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3D Bioprinting for Tissue Engineering and Regenerative Medicine

**Bioink formulation using
synthetic biomaterials**

**Bioprinting of biologically
active construct**

Towards biologically relevant synthetic designer matrices in 3D bioprinting for tissue engineering and regenerative medicine

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Keywords: 3D bioprinting; bioinks; synthetic scaffolds; tissue reconstruction; hydrogels; scaffold functionalization; biomedical engineering.

Abstract

3D bioprinting is one of the most promising technologies in tissue engineering and regenerative medicine. As new printing techniques and bioinks are getting developed, new cellular constructs with high resolution and functionality arise. Different to bioinks of animal, algal or plant origin, synthesized bioinks are proposed as superior biomaterials because their characteristics are fully under control. In this review, we will highlight the potential of synthetic biomaterials to be used as bioinks in 3D bioprinting to produce functionally enhanced matrices.

Introduction

There is a global trend in the advancement of 3D bioprinting technologies for applications in the field of tissue engineering and regenerative medicine and this is foreseen to revolutionize our future healthcare system. With the increasing world's population, there is a global demand for organs as well as for alternatives for drug screening, disease modeling and diagnostics. By the end of 2015, more than 130,000 people were waiting for an organ transplant in EU and USA alone [1]. This demand will keep rising to the point where conventional methods such as transplants, prosthetics, animal models or traditional *in vitro* techniques will no longer be regarded as viable or cost effective options. 3D bioprinting emerged as a promising solution to address the quest for tissues/organs by the use of biocompatible materials combined with bioactive molecules and viable cells to create *de novo* functional structures [2]. The idea of bioprinting was firstly explored in the early 2000s by Boland and Mironov as well as by Nakamura (the first two sharing a group other than the third), upon using a modified inkjet printer to print living cells [3–7]. Soon after, publications on bioprinting steadily increased every year, new printing strategies and materials were discovered, and bioprinting startup companies began

to blossom, giving rise to predictions that the 3D bioprinting market will reach an estimated market size of 1.82 billion dollars by 2022 [8].

The main focus of the majority of 3D bioprinting research and development lies in the fabrication of human tissues/organs that structurally and functionally resemble their native counterparts. To achieve this, different biocompatible materials ranging from naturally-derived to chemically-synthesized polymers - including their modifications and composite materials - are being extensively investigated as potential bioinks for 3D bioprinting [9,10]. Naturally-derived matrices, such as Matrigel® or alginate, are currently the only commercially available option for tissue engineering, although these materials are not able to fully reconstitute natural tissue. Furthermore, Matrigel® as itself is not suitable as a bioink, requiring modifications or blending with other compounds such as agarose for 3D bioprinting [11]. Therefore, the development of fully synthetic designer matrices that can successfully be used as bioinks, poses as a new hallmark in the field of bioprinting. These synthetic designer matrices not only can be manipulated to reassemble specific biological matrices, but can also be made “smart” by changing their structural or functional properties as a response to different biological cues, which are important for cellular proliferation, matrix remodeling or disease progression [12]. These functionalized materials, combined with 3D bioprinting technologies, can revolutionize the way of achieving the complex architecture of tissues/organs, and could also improve the integration, degradability or even the function of the newly synthesized construct.

In this review, we will only briefly discuss the currently used techniques in 3D bioprinting, and rather focus on promising novel biomaterials that can be functionalized and used for more advanced tissue engineering and regenerative medicine applications. We will further discuss different approaches to obtain smart and responsive scaffolds, and will finally mention current endeavors to bring 3D bioprinting from an *in vitro* to an *in situ* approach.

Current challenges and strategies

Various biomaterials, which are suitable as scaffolds for 3D cell culture in regenerative medicine, have been investigated in their ability to mimic the complex, multilayered organization inherent to tissues/organs in an accurate manner. 3D bioprinting requires the merge of biomaterials with cells and their subsequent deposition in a controlled and layered fashion without compromising the cell structure and functionality. This mixture of soft material, i. e. the bioink, and cells allows for the development of complex 3D constructs, often comprising more than one material, cell type or bioactive molecule. The development of a suitable bioink for 3D bioprinting is extremely challenging since the material is in contact with living cells right from the beginning of the process, expanding the set of requirements needed for a viable construct. An ideal biomaterial for 3D bioprinting has necessarily to be biocompatible, biodegradable, and non-immunogenic. It has to promote cell adhesion and possess optimal physical properties, be printable in high resolution, exert phase transitions between the liquid and solid state while keeping high mechanical stability and integrity, should be degradable at a rate concomitant with the regeneration process and should be solidified in a non-cytotoxic way.

