importantly, such self-organizing features that are characteristic of living materials are virtually unattainable using conventional cell-laden hydrogel/fibre biomaterials, providing added functionalities and bioactivities that may ultimately unlock more effective clinical therapeutic actions. In addition, this highly versatile synNotch technology can be used to encode mammalian cells with 'AND' Boolean logic gates that produce different responses according to the presence/absence of multiple orthogonal inputs [36]. Cascades of synNotch receptors have also enabled the build-up of self-rearrangeable living materials with tailored spatial organization and user-defined spatial transdifferentiation (i.e., fibroblasts into myotubes) driven by engineered cellular interactions [36]. Remarkably, instead of expressing membrane-bound motifs in response to cell-cell interactions, diffusible synNotch systems have been recently developed that engineer secretor, anchor and receiver cells for enabling long-range spatial distribution of programmable soluble morphogens [37].

2.3. On-demand control over Living Materials Assembly or Disassembly

Although enabling programmable and spatially-controlled tissue build-up due to selective cell-cell interactions, previous constructs were generated through passive and autonomous assembly. In this context, cell engineering technologies can be additionally exploited for installing on-demand control of living materials assembly/disassembly stages, thereby programming their manufacturing not only in a selective mode, but also in a coordinated manner. For instance, using a combination of metabolic glycoengineering and direct chemical functionalization, researchers produced cells bearing β-cyclodextrins that can self-assemble via photoreversible host-guest interactions with azobenzene-PEG-azobenzene crosslinking agents [38]. Under ultraviolet light exposure (λ = 365 nm), cell-cell interactions are dissociated due to azobenzene photoisomerization from *trans*- to *cis*-isomer that is unable to fit within β-cyclodextrins, while exposure to visible light reverses this configuration and re-enables cell-cell assembly. Also, such azobenzene groups were linked to cell-selective aptamers for enabling reversible heterotypic cell-cell interactions. In another work, liposomal fusion was employed for installing photocleavable linkages between complementary cells bearing oxime and ketone groups, which allowed for remote controlled tissue disassembly following exposure to ultraviolet light stimuli (λ = 365 nm) [39].

In an inspired approach, researchers genetically engineered cells expressing cryptochrome 2 photoreceptors or their complementary interaction partner CIBN, which resulted in blue light switchable cell-cell crosslinking into large clusters [40]. Because such cell-cell interactions can be reversed in the dark, the generated cellular assemblies could be repeatedly switched under an ON/OFF cyclic mode. Besides being employed for actively controlling cell-cell interactions in living materials, optogenetic tools have recently unlocked the use of photoreversible (λ = 660 and 740 nm) cell-matrix interactions, which was achieved by combining red light-switchable phytochrome B-functionalized matrix with living cells expressing integrin $\alpha V\beta 3$ ligands engineered with phytochrome-interacting factor domain [41].

Apart from light-induced disassembly, researchers have recently exploited metal ion- dependent DNAzymes cleavage of their ribonucleotide substrates to trigger cell-cell disassembly [42]. Following this rationale, cells were initially engineered via lipophilic insertion of different DNAzymes (i.e., Zn^{2+}- and Mg^{2+}-specific) and their respective substrate strands, which enabled autonomous cell-cell assembly through selective hybridization involving DNAzyme-substrate molecular recognition events. Due to metal ion-dependent DNAzyme activities, Zn^{2+} and Mg^{2+} were used as orthogonal inputs for controlling the assembly/disassembly stage between multiple 3D spheroid building blocks. In addition, such microtissues were engineered to disassemble under different Boolean logic operations, such as those requiring the presence of (i) both Zn^{2+} and Mg^{2+} input signals ('AND' operator), and (ii) either Zn^{2+} or Mg^{2+} input signals ('OR' operator).

2.4. Programming Living Materials Behaviour

Beyond enabling the assembly of living materials, cell engineering technologies can also be explored for installing molecular components that shape the performance of these constructs chronologically. In particular, the evermore accumulated know-how in synthetic biology tools can further expand the customization and control over cellular behaviour/responses beyond those naturally-occurring, thereby unlocking the possibility to program living materials with unprecedented functionalities [6]. As aforementioned, versatile genetic engineering toolkits unveil opportunities for designing the decision-making framework of cells in a pre-programmed mode, subsequently encoding living materials with numerous response modes, such as: (i) constitutive expression of exogenous genes, or (ii) bioresponsive/

remote-controlled expression of exogenous genes [43]. Moreover, synthetic networks can be integrated within living materials to dampen, amplify or completely alter the thresholds required for outputting their endogenous genes. This results in engineered biological constructs with tailored secretion of protein therapeutics or other biologically-relevant molecules to meet tissue-specific functions or enhance their regenerative activities. For instance, researchers have recently installed mechanogenetic circuits in chondrocytes that elicit the production of anti-inflammatory drug interleukin-1 receptor antagonist following mechanical stimuli [44]. These engineered cells were integrated in tissue constructs that autonomously responded to physiologically-relevant mechanical loading by secreting the therapeutic protein and protecting against inflammatory insult, thus serving as highly promising platforms for long-term delivery in osteoarthritic joints.

Apart from controlling the secretion of soluble molecules or surface-expressed motifs, recent endeavours in synthetic biology have tapped into the potential of customizing the material synthesis of living cells. In a remarkable strategy combining genetic engineering and polymer chemistry, researchers have genetically-targeted hippocampal neuron cells to synthesize and assemble electroactive materials *in vivo* [45]. In another perspective, genetic engineering tools can be used to produce cells exhibiting novel behaviours that are characteristic of certain types of materials (i.e., organic polymers or inorganic nanomaterials). For instance, research in optogenetics delves into encoding light-responsive cellular circuits to spatiotemporally control cell-cell interactions, cellular processes and their biochemical outputs, or triggering their contraction/locomotion [46]. On the other hand, the nascent field of magnetogenetics offers the opportunity to genetically encode magnetic-responsiveness in living cells, a behaviour that has been otherwise exclusive to certain classes of inorganic nanomaterials (i.e., iron oxide-based) [47]. Such programmability ultimately unlocks the ability to design living materials with exotic response modes that are naturally elusive to obtain in most living matter.

Alternatively, although operating on a lower level of programmability, outer functionalization cell engineering strategies also enable the selective conjugation of nanoparticles, peptides, bioactive molecules and other synthetic components to living cells, which can further modulate the behaviour of biological constructs. Moreover, non-living components (i.e., proteins, polymers and inorganic materials) can also be engineered to encode further bioactivity or function as anchoring hotspots for installing additional adaptable behaviours in living materials [26,48].

