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From molecular design to 3D printed life-like materials with unprecedented properties

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Abstract

Stimuli-responsive synthetic materials have gained interest as biomaterials due to their ability to transform upon external stimuli. In order to mimic the cellular microenvironment synthetically, it is proposed that stimuli-responsiveness needs to be coupled with hierarchical supramolecular assembly of the materials applied. Additionally the mechanical properties of the microenvironment, i.e. the extracellular matrix, determine the nature of the tissue, which also needs to be mimicked in the material. Full control of mechanical properties, stimuli-responsiveness and hierarchical structure formation is proposed to be achieved by 3D printing of supramolecular systems into hierarchical structures that are able to react and adapt to stimuli, using metamaterial concepts.

Therefore, in this review we discuss 3D printing of stimuli-responsive materials, and the design and development of metamaterials. Combination of these concepts with supramolecular chemistry is proposed to result in the design and synthesis of life-like biomaterials with unprecedented properties.

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Introduction

In nature, the behavior of living cells is largely regulated by complex assemblies of various bioactive compounds, both inside and outside cells. The extracellular matrix (ECM) is a large supramolecular assembly functioning outside the cell, which is dynamic and responds to changes in the local environment. In this way the ECM contributes to the control of cell behavior via intracellular pathway signaling, resulting in a cascade response with various feedback mechanisms [1]. This bidirectional signaling, in which cells respond to the ECM and the ECM assemblies adjust to the cell, is referred to as 'dynamic reciprocity'. In the complex multicellular process of tissue regeneration and formation, spatial and temporal guidance of bioactive compounds in a dynamic way is needed to recapitulate the structure, shape and function of native tissue [2]. It is proposed that dynamic reciprocity can be introduced into synthetic three-dimensional (3D) scaffolds [3–5] via dynamic modification with bioactive components that mimic the natural biophysical and biochemical environment of the cell. Eventually, such bioactive scaffolds can be applied for *in situ* regeneration, in which the body's own reparative capability is used to build new tissues, i.e. by recruiting cells to the site of injury using bioactive biomaterials [6].

In recent years, scaffold processing techniques have been automated with additive manufacturing (AM) techniques in order to achieve precise control over specific design properties in a layer-by-layer fashion [7–9]. These 3D printing techniques enable vast unlimited freedom in design to easily and rapidly develop scaffolds with complex architecture. Complex architecture scaffolds can be developed from multiple material layers using 3D printing techniques and the production can be scaled up with relative ease. Despite the benefits of 3D printing, the major drawback is that the processed scaffolds are static and inanimate. Introduction of dynamics and movement can be performed via 3D printing of stimuli-responsive materials that can mimic the dynamics and function of native tissues [1,10,11]. This enables the possibility to control and guide cell behavior that mimics, for example, the functions that resembles muscle tissue or heart tissue. Importantly, cells also respond to the environment, which mechanically differs for each tissue such as bone, cartilage or skin. Therefore, it is important to also mimic the mechanical property of native tissue to create an accurate synthetic scaffold. It has been known that the topology of mechanical metamaterials can influence the mechanical properties. Changes and control of advanced geometry can be introduced by 3D printing in order to mimic the mechanical property in native tissues.

In this review, we propose to introduce supramolecular chemistry, into the 3D printing field, to implement stimuli-responsiveness into complex architectures in a

dynamic way to create a functional life-like material with desired mechanical properties. We present a few elegant examples of stimuli-responsive materials that can be used to control dynamic responsive scaffolds at the microscale. Moreover, complex structures that define the mechanical properties at the macroscale are illustrated with examples. Finally, we provide our perspective of the realization of a dynamic and controlled system that leads to true dynamic reciprocity with full control of the bioactive material with desired mechanical properties at different length scales (Figure 1).

Stimuli-responsive polymers

Stimuli-responsive polymers are smart polymers that can transform over time due to the coupling or conversion of energy between different physical domains under defined environmental stimuli [12]. 3D printing of stimuli-responsive materials has been performed to introduce the ability of shape and/or function transformation over time [13–15]. Conformational and chemical changes after 3D printing are induced by variety of stimuli, such as light irradiation, water or temperature, as summarized (Table 1).