Moreover, the biomaterial also needs to be chemically defined, easily and affordably manufactured, and, ideally, commercially available. Finally, it would be easily functionalized, in order to expand its range of applicability and biomimicry in different tissues/organs. Because of these challenges, we still lack the perfect bioink for 3D bioprinting. A few recent reviews have covered extensively the more commonly used bioinks, both naturally derived and synthetic [13,14]. Our aim is to focus on the emerging trend in the development of synthetic designer matrices for 3D bioprinting.

Synthetic designer matrices

Poly(ethylene glycol) or PEG is a water soluble synthetic hydrophilic polyether widely used for biomedical applications[13]. The mechanical properties of PEG can be manipulated by altering its chemical structure or via regulation of UV exposure during the photocrosslinking process[13]. Due to its hydrophilic nature, similar to alginate, it has poor adsorption properties and is usually functionalized with cell adhesion components when used for cell culture applications. Although the traditional structures and hydrogels created when using PEG have structural and stiffness issues, new developments have shown that PEG-modified hydrogels can be extremely versatile and even mimic natural gelation processes. The transglutaminase-crosslinked PEG (TG-PEG) hydrogel platform was reported as a versatile synthetic designer matrix. This system relies on 8-PEG-VS that is functionalized with two complementary short glutamine or lysine peptides, which serve as defined substrates for thrombin activated transglutaminase factor XIII (FXIIIa) (Fig.1 A)[15,16]. This flexible building block strategy not only allows further functionalization to be added to the resulting polymer but also results in a fully synthetic, fibrin mimetic, FXIIIa-crosslinked hydrogel that is highly biocompatible. The crosslinking can be formed under physiological conditions and don't require any harsh chemical or photocrosslinking strategy, although, it needs the presence of the FXIIIa protein. Moreover, unlike fibrin, it has the advantage of being synthetic as compared to the animal derived product. Due to its temporary role in the natural environment, the gelation of fibrin is irreversible, and the degradation rates are rapid, limiting its mechanical properties and shear-thinning behavior [13]. On the other hand, this functionalized PEG can support a tunable degradability (Fig.1 B-C)[17]. The similarity on the polymerization and gelation behavior of this synthetic polymer and the one observed in fibrin hydrogels - already proven to be suitable for bioprinting - open a new promising door for the use of this material in a similar manner.

Similarly to PEG, synthetic self-assembling peptides are regarded as attractive candidate bioinks [18–20]. Specifically, ultrashort synthetic peptides can self-assemble into nanofibrous hydrogels under physiological conditions. After self-assembly, the resulting hydrogel retains high amounts of water, and its fibrous structure closely resembles collagen fibers from the native extracellular matrix (ECM), which makes it highly biocompatible and biodegradable and enables a suitable environment for cells to grow (Fig.2 A)[21]. Moreover, peptides can be chemically synthesized, ranging from ultrashort peptides (2-7 aa in size) to regular sized peptides (~20aa and longer). This approach allows a precise information

about the peptide composition and minimizes batch-to-batch variation. The gelation time and mechanical properties can also be tuned by adjusting intrinsic factors (such as amino acid sequence or peptide concentration) or external factors (such as pH, temperature or salt concentration), making these hydrogels potentially suitable for 3D bioprinting. Used as a bioink, peptide hydrogels were printed in a droplet array format, formed continuous threads when extruded from a syringe (Fig.2 B) and were preliminarily printed using an extrusion-based bioprinter [18,20].