On another level, it is becoming increasingly established that tissues morphogenesis occurring during development is guided not only by cell-cell interactions but also by the continuous and dynamic self-presentation of their extracellular microenvironment [1,49]. In fact, living cells are in constant bidirectional communication with the ECM that they synthesize, reprogram and remodel, which in turn play a key role in influencing cellular fate, function and plasticity in biological tissues [50]. In general, different ECM characteristics have been demonstrated to drive cellular decision events beyond those already pre-programmed in native morphogenesis, such as (i) matrix composition, concentration and enzymatic degradability, (ii) cell-ligand interactions and ligand mobility/dynamics, (iii) stiffness, (iv) topography (i.e., conveyed through geometrical organization or anisotropic fibre alignments), and (v) viscoelasticity and stress-relaxation properties [50]. Considering the importance of such factors in modulating cellular behaviours, researchers have been exploring the use of ECM-mimetic materials and decellularized ECMs (in either their close-to native state or additionally bioengineered with chemically-active functional groups) and incorporating these components in the assembly stages of living materials [51]. Also, because each type of tissue is characterized by a unique combination of ECM components, there is an increasing interest in employing organotypic decellularized ECMs to further direct the activities of naïve cells towards a specific tissue/organ function or for modelling a particular disease stage [52]. For instance, researchers have recently employed a combination of skeletal muscle and vascular tissue-derived decellularized ECMs as bioinks for the 3D embedding bioprinting of pre-vascularized large skeletal muscle constructs [53]. Compared to cell-laden decellularized ECM hydrogels and cell-seeded decellularized ECM sponges, such tissue-specific cell-laden materials presented vastly improved biofunctionality in terms of myotube formation, as well as significantly enhanced the production of *de novo* muscle fibres, vascularization and innervation in *in vivo* volumetric muscle loss injuries, achieving 85% functional recovery. As characteristic of biological muscle tissues, such constructs could also respond to incoming electric stimuli with whole-tissue mechanical contraction, indicating efficient bioelectrical communication. Alternatively, numerous other works have disclosed the superior organotypic biofunctionality that arises from incorporating tissue-specific dECMs within the development of cell-laden materials, which have yielded functional microtissue analogues across a broad range of human tissues: such as (i) cornea, (ii) oesophagus, (iii) peripheral nerves, (iv) blood vessels, (v) heart, (vi) cartilage, (vii) adipose tissue, (viii) liver, (ix) lung, (x) pancreas, and (xi) kidneys [51,54,55]. In addition, aided by recent advances in tools dedicated to omics analysis, researchers are progressively identifying key components present in such organotypic dECMs that are the major drivers of organogenesis and/or responsible for augmenting the constructs bioperformance beyond those of naïve cell assemblies [50]. In the foreseeable future, these strategies will unveil opportunities for customizing the composition of living materials with specific ECM biomolecular cues in order to refine their biofunctionality toward physiologically-relevant and organotypic actions.

Ultimately, the behaviour of living materials can be programmed to transform their innate biosynthesis into pre-scripted biomolecule and biomaterial biofactories, thus functioning as highly specialized therapeutics, tissue manufacturers or as next-generation biosensing modules for advanced theranostic endeavours.

3. Progress on Living Materials as Therapeutic Platforms for Tissue Engineering

In this rapidly emerging and exciting field, mammalian cells represent fundamental building blocks and customizable canvas that can be leveraged to generate multifunctional living materials inheriting many of the characteristic hallmarks of living systems, namely: (i) compartmentalization of precursors and functionalities, (ii) information recognition and processing, (iii) cascaded signal transduction and transmission, (iv) adaptability and actuation, as well as (v) proliferation and maturation [56]. This subsection aims to overview recent advances of living materials as biologically-instructive and therapeutic platforms for biomedical applications, while critically discussing their progress as this field moves closer to translational and clinically-relevant activities.

Cell sheets are thin layers of microtissues assembled from native cell-cell interactions and autonomously reinforced along time through *de novo* ECM deposition [1]. Multiple cell types can be combined during the layer assembly to yield multifunctional tissues with enhanced bioactivities arising from synergistic cell-cell crosstalks [18]. In addition, their ability to self-merge with other layers unlocks the possibility to stack different cell sheets in a modular manner to produce thicker and hierarchical biological constructs [57]. In this context, multi-layered human cell-based cell sheets containing adipose tissue-derived mesenchymal stromal cells (hASCs) and umbilical vein endothelial cells (HUVECs) were recently fabricated with heterotypic (hASCs/HUVECs/hASCs) and homotypic configurations (hASCs/hASCs), in which the heterotypic constructs attained improved matrix mineralization and osteogenic markers expression

(i.e., ALP activity, osteocalcin and osteopontin) [18] (**Figure 1a**). Moreover, such multicellular crosstalk also led to enhanced *in vivo* angiogenesis, while their living behaviour resulted in the migration and integration with chick embryo vasculature. Other than generating planar multilayered tissues, cell sheets can be rolled to produce three-dimensional biofunctional tubular constructs, but processing these living materials into other architectures is difficult due to their biomechanical fragility [1]. Alternatively, researchers have recently developed magnetic-responsive cell sheets that can be instructed to assemble under the presence of external magnetic fields into a broad variety (i.e., discs, rings and concave-shaped geometries) of complex constructs [58]. Considering cell sheets were originally reported back in 1990, they represent one of the most established living materials in the clinic [59]. Such autologous constructs have been clinically-approved for regenerating several tissues, such as blood vessels (Lifeline®), cornea (Holoclar®), oesophagus (CellSeed Inc), heart (HeartSheet®) and skin (Epicel®), and there is also a large pipeline of commercially-available products currently undergoing clinical trials