Light irradiation promoted material folding

Photochemical treatment can be used to control the self-folding mechanism of a material. Molecular control in combination with light irradiation results in bending and/or folding of polymers upon irradiation. Pentaerythritol tetra(3-mercaptopropionate) (PETMP), 2-methylene-

Figure 1
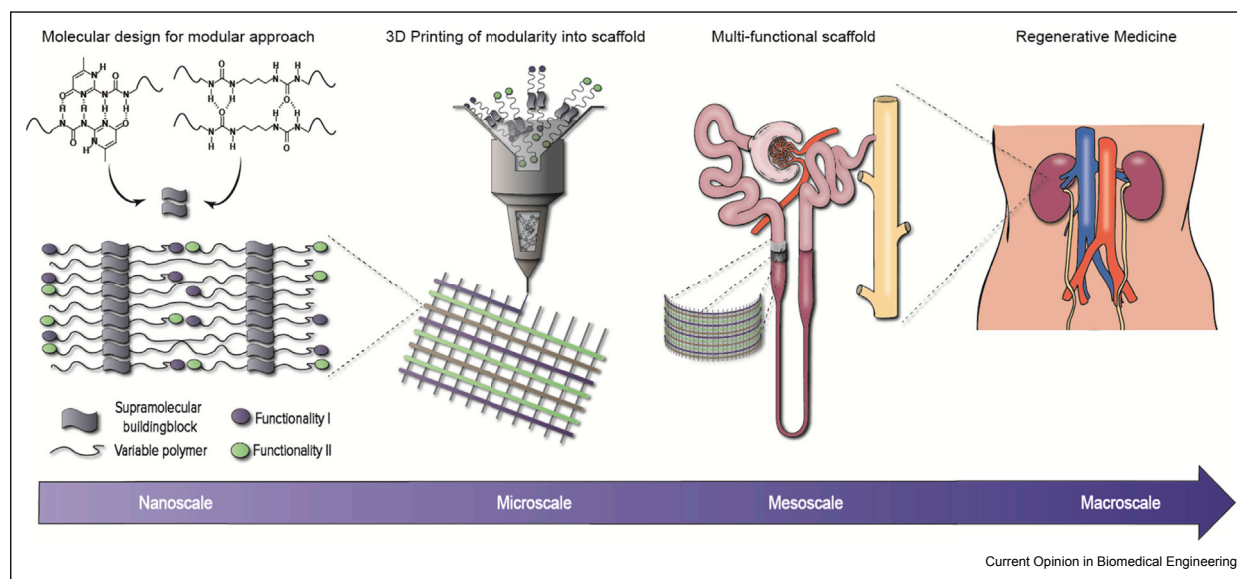
From molecular design to regenerative medicine. The molecular design at nanoscale using supramolecular polymers enables modularity in biomaterials. The incorporation of different polymer backbone can change the polymer properties. Additionally, bioactive components and stimuli-responsive molecules can be coupled to the monomers to functionalize the material. By 3D printing this modular biomaterial and depositing the specific functionality at desired positions, a gradient in functionality can be achieved at the microscale. The implantation of such a bioactive scaffold (e.g. as nephron) at mesoscale is proposed to promote regeneration of tissues and the stimulation of neo-tissue formation at the macroscale (e.g. as application in the kidney).

Table 1

Summary of the stimuli responsive materials, their biomedical applications and future perspectives.

Stimulus	Materials	Biomedical application	Future perspective	Reference
Light	PETMP; MDTVE; EGDMP	Not applicable	Cell culture on flat sheet and folding into 3D structure afterwards	[16]
Water	Polystyrene films; black ink	Drug delivery	Target specific drug delivery systems	[17,18]
	Various molecular weight PEG	In vitro bio-origami β-TC-6 cell culture	Self-folding vascularized tissue constructs	[19]
Temperature	Acrylamide; cellulose fibrils	Not applicable	Shape-shifting architectures; vascular regeneration	[20]
	Tangoblack; Verowhite	Not applicable	Specific hinges folding to create complex architecture scaffolds; heart valves	[21,22]
	PNIPAM-ABP; PCL	Capture and release of yeast cells in vitro	Origami of bilayer scaffolds to introduce curvatures for cell culture	[23,24]