Existing printing strategies

Printing of biologically relevant constructs is the ultimate goal of 3D bioprinting, and several printing techniques rely on different principles to achieve similar goals. In general, to bioprint a 3D tissue construct, first a model of the structure needs to be created using computer-aided design (CAD) software, that can modulate and define 3D structures either by creating a *de novo* model or via image reconstruction using CT scan or MRI data from templates or defective tissue. The resulting model is then converted to a format that can be used by a 3D bioprinter to pattern cellular layers that will ultimately fuse together and create bigger and more complex structures. We can classify bioprinting techniques in three broad categories: microextrusion-based bioprinting, inkjet-based bioprinting, and laser-assisted bioprinting.

In microextrusion-based systems, the printed cell-containing material is extruded through a nozzle or needle onto a substrate in a layer-by-layer mode of action. Because of the continued extrusion and filament formation, the bioink should exert enough viscosity for the printing process to happen. Finally, the mechanical integrity of the 3D structure is achieved by UV/visible light or chemical crosslinking methods [14]. Microextrusion allows good control over the material flow, the printing space and can utilize bioinks with very high cell densities. Although being the most used technique for bioprinting, it still faces challenges, particularly the lack of resolution and the shear stress at the tip of the nozzles, that causes cell deformation and cell death[14].

Inkjet-based systems function in a similar way, but instead of relying on a continuous flow of bioink, they work mostly in a drop-on-demand fashion, ejecting bioink droplets onto the substrate. The drop formation can be achieved through thermal, electrostatic, piezoelectric, electrohydrodynamic, acoustic or solenoid valve action at the nozzle. Upon drop formation, the printing can be performed through a continuous and uninterrupted stream of droplets or by drop-on-demand. It is a relatively low-cost method and allows the creation of gradients of cells or other biomolecules incorporated in the bioink via drop size or density [22]. Other than tissue engineering, the application of this technique is particularly relevant to the fabrication of biosensors and biochips. Due to the force required to eject the droplets, this method is limited by the viscosity of the material used. Also, because the biomaterial needs to be in a liquid state during printing, it has to form a stable 3D structure upon crosslinking immediately after deposition, which often slows down the printing process. Finally, this technique is not compatible with biologically relevant cell concentrations, but only feasible when using low cell densities[22,23].

Laser-assisted bioprinting relies on the laser-induced forward transfer of the bioink onto the substrate [24]. The system consists of a laser beam and a focusing module, a donor transport support, a ribbon of titanium or gold that adsorbs the bioink, and the layer of bioink to be printed in a liquid form. This technique allows high-resolution printing and can achieve deposition of a single cell per drop while keeping a good printing speed. Since it is a nozzle-free printing, there are no shear stress and clogging problems, which results in negligible effects on cell viability. Despite all the advantages, there are still considerable drawbacks, namely the need for preparation of multiple ribbons if more than one cell type or material are required to be printed, which is time-consuming. Also, due to the metallic nature of the absorbing layer, metallic residues contaminate the final product. Finally, the high resolution achieved during printing needs a rapid gelation of the bioink, which in turn slows down the printing flow[23].

Each of these printing categories provide several different mechanisms by which the printing process can be accomplished. Different reviews that are extensively covering the different mechanisms of each technique are available in the current literature [14,22,24].

The path towards 3D printed synthetic matrices in tissue engineering

There has been a growing trend in publications regarding 3D bioprinting of tissues and organs in the last few years. Table 1 summarizes the most prominently used bioink materials, their modifications and mention the cell/tissue used[17,18,20,25–35,11,36–38]. Unfortunately, so far only few publications were able to tackle the usage of fully synthetic materials for 3D bioprinting of functional constructs. Still, there are existing strategies and composite materials that have achieved remarkable results in tissue engineering and might potentially be adapted for 3D bioprinting in the future. We now highlight applications from a few of these designer matrices with potential utility for creating 3D bioprinted structures.