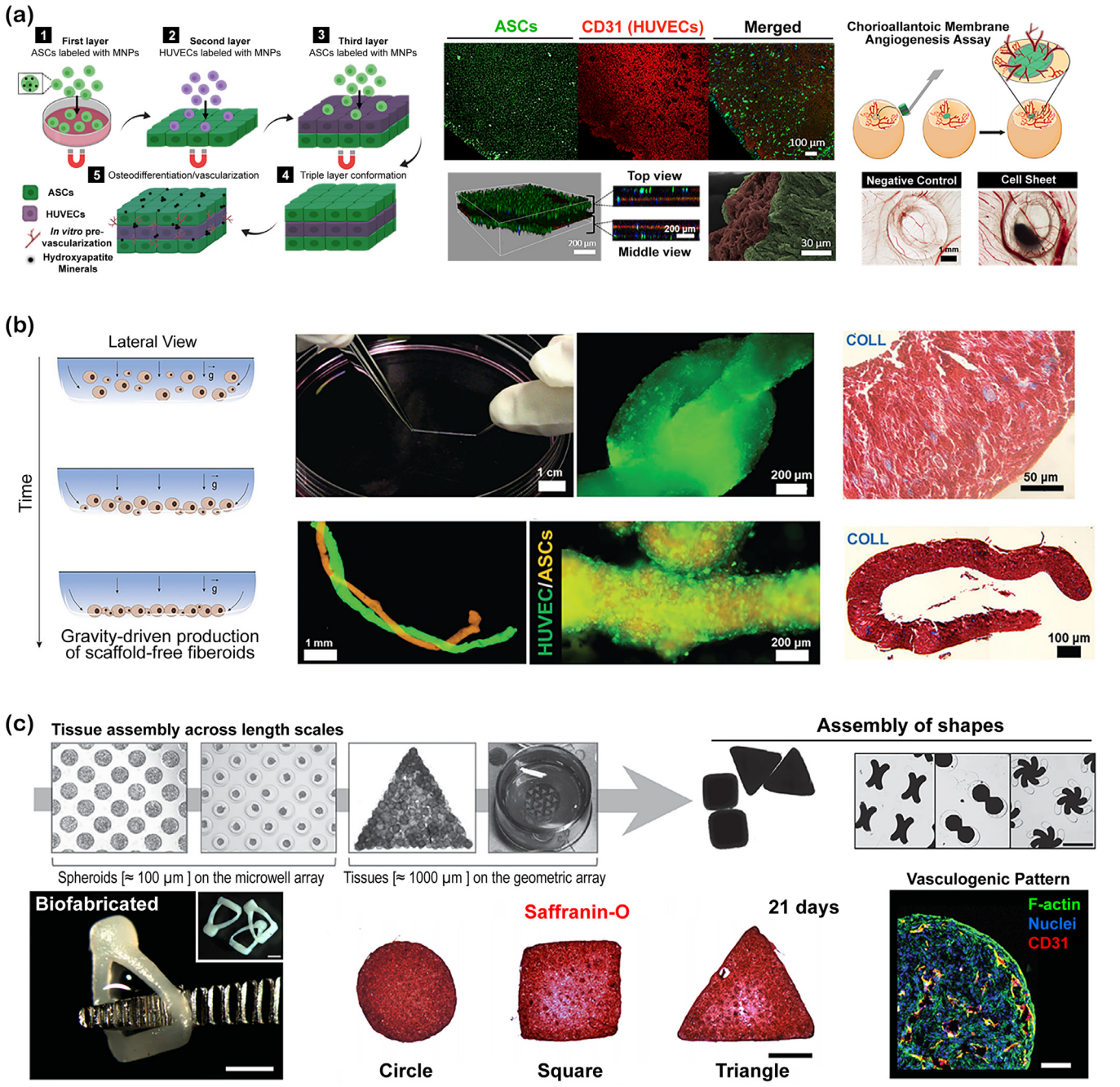


Figure 1. Controlled assembly of modular living materials in different architectural configurations. **(a)** Magnetic nanoparticles were introduced into human cells (i.e., adipose tissue-derived mesenchymal stromal cells – hASCs, and umbilical vein endothelial cells – HUVECs) to render them magnetic-responsive and driving their assembly under the application of external magnetic fields. Multi-layered cell sheets were generated with hierarchical organization and heterotypic compositions, which led to synergistic cell crosstalks potentiating their pro-angiogenic activities. Adapted with permission from [18]. Copyright 2020, Elsevier. **(b)** Hanging column strategy for assembling fibre-shaped constructs that were mechanically-handleable and could be intertwined together to form multi-fiberoid structures comprising different human cell types (i.e., hASCs and HUVECs). Fiberoid microtissues exhibited extensive nascent collagen deposition following maturation in physiological conditions. Adapted with permission from [19]. Copyright 2019, Wiley-VCH. **(c)** Geometrical confinement strategy of spheroid building blocks for high-throughput manufacturing of microtissues with user-defined shapes and customizable cellular compositions. Following long-term maturation, continuous cartilage-like tissues were assembled from bovine chondrocytes, while multicellular constructs comprised by human mesenchymal stromal cells and HUVECs developed vasculogenic patterns. Adapted with permission from [20]. Copyright 2016, Wiley-VCH.

for numerous applications [60]. This success attests to the potential of living materials in translational activities and stimulates continuous efforts in this field to develop alternative iterations of these systems that will eventually find their place in the clinic.

Moving up from planar microtissues, researchers have recently developed centimetre-long cell-only fibres (i.e., fiberoids) assembled from native cell-cell interactions (**Figure 1b**) [19]. These structures were assembled through a modified hanging drop strategy – hanging column - by adding cellular suspensions into superhydrophobic surfaces patterned with wettable stripe-like regions. Such constructs could be generated from distinct cell types (i.e., hASCs or MC3T3-E1) and/or in different differentiation stages (i.e., undifferentiated or osteodifferentiated hASCs), being readily handleable and mechanically-compliant following biological maturation under physiological conditions, enabling the interweaving of multiple fiberoids together. Due to their customizable composition, heterotypic fiberoids (hASCs/HUVECs) were produced and displayed significant pro-angiogenic activity in a chick embryo chorioallantoic membrane model. Such living materials are highly attractive to be explored in advanced regenerative strategies considering their capacity to integrate biological tissues and assist in the formation of vascularized nascent tissues. In order to further expand their applicability in the future, such fibre-based materials can be architecturally manipulated via textile tissue engineering techniques (i.e., knitting, braiding, weaving and winding) to create large-scale interconnected multifibre constructs displaying improved biomechanical properties and mirroring human tissues with fibrillar structures [1].

In a pursuit for larger and truly three-dimensional living materials, researchers employed a microfabrication approach based on non-adherent hydrogel templates for pooling and clustering together multiple cells or spheroids, taking advantage of their autonomous assembly (**Figure 1c**) [20]. Geometrical confinement of these building blocks led to a self-governed generation of continuous tissue constructs up to the centimetre scale and presenting a broad range of user-defined architectural configurations (i.e., squares, triangles, circles, multi-branched and stapes). Constructs assembled from different cell types and biologically matured along time presented distinct phenotypes and behaviour. For instance, bovine chondrocytes yielded Safranin O-positive continuous cartilage-like tissues, while multicellular constructs assembled from human mesenchymal stromal cells and HUVECs developed vasculogenic patterns resembling vessels when matured in the presence of morphogen Sonic hedgehog.

As an alternative to micro-molding technologies, acoustic fields have been used to drive the spatial rearrangement of suspended cells to yield ring-shaped tissues, which could then be re-instructed later on to acoustically-assemble into large-scale multi-ring bracelets and concentric structures (**Figure 2a**) [61]. Inspired by the native brain structure, the authors leveraged this technology to develop concentric constructs exhibiting a neuron-rich outer ring and a smaller inner layer densely populated with glial cells. Such brain-like living materials presented two-layered structures recapitulating the directional neurite alignment found in mouse brain, while also displaying the ability to respond through synchronized calcium spiking when sensing GABAergic inhibitor picrotoxin and neurotransmitter glutamate. In a different strategy, researchers have developed cell-rich hydrogels by crosslinking surface-modified mouse myoblasts (i.e., C2C12) with a complementary polymer (i.e. PEG-branched alginate) [33]. In these systems, cell division processes reduce the number of crosslinks, which leads to swelling and disassembly of the microtissues. Such cell-rich materials could selectively adhere to collagen-coated dishes and reversibly detach under trypsin.