propane-1,3-di(thioethylvinylether) (MDTVE) and ethylene glycol di(3-mercaptopropionate) (EGDMP) have been mixed in 1:4:5 wt% ratio with two photo-initiators and a photo-absorber [16]. Irradiation with light with a wavelength of 365 nm activates the photo-initiator which is able to react with MDTVE resulting in the formation and rearrangement of the network. This process macroscopically results in stress relaxation without altering the crosslink density in the network. There are two ways to deform a polymer sheet: bending and folding. Irradiation of a large area of the sheet resulted in bending of the sheet due to the stress relaxation gradient. The hinge angle was in proportion with the photomask width, which was decreased when the surface of the irradiation area increased resulting in

de formation of a curvature. When a localized narrow region was irradiated, the sheets were folded with an increased hinge angle.

Another light-activated material was made using pre-stressed polystyrene films on which black ink was printed as hinges using a desktop laser printer [17]. The hinges covered with black ink were irradiated with infrared light, and as a result of that were heated up above their glass transition temperature more rapidly than the rest of the polystyrene film. In this way the film bended towards the direction of the light (see Figure 2A). This self-folding technique by light activation is an effective method to control shape behavior at mesoscale. This has been applied for many

Figure 2

Current Opinion in Biomedical Engineering
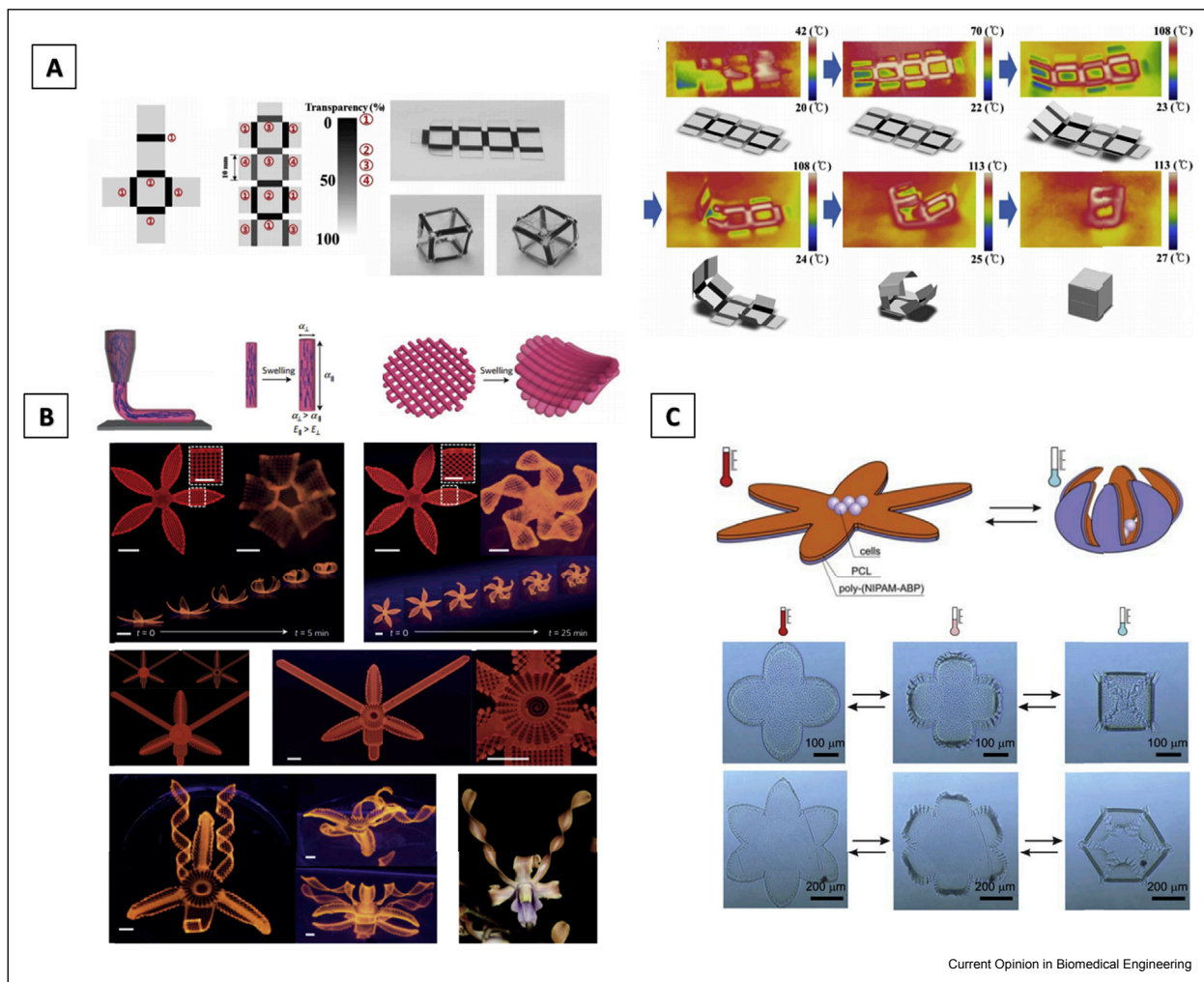
Transformation of stimuli-responsive objects. **A.** Folding of hexahedral shape polystyrene sheet with black hinges due to infrared irradiation. Deformation process was visualized using thermographic images [17]. **B.** Printing of shear-induced alignment of cellulose fibrils, which introduced curvature in flower structures due to swelling effects in solution. Orientation of the bilayers influences the angle of curvature to design different morphologies. (Reprinted by permission from Macmillan Publishers Ltd: Nature Materials [20], copyright 2016.) **C.** Bilayer of star-shape polymer from PCL and poly-(NIPAM-ABP). The poly-(NIPAM-ABP) swells and folds due to cooling, and shrinks at elevated temperature. (Reproduced from Ref. [23] with permission of The Royal Society of Chemistry.)