Hauser and co-workers were the first group to propose ultrashort solely aliphatic lysine-containing self-assembling hexapeptide hydrogels as bioinks suitable for bioprinting, following their first discovery and promising results as promoters of skin regeneration, when used as dressings in burnt wounds [39,40]. These ultrashort peptide hydrogels were able to maintain organotypic cultures when used in combination with Caco2 intestinal epithelial cells, showing huge potential to be used in organoid 3D cultures. Embryonic stem cells (ESC) were also able to retain high levels of viability as well as their pluripotency markers (Fig.3 A, B) [18]. Nevertheless, encapsulated mesenchymal stem cells were successfully induced to undergo adipogenesis upon appropriate stimulus, showing that encapsulated cells can still respond to stimuli and can differentiate [18]. On the other hand, although not by bioprinting, Wang *et al.* recently showed, using different self-assembling peptides, that fluorenyl-9-methoxycarbonyl (Fmoc)-diphenylalanine (Fmoc-FF) and Fmoc-arginine-glycine- aspartate (Fmoc-RGD) hydrogel scaffolds fostered proliferation and multi-differentiation of human mesenchymal stem cells (MSCs) to an osteogenic, adipogenic or chondrogenic lineage upon specific induction, in an *in vitro* and *in vivo* mouse model [41]. Gao *et al.* were able to combine the printability potential of PEG-based hydrogels with high

functionalization using an inkjet printing technique. By crosslinking PEG with acrylated RGD and matrix metalloproteinase (MMP)-sensitive peptides, MSC were encapsulated and printed as a 3D construct. When cultured in either osteogenic or chondrogenic medium, printed structures showed sustained differentiation to bone and cartilage-like tissues, with changes in the expression profiles and ECM-specific deposition and remodeling [26]. Similarly, using a copolymer comprising methacrylated PEG, poly[N-(2-hydroxypropyl) methacrylamide mono/dilactate] (polyHPMA-lac), and methacrylated hyaluronic acid (HA) and chondroitin sulfate (CS), Abbadessa *et al.*, were able to create a synthetic thermoresponsive hydrogel that nurtured the formation of cartilage-like tissue for up to 42 days. Moreover, when printed, the final construct sustained 95% of chondrocyte's survival [42]. By using a chemically defined, synthetic and responsive matrix, Gjorevski *et al.* showed promising results towards organoid culture. The group used the earlier mentioned TG-PEG hydrogel platform for the culture of intestinal stem cells (ISC) with the aim of creating an organoid with an architecture and cell diversity close to the respective organ (Fig.3 C). During organogenesis, cell proliferation and tissue maturation through matrix remodeling are highly dependent on changes in the stiffness of the scaffold [17]. By creating a hybrid hydrogel comprising a stable PEG backbone mixed with a modified hydrolytically degradable variant and further functionalized with RGD peptides and laminin-111, Gjorevski *et al.* described a way to create a dynamic 3D setting that allowed ISC expansion in a stiffer environment and subsequent organoid formation upon controlled scaffold softening (Fig.3 D)[17]. In another recent attempt, using a similar principle, Anjun *et al.* created a responsive glucosaminoglycan (GAG)-based natural-synthetic hybrid hydrogel that can also act as a tunable delivery system for biomolecules. Moreover, further functionalization of this system with CS and MMP-sensitive peptides allowed to create a chondroitinase and protease-responsive hydrogel, tailoring its degradability, adhesion, biomolecule binding ability and mechanical properties (Fig.1 D). As a proof of concept, bone morphogenic protein-2 (BMP-2) was added to the polymer solution and MSC were encapsulated in the material. When compared to encapsulated cells treated with BMP-2 added to the culture medium, CS-8PEG-BMP2 encapsulated MSC showed a higher osteogenic differentiation profile due to controlled release of BMP-2 upon protease-dependent matrix remodeling [27]. Both of these studies involving synthetic matrices did not perform bioprinting experiments. Thus, printing needs to be done to verify if similar results can be achieved when using this bioink in combination with MSCs. However, it was clearly demonstrated that complex tissue constructs can be achieved without the use of animal-derived molecules for further functionalization. Using these types of synthetic matrices for 3D bioprinting could potentially open up opportunities for further improvement in 3D constructs by deposition of multiple variations and functionalization of the same polymer, creating a gradient of different microenvironments that could lead to controlled stem cell differentiation and maturation in a stratified but concise way. Finally, although some of the currently used composites still rely on some molecules from animal origins, such as chondroitin, it is possible to fully synthesize this compound and/or other

oligosaccharides with biological relevance, enabling adaptation to fully synthetic materials [43].

