Materials Today Bio 16 (2022) 100352

Contents lists available at ScienceDirect

Materials Today Bio

journal homepage: www.journals.elsevier.com/materials-today-bio

Biohybrid materials: Structure design and biomedical applications

Chong Wang, Zhuohao Zhang, Jiali Wang, Qiao Wang, Luoran Shang *

Shanghai Xuhui Central Hospital, Zhongshan-Xuhui Hospital, And the Shanghai Key Laboratory of Medical Epigenetics, The International Co-laboratory of Medical Epigenetics and Metabolism (Ministry of Science and Technology), Institutes of Biomedical Sciences, Fudan University, Shanghai, 200032, China

A R T I C L E I N F O

Keywords:
Biohybrid sheet
Biohybrid fiber
Biohybrid microgel
Biohybrid microcapsule
Biohybrid scaffold

A B S T R A C T

Biohybrid materials are proceeded by integrating living cells and non-living materials to endow materials with biomimetic properties and functionalities by supporting cell proliferation and even enhancing cell functions. Due to the outstanding biocompatibility and programmability, biohybrid materials provide some promising strategies to overcome current problems in the biomedical field. Here, we review the concept and unique features of biohybrid materials by comparing them with conventional materials. We emphasize the structure design of biohybrid materials and discuss the structure-function relationships. We also enumerate the application aspects of biohybrid materials in biomedical frontiers. We believe this review will bring various opportunities to promote the communication between cell biology, material sciences, and medical engineering.

1. Introduction

Over the past few decades, fantastic achievements have been made in biomedical fields, which takes advantage of not only life science progress but also the rapid development of materials science. In particular, many novel materials have emerged and are functionalized with “smart” properties in that they can sense and respond to external environmental stimuli [1]. With the development in multidisciplinary aspects, smart materials have been widely employed in biosensing, drug delivery, tissue engineering, wearable devices, etc [2–7]. Smart materials are generally composed of various active components, among which the living cells are extraordinarily attractive since they could respond to even weak stimuli and produce highly complex biological activities [2]. Apart from sensing, living cells can also serve as living factories and synthesize materials with special functions [6,7]. As such, researchers have tried to harness living cells and directly integrate them with synthetic materials to realize greater control of material properties and functionalities [2,5,8]. The synergistic combination of living cells with nonactive materials is known as biohybrid materials, which has become a new paradigm for the design of smart materials. On one hand, synthetic materials provide protection and substrate to the cells [8]. On the other hand, living cells exercise their functions of sensing, synthesis, secretion, etc., thus modulating the physicochemical properties of the composite materials [6,8]. Since the advantages of cells and synthetic materials are combined, these biohybrid materials enable living cells to perform desired functions and facilitate the assembly of artificial biological systems, which have revolutionized synthetic biology and materials chemistry, and promoted the progress of the biomedical field [9,10].

For the fabrication of biohybrid materials, various types of living cells have been explored, including mammalian cells, insect cells, and microbial cells [2,5,8,11]. In terms of synthetic materials, polymers, nanoparticles, and other functional ingredients have been incorporated, whose biocompatibility and mechanical behaviors are guaranteed appropriate to couple with cells [5,12]. These materials could act as coatings on cell surface, microcarriers for cell growth, scaffolds for cell organization, etc [8,13]. To precisely control the structure of the biohybrid materials, many efforts have been made in the fabrication methods, inducing molding, electrospinning, microfluidics, 3D printing, to list a few. Especially, owing to the ability of easy operating and flexible control of the architecture of the products, microfluidics and 3D printing play significant roles in the fabrication of biohybrid materials. With these methods, biohybrid materials could be processed into various configurations, including microgels, microparticles/capsules, fibers, films, and scaffolds [14–17]. The differently structured biohybrid materials and devices hold great potential in the biomedical field.

In this paper, we present a concise overview of the structure design of biohybrid materials and their biomedical applications in biomedical fields (Fig. 1). Although there are many reviews about biohybrid materials [1,3,10,12], few of them have emphasized the correlation between their structures and biomedical applications. Considering that structure property is an essential factor for the development of biomaterials, we take it as a criterion for the design of biohybrid biomaterials. Herein, we

* Corresponding author.
E-mail address: luoranshang@fudan.edu.cn (L. Shang).

https://doi.org/10.1016/j.mtbio.2022.100352
Received 29 April 2022; Received in revised form 1 July 2022; Accepted 2 July 2022
Available online 8 July 2022

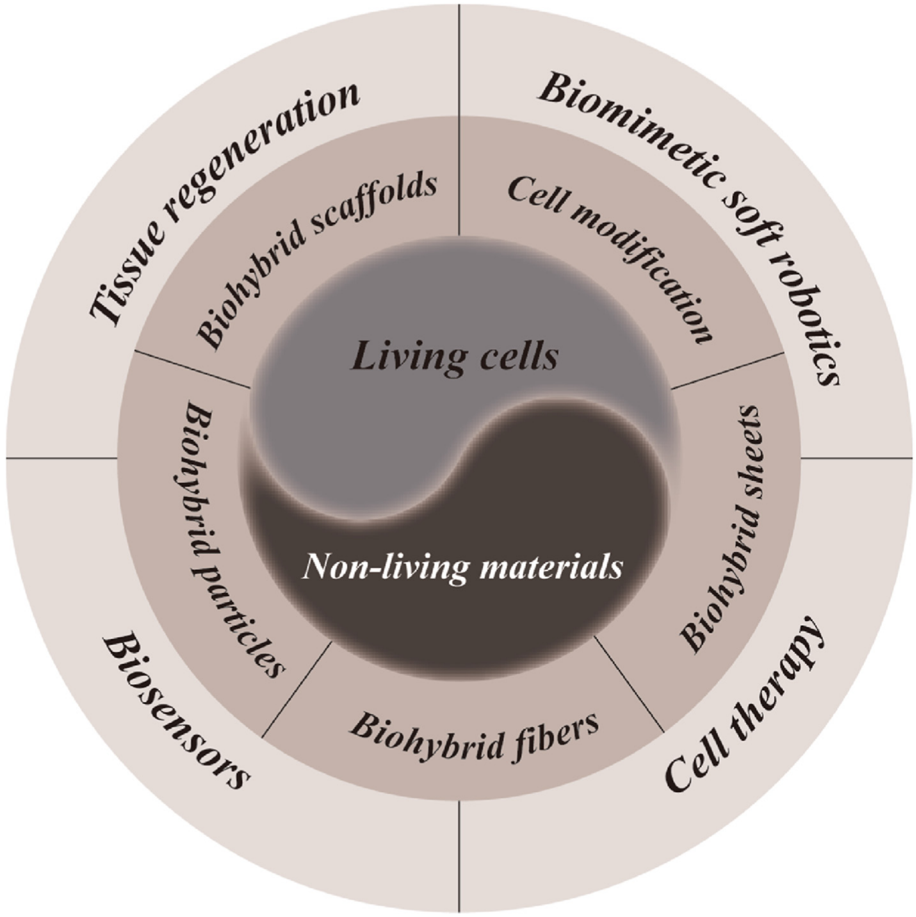

Fig. 1. Overview of biohybrid materials for biomedical applications.

first introduce the concept of biohybrid materials and summarize the properties of the "bio" parts and the synthetic parts. Subsequently, we shift to the different architectures of biohybrid materials and discuss the correlation between their structures and functions. Following these sections, we enumerate the applications of biohybrid materials in biomedical fields and elaborate on specific examples as to which aspect of the structure features contribute to the functional performances. Finally, we provide critical perspectives regarding the current challenges and the unique opportunities of biohybrid materials. We expect that this review would inspire the design of novel biohybrid materials and extend their application values in biomedical areas.

2. Concept of biohybrid materials

Biohybrid materials are composite materials composed of living cells together with non-living substances [3]. On one hand, living cells are sensitive to external stimuli, and the introduction of living cells endows the biohybrid materials with "smart" functions that mimic some natural biological activities [6]. On the other hand, although living cells have many fantastic features since most of them are vulnerable to the external environment, they can only survive and function in relatively mild conditions. Therefore, it is vitally important to create certain protective barriers for living cells to achieve specific properties [1]. Additionally, materials with tailorable physicochemical properties could mediate bio-interface features and may augment cellular functions [10]. Besides, with proper design and assembly of these biohybrid materials, more complex and bioactive behaviors could be implemented [18–20]. Taken together, biohybrid materials achieve highly controllable and distinctive functionalities by combining living cells and non-living materials and taking advantage of the two parts.

2.1. Features of the bio-part

In recent decades, with the deepening understanding of cell behavior and functionality, a large variety of living cells (including microbial cells, insect cells, and mammalian cells) have been used to design and synthesize biohybrid materials [5,6]. As a typical example, some bacteria show distinctive motility functions in response to light, temperature, magnetic fields, electric fields, chemical gradients or oxygen concentration [2]. The motility cells could be directly harnessed as actuators to construct biohybrid robotics [1,5]. This also includes alternative motile cells, for example, sperm cells, as well as motile tissues such as cardiomyocytes, skeleton muscles, and insect-contractile tissues [21–23]. Benefiting from the utilization of living bio-parts, such biohybrid robotics enable controllable motion and bring extraordinary properties hardly achievable in traditional electromechanical-based robotics such as miniaturization, environment-friendliness, self-healing performance, etc. Apart from motility, some living cells could perform therapeutic activities, such as stem cells, natural killer (NK) cells, islet cells, probiotics, etc [24–26]. By combining with carrier materials that could support cell viability and extend cellular functions, these biohybrid materials could be utilized for disease treatment and tissue regeneration [27,28]. Besides, some microbial strains, such as *Escherichia* coli, could be genetically modified to secret novel substances with structural patterning and programable functions [29].