drug delivery systems [18]. An important drawback of this technique to be applied *in vivo* is that the application is restricted to the use of wavelengths that can penetrate tissue.

Water affected material behavior

Dynamic movement at mesoscopic length scale can be introduced by exploring interactions with water. This has been done by using polyethylene glycol (PEG) with two different molecular weights that are photo-crosslinked into PEG bilayer scaffolds [19]. The self-folding process of the scaffolds was driven by differential swelling in aqueous solutions. The materials made consisted of various bilayer thicknesses with different molecular weight combinations of PEG (700 Da/4 kDa, 700 Da/10 kDa, and 4 kDa/10 kDa). These bilayers were investigated to create particular radii of scaffold curvature. B-TC-6 islet cells were encapsulated within the bilayer scaffolds, which were able to self-fold into cylinders. These cells significantly provided higher insulin release in the tubular geometries compared to flat geometries. In a different study, local anisotropic swelling of a biomimetic hydrogel composite was investigated via introduction of a spatio-temporally controllable bilayer architecture [20]. This approach made use of stiff cellulose fibrils embedded within a soft acrylamide matrix, which was inspired by the external stimuli of the non-directional response from plants. Curvatures in bilayer structures were introduced by controlling the anisotropic swelling and the forced contact between the two layers along the entire mid plane. The curvature strain tensor and the differential swelling must remain identical to enable the controlled dynamic rearrangement in architecture (see Figure 2B). These stimuli-responsive materials have great potential in the development of active functional scaffolds since the materials are able to adapt in aqueous environment.

### Temperature induced conformational changes

Thermal responsive materials have been used in the field of drug delivery and tissue regeneration. These smart materials are able to shrink, swell, or fold due to a change in temperature. For instance, printed active composites composed of glassy polymer fibers with thermomechanical shape memory properties, have been developed to reinforce elastomeric matrices to enable controlled shape changes [21]. In this exemplar study, two material composites were used. One material consisted of Tango black (a rubbery material at room temperature), which contains urethane acrylate oligomer, *exo*-1,7,7-trimethylbicyclo, hept-2-yl acrylate, methacrylate oligomer, polyurethane resin and a photo-initiator. Another material, Verowhite (a rigid plastic at room temperature) consisted of isobornyl acrylate, acrylic monomer, urethane acrylate, epoxy acrylate, acrylic oligomer and photo-initiator. After cooling, plastic deformation occurred while the strain was