3D printed composites and functionalization of naturally-derived bioinks

Apart from synthetic matrices, it is also important to highlight some of the recent research work involving bioinks composed of natural biomaterials. For example, by using a microextrusion-based system and a bioink comprising arginylglycylaspartic acid (RGD) modified gamma-irradiated alginate, polycaprolactone (PCL) and MSCs, Daly *et al.* printed a structure with high chondrogenic potential *in vitro* that also achieved an enhanced level of endochondral bone formation *in vivo* [30]. While PCL served as a scaffold to confer the structural support that alginate lacks, the modified alginate bioink provided a cell-friendly environment for tissue development and maturation. Nonetheless, the influence of immunological compromising factors from animal or plant origin remains as a major issue. Moreover, Jia and colleagues recently used a bioprinting technique, based on a multilayered coaxial nozzle extrusion device combined with a cell-laden blended bioink containing alginate, gelatin methacryloyl (GelMA) and 4-arm poly(ethylene glycol)-tetra-acrylate (PEGTA). This technique used a two-step crosslinking strategy to give structural stability to the 3D constructs. The first crosslinking step involved calcium-induced complexation of alginate to allow enough structural stability during the printing process, whereas in the second step, UV exposure was used to covalently photocrosslink GelMA, which permanently fixed the printed structure. By including endothelial and stem cells in the bioink, this approach was able to create highly organized perfusable vessels, biomimeticizing vasculature [44]. However, the influence of UV exposure on the fate of the cells remains to be investigated, particularly for long term applications. In another study, the lack of structural fidelity of alginate was reduced by blending it with nanocellulose, which allowed to print a suitable hydrogel capable of supporting chondrocyte growth and proliferation, using an electromagnetic inkjet printing technique [45]. Chung and his group intriguingly showed that an alginate-based bioink could be functionalized by combining it with M13 phages modified to display integrin-binding and calcium binding domains in their coat proteins. The modified phage suspension was also able to polymerize alone upon calcium crosslinking due to the calcium binding domain incorporation and was stable for weeks when incorporated in alginate gels. This composite was printed by pneumatic microextrusion with high fidelity while keeping high cell viability and proliferation, due to the added adhesion domain, and promoting osteogenic differentiation of bone precursor cells [46]. Again, the use of viral phages, although modified to nonpathogenic ones, is not ideal for human use. By using human plasma as a source of fibrin, Cubo & Garcia combined it with human fibroblast and keratinocytes to achieve a printed bilayered skin structure. Constructs were printed using a microextrusion piston-based system comprising four syringes. By mixing the plasma with fibroblasts and CaCl₂ solution in the extrusion head, a fibrin hydrogel was formed, followed by deposition of a monolayer of keratinocytes. The constructs were transplanted into immunodeficient mice and showed high integration with the native tissue [34]. Using a similar approach, but with fibrinogen isolated from

bovine plasma mixed with hyaluronic acid, approtinin and factor XIIIa, England *et al.* printed rat Schwann cells for neuroregeneration. The printed bioink was able to sustain cellular growth and provided physical guidance for the glial cells to elongate their neurites across the printed fibrin-factor XIII-HA strands [33].