Rather than simply driving the coalescence of single cells into large constructs, researchers are exploring new routes employing organoids as building blocks, since they represent self-organizing multicellular modules that are inherently more biofunctional and representative of biological tissues [63]. For instance, organoid-forming stem cell inks have been recently bioprinted into centimetre-long tissues with microarchitectural features arising from cellular rearrangement as they proliferate, interact and self-organize into multiple and continuous organoids (**Figure 2b**) [62]. Under this strategy, the combination of stomach- and colon-derived stem cells resulted in the formation of large tubes featuring organ-specific morphologies from the gastrointestinal junction, such as smooth gastric zones and intestinal compartments filled with tissue-like crypts and villus domains. Alternatively, constructs assembled from endothelial cells readily self-organized into branched vascular tubes containing perfusable lumens, while the inclusion of stromal cells in intestinal tubes accelerated lumen formation and doubled its diameter, allowing these co-cultured tissues to be connected to a liquid perfusion system. Importantly, this method combines the macroscopic structural control offered by bioprinting technologies with the microscopical features emerging from organoid self-organization, which are expected to lead to increasingly bioactive and bioresponsive living materials. In another approach, human periosteum microspheroids were differentiated into multiple callus organoids, which could then be assembled into multimodular constructs that formed large-scale ectopic bones *in vivo* and were able to heal murine critical-sized long bone defects [64]. Interestingly, bone tissues regenerated via these living constructs presented intricate bone marrow compartments and exhibited morphologies similar to those of native tibia.

In order to further increase the complexity of mammalian living materials, researchers have recently disclosed a one-pot orthogonal differentiation platform for generating programmable multicellular organoids and organ-specific constructs [65]. This technology was leveraged for producing vascularized cortical organoid constructs patterned on-demand with distinct neural regions comprising neural stem cells, endothelium and neurons. Adding to this, biomanufacturing strategies have been recently developed for robust vascularization of large-scale living materials presenting physiological cell densities, thus overcoming the natural nutrient/oxygen diffusion limits that hinder the long-term viability of tissue-dense constructs (**Figure 2c**) [3]. As researchers continuously advance the development of pre-vascularized living materials, this field rapidly progresses from limited micro-scale assemblies towards anatomically-sized constructs with evermore close-to-native biofunctionality.

4. Future Outlook

The ultimate goal of biomedical engineering viewed in the light of living materials philosophy is to take a leap from exploring biocompatible, passive biomaterials that unidirectionally instruct and/or are biodegraded within the body, toward developing materials capable of: (i) actively exchanging information, (ii) dynamically adapt to their surroundings, and (iii) functionally integrate with biological tissues; giving rise to additional cooperative/synergistic functions that may chronologically evolve along time while retaining biological memory of their received/sent stimuli [66].

Building on this unique potential, herein we identify a set of technologies that can be exploited for engineering mammalian cells as the building block precursors of living materials, presenting physiological functionalities with increasing degrees of organizational complexity, ultimately pushing the field closer to recreating tissue analogues that can operate as (bio)governed clinically-relevant therapeutics. In this line, the rapid advances being achieved in gene editing tools (i.e., CRISPR/Cas9) may contribute for paving new avenues in the engineering of increasingly programmable and biofunctional living materials.

By now, it is becoming increasingly evident that living materials have the potential to regenerate tissues that is far beyond the reach of conventional natural/synthetic drugs, biologics or acellular biomaterial scaffolds. Still, as researchers continuously take steps toward