2.2. Features of the non-living part

The coupling of non-living materials with live cells could be accomplished by coating cell surfaces or embedding cells inside a material matrix [30,31]. These strategies are usually adopted to adjust and enhance the functionality of living cells [18,32]. For instance, aiming for enhancing the stress resistance of living cells and expanding their applications, non-living materials with tunable mechanical properties are incorporated to serve as membrane guards or matrices [31]. These materials give structural support to cells, thus keeping cells alive, supporting cell proliferation, and protecting cells from immunological agents or harsh environments [1]. In addition, desirable biocompatibility and porous structure are also favored for enabling the translation of gas, nutrients, and metabolism substances [26,33,34]. To meet these requirements, a variety of cytocompatible materials, such as collagen, sodium alginate, gelatin, silk fibroin, agarose, etc., have been commonly applied in biohybrid materials [6,9,24,30]. Besides, the intrinsic properties of the non-living components, especially the stimuli-responsive features can offer great manipulability to the biohybrid materials [35].

3. Structures of biohybrid materials

Since the functions of materials are closely related to their structures, there is a growing understanding of cell function and cell-materials interaction. This promotes us to exploit some specific functions of living cells by specially designing the structure of biohybrid materials. For instance, biohybrid materials with different forms, such as engineered cell modification, cell-encapsulated microparticles, cell-laden fibers, biohybrid sheets and 3D biohybrid scaffolds, are prevalent in biomedical applications. Besides, each of them holds unique advantages in certain aspects, which will be introduced in the following section.

3.1. Cell modification

Engineered cell modification is currently a hotspot of biomedical research due to its great potential in cell protection, targeting delivery, and cell therapy [18,32,36]. Specifically, cell modification aims to enhance or mediate cell functions via physical or chemical modifications as well as genetic engineering. Among various strategies, the cell coating method is commonly used to protect living cells and enhance their functionalities [18,32,36]. For example, Zhu et al. presented "Supra-Cells" showing great resistance to the external environment by coating mammalian cells with a layer of nanoparticle-based exoskeletons, which was accomplished by the physical interactions between nanoparticles and cell membranes [32], as shown in Fig. 2a and b. Differently, a chemical modification strategy was adopted by Maciel et al. to coat a layer of partial silica on the surface of living cells [36] (Fig. 2c). With this method, the modified adherent cells held greater stress resistance and could survive longer in a suspension environment. Apart from coating nanoparticles on the cell surface to enhance cell resistance, a similar cell modification strategy was introduced to protect cells from the recognition and attack of the host's immune system. This was achieved by coating a layer of polysialic acid (PSA)-tyramine nanogel framework on the surface of living cells [18]. It was demonstrated that the nanogel framework could successfully shield the epitopes on the surface of Rhesus D (RhD)-positive red blood cells and completely blocks the antigen-antibody recognition, which reduced the rejection reactions and given new opportunities to overcome the urgent shortages of RhD-negative blood.

3.2. Cell-encapsulated microparticles

In addition to nanoparticle coating, biocompatible hydrogels with tunable physicochemical properties are widely used for cell encapsulation due to their similar structure and functions to the extracellular matrix [37,38]. Particularly, single-cell level encapsulation offers great advantages to precisely controlling environmental cues as well as cellular

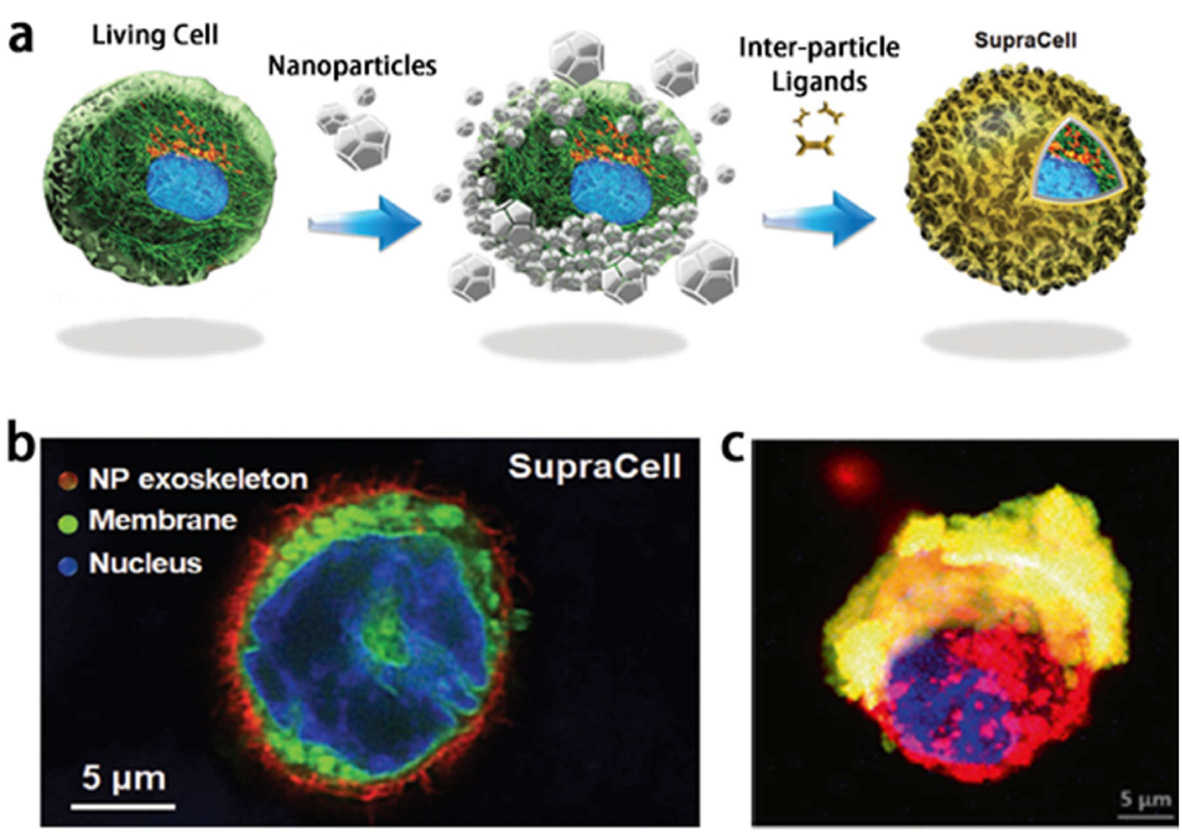

Fig. 2. Engineered cell modification. (a) Schematic showing the formation procedure of the "SupraCells" [32]. Copyright 2019, Wiley VCH. (b) Confocal image of the "SupraCell" [32]. Copyright 2019, Wiley VCH. (c) Representative confocal image showing a layer of partial silica coating on the surface of living cells [36]. Copyright 2021, Wiley VCH.

behaviors [31,39–41]. Notably, following the principle of Poisson distribution, droplet microfluidics has been well established for producing single-cell laden microgels, which is accomplished by diluting the cell suspension to an extremely low concentration and ensuring that most droplets contain no more than one cell [31].

Since the encapsulated cell tends to locate at or escape from the interface of droplets, some special strategies have been proposed to match the cell encapsulating and droplet crosslinking processes [14]. Leijten and coworkers presented a dual photo-crosslinker droplet microfluidic system by dissolving Irgacure 2959 and Irgacure 651 in the inner aqueous phase and outer organic phase, respectively [39]. After ultraviolet (UV)-induced crosslinking and subsequent fluorescence-activated cell sorting (FACS), single-cell laden poly (ethylene glycol) diacrylate (PEGDA) microparticles are fabricated and enriched (Fig. 3a). Similarly, enzymatically crosslinked microgels encapsulating single cells were formed in a microfluidic droplet generator with the aid of a delaying crosslinking strategy [14].

In addition to single-cell encapsulation, there are cases where a population of cells are encapsulated, and the resultant biohybrid microparticles could be used for cell culturing, delivery, etc [42–48]. Biohybrid microparticles could be generated from microfluidic droplets, where maintaining a high viability of cells is a prerequisite [46]. As such, Headen et al. presented a flow-focusing device, by which cells could be encapsulated in droplets that undergo a cytocompatible covalent cross-linking process [45]. Alternatively, Weitz and co-workers generated cell-laden microgels through water-in-oil-in-water (w/o/w) emulsion droplets with an ultrathin oil layer. After UV irradiation, the aqueous core of the droplets, which was a pregel solution containing cells, were selectively cross-linked and cells were immobilized in the gel matrix. Subsequently, by collecting the microparticles in an aqueous solution, the ultrathin oil shell dewetted from the surface of the microparticles, leading to the formation of cell-laden microgels [45]. This strategy enables rapid production of cell-laden microparticles and minimized the contact of living cells with organic solvents.