maintained constant. A 2D sheet from different layers of material was printed and the temperature induced contraction of the hinges promoted the folding into a 3D box shape upon increase of temperature [22]. Furthermore, the bilayer construct of the thermo-responsive hydrogel poly(*N*-isopropylacrylamide) contained 1 mol % of 4-aryloylbenxophenone comonomer (poly(NIPAM-ABP)) and poly(ε-caprolactone) (PCL) with 6 mol% of 4-hydroxybenzophenone, which was crosslinked with UV light. This bilayer was used to (un)fold the capsule upon temperature change [23]. The poly(NIPAM-ABP) hydrogel was able to swell and shrink upon temperature changes without any residual deformation. The thickness of the PCL layer determines the radius of the curvature. The conformational changes induced by temperature, demonstrated a reversible temperature response to control the encapsulation and release of yeast cells within the bilayer scaffold (see Figure 2C). It is proposed that this is an interesting approach to develop a functional scaffold that can transform its geometry at physiological temperatures. However, Stoychev G et al. demonstrated in recent work, to manage the tuning of the timescale of this thermosensitive actuation. The slow water diffusion into the hydrogels resulted in a broad range of actuation time, which was determined to be seconds to hours. It was shown that the response time could be decreased to milliseconds by using two approaches, i.e. via a porous hydrogel or by size reduction of the hydrogel [24]. This finding opens a new perspective for the development of fast thermosensitive poly(NIPAM) materials for biomedical applications.

Mechanical metamaterials

3D printing of synthetic materials into specific advanced geometries can tailor and manipulate the mechanical properties at the mesoscale. These types of synthetic materials are known as mechanical metamaterials, which are associated with artificial engineered micro- and nano-architecture based materials with unprecedented properties and performance [25–27]. The design of these structures can determine enhanced stiffness, flexure, movement and strength of the material. The modification of mechanical metamaterials can be realized using origami techniques. The folding of a 2D sheet into a 3D architecture provides an elegant source for designing mechanical metamaterials [28]. This material transformation has been utilized in biomedical applications such as the application of heart stents or other laparoscopic surgery methods, where large sheet of materials can be implanted using minimal invasive surgeries (see Figure 3A) [28–30]. Other types of mechanical metamaterials are the auxetic metamaterials, which are materials in which the mechanical properties are manipulated by small-scale topologies and which exhibit a negative Poisson's ratio [31]. This means that stretching of such materials result in lateral expansion

Figure 3
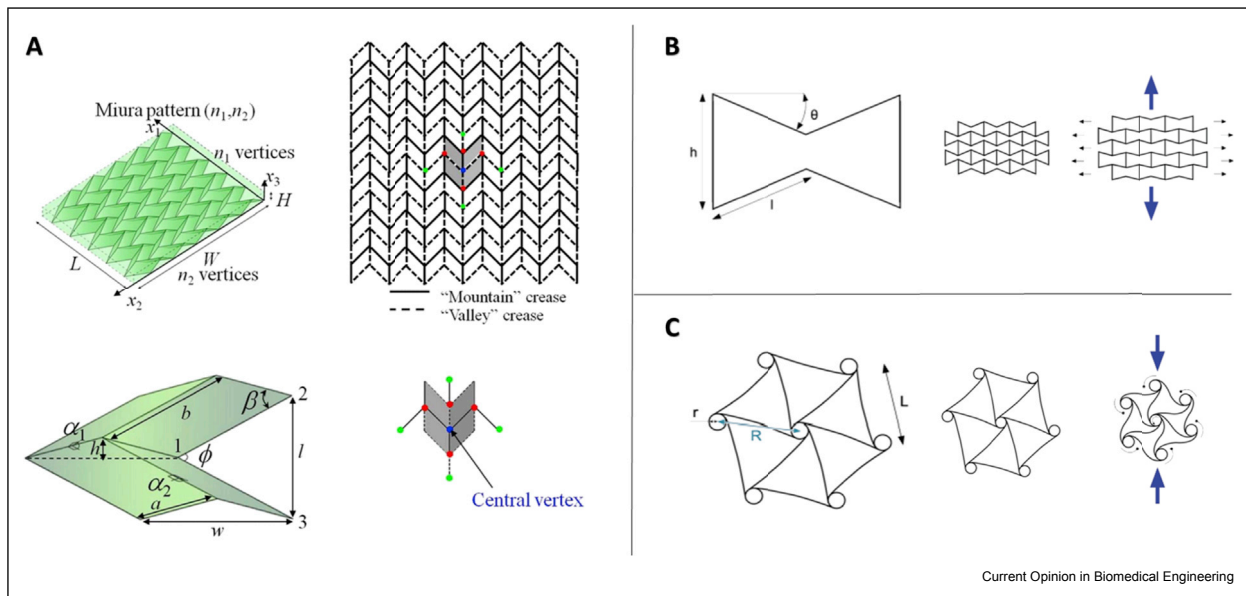
Mechanical metamaterials. **A.** Origami of metamaterials via folding of a 2D sheet material into a 3D architecture. Different folding patterns can manipulate the property of the 2D sheet. (Reprinted by permission from Macmillan Publishers Ltd: Scientific Reports [28], copyright 2014.) **B.** Auxetic metamaterials with re-entrant structures [32] or **C.** chiral structures to influence the mechanical properties of the material [32].