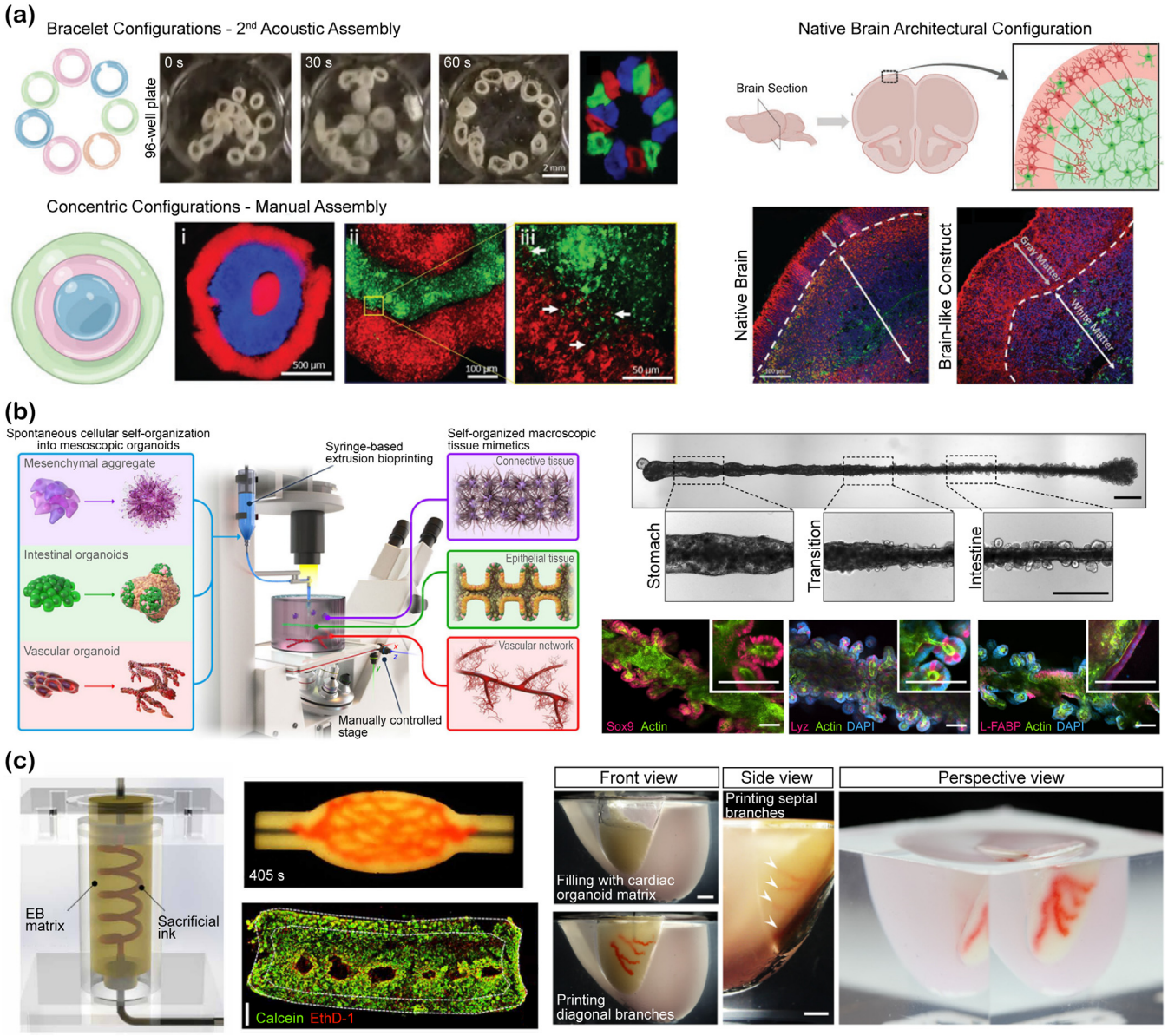


Figure 2. Assembly of large-scale living materials under different technologies. **(a)** Acoustic fields were used to generate ring-shaped cellular constructs, which could then be guided under a second acoustic stimulus to assemble into multi-ring bracelets and concentric configurations. This approach allowed for the build-up of brain-like tissues presenting two-layered architectures consisting in neuron-rich outer rings and glial cell-rich inner layers. Adapted with permission from [61]. Copyright 2019, Wiley-VCH. **(b)** Extrusion-based bioprinting of organoid-forming cell bioinks (i.e., mesenchymal stromal cells, umbilical vein endothelial cells, intestinal stem cells and intestinal mesenchymal cells) to assemble self-organizing and organ-specific tissues, such as epithelial tubes, connective tissues and perfusable vascular networks with user-defined configurations. Adapted with permission from [62]. Copyright 2020, Springer Nature. **(c)** Biomanufacturing technology for sacrificial writing into functional tissue (SWIFT) for robust vascularization of large-scale constructs with high cell density. Patient-specific–induced pluripotent stem cell–derived organoids were used as the building blocks of the self-healing and viscoelastic tissue matrix. Under this strategy, branched and perfusable intricate vascular networks were embedded within cardiac tissue constructs prior to their compaction and maturation, which assured long-term viability and enhanced their biofunctionality. Adapted under the terms of the CC-BY-NC Creative Commons Attribution license [3]. Copyright 2019, American Association for the Advancement of Science.

establishing the evolution of living materials to the clinic, there are several translational roadblocks posed by in-human applications of materials containing living cells - either in native or engineered form - that must be addressed to guarantee safety and future clinical approval [67,68]. Due to their human origin, such biological constructs present fewer biosafety issues over prokaryotic living biohybrids, but are still mandated to comply with *in vitro* expansion and culture in xeno-free conditions [69], which may affect the assembly of many of these constructs, and more importantly, whether their biofunctionality and long-term stability are retained. In addition, apart from well-established concerns pertaining to the loss of native cell phenotypes and altered gene expression profiles during *in vitro* culture, traditional 2D cell expansion is considered widely ineffective for large-scale manufacturing. On this note, additional research efforts are urgently required for accelerating the transition to 3D cell expansion technologies that promise to drastically improve cell production volumes and offer more close-to-native cell culturing conditions. Another crucial point that warrants further discussion in the roadmap to clinical translation is the source of the cellular building blocks to be employed. Although patient-derived cells are ideal candidates for matching patients' tissues in a fully personalized manner, the decision to use autologous cells has large consequences in the production pipeline, vastly increasing commercialization costs and the complexity of the process by introducing multiple steps, such as patient cell harvesting, isolation, validation and expansion. This leads to a lengthy

manufacturing process that can be completely incompatible with the needs of patients facing acute injuries/diseases.

Furthermore, the high inter-patient variability may greatly compromise the biofunctionality of the living materials, since cells obtained from distinct donors will have different regenerative efficacies. Also, different genetic and phenotypic factors could potentially alter the assembly and maturation of living materials, which adds another layer of variability in terms of accurately predicting their behaviour once implanted within the body. In the short-term, from a practical standpoint, it will be interesting to actively research potentially universal cell sources as building blocks for living materials. For instance, researchers have recently developed hypoimmunogenic human induced pluripotent stem cells (iPSCs) by overexpressing CD47 and inactivating major histocompatibility complex (MHC) class I and II genes [70] Notably, cardiomyocytes, endothelial and smooth muscle cells derived from hypoimmunogenic iPSCs can successfully achieve long-term survival without the use of immunosuppressants in completely MHC-mismatched allogeneic hosts [70]. From a medical perspective, such strategy may facilitate the widespread use of human-based living materials by completely avoiding immune rejection in implantation, as well as streamlining the production and biofunctional validation of biological constructs down to a single cell source. Due to their pluripotency, such cells can be differentiated into the main components of a broad range of human tissues, unlocking the development of truly universal living material products and bringing this field closer to feasible clinical translation in the foreseeable future.

Also, considerable attention should be placed on promoting the utilization of scalable and high-throughput cell bioengineering technologies, which would streamline the availability of cellular building blocks for the manufacturing of living materials. Such advances are also critical for overcoming cell expansion/functionalization bottlenecks that entail long processing periods with low modification efficiencies. Moreover, the impact of cell engineering technologies must be thoroughly investigated with regard to changes in secretome expression, genomic stability and long-term interaction with native cells [71]. On another level, the current development of some living materials entails a high degree of biomanufacturing and processing complexity, which hinders scalability and subsequently their widespread use [72]. Uncovering versatile biofabrication strategies that are rapidly scalable across several length scales (i.e., from micro- to centimetre-sized constructs) and are tolerant to the incorporation of different cell types and biomaterials, will also play a major role in progressing this field towards clinically-relevant scenarios. On another angle, it will be critical to guarantee that living materials can collectively match the slower pace of tissue turnover in adults and that are unable of outgrowing into the surrounding tissues and organs. In the upcoming future, it will be vital to develop advanced *in situ* medical bioimaging technologies for monitoring the development of implanted living materials as whole nascent tissues, as well as turning to high-resolution biosensing strategies for providing maps of individual cellular status/bioperformance [73].

Advancing the development of living materials as functional tissue analogues would not only streamline the availability of organs personalized to match patients omics, but also create the opportunity to supply patients with a potentially more favourable organ in terms of pharmacogenetic responses. To guide these concepts, recent breakthroughs in spatial omics can inform researchers about the fundamental ingredients to generate functional living materials mirroring a particular tissue [74]. Integrating this know-how with computational biology tools powered by artificial intelligence could be used for modelling *in silico* the assembly and evolution of living materials, as well as potentially predict their underlying biofunctionalities through an information-driven process [75]. Such strategies offer the opportunity to streamline the development of tissue-specific biofunctional modules including a pre-screened selection of the essential components responsible for tissue/organ function. This constitutes a far more realistic and practical approach in the short-term, in comparison to the still elusive concept of fully recreating human organs, which although ideal, is still currently hindered by the incomplete understanding of the dynamic composition and complex biochemical interactions that permeate native tissues. In the foreseeable future, identifying the key drivers of tissue performance can also inspire the build-up of new synthetic tissues and organs with exotic functions. Adding to this, due to their modular bottom-up nature and customizable composition, the greatest conceptual leap of living materials may be to assemble digitally-integrated organs with information processing and reporting capabilities [76]. Moreover, recent advances in bioelectronics have demonstrated the use of the human body as a medium for powering multiple skin-interfaced wearables from a single device (e.g., smartphone) [77]. Through this concept, multiple independent electrobiological materials can communicate and be powered wirelessly, bypassing the need for batteries and paving the future for the next-generation of living materials operating as tissue-machine interfaces, programmable biosensors and digitally-responsive clinical therapeutics.

5. Outstanding Questions

To expand the relevance of living materials in translational practices, several questions must be addressed in future research. For instance, how can researchers benchmark age-matched living materials for different patient age groups, and whether such constructs can grow together with younger patients in development and adapt their performance when reaching adult stages. Also, it will be important to integrate computational biology with machine learning in order to predict the response of living materials when implanted.