Besides, all-aqueous phase droplets, derived from immiscible aqueous solutions with sufficiently high concentrations, have been exploited for the generation of biohybrid particles due to their superior biocompatibility. Based on this, Zhao and colleagues presented a three-aqueous-phase microfluidic platform to achieve cell capsulation [43]. The continuous phase was a poly (ethylene glycol) (PEG) solution, the middle phase was an aqueous solution of sodium alginate and dextran, and the inner phase was a cell-containing sodium carboxymethylcellulose solution. Since the interfacial tension between the aqueous phases is ultra-low, a solenoid valve was introduced to facilitate the generation of all-aqueous double emulsion droplets. The droplets were then collected in a calcium chloride solution, which resulted in the formation of cell-laden all-aqueous microcapsules. Vilabril et al. utilized the electrospray strategy to produce cell-laden microcapsules by the complexation of oppositely charged alginate and ∊-poly-L-lysine (PLL), which were subsequently proved biocompatible [44]. Shum and coworkers prepared cell-laden alginate-dextran droplets through electrospray [42]. The droplets were solidified in a continuous phase containing Ca^{2+}, leading to the formation of cell-laden alginate microgels (Fig. 3b).

3.3. Cell-laden fibers

Fiber-shaped biohybrid materials are attractive and useful in creating complex biohybrid objects due to their flexible structure and large aspect ratio, and these features facilitate the assembly of biohybrid fiber materials to a larger scale and higher order. Interestingly, fibrous structures

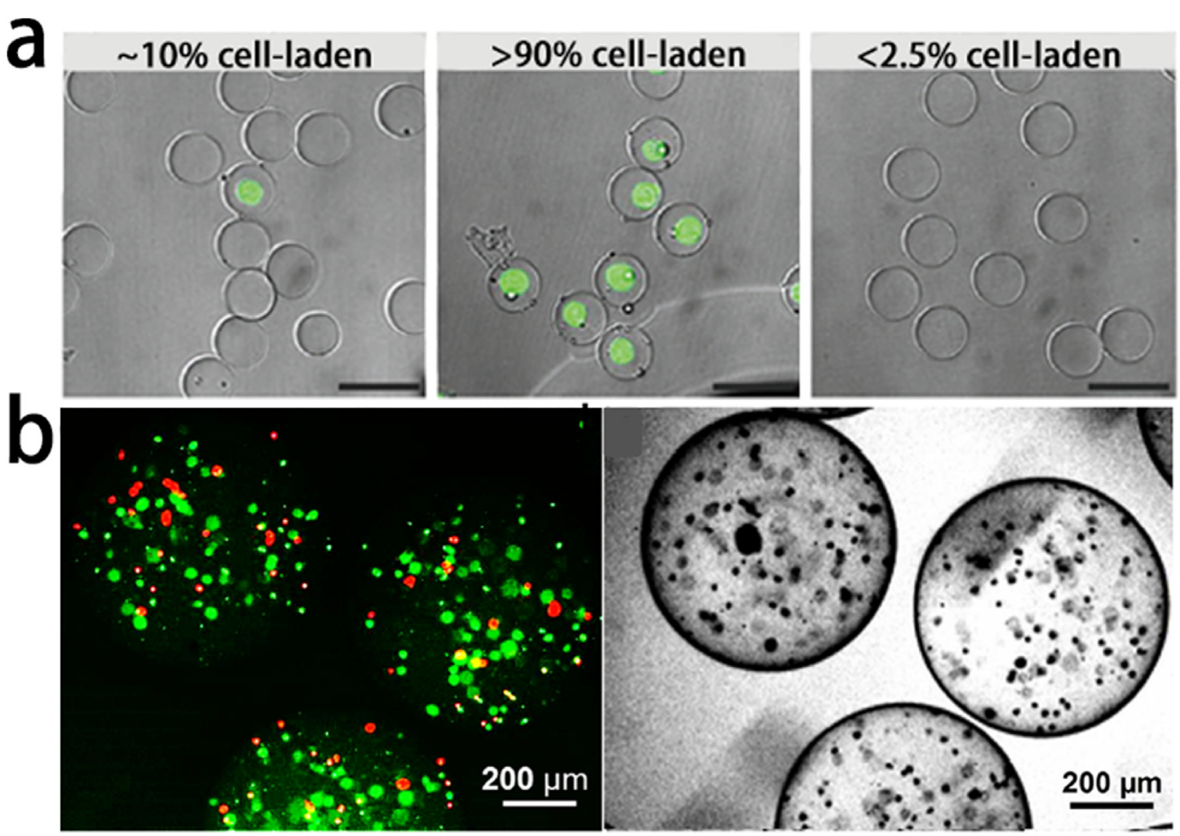

Fig. 3. Biohybrid microparticles. (a) Enrichment of single-cell-laden microgels by FACS. The live cells were stained with green fluorescence [39]. Copyright 2012, Wiley VCH. (b) Fluorescent (left) and bright-field (right) microscopic images of biohybrid microgels [42]. Red and green fluorescence represents live and dead cells, respectively. Scale bars are 50 μm in (a). Copyright 2015, American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

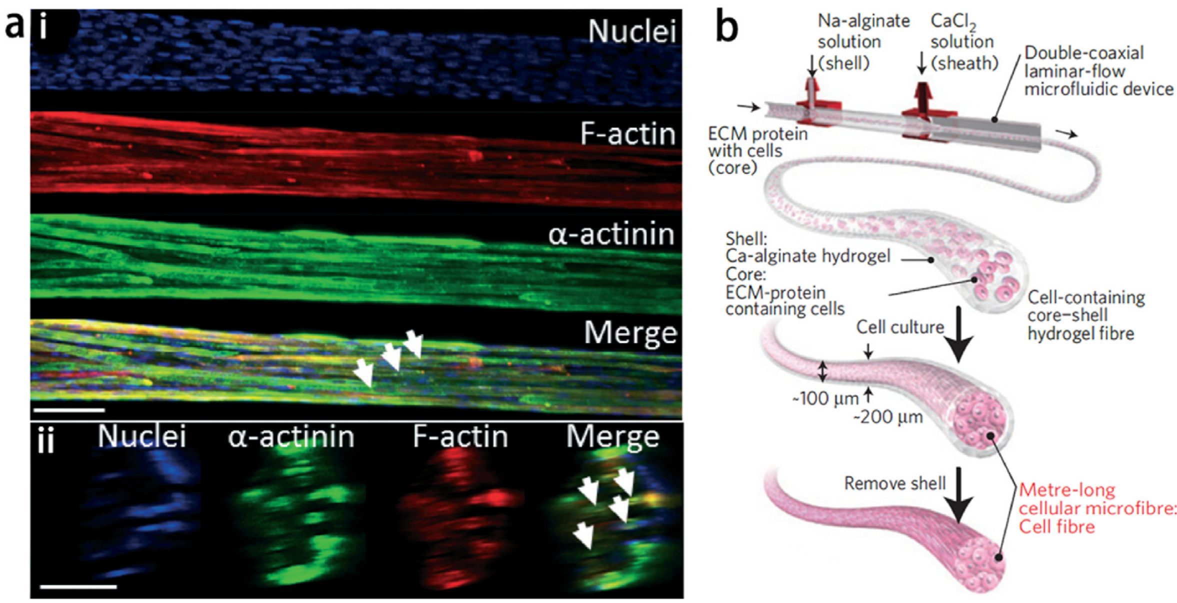

Fig. 4. Construction and fabrication of biohybrid fibers. (a) Confocal images of longitudinal (i) and axial (ii) sections of the fascicle-like biohybrid constructs [50]. Copyright 2014, Royal Society of Chemistry. (b) Schematic diagram showing the fabrication procedures of the biohybrid meter-long fibers [51]. Scales bar are 50 μm in (a). Copyright 2013, Springer Nature.

exist widely in living body tissues, such as blood vessels, muscles, and nerves. These constructs inspire us to build biohybrid fibrous materials [49–51]. The molding technique is a facile method for the fabrication of biohybrid fibers. For example, Neal et al. presented an elongated fascicle-inspired biohybrid material with aligned cells by utilizing a sacrificial outer molding approach [50]. Specifically, a polydimethylsiloxane (PDMS) mold was first generated containing a through-hole. A fiber-like cavity tube was formed with the help of a cylindrical steel pin and a sacrificial material, gelatin. A mixture of fibrinogen and cells was seeded in the cavity tube. By raising the temperature, gelatin melted to release a gelling agent, which caused fibrinogen to solidify and finally resulted in the formation of fascicle-like constructs (Fig. 4a).

Hiroaki Onoe et al. presented a strategy to fabricate meter-long cell-laden fibers by microfluidic spinning [51]. To improve the biocompatibility of the fibers and the cell viability, the microfluidic device was designed that enabled to generate fibers consisting of a rapidly gelled tubular Ca-alginate shell and an extracellular matrix (ECM) core containing cells. After the slow gelation of ECMs, the outer Ca-alginate shell was decomposed and removed by adding alginate lyase, and the cell-laden fiber was finally obtained, as shown in Fig. 4b. The versatility of this strategy was demonstrated by accommodating different types of cells in the fibers. In another attempt, biohybrid fibers with a core-shell construction have been fabricated by applying coaxial electrospinning [49]. The resultant fibers contained an aqueous core with a water-soluble polymer, which facilitated the survival and proliferation of yeast cells, and an insoluble polymer shell, which provided physical durability and supported the water-based application of the fibers.