instead of contraction. An example of a well-established basic deformation mechanism is the re-entrant structure, which is known as foam or honeycomb structure that directs inward or has a negative angle (see Figure 3B) [32]. Another example is the chiral structure, which has a central cylinder structure that is chiral or anti-chiral (i.e. not superimposable on its mirror image) (see Figure 3C) [32]. Under mechanical loading, these cylinders rotate and result in the folding or unfolding of the composing ligaments.

The combination of smart materials and well-designed topology in the additive manufacturing field offers promising possibilities for next generation functional biomaterials. The above examples demonstrate the importance of the molecular design of the materials combined with the specific design of well-ordered scaffold architecture, in order to create predictable functionality at the macroscale. With this understanding, it is proposed that novel self-assembling and programmable multifunctional architectures can be developed in combination with intrinsic bioactivity and tunable mechanical properties, possibly controlled at different length scales.

Perspective

Specific dynamic movement of a bioactive scaffold is proposed to be controlled at the nanoscale via molecular assembly. Dynamic control at the molecular scale is necessary to obtain unprecedented material properties that mimic the functional native microenvironment using fully synthetic materials. Additionally, the

mechanical properties or tissues need to be recapitulated in synthetic materials in order to be able to regenerate tissue. It is proposed that, the reconfiguration of topology using 3D printing can influence the mechanical performance of the synthetic material at mesoscale to mimic the native tissue.

Despite the benefit of stimuli-responsive polymers and metamaterials, these polymers lack modularity, which is a challenge for the introduction of novel functionalities and bioactive components into the materials. In order to implement this modularity into these stimuli-responsive metamaterial scaffolds, we propose to use supramolecular polymer chemistry. Supramolecular polymers consist of monomers that are held together via specific, directed non-covalent interactions [33,34]. The structural and architectural design of supramolecular polymers are widely explored and are shown to be highly controlled and dynamic in nature [33–35]. Moreover, the incorporation of bioactive components within supramolecular systems provide endless possibilities [36,37]. As such, the incorporation of e.g. drugs or growth factors in combination with stimuli-responsive behavior and metamaterial properties could lead to synthetic materials with intrinsic and adjustable bioactivity and mechanical function. Interesting examples include the development of scaffolds that *in vivo* respond to infection or disease progression affecting tissue regeneration processes, e.g. scavenging of free radicals or neutralizing inflammatory compounds, or mechanically unloading tissue areas to prevent wound contraction and scar formation.

We propose that the introduction of dynamic and modular synthetic supramolecular polymers that are able to self-assemble in hierarchical structures, into the field of 3D printing, can support the bottom-up fabrication of novel bioactive scaffolds. In this way true dynamic reciprocity at various length scales is proposed to be introduced into 3D printed synthetic scaffold materials.

Acknowledgements

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- of special interest
- of outstanding interest

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