Another crucial point lies in their availability to practicing clinicians, as current iterations of living material products require extensive processing steps and time-consuming maturation periods. In this context, further research into novel cryopreservation methodologies should be pursued to transform living materials into off-the-shelf tissues and organ products. However, as we move towards increasingly large constructs and biofunctionally-complex systems, attaining this goal will be progressively more difficult since so far, no organs have been cryopreserved and successfully revived. Recognizing and addressing these overarching questions is the first step toward accelerating the translation of such living systems toward clinical applications. Recently, researchers have demonstrated that alginate-based hydrogels are able to confine ice crystal nucleation/growth and decrease osmotic stress during the cryopreservation of cell-biomaterial constructs as well as individual pancreatic islets [78]. Despite promising, this strategy is still insufficient for the cryopreservation of large-volume constructs, particularly those containing complex bioarchitectures interwoven in vascular systems [79]. A major roadblock is attaining uniform and rapid volumetric warming to inhibit ice recrystallization and devitrification during thawing, which causes deleterious injuries and undermines their viability. Concerning this, recent innovations in magnetic field thawing have been demonstrated to be attractive for overcoming intrinsic heterogeneities in the characteristics of cells and their microenvironment, thus reducing the amount of cryoprotectants and improving cryopreservation outcomes in dental pulp microtissues [78]. In the upcoming future, it will be paramount to improve and scale-up precision preservation to large-volume constructs (i.e., by combining multiple technologies to attain synergistic thawing), as well as to develop biosensing tools to monitor the success rates and the biological outcomes (i.e., changes in cellular functions, chromosomal instabilities/aberrations, and epigenetic alterations) of thawed living materials to ensure that their cytogenetic status remains unaffected [78,79]. The path that living materials must travel to benefit from rapid and widespread clinical applications, will require a multidisciplinary effort

from the scientific community to ensure safety, feasibility and reliability of the final products, while considering affordability and overcoming the necessary regulatory and ethical challenges.

6. Search strategy and selection criteria

Data for this Review were identified by searches of Scopus, Web of Science, Google Scholar, and references from relevant articles using the search terms “living materials”, “scaffold-free”, “cell surface engineering”, “genetic engineering”, and “cell-cell interactions”. Only articles published in English between 2000 and 2021 were included, with particular emphasis on the advances from the last 5 years.

Contributors

P. L., V. M. G. and J. F. M. conceptualized, prepared and discussed the review outline. P.L. wrote the manuscript and prepared the corresponding figure panels. V. M. G and J. F. M. reviewed and imparted additional feedback and considerations to the whole manuscript. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

The authors have no competing interests to declare.

Acknowledgments

Pedro Lavrador acknowledges an individual PhD fellowship from the Portuguese Foundation for Science and Technology (SFRH/BD/141834/2018). Vítor Gaspar also acknowledges the financial support by the Portuguese Foundation for Science and Technology through an individual Junior Researcher contract (CEEC/1048/2019). This work was also supported by the Programa Operacional Competitividade e Internacionalização (POCI), in the component FEDER, and by national funds (OE) through FCT/MCTES, in the scope of the projects Margel (PTDC/BTM-MAT/31498/2017) and PANGEIA (PTDC/BTM-SAL/30503/2017). This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. The funders were not involved in paper design, literature collection, literature analysis, interpretation or writing of the paper.