3.4. Biohybrid sheets

Biohybrid sheets hold great potential in manipulating the behavior of living cells and could act as building blocks to reconstruct complex architectures of living tissue models. Biohybrid sheets are commonly used in the research of cell-cell/cell-matrix interactions, cell culture, and cell therapy [52–54]. Basically, biohybrid sheets are made up of a layer of hydrogel matrix embedded with living cells. For instance, Son et al. introduced a mesh-like PDMS mold to fabricated cell-laden mesh-like sheets with hepatic lobule-like architecture [55]. By using a PDMS drainage well, the sheets could be further stacked, as shown in Fig. 5a. Besides, some biohybrid sheets with complex constructions are produced with the help of microfluidics and microfabrication techniques. Leng et al. developed a multilayer microfluidic system under programmed valve actuation to produce mosaic biohybrid sheets [52]. Biopolymers together with payloads flowed in the microfluidic device to form a planar fluid network, which was then crosslinked to form a mosaic hydrogel sheet with well-controlled properties. The hydrogel sheets could be stacked or collected continuously through a rotating drum, as shown in Fig. 5bi. Accordingly, by selecting cells as payloads, biohybrid hydrogel sheets were formed with uniformly distributed cardiomyocytes or patterned spots of fibroblasts (Fig. 5b ii,iii,iv). Apart from embedding living cells within hydrogel layers, biohybrid cell sheets can also be constructed by labeling different living cells with magnetic nanoparticles and subsequently incubating them layer-by-layer under the control of mild magnetic force. This strategy shows remarkable outcomes in easily fabricating homotypic or heterotypic cell sheets, which might be applied in the study of cell-cell interactions and tissue regeneration [56].

3.5. 3D biohybrid scaffolds

Biohybrid scaffolds, with the aim of generating tissue analogous *in vitro*, are widely used in regenerative medicine and organ reconstruction [57]. The perfusable feature of biohybrid scaffolds is vital for their clinical applications. For this purpose, a variety of strategies have been put forward [35,58–60]. As an example, a casting approach was used by Huang and coworkers in the fabrication of cell-laden collagen vascular-like networks [59]. By casting a fibroblasts-laden collagen suspension into a vascular-like gelatin mold, the cell-laden collagen scaffold with a vascular-like construct was successfully achieved by the cross-linking of collagen. The vascular-like scaffold can be released from the mold by melting gelatin at 37 °C. Further, the same group fabricated perfusable biohybrid hydrogel constructs through a sacrificial molding strategy [58]. Specifically, a reservoir containing a gelatin-based precursor matrix was first prepared, with gellan- or gelatin-based microgels used as fillers to control the rheological property. A sacrificial material was extruded into the reservoir, which was then trapped in the matrix by the jammed microgels. The sacrificial material was removed after gelation of the matrix, thereby generating voids or channel structures. Based

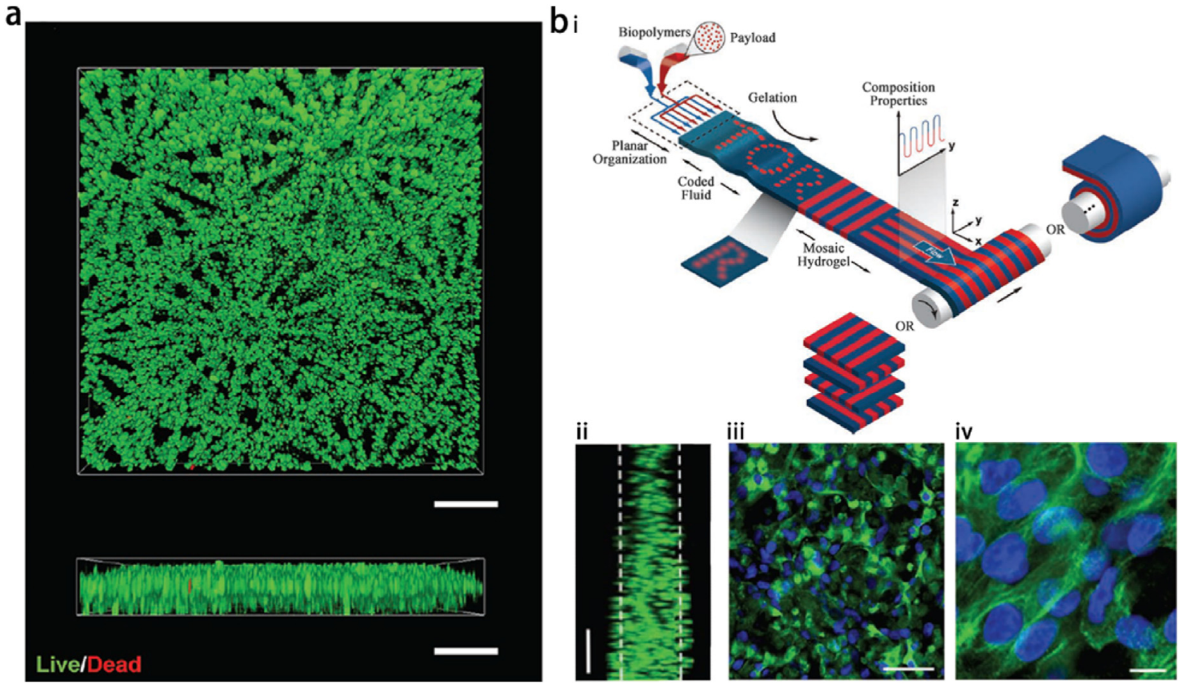

Fig. 5. Cell-laden biohybrid sheets. (a) Confocal images showing a stack of four cell-laden hepatic lobule-like sheets [55]. Copyright 2016, Wiley VCH. (b) Cell-laden mosaic hydrogel sheets. (i) Schematic illustration of the production of mosaic sheets; (ii) Confocal fluorescence image showing the location of cardiomyocytes in the biohybrid sheet. (iii,iv) Confocal fluorescence images showing the biohybrid sheet incorporated with fibroblasts [52]. Copyright 2012, Wiley VCH. Scale bars are 500 μm (a), 200 μm (b.ii), 50 μm (b.iii), and 10 μm (b.iv).

on this, living cells could be embedded in the matrix, and perfusable biohybrid constructs could be achieved therefrom.

Moreover, 3D bioprinting has been extensively explored in the fabrication of biohybrid 3D scaffolds due to its intrinsic advantages in creating complex structures. Burdick and coworkers developed an "*in situ*-crosslinking" strategy for 3D bioprinting [60]. The printing setup included a photo-permeable capillary, through which the printed filaments could be photo-crosslinked during extrusion. This design implied a cell-protective printing environment and could be implemented for the printing hydrogel ink of different formulations. Fibroblasts encapsulated in the ink could maintain high viability after printing regardless of the filament size. Besides, lattice- and macro nose-structured constructs were printed, both of which preserved a high cell viability, as shown in Fig. 6a. Taking advantage of microfluidics and 3D bioprinting, Wang et al. prepared biohybrid scaffolds with a similar multi-channel lattice structure [35]. The scaffolds were endowed with near-infrared (NIR) responsiveness ability, which facilitated the infiltration of cells (Fig. 6b). Moreover, Huang et al. printed living material based on biofilms of genetically modified *Bacillus subtilis* [17]. The engineered biofilms exhibited viscoelastic behaviors and can be programmed into complex patterns, as shown in Fig. 6c.

4. Applications of biohybrid materials

Compared with conventional synthetic materials, biohybrid materials are "smarter" and thus hold more ability to mimic biological functions [1]. Due to the integration of living cells and non-living materials, these biohybrid materials could perform biological functions of living cells under the protection and mediation of materials [28]. Moreover, with the deeper integration of material design and cell biology, the structure of biohybrid materials can cater to their application scenarios better. In brief, biohybrid materials with smaller volumes (such as modified living cells and biohybrid microparticles) are popular in the field of cell delivery, while the larger ones are more suitable for applications such as tissue repairing. Recently, the rapid rise of biohybrid materials promotes the development of the biomedical field, including biomimetic soft robotics, biosensors, cell therapy, tissue engineering, etc [23,33,61,62].