Future perspectives

The 3D bioprinting field has come a long way since its primordial validation stage, being more accessible to the scientific community, in part due to low cost printing devices, and used as a valuable tool to validate tissue engineering approaches. Nevertheless, we have still to achieve the transition of this method from bench to bed-side and its routine use in clinical applications. In the meantime, printing techniques keep evolving to be faster, more reliable and affordable. There are already commercially available robotic-based systems such as the BioAssemblyBot® printer, which relies on a 6-axis robotic arm and up to 8 bioinks to achieve highly complex constructs in a quick and controlled way. Also, Kang and colleagues recently showed their integrated tissue-organ printer (ITOP) system, able to fabricate real scale tissue constructs of any shape, using a multi-dispensing module approach of synthetic biodegradable polymers to confer mechanical support and composite hydrogels for cell delivery, all in an integrated and user-friendly procedure [47]. Exploring the full potential of 3D bioprinting, the field has already branched to higher and more complex strategies. 4D bioprinting adds “time” as a factor in the printing process, and not time of printing, but rather the ability of constructs to respond and behave in a programmed way over time, after printing. Using approaches as cell-origami or tunable sol-gel transitions, printed materials are able to self-assemble into 3D structures over time in response to pH, temperature or osmolarity and have a wide range of biomedical applications [48]. Using such strategies already enabled the creation of structures using living cells to self-assemble bilayered “vessel-like” constructs or structures able to self-fold/unfold for drug or cell delivery [49,50]. As medicine gets more and more personalized and targeted, *in situ* printing will appear in the near future. Although only attempted a few times, either by printing *in vivo* on a mouse calvaria defect or by filling a defect on a bovine femoral substrate emulating an osteochondral lesion, the proof of concept of applying 3D bioprinting techniques to *in situ* use was already accomplished, opening exciting paths toward what will be possible in the future [51,52].

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Papers of particular interest, published within the period of review, have been highlighted as:

•• of outstanding interest

[17] This paper reports, for the first time, the development of a well characterized modular synthetic hydrogel that is able to change its mechanical properties. Upon intestinal stem cell culture in this matrix, the hydrogel environment fosters separate stages of organoid formation by keeping an adequate softening rate, pointing to the importance of scaffold stiffness on cell fate.

[18] This paper shows the potential of self-assembling ultrashort peptides to be used as fully synthetic bioinks for 3D bioprinting. Through several regenerative medicine approaches (stem cell encapsulation, differentiation and organotypic cultures) as well as printing applications (droplet arrays, sequential deposition and extrusion), the authors show the possibilities of this unexplored biomaterial.

• of special interest

[13] This extensive review describes the most commonly used bioinks and evaluates their printability, biomimicry, resolution, affordability, scalability, practicality, mechano-structural integrity and tissue formation post-implantation.

[24] This recent book chapter reviews bioprinting techniques and all their sub processes and mechanisms. It also provides a comparison between printing processes and gives examples of printed cells and tissues in combination with different bioinks.

[27] This paper describes an enzymatically formed hybrid hydrogel, comprising fibrin-mimetic, TG-PEG and chondroitin sulfate. This hydrogel closely reassembles the GAG and protein properties of natural ECM. Further functionalization is achieved by tuning growth factor binding and release as well as by controlling degradability.

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Figure Legends

Figure 1 – **(a)** Synthesis of two multiarm PEG-peptide conjugates, n-PEG-MMP-Lys and n-PEG-Gln via Michael-type addition reaction. These two precursors differ in their attached transglutaminase peptide substrates that enable the subsequent formation of PEG hydrogels via factor XIIIa. **(b)** A scheme illustrating the chemical basis of functionalization of mechanically dynamic PEG hydrogels. **(c)** Mechanical characterization of hybrid sPEG–dPEG gels. For each condition, graph shows

individual data points derived from $n = 3$ independently prepared gels and means. G' , shear modulus. **(d)** Factor XIII mediated CS-PEG hydrogel formation. The functionalization of (i) 8-PEG-VS with TG-Gln peptides resulted in 8-PEG-Gln and (ii) CS-maleimide with CS-MMP-Lys in CSn-MMP-Lys. (iii) Formulations of 8-PEG-Gln, CS-MMP-Lys and the cell adhesion ligand TG-RGD-Lys are crosslinked by FXIIIa in presence of cells to form 3D cellular microenvironments. Reprinted with permission from [16] (a), [17] (b, c) and [27] (d).

Figure 2 - (a) Aliphatic ultrashort peptides self-assemble into nanofibrous hydrogels. Self-assembly is driven by a characteristic amphiphilic motif. The peptides are proposed to assemble in an antiparallel fashion, giving rise to α -helical intermediate structures (red line, graph) detected by circular dichroism. Peptide dimers and multimers subsequently stack in β -fibrils (blue line, graph) along the fiber axis that condensate further to finally arrange into nanofibers and sheets. Field emission scanning the resulting hydrogels, resembling extracellular matrix structures. **(b)** The peptides demonstrate salt-enhanced gelation, forming stiffer hydrogels at lower peptide concentrations in saline solutions as compared to water. Exploiting this property, it was possible to prepare (i) hydrogel noodles by extruding peptide solution into a concentrated salt bath and (ii & iii) print droplet arrays via sequential deposition of peptide and PBS containing cells and/or small. Reprinted with permission from [40](a) and [18](b).