References

[1] Gaspar VM, Lavrador P, Borges J, Oliveira MB, Mano JF. Advanced Bottom-Up Engineering of Living Architectures. Adv Mater 2020 Feb 13;32(6):1903975.
[2] Webber MJ, Khan OF, Sydlik SA, Tang BC, Langer R. A Perspective on the Clinical Translation of Scaffolds for Tissue Engineering. Ann Biomed Eng 2015 Mar 9;43(3):641–56.
[3] Skylar-Scott MA, Uzel SGM, Nam LL, Ahrens JH, Truby RL, Damaraju S, et al. Biomanufacturing of organ-specific tissues with high cellular density and embedded vascular channels. Sci Adv. 2019 Sep 6;5(9):eaaw2459.
[4] Daly AC, Davidson MD, Burdick JA. 3D bioprinting of high cell-density heterogeneous tissue models through spheroid fusion within self-healing hydrogels. Nat Commun 2021 Dec 2;12(1):753.
[5] Chen AY, Zhong C, Lu TK. Engineering Living Functional Materials. ACS Synth Biol 2015 Jan 16;4(1):8–11.
[6] Burgos-Morales O, Gueye M, Lacombe L, Nowak C, Schmachtenberg R, Hörner M, et al. Synthetic biology as driver for the biologization of materials sciences. Mater Today Bio. 2021 Jun;11:100115.
[7] Shang L, Shao C, Chi J, Zhao Y. Living Materials for Life Healthcare. Accounts Mater Res 2021 Jan 22;2(1):59–70.
[8] Gilbert C, Tang T-C, Ott W, Dorr BA, Shaw WM, Sun GL, et al. Living materials with programmable functionalities grown from engineered microbial co-cultures. Nat Mater 2021 May 11;20(5):691–700.
[9] Wang Y, An B, Xue B, Pu J, Zhang X, Huang Y, et al. Living materials fabricated via gradient mineralization of light-inducible biofilms. Nat Chem Biol 2021 Mar 21;17(3):351–9.
[10] An B, Wang Y, Jiang X, Ma C, Mimee M, Moser F, et al. Programming Living Glue Systems to Perform Autonomous Mechanical Repairs. Matter 2020 Dec;3(6):2080–92.
[11] Hay JJ, Rodrigo-Navarro A, Petaroudi M, Bryksin AV, García AJ, Barker TH, et al. Bacteria-Based Materials for Stem Cell Engineering. Adv Mater 2018 Oct 25;30(43):1804310.
[12] Huang J, Liu S, Zhang C, Wang X, Pu J, Ba F, et al. Programmable and printable Bacillus subtilis biofilms as engineered living materials. Nat Chem Biol 2019 Jan 3;15(1):34–41.
[13] Tang T-C, An B, Huang Y, Vasikaran S, Wang Y, Jiang X, et al. Materials design by synthetic biology. Nat Rev Mater 2021 Apr 23;6(4):332–50.
[14] Nguyen PQ, Courchesne N-MD, Duraj-Thatte A, Praveschotinunt P, Joshi NS. Engineered Living Materials: Prospects and Challenges for Using Biological Systems to Direct the Assembly of Smart Materials. Adv Mater 2018 May 10;30(19):1704847.
[15] Gilbert C, Ellis T. Biological Engineered Living Materials: Growing Functional Materials with Genetically Programmable Properties. ACS Synth Biol 2019 Jan 18;8(1):1–15.
[16] Park J, Andrade B, Seo Y, Kim M, Zimmerman SC, Kong H. Engineering the Surface of Therapeutic “Living” Cells. Chem Rev 2018 Feb 28;118(4):1664–90.
[17] Cooper GM. Cell-Cell Interactions. The cell: a molecular approach. 2nd ed. Sunderland: Sinauer Associates; 2000.
[18] Silva AS, Santos LF, Mendes MC, Mano JF. Multi-layer pre-vascularized magnetic cell sheets for bone regeneration. Biomaterials 2020 Feb;231:119664.
[19] Sousa AR, Martins-Cruz C, Oliveira MB, Mano JF. One-Step Rapid Fabrication of Cell-Only Living Fibers. Adv Mater 2020 Jan 26;32(2):1906305.
[20] Vrij E, Rouwkema J, LaPointe V, van Blitterswijk C, Truckenmüller R, Rivron N. Directed Assembly and Development of Material-Free Tissues with Complex Architectures. Adv Mater 2016 Jun 1;28(21):4032–9.
[21] Matsusaki M, Kadowaki K, Nakahara Y, Akashi M. Fabrication of Cellular Multilayers with Nanometer-Sized Extracellular Matrix Films. Angew Chemie Int Ed 2007 Jun 18;46(25):4689–92.
[22] Sasaki K, Akagi T, Asaoka T, Eguchi H, Fukuda Y, Iwagami Y, et al. Construction of three-dimensional vascularized functional human liver tissue using a layer-by-layer cell coating technique. Biomaterials 2017 Jul;133:263–74.
[23] Cachat E, Liu W, Martin KC, Yuan X, Yin H, Hohenstein P, et al. 2- and 3-dimensional synthetic large-scale de novo patterning by mammalian cells through phase separation. Sci Rep. 2016 Aug 9;6(1):20664.
[24] Jeon O, Bin Lee Y, Jeong H, Lee SJ, Wells D, Alsberg E. Individual cell-only bioink and photocurable supporting medium for 3D printing and generation of engineered tissues with complex geometries. Mater Horizons 2019;6(8):1625–31.
[25] Patrício SG, Sousa LR, Correia TR, Gaspar VM, Pires LS, Luís JL, et al. Freeform 3D printing using a continuous viscoelastic supporting matrix. Biofabrication 2020 May 15;12(3):035017.
[26] Maciel MM, Correia TR, Gaspar VM, Rodrigues JMM, Choi IS, Mano JF. Partial Coated Stem Cells with Bioinspired Silica as New Generation of Cellular Hybrid Materials. Adv Funct Mater 2021 Mar 9:2009619.
[27] Kim BJ, Cho H, Park JH, Mano JF, Choi IS. Strategic Advances in Formation of Cell-in-Shell Structures: From Syntheses to Applications. Adv Mater 2018 Apr 5;30(14):1706063.
[28] Oliveira MB, Hatami J, Mano JF. Coating Strategies Using Layer-by-layer Deposition for Cell Encapsulation. Chem - An Asian J 2016 Jun 21;11(12):1753–64.
[29] O’Brien PJ, Luo W, Rogozhnikov D, Chen J, Yousaf MN. Spheroid and Tissue Assembly via Click Chemistry in Microfluidic Flow. Bioconjug Chem 2015 Sep 16;26(9):1939–49.
[30] Gartner ZJ, Bertozzi CR. Programmed assembly of 3-dimensional microtissues with defined cellular connectivity. Proc Natl Acad Sci 2009 Mar 24;106(12):4606–10.
[31] Agatemor C, Buettner MJ, Ariss R, Muthiah K, Saeui CT, Yarema KJ. Exploiting metabolic glycoengineering to advance healthcare. Nat Rev Chem 2019 Oct 6;3(10):605–20.
[32] Li W, Pan H, He H, Meng X, Ren Q, Gong P, et al. Bio-Orthogonal T Cell Targeting Strategy for Robustly Enhancing Cytotoxicity against Tumor Cells. Small 2019 Jan 25;15(4):1804383.
[33] Nagahama K, Kimura Y, Takemoto A. Living functional hydrogels generated by bioorthogonal cross-linking reactions of azide-modified cells with alkyne-modified polymers. Nat Commun 2018 Dec 6;9(1):2195.
[34] Toda S, Blauch LR, Tang SKY, Morsut L, Lim WA. Programming self-organizing multicellular structures with synthetic cell-cell signaling. Science (80-) 2018 May 31;162(July):eaat0271.
[35] Santorelli M, Lam C, Morsut L. Synthetic development: building mammalian multicellular structures with artificial genetic programs. Curr Opin Biotechnol 2019 Oct;59:130–40.
[36] Morsut L, Roybal KT, Xiong X, Gordley RM, Coyle SM, Thomson M, et al. Engineering Customized Cell Sensing and Response Behaviors Using Synthetic Notch Receptors. Cell 2016 Feb;164(4):780–91.
[37] Toda S, McKeithan WL, Hakkinen TJ, Lopez P, Klein OD, Lim WA. Engineering synthetic morphogen systems that can program multicellular patterning. Science (80-) 2020 Oct 16;370(6514):327–31.
[38] Shi P, Ju E, Yan Z, Gao N, Wang J, Hou J, et al. Spatiotemporal control of cell-cell reversible interactions using molecular engineering. Nat Commun 2016 Oct 6;7:1–9.
[39] Luo W, Pulsipher A, Dutta D, Lamb BM, Yousaf MN. Remote control of tissue interactions via engineered photo-switchable cell surfaces. Sci Rep 2014 Sep 10;4:1–8.
[40] Yüz SG, Rasoulinejad S, Mueller M, Wegner AE, Wegner SV. Blue Light Switchable Cell–Cell Interactions Provide Reversible and Spatiotemporal Control Towards Bottom-Up Tissue Engineering. Adv Biosyst 2019 Apr 18;3(4):1800310.