4.1. Biomimetic soft robotics

Living cells can sense and respond to external stimuli; by integrating with non-living materials, biomimetic soft robotics could be built with living cells offering biological actuation [5,63]. Typically, muscle cells have caught a lot of attention for assembling soft robots and mimicking biological behaviors [1]. For instance, Parker and colleagues manufactured a freely swimming soft robot by assembling a muscle tissue sheet on an elastomer layer [64]. Specifically, a jellyfish-like device was developed by culturing a layer of rat cardiomyocytes with a specific orientation on a jellyfish-shaped PDMS film. Taking advantage of the responsive contractility of cardiomyocytes, freely swimming behavior was achieved by adding an external electric field stimulation. In another work, the same group presented an optical-responsive ray-mimicking soft robot [65]. The device was composed of four layers, where engineered rat cardiomyocytes capable of responding to the external optical stimulation were arranged onto a PDMS elastic body (Fig. 7a). The behavior of rays was replicated by applying periodical optical stimulation, which led to sustained forward movements. More recently, a biohybrid fish that can autonomously swim was developed using cardiomyocytes derived from human stem cells [66]. This biohybrid fish mainly contained a five-layer structure (Fig. 7b), in which two independently activated engineered muscle layers were mutually antagonistic to each other upon alternate stimulation of blue-and-red light, which eventually contributed to body propulsion. Apart from aquatic organism-like robotics, muscle cells can also be applied to fabricate biohybrid flagellum and biological machines by coupling them with hydrogels, elastomers, etc [21,22,67].

4.2. Biosensors

Since living cells are sensitive to the external environment, biohybrid materials could be employed as sensors for detecting trace analytes [2].

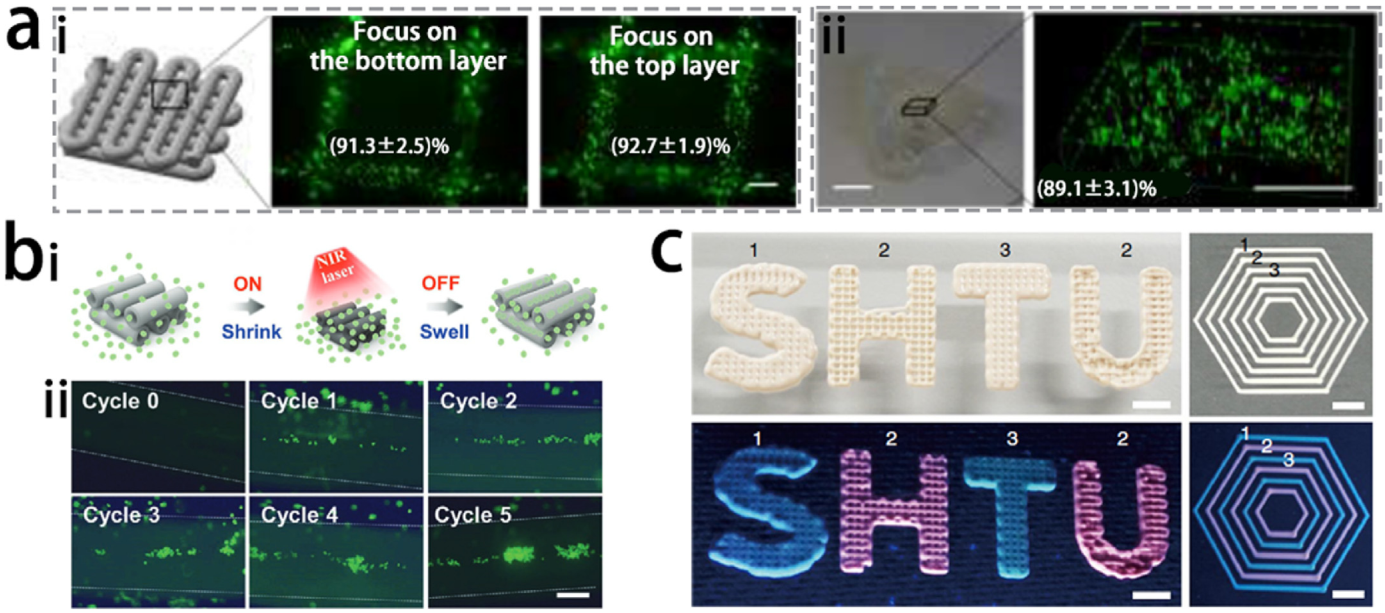

Fig. 6. Cell-laden 3D scaffolds. (a) In-situ 3D-bioprinted biohybrid scaffold. Fluorescence images indicating cell viability in a (i) 3D-bioprinted lattice scaffold and a (ii) nose-like scaffold [60]. Copyright 2017, Wiley VCH. (b) Cell enrichment in the responsive scaffold during the irradiation of NIR. (i) Schematic of the enrichment capability of the responsive scaffolds. (ii) Fluorescent images of living cells in the 3D biohybrid scaffolds after periodic irradiation of NIR [35]. Copyright 2021, Wiley VCH. (c) Pictures of 3D printed biofilms under normal (top) and UV light (bottom). The number from 1 to 3 refers to the biofilms with blue quantum dots (QDs), green QDs, and red QDs, respectively [17]. Copyright 2019, Springer Nature. Scale bars are 500 μm (a), 300 μm (b.ii), and 5 mm (c). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

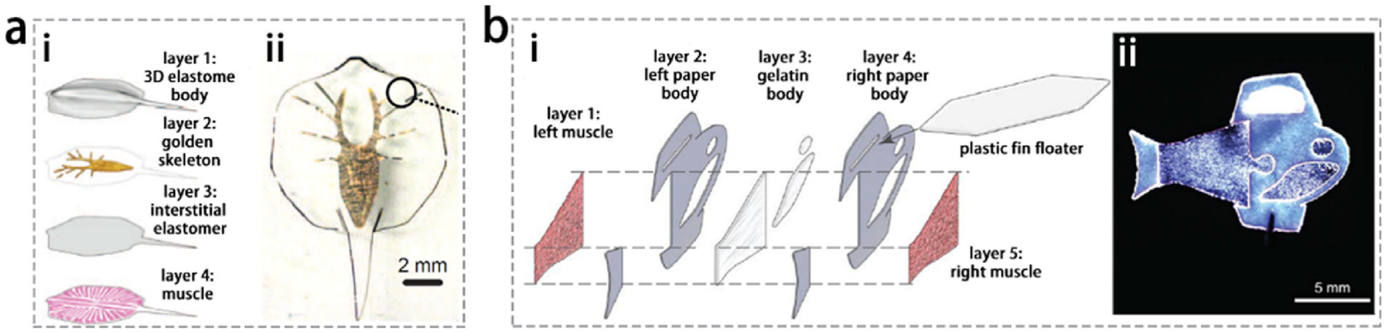

Fig. 7. Biohybrid soft robotics. (a) Biohybrid ray: (i) schematic showing the four layers structure of the biohybrid ray; (ii) the picture of the biohybrid ray [65]. Copyright 2016, The American Association for the Advancement of Science. (b) Biohybrid fish constructed using human cardiomyocytes: (i) schematic showing the five-layer structure of the biohybrid fish; (ii) image of the biohybrid fish [66]. Copyright 2022, The American Association for the Advancement of Science.

At the same time, the energy conversion accompanying the response process could be leveraged to construct devices such as actuators and energy harvesters [68–70]. For instance, Sahin and colleagues presented a nanogenerator based on the natural instinct of *Bacillus subtilis* spores to respond to a constantly changing relative humidity [68]. The spores were assembled into a dense monolayer and coated on a substrate. Along with the change of water potential, the spores changed their diameter and caused variation in the curvature of the substrate, as shown in Fig. 8a. To expand the application of such bio-hybrid hypomorph actuator, it was coupled with an electromagnetic generator. Under alternately applied dry and humid air, the deformation of the actuator activated the electromagnetic generator, which can provide an average power of 0.7 μW with only 3 mg of the spores (Fig. 8b). Using similar principles, evaporation-driven oscillatory engines and hygroscopy-driven artificial muscles were produced in the following work [69]. These devices could be helpful to drive the motion of soft robotics. Besides, some biosensors were developed by coupling eukaryotic cells with non-living materials. For example, a *Chlorella*-based biosensor is designed to detect metal irons in water by amplifying the photoelectrical efficiency with the aid of Cu nanoparticles and Cu electrodes [71].

Apart from physical factors in the environment, biohybrid materials also hold great potential to sense and respond to chemicals [72,73]. For instance, genetically engineered cells, especially bacteria, are commonly assembled with non-living materials to achieve chemical sensing. Zhao et al. introduced a hydrogel-elastomer hybrid matrix embedded with engineered living bacteria [73], as shown in Fig. 8c. The matrix combined the advantages of both hydrogels and elastomers and could support cell growth and proliferation. Multiple bacteria strains were used that could express green fluorescent proteins (GFP) when a corresponding chemical inducer was present. With that, the chemical sensing ability of the biohybrid matrix was demonstrated and the feasibility of applying this sensor in wearable devices was also confirmed.