Figure 3 - (a) Ultrashort peptide hydrogels encourage proliferation of encapsulated human H1 embryonic stem cells which retain their pluripotency, as reflected by the staining of nuclear transcription factors Oct4 and Nanog (red) and surface biomarkers Tra-I-60 and Tra-I-81 (green). **(b)** Human mesenchymal stem cells (hMSCs) encapsulated in hydrogel droplets and cultured on hydrogel films elongated along the peptide fibers, as reflected by the staining of their actin cytoskeleton (green). **(c)** Organoids formed in PEG hydrogels contain differentiated intestinal cells. **(d)** Summary of the rules governing different stages of organoid formation within an artificial matrix. Scale bars, 50 μ m. Reprinted with permission [18] (a, b) and [17] (c, d).

Table legends

Table 1- List of recently used synthetic and naturally-derived biomaterials, using modifications or distinct features, with verified or potential printability.

Origin	Biomaterials	Modification/Feature	Cells	Bioprinted?	Reference
Synthetic	Self-assembling peptides	Ultrashort peptides	hMSCs	Yes	Loo et al. 2015
		Short peptides	Bovine nucleous pulposus cells	No	Wan et al. 2016
		Short peptides	Murine mammary epithelial cells	Yes	Raphael et al. 2016
	PEG	Acrylated PEG + acrylated RGD and MMP-sensitive peptide	Human BM-MSCs	Yes	Gao et al. 2015
		FXIIIa Transglutaminase crosslinked PEG with RGD and laminin 111	Murine intestinal stem cells (organoid)	No	Gjorevski et al. 2016
		FXIIIa Transglutaminase crosslinked PEG with RGD, chondroitin sulphate and MMP-sensitive peptide	Human BM-MSCs	No	Anjum et al. 2016
	Pluronic®	Added acrylated hyaluronan	Bovine chondrocytes	Yes	Müller et al. 2015
Naturally-derived	Collagen	Crosslinked with genipin	Human osteoblasts and adipose-derived SCs	Yes	Kim et al. 2016
	Alginate	RGD modified gamma-irradiated alginate + PCL	Pig BM-MSCs	Yes	Daly et al. 2016
	Gelatin	Methacryloyl gelatin (GelMA) + nanocrystalline hydroxyapatite	Human osteoblasts and MSCs	Yes	Zhou et al. 2016
		Methacryloyl gelatin (GelMA) + gold nanorods and alginate	Rat cardiomyocytes and cardiac fibroblasts	Yes	Zhu et al. 2017
	Fibrin	Fibrin-factor XIII-hyaluronan composite	Rat Schwann cells	Yes	England et al. 2016
		Two-cell layer system	Human fibroblasts and human keratinocytes	Yes	Cubo et al. 2016
	Chitosan	Composite of chitosan and collagen type I	Human hepatocytes	Yes	Zhong et al. 2016
	Agarose	Added Matrigel®	Human colonic epithelial cells	Yes	Fan et al. 2016

	Hyaluronan	Methacrylated hyaluronan+ poly(N-isopropylacrylamide) + methacrylated chondroitin sulphate	Bovine chondrocytes	Yes	Kesti et al. 2015
	Matrigel®	Two-cell layer system	Human alveolar epithelial type II cells and EA.hy926 hybrid cells	Yes	Horváth et al. 2105
		Matrigel® bioink + PCL/Borate glass composite scaffold	Human adipose-derived SCs	Yes	Murphy et al. 2017

Table 1 List of recently used synthetic and naturally-derived biomaterials, using modifications or distinct features, with verified or potential printability.

(a)

Highlights:

- Importance of fully synthetic bioinks for 3D bioprinting.
- Composition of synthetic matrices.
- Functionalization of bioinks to create smart responsive matrices.
- Bioprinted 3D tissue constructs.