[41] Baaske J, Mühlhäuser WWD, Yousefi OS, Zanner S, Radziwill G, Hörner M, et al. Optogenetic control of integrin-matrix interaction. Commun Biol 2019 Dec 8;2(1):15.
[42] Qian R-C, Zhou Z-R, Guo W, Wu Y, Yang Z, Lu Y. Cell Surface Engineering Using DNAzymes: Metal Ion Mediated Control of Cell−Cell Interactions. J Am Chem Soc 2021 Apr 21;143(15):5737–44.
[43] Hoffman T, Antovski P, Tebon P, Xu C, Ashammakhi N, Ahadian S, et al. Synthetic Biology and Tissue Engineering: Toward Fabrication of Complex and Smart Cellular Constructs. Adv Funct Mater 2020 Jun 3;30(26):1909882.
[44] Nims RJ, Pferdehirt L, Ho NB, Savadipour A, Lorentz J, Sohi S, et al. A synthetic mechanogenetic gene circuit for autonomous drug delivery in engineered tissues. Sci Adv 2021 Jan 27;7(5):eabd9858.
[45] Liu J, Kim YS, Richardson CE, Tom A, Ramakrishnan C, Birey F, et al. Genetically targeted chemical assembly of functional materials in living cells, tissues, and animals. Science (80-) 2020 Mar 20;367(6484):1372–6.
[46] Aydin O, Zhang X, Nuethong S, Pagan-Diaz GJ, Bashir R, Gazzola M, et al. Neuromuscular actuation of biohybrid motile bots. Proc Natl Acad Sci 2019 Oct 1;116(40):19841–7.
[47] Li TL, Wang Z, You H, Ong Q, Varanasi VJ, Dong M, et al. Engineering a Genetically Encoded Magnetic Protein Crystal. Nano Lett 2019 Oct 9;19(10):6955–63.
[48] Lavrador P, Esteves MR, Gaspar VM, Mano JF. Stimuli-Responsive Nanocomposite Hydrogels for Biomedical Applications. Adv Funct Mater 2021 Feb 18;31(8):2005941.
[49] Rosales AM, Anseth KS. The design of reversible hydrogels to capture extracellular matrix dynamics. Nat Rev Mater 2016 Feb 2;1(2):15012.
[50] Zhang Y, Rathnam C, Pongkulapa T, Lee K. Bioengineering Approaches for the Advanced Organoid Research. Adv Mater 2021 Nov 11;33(45):2007949.
[51] Kim BS, Das S, Jang J, Cho D-W. Decellularized Extracellular Matrix-based Bioinks for Engineering Tissue- and Organ-specific Microenvironments. Chem Rev 2020 Oct 14;120(19):10608–61.
[52] Ferreira LP, Gaspar VM, Mano JF. Decellularized Extracellular Matrix for Bioengineering Physiomimetic 3D in Vitro Tumor Models. Trends Biotechnol 2020 Dec;38(12):1397–414.
[53] Choi Y-J, Jun Y-J, Kim DY, Yi H-G, Chae S-H, Kang J, et al. A 3D cell printed muscle construct with tissue-derived bioink for the treatment of volumetric muscle loss. Biomaterials 2019 Jun;206:160–9.
[54] Wang T, Han Y, Wu Z, Qiu S, Rao Z, Zhao C, et al. Tissue-Specific Hydrogels for 3D Printing and Potential Application in Peripheral Nerve Regeneration. Tissue Eng Part A 2021 Jul 26.
[55] Ha D-H, Chae S, Lee JY, Kim JY, Yoon J, Sen T, et al. Therapeutic effect of decellularized extracellular matrix-based hydrogel for radiation esophagitis by 3D printed esophageal stent. Biomaterials 2021 Jan;266:120477.
[56] Yewdall NA, Mason AF, van Hest JCM. The hallmarks of living systems: towards creating artificial cells. Interface Focus 2018 Oct 6;8(5):20180023.
[57] Sakaguchi K, Shimizu T, Okano T. Construction of three-dimensional vascularized cardiac tissue with cell sheet engineering. J Control Release 2015;205:83–8.
[58] Santos LF, Sofia Silva A, Mano JF. Complex-shaped magnetic 3D cell-based structures for tissue engineering. Acta Biomater 2020 Dec;118:18–31.
[59] Lu Y, Zhang W, Wang J, Yang G, Yin S, Tang T, et al. Recent advances in cell sheet technology for bone and cartilage regeneration: from preparation to application. Int J Oral Sci 2019 Jun 21;11(2):17.
[60] De Pieri A, Rochev Y, Zeugolis DI. Scaffold-free cell-based tissue engineering therapies: advances, shortfalls and forecast. npj Regen Med 2021 Dec 29;6(1):18.
[61] Ren T, Chen P, Gu L, Ogut MG, Demirci U. Soft Ring-Shaped Cellu-Robots with Simultaneous Locomotion in Batches. Adv Mater 2020 Feb 27;32(8):1905713.
[62] Brassard JA, Nikolaev M, Hübscher T, Hofer M, Lutolf MP. Recapitulating macroscale tissue self-organization through organoid bioprinting. Nat Mater 2021 Jan 21;20(1):22–9.
[63] Hofer M, Lutolf MP. Engineering organoids. Nat Rev Mater 2021 May 19;6(5):402–20.
[64] Nilsson Hall G, Mendes LF, Gklava C, Geris L, Luyten FP, Papantoniou I. Developmentally Engineered Callus Organoid Bioassemblies Exhibit Predictive In Vivo Long Bone Healing. Adv Sci 2020 Jan 10;7(2):1902295.
[65] Skylar-Scott MA, Huang JY, Lu A, Ng AHM, Duenki T, Nam LL, et al. An orthogonal differentiation platform for genomically programming stem cells, organoids, and bioprinted tissues. bioRxiv. 2020 Jan 1 2020.07.11.198671.
[66] Tibbitt MW, Langer R. Living Biomaterials. Acc Chem Res 2017 Mar 21;50(3):508–13.
[67] Wang LL, Janes ME, Kumbhojkar N, Kapate N, Clegg JR, Prakash S, et al. Cell therapies in the clinic. Bioeng Transl Med 2021 May 26;6(2):1–36.
[68] Chen YY, Smolke CD. From DNA to Targeted Therapeutics: Bringing Synthetic Biology to the Clinic. Sci Transl Med 2011 Oct 26;3(106):106ps42.
[69] Aijaz A, Li M, Smith D, Khong D, Leblon C, Fenton OS, et al. Biomanufacturing for clinically advanced cell therapies. Nat Biomed Eng 2018 Jun 11;2(6):362–76.
[70] Deuse T, Hu X, Gravina A, Wang D, Tediashvili G, De C, et al. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. Nat Biotechnol 2019 Mar 18;37(3):252–8.
[71] Csizmar CM, Petersburg JR, Wagner CR. Programming Cell-Cell Interactions through Non-genetic Membrane Engineering. Cell Chem Biol 2018 Aug 16;25(8):931–40.
[72] Place ES, Evans ND, Stevens MM. Complexity in biomaterials for tissue engineering. Nat Mater 2009 Jun;8(6):457–70.
[73] Zhang P, Shao N, Qin L. Recent Advances in Microfluidic Platforms for Programming Cell-Based Living Materials. Adv Mater 2021 Nov 18;33(16):2005944.
[74] Bingham GC, Lee F, Naba A, Barker TH. Spatial-omics: Novel approaches to probe cell heterogeneity and extracellular matrix biology. Matrix Biol 2020 Sep;91−92:152–66.
[75] Goecks J, Jalili V, Heiser LM, Gray JW. How Machine Learning Will Transform Biomedicine. Cell 2020 Apr;181(1):92–101.
[76] Orive G, Taebnia N, Dolatshahi-Pirouz A. A New Era for Cyborg Science Is Emerging: The Promise of Cyborganic Beings. Adv Healthc Mater 2020 Jan 28;9(1):1901023.
[77] Li J, Dong Y, Park JH, Yoo J. Body-coupled power transmission and energy harvesting. Nat Electron 2021 Jun 10;4:530–8.
[78] Huang H, He X, Yarmush ML. Advanced technologies for the preservation of mammalian biospecimens. Nat Biomed Eng 2021 Aug 23;5(8):793–804.
[79] Chang T, Zhao G. Ice Inhibition for Cryopreservation: Materials, Strategies, and Challenges. Adv Sci 2021 Mar;8(6):2002425.