4.3. Cell therapy

In recent years, advances in clinical medicine and cell biology have demonstrated the increasing significance of cell therapy, which offers an option of disease treatment alternative to drug therapy [3]. However, living cells are vulnerable to harmful environments such as shear forces and the host immune system, which leads to unexpected cell damage and

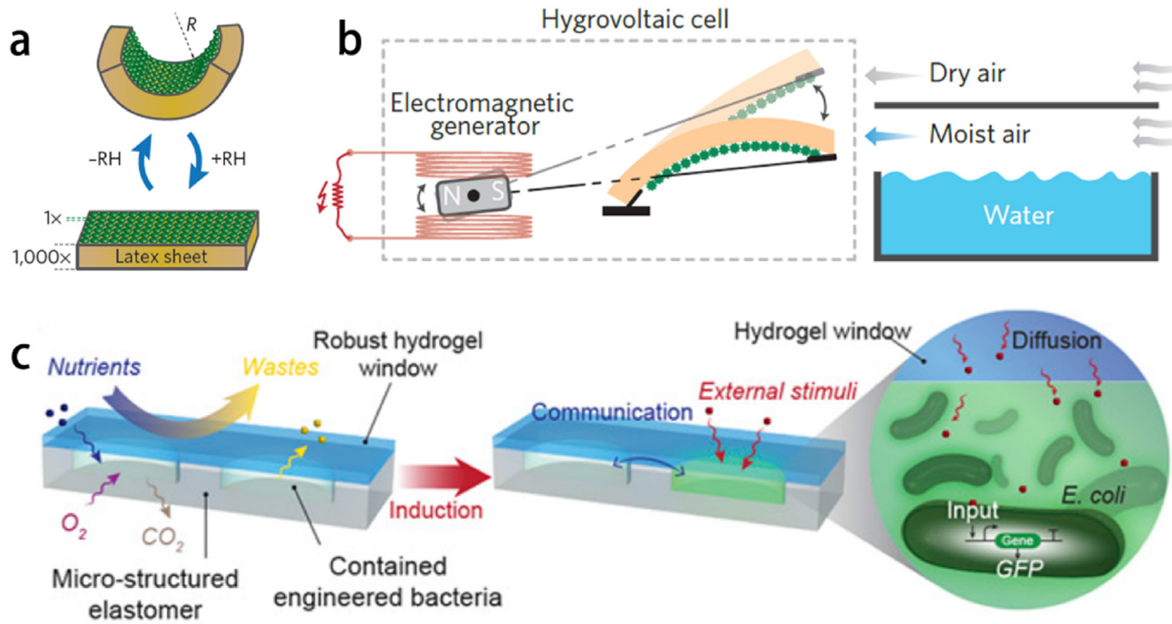

Fig. 8. Biohybrid sensors. (a) Schematic showing the bending of the biohybrid hypomorph actuator caused by relative humidity (RH) changes [68]. Copyright 2014, Springer Nature. (b) Schematic of the biohybrid nanogenerator driven by humidity variations [69]. Copyright 2015, Springer Nature. (c) Schematic illustrating the biohybrid matrix with capabilities of substance exchange, cell/cell and cell/environment communication, and chemical sensing [73]. Copyright 2017, National Academy of Sciences.

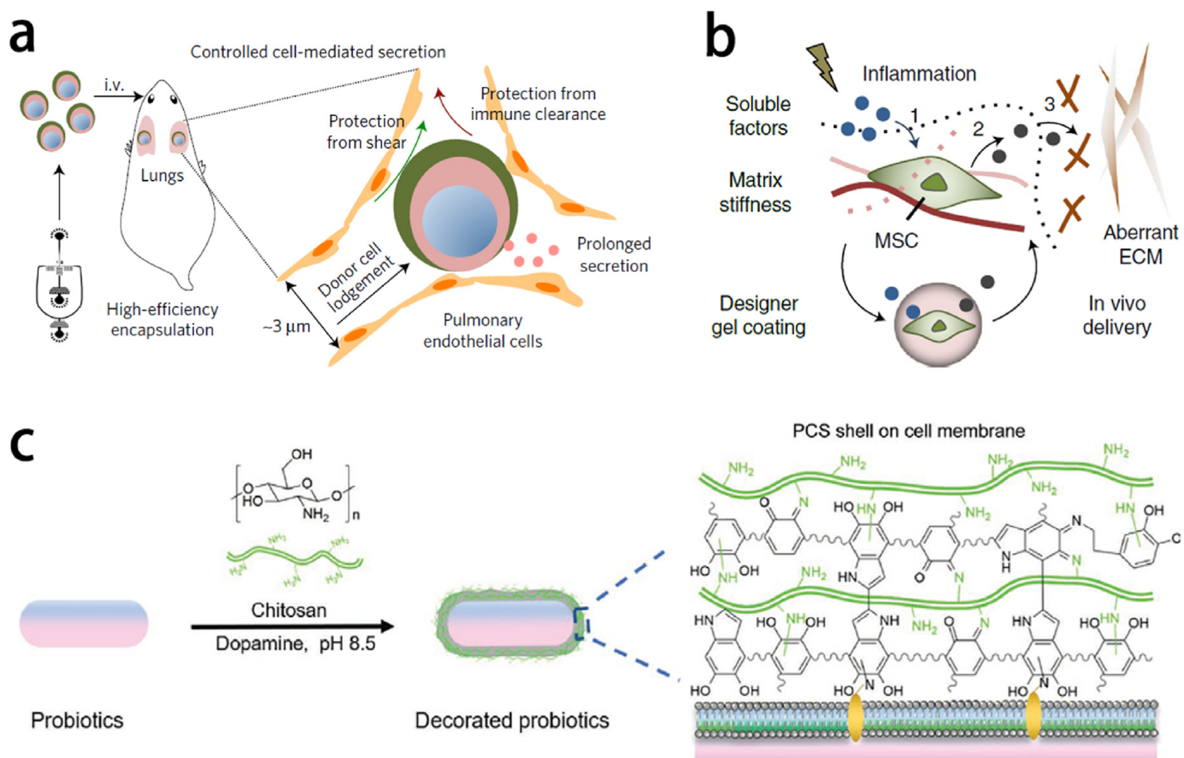

Fig. 9. Biohybrid therapeutic agents. (a) Schematic of the therapeutic strategy using single-cell encapsulated biohybrid microgel [31]. Copyright 2017, Springer Nature. (b) Schematic of the single cell-loaded microgel (1) receiving soluble factors, (2) producing paracrine factors, and (3) correcting extracellular matrix remodeling [19]. Copyright 2022, Springer Nature. (c) Modification of probiotics with co-deposition of dopamine and chitosan [75]. Copyright 2021, Wiley VCH.

insufficient therapeutic efficacy. Biohybrid materials play a role in protecting the donor cells and enhancing the efficacy of cell therapy [74]. For example, Mao et al. presented single cell-loaded biohybrid microgels with delayed clearance kinetics of marrow stromal cells (MSCs) [31]. By precisely controlling the encapsulation of living cells in alginate microgels, the cells exhibited high viability and more prolonged secretion of Interleukin-6 (IL-6) compared with uncoated cells (Fig. 9a). The same strategy was further adopted by Wong et al. who presented single cell-loaded biohybrid microgels with specific chemomechanical cues to inhibit aberrant tissue remodeling [19], as shown in Fig. 9b. Similarly, to

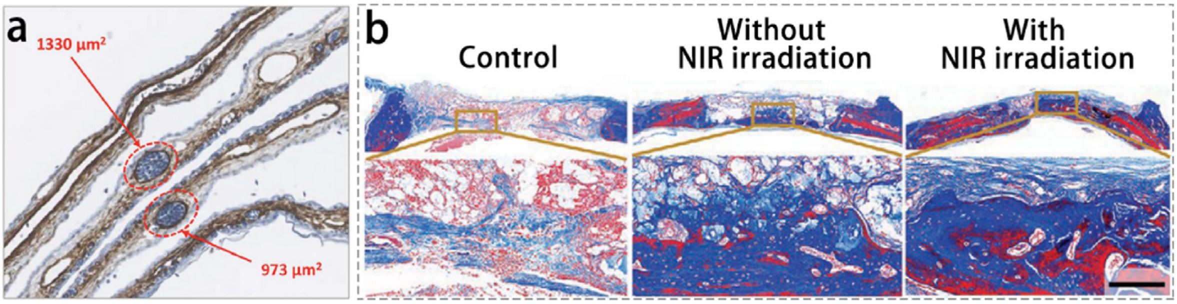

Fig. 10. Biohybrid materials promoting tissue regeneration. (a) Histological image of the chick chorioallantoic membrane (CAM) assay of the angiogenic biohybrid patch. Cell nucleus staining (blue) and alpha-smooth actin staining (brown) was applied. Two red arrows highlight the vessel areas [77]. Copyright 2016, Wiley VCH. (b) Masson's trichrome staining indicating the bone regeneration capacity of the biohybrid scaffold under NIR stimulation [35]. Copyright 2021, Wiley VCH. Scale bar is 200 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

improve the immunotherapy effect of natural killer (NK) cells, biohybrid NK cell-laden microspheres with a porous structure were designed and fabricated [24]. The microspheres could protect the NK cells from the external environment and achieve enhanced cell proliferation and sustained release of perforin and granzyme, thus facilitating tumor killing. Moreover, during the proliferation, the porous structure enabled the NK cells to bud from the microspheres and migrate to the surrounding tumor tissues, which further enhanced the therapeutic effect.

Apart from mammalian cells, some functionalized microbial cells also hold great potential for enhancing cell therapy [26]. For instance, living probiotics can be functionalized by co-depositing dopamine with various functional molecules at the cell surface [75]. Based on this method, an *Escherichia coli* strain Nissle 1917 (EcN) was coated with dopamine and chitosan at the cell surface, which showed enhanced tolerance to the surrounding environment and improved survival in the stomach and gut. Moreover, the decorated probiotics proved to be helpful for the targeted treatment of colitis (Fig. 9c).

4.4. Tissue regeneration

As an interdisciplinary field, tissue engineering aims to restore diseased and damaged tissues/organs without dealing with the problem of insufficient donor supply and immune rejection. Biohybrid materials could serve as tissue scaffolds due to their unique advantages in regulating cell behavior and supporting cell proliferation [74,76]. A typical case was presented with NIH/3T3 fibroblast cells encapsulated into porous 3D PEG-based hydrogel patches to encourage vascular regeneration [77]. These patches could protect the cells and a microchannel design was implemented to augment vascular endothelial growth factor (VEGF) secretion, which further proved to be beneficial for the generation of blood vessels (Fig. 10a). In another case, biodegradable collagen was introduced to fabricate biohybrid microcapsules and carry mesenchymal stromal cells [30]. The resultant biohybrid microcapsules were able to improve cardiac function in a murine model by intramyocardial injection.

Apart from protecting cells from the external environment, non-living materials also provide an opportunity to bring additional functions. As a highly adhesive biomaterial, dopamine is widely used in surface modification of non-living material and living cells. Based on this property, a type of tissue adhesive dopamine-grafted hydrogel was prepared with human adipose-derived stem cells encapsulated within and limbal epithelial stem cells seeded on the surface [53]. The biohybrid hydrogel has been proved to have excellent transparency and biocompatibility, thus could be implanted for corneal regeneration. Moreover, by combining responsive materials with living cells, these “smart” biohybrid materials have unique features for controlling cell behavior and facilitating tissue regeneration. For example, Zhao and coworkers designed and fabricated responsive biohybrid scaffolds with biomimetic enrichment channels to promote bone regeneration [35]. In this work, *N*-isopropyl acrylamide (NIPAM) and black phosphorus (BP) were incorporated into the scaffold and offered a NIR-responsive capacity to promote human umbilical vein endothelial cells (HUVECs) infiltrating into the channels. This feature, together with the role of BP in promoting osteogenic differentiation, facilitated the regeneration of bone tissues in a rat model (Fig. 10b).

5. Conclusion

In this manuscript, we review the features of biohybrid materials and their recent applications in the biomedical field. Specially, we emphasize the structure of different biohybrid materials and list some common structures and their corresponding applications in biomedical fields. Integrating living cells and non-living materials together, biohybrid materials enhance their capabilities and further promotes the communication between cells and synthetic materials [6]. On one hand, the sensory and responsive functions of living cells make biohybrid materials smart. The “smart” properties of biohybrid materials enable them to mimic biological functions and act as living factories [1]. On the other hand, since non-living materials tend to protect living cells, they can support and even enhance cell functions [8]. In addition, benefiting from evolving fabrication technologies such as 3D bioprinting and microfluidics, the structures of biohybrid materials can be precisely designed and fabricated to perform corresponding functions [17,52]. Moreover, taking advantage of the combination of living cells and non-living materials, these biohybrid materials are generally applied in biomedical science and engineering, such as biosensors, soft robotics, tissue engineering, and cell therapies [19,22,31,73].

Despite the great advances and encouraging achievements in the biomedical field, there are still some challenges in realizing a broader application of biohybrid materials. For this purpose, subsequent research may focus on the following aspects. First, since biohybrid materials contain both living cells and non-living substances, the communication between the two needs to be more thoroughly investigated[3]. Specifically, the safety of cell surface engineering for clinical utility remains to be systemically evaluated [31]. Secondly, commonly used non-living substances in biohybrid materials include hydrogel, nanoparticles, etc [31,36,51]. Future biohybrid materials could exploit other types of biocompatible materials and intricate design of their surface structure and chemical moieties is anticipated. The third point is manufacturing strategies of biohybrid materials, such as cell encapsulation, cell-involved molding, casting, and printing. All of the above techniques require precise control of the operation parameters, and the change of material properties due to the incorporation of cells should be taken into account. For example, in 3D bioprinting, rheology behaviors of the cell-embedded ink should be optimized to improve the printability while minimizing cell damage, and this is vital when introducing a new biohybrid material system [78].

Overall, the combination of cells and non-living substances enables biohybrid materials to mimic living organisms and achieve dynamic smart performances. It's foreseeable that the great biocompatibility and flexibility of biohybrid materials will enable them to get great achievement in multiple aspects. In view of the exhilarating progress in material science and cell biology, we expect that biohybrid materials would continue pushing the frontiers of the biomedical sciences and engineering.

Credit author statement

The authors claim that they have no conflict of interest.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2020YFA0908200), the National Natural Science Foundation of China (22002018), the Innovative Research Team of High-level Local University in Shanghai, and the Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtbio.2022.100352.

References

[1] P.Q. Nguyen, et al., Engineered living materials: prospects and challenges for using biological systems to direct the assembly of smart materials, Adv. Mater. 30 (19) (2018), e1704847.
[2] L.K. Rivera-Tarazona, et al., Stimuli-responsive engineered living materials, Soft Matter 17 (4) (2021) 785.
[3] Y. Yu, et al., Living materials for regenerative medicine, Eng. Regen. 2 (2021) 96.
[4] J. Cao, et al., Artificial bioaugmentation of biomacromolecules and living organisms for biomedical applications, ACS Nano 15 (3) (2021) 3900.
[5] C. Appiah, et al., Living materials herald a new era in soft robotics, Adv. Mater. 31 (36) (2019), e1807747.
[6] A. Rodrigo-Navarro, et al., Engineered living biomaterials, Nat. Rev. Mater. 6 (12) (2021) 1175.
[7] D. Wangpraseurt, et al., Biomimetic 3D living materials powered by microorganisms, Trends Biotechnol. (2022), https://doi.org/10.1016/j.tibtech.2022.01.003 available from:.
[8] L. Ouyang, et al., Assembling living building blocks to engineer complex tissues, Adv. Funct. Mater. 30 (26) (2020), 1909009.
[9] T.C. Tang, et al., Materials design by synthetic biology, Nat. Rev. Mater. 6 (4) (2020) 332.
[10] R. Raman, et al., Biohybrid design gets personal: new materials for patient-specific therapy, Adv. Mater. 32 (13) (2020), e1901969.
[11] M.A. Daniele, et al., Rapid and continuous hydrodynamically controlled fabrication of biohybrid microfibers, Adv. Funct. Mater. 23 (6) (2013) 698.
[12] K. Bente, et al., Biohybrid and bioinspired magnetic microswimmers, Small (2018), e1704374.
[13] J. Lee, et al., Cytoprotective silica coating of individual mammalian cells through bioinspired silicification, Angew. Chem., Int. Ed. Engl. 53 (31) (2014) 8056.
[14] T. Kamperman, et al., Centering single cells in microgels via delayed crosslinking supports long-term 3D culture by preventing cell escape, Small 13 (22) (2017), 1603711.
[15] M. Yeo, et al., Anisotropically aligned cell-laden nanofibrous bundle fabricated via cell electrospinning to regenerate skeletal muscle tissue, Small 14 (48) (2018), e1803491.
[16] X. Ma, et al., Deterministically patterned biomimetic human iPSC derived hepatic model via rapid 3D bioprinting, Proc. Natl. Acad. Sci. U. S. A 113 (8) (2016) 2206.
[17] J. Huang, et al., Programmable and printable Bacillus subtilis biofilms as engineered living materials, Nat. Chem. Biol. 15 (1) (2019) 34.
[18] Y. Zhao, et al., Surface-anchored framework for generating RhD-epitope stealth red blood cells, Sci. Adv. 6 (12) (2020), eaaw9679.
[19] S.W. Wong, et al., Inhibition of aberrant tissue remodelling by mesenchymal stromal cells singly coated with soft gels presenting defined chemomechanical cues, Nat. Biomed. Eng. 6 (1) (2022) 54.
[20] A.V. Singh, et al., Microemulsion-based soft bacteria-driven microswimmers for active cargo delivery, ACS Nano 11 (10) (2017) 9759.
[21] B.J. Williams, et al., A self-propelled biohybrid swimmer at low Reynolds number, Nat. Commun. 5 (2014) 3081.
[22] C. Cvetkovic, et al., Three-dimensionally printed biological machines powered by skeletal muscle, Proc. Natl. Acad. Sci. U. S. A 111 (28) (2014), 10125.
[23] H. Xu, et al., Sperm-hybrid micromotor for targeted drug delivery, ACS Nano 12 (1) (2018) 327.
[24] D. Wu, et al., NK-Cell-Encapsulated porous microspheres via microfluidic electrospray for tumor immunotherapy, ACS Appl. Mater. Interfaces 11 (37) (2019), 33716.
[25] D. Delcassian, et al., Magnetic retrieval of encapsulated beta cell transplants from diabetic mice using dual-function MRI visible and retrievable microcapsules, Adv. Mater. 32 (16) (2020), e1904502.
[26] A. González, et al., Entrapping living probiotics into collagen scaffolds: a new class of biomaterials for antibiotic-free therapy of bacterial vaginosis, Adv. Mater. Technol. 5 (7) (2020), 2000137.
[27] Y. Wan, et al., Recent advances in biohybrid materials for tissue engineering and regenerative medicine, J. Mol. Eng. Mater. 4 (1) (2016), 640001.
[28] W. Jiang, et al., Cell-laden microfluidic microgels for tissue Regeneration, Lab Chip 16 (23) (2016) 4482.
[29] L.C. Gerber, et al., Incorporation of penicillin-producing fungi into living materials to provide chemically active and antibiotic-releasing surfaces, Angew. Chem., Int. Ed. Engl. 51 (45) (2012), 11293.
[30] E. Jacques, et al., Collagen-based microcapsules as therapeutic materials for stem cell therapies in infarcted myocardium, ACS Biomater. Sci. Eng. 6 (8) (2020) 4614.
[31] A.S. Mao, et al., Deterministic encapsulation of single cells in thin tunable microgels for niche modelling and therapeutic delivery, Nat. Mater. 16 (2) (2017) 236.
[32] W. Zhu, et al., SupraCells: living mammalian cells protected within functional modular nanoparticle-based exoskeletons, Adv. Mater. 31 (25) (2019), e1900545.
[33] M. Jamal, et al., Bio-origami hydrogel scaffolds composed of photocrosslinked PEG bilayers, Adv. Healthc. Mater. 2 (8) (2013) 1142.
[34] L.C. Gerber, et al., Incorporating microorganisms into polymer layers provides bioinspired functional living materials, Proc. Natl. Acad. Sci. U. S. A 109 (1) (2012) 90.
[35] X. Wang, et al., Microfluidic 3D printing responsive scaffolds with biomimetic enrichment channels for bone regeneration, Adv. Funct. Mater. 31 (40) (2021), 2105190.
[36] M.M. Maciel, et al., Partial coated stem cells with bioinspired silica as new generation of cellular hybrid materials, Adv. Funct. Mater. 31 (29) (2021), 2009619.
[37] L. Bian, et al., Hydrogels that mimic developmentally relevant matrix and N-cadherin interactions enhance MSC chondrogenesis, Proc. Natl. Acad. Sci. U. S. A 110 (25) (2013), 10117.
[38] S. Allazetta, et al., Cell-instructive microgels with tailor-made physicochemical properties, Small 11 (42) (2015) 5647.
[39] T. Kamperman, et al., Single cell microgel based modular bioinks for uncoupled cellular micro- and macroenvironments, Adv. Healthc. Mater. 6 (3) (2017), 1600913.
[40] P.S. Lienemann, et al., Single cell-laden protease-sensitive microniches for long-term culture in 3D, Lab Chip 17 (4) (2017) 727.
[41] T. Kamperman, et al., Single-cell microgels: technology, challenges, and applications, Trends Biotechnol. 36 (8) (2018) 850.
[42] Y. Song, et al., All-aqueous electrosprayed emulsion for templated fabrication of cytocompatible microcapsules, ACS Appl. Mater. Interfaces 7 (25) (2015), 13925.
[43] K. Zhu, et al., All-aqueous-phase microfluidics for cell encapsulation, ACS Appl. Mater. Interfaces 11 (5) (2019) 4826.
[44] S. Vilabril, et al., One-Step all-aqueous interfacial assembly of robust membranes for long-term encapsulation and culture of adherent stem/stromal cells, Adv. Healthc. Mater. 10 (10) (2021), e2100266.
[45] D.M. Headen, et al., Microfluidic-based generation of size-controlled, biofunctionalized synthetic polymer microgels for cell encapsulation, Adv. Mater. 26 (19) (2014) 3003.
[46] C.H. Choi, et al., One-step generation of cell-laden microgels using double emulsion drops with a sacrificial ultra-thin oil shell, Lab Chip 16 (9) (2016) 1549.
[47] M.G.A. Mohamed, et al., An integrated microfluidic flow-focusing platform for on-chip fabrication and filtration of cell-laden microgels, Lab Chip 19 (9) (2019) 1621.
[48] H.F. Chan, et al., Efficient one-step production of microencapsulated hepatocyte spheroids with enhanced functions, Small 12 (20) (2016) 2720.
[49] I. Letnik, et al., Living composites of electrospun yeast cells for bioremediation and ethanol production, Biomacromolecules 16 (10) (2015) 3322.
[50] D. Neal, et al., Formation of elongated fascicle-inspired 3D tissues consisting of high-density, aligned cells using sacrificial outer molding, Lab Chip 14 (11) (2014) 1907.
[51] H. Onoe, et al., Metre-long cell-laden microfibres exhibit tissue morphologies and functions, Nat. Mater. 12 (6) (2013) 584.
[52] L. Leng, et al., Mosaic hydrogels: one-step formation of multiscale soft materials, Adv. Mater. 24 (27) (2012) 3650.
[53] L. Koivusalo, et al., Tissue adhesive hyaluronic acid hydrogels for sutureless stem cell delivery and regeneration of corneal epithelium and stroma, Biomaterials 225 (2019), 119516.
[54] S.H. Yang, et al., Cytocompatible in situ cross-linking of degradable LbL films based on thiol–exchange reaction, Chem. Sci. 6 (8) (2015) 4698.
[55] J. Son, et al., Freestanding stacked mesh-like hydrogel sheets enable the creation of complex macroscale cellular scaffolds, Biotechnol. J. 11 (4) (2016) 585.

[56] S.S. Ana, et al., Multi-layer pre-vascularized magnetic cell sheets for bone regeneration, Biomaterials 231 (2020), 119664.
[57] A.K. Capulli, et al., JetValve: rapid manufacturing of biohybrid scaffolds for biomimetic heart valve replacement, Biomaterials 133 (2017) 229.
[58] A.M. Compaan, et al., Cross-linkable microgel composite matrix bath for embedded bioprinting of perfusable tissue constructs and sculpting of solid objects, ACS Appl. Mater. Interfaces 12 (7) (2020) 7855.
[59] Y. Jin, et al., Fabrication of stand-alone cell-laden collagen vascular network scaffolds using fugitive pattern-based printing-then-casting approach, ACS Appl. Mater. Interfaces 10 (34) (2018), 28361.
[60] L. Ouyang, et al., A generalizable strategy for the 3D bioprinting of hydrogels from nonviscous photo-crosslinkable inks, Adv. Mater. 29 (8) (2017), 1604983.
[61] J. Pu, et al., Virus disinfection from environmental water sources using living engineered biofilm materials, Adv. Sci. 7 (14) (2020), 1903558.
[62] S. Sankaran, et al., Optoregulated drug release from an engineered living material: self-replenishing drug depots for long-term, light-regulated delivery, Small 15 (5) (2019), e1804717.
[63] V. Magdanz, et al., Development of a sperm-flagella driven micro-bio-robot, Adv. Mater. 25 (45) (2013) 6581.
[64] J.C. Nawroth, et al., A tissue-engineered jellyfish with biomimetic propulsion, Nat. Biotechnol. 30 (8) (2012) 792.
[65] S. Park, et al., Phototactic guidance of a tissue-engineered soft-robotic ray, Science 353 (6295) (2016) 158.
[66] K.Y. Lee, et al., An autonomously swimming biohybrid fish designed with human cardiac biophysics, Science 375 (6581) (2022) 639.
[67] Y. Morimoto, et al., Biohybrid robot with skeletal muscle tissue covered with a collagen structure for moving in air, APL Bioeng. 4 (2) (2020), 026101.
[68] X. Chen, et al., Bacillus spores as building blocks for stimuli-responsive materials and nanogenerators, Nat. Nanotechnol. 9 (2) (2014) 137.
[69] X. Chen, et al., Scaling up nanoscale water-driven energy conversion into evaporation-driven engines and generators, Nat. Commun. 6 (2015) 7346.
[70] W. Wang, et al., Harnessing the hygroscopic and biofluorescent behaviors of genetically tractable microbial cells to design biohybrid wearables, Sci. Adv. 3 (5) (2017), e1601984.
[71] D.N. Roxby, et al., Microalgae living sensor for metal ion detection with nanocavity-enhanced photoelectrochemistry, Biosens. Bioelectron. 165 (2020), 112420.
[72] C. Gilbert, et al., Living materials with programmable functionalities grown from engineered microbial co-cultures, Nat. Mater. 20 (5) (2021) 691.
[73] X. Liu, et al., Stretchable living materials and devices with hydrogel–elastomer hybrids hosting programmed cells, Proc. Natl. Acad. Sci. U. S. A 114 (9) (2017) 2200.
[74] W. Ji, et al., 3D Bioprinting a human iPSC-derived MSC-loaded scaffold for repair of the uterine endometrium, Acta Biomater. 116 (2020) 268.
[75] C. Pan, et al., Polymerization-mediated multifunctionalization of living cells for enhanced cell-based therapy, Adv. Mater. 33 (13) (2021), e2007379.
[76] S. Chen, et al., A conductive cell-delivery construct as a bioengineered patch that can improve electrical propagation and synchronize cardiomyocyte contraction for heart repair, J. Contr. Release 320 (2020) 73.
[77] R. Raman, et al., High-resolution projection microstereolithography for patterning of neovasculature, Adv. Healthc. Mater. 5 (5) (2016) 610.
[78] S. Vanaei, et al., An overview on materials and techniques in 3D bioprinting toward biomedical application, Eng. Regen. 2 (2021) 1.