Accepted Manuscript

Current Developments in Multifunctional Smart Materials for 3D/4D Bioprinting

Nathan J. Castro, Christoph Meinert, Peter Levett, Dietmar W. Hutmacher

PII: S2468-4511(17)30004-1

DOI: [10.1016/j.cobme.2017.04.002](https://doi.org/10.1016/j.cobme.2017.04.002)

Reference: COBME 13

To appear in: *Current Opinion in Biomedical Engineering*

Received Date: 18 April 2017

Accepted Date: 19 April 2017

Please cite this article as: N.J. Castro, C. Meinert, P. Levett, D.W. Hutmacher, Current Developments in Multifunctional Smart Materials for 3D/4D Bioprinting, *Current Opinion in Biomedical Engineering* (2017), doi: 10.1016/j.cobme.2017.04.002.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Current Developments in Multifunctional Smart Materials for 3D/4D Bioprinting

Nathan J. Castro¹, Christoph Meinert¹, Peter Levett¹ and Dietmar W. Hutmacher¹

Institute of Health and Biomedical Innovation, Queensland University of Technology,
Queensland, Australia 4059

Abstract:

Tissue engineering and regenerative medicine (TE&RM) has made continuous advancements by extending three-dimensional (3D) printing towards the development and fabrication of biomimetic scaffolds composed of biomimetic natural and synthetic materials. Although several clinical applications of 3D-printed scaffolds made of classical biomaterials have yielded promising results, current basic research trends have shifted towards rendering scaffolds “smart” or dynamically responsive where shape changes are induced by external stimulation (ie. electromagnetic radiation, mechanical stress, heat) or physiological changes in the local microenvironment through pre-designed mechanisms of action. Although a bulk of the research efforts have led to high-impact publications demonstrating the capabilities of these technologies, hardly any studies have been published illustrating the application of these research efforts in a clinical context. Herein, we will discuss current trends and advancements of 3D printable bioinks and highlight the most recent developments (2015-present) of 3D-printed smart materials also commonly referred to as 4D printing/programmable matter. We will conclude with a prospective opinion of this research field where innovation is synonymous with risk-taking. High-risk, high-reward research founded on revolutionary innovations are often disruptive and lead to new paradigms; conclusively the 4D printing can be disruptive, because it has the potential to change the current paradigm by changing the question from “what can we do with these materials/technologies?” to “how can we move the concept/technology forward to achieve what we need”.

Highlights:

- Programmable matter needs to translate its potential to be a disruptive technology via high quality applied research.
- The current paradigm needs to be changed from “what can we do with these materials/technologies?” to “how can we move the concept/technology forward to achieve what we need”.
- From a clinical point of view physiological and biomechanical constraints based on host tissue environment are not typically considered in smart material design.
- Currently the lack of in-depth follow-up studies have resulted in poor clinical translation of smart material concepts.
- Global collaborative efforts spur and facilitate development, characterisation and validation of new bioinks.

Keywords (3 - 5 words): 4D Printing, Programmable matter, Gelatin methacryloyl (GelMA), Bioink, Smart materials

Introduction:

The rise in four-dimensional (4D) printing can largely be attributed to a widely broadcast 2013 TED (Technology, Entertainment, Design LLC, a non-profit company devoted to idea dissemination) talk in which shape-changing 3D printed structures were presented [1-3]. Since that day, the concept of 4D printing has rapidly developed and expanded its breadth and depth leading to an increase of high-impact original research introducing novel manufacturing approaches/materials. Unfortunately, few follow-up publications have extended the understanding of these materials in depth or evaluated reproducibility and up-scaling on these techniques and approaches.

Dynamic shape changing materials are typically fabricated by multi-material 3D printing techniques, but the definition of “4D printing” has quickly become ambiguous and broad. As a result, a more detailed definition specifically stating the constraint of requiring shape or functional change be inducible directly after printing. Other criteria have also been established as reported by The Atlantic Council of the United States where 4D printing is defined as: 3D printing of objects which can self-transform in form or function when exposed to a predetermined stimulus, including osmotic pressure, heat, current, ultraviolet light, or other energy sources [4-6]. Even so acclaimed by the early publications it needs to be shown if the technology holds great potential in a myriad of industries and applications including TE&RM. Hence, in the following we will critically review, highlight and discuss novel programmable matter formulations and 3D printing strategies in the context of what is defined as bioprinting for potential use in TE&RM.

Osmotic pressure-driven hydrogel-based bioinks

A simple mechanism facilitating 4D temporal shape transformation of 3D printed materials for bioprinting is the utilization of the intrinsic swelling characteristics of hydrogels. A pioneering example demonstrating this fundamental principle was the fabrication of bi-layered constructs composed of photocrosslinkable polyethylene glycol (PEG) of varying molecular weights [7] (Figure 1a). When placed into an aqueous environment, pre-designed folding of the constructs was induced by the differential swelling of the bonded hydrogel layers (Figure 1a), resulting in a well-predictable and controllable curvature to form micropatterned spheres (Figure 1b), helices (Figure 1c), or cylinders (Figure 1d). This principle was later adapted for 4D printing applications via the stimulus-initiated shape transformation of printed planar structures upon immersion in water [8].

In this example, shape-change was driven by bi-layered sheets consisting of a profoundly swelling hydrogel and a non-swelling, flexible material which were used to form joints between rigid linear structures. The joint folding direction was controlled by the radial orientation of the sheets with respect to the adjacent structures, while the degree of folding was fine-tuned by controlling the spacing and diameter of disk-shaped angle limiters which provided a physical stop once the desired folding angle was reached (Figure 1e). Using this approach, various structures were fabricated such as planar grids which transformed into sinusoidal waves (Figure 1f), hyperbolic or double-curved (convex and concave) surfaces (Figure 1g). More recently, Lewis *et al.* demonstrated well-predictable swelling-induced

curvature of planar structures fabricated using a single printable ink as opposed to multiple materials with distinct swelling properties [9]. This approach relied on the anisotropic swelling behaviour of the hydrogel composite ink developed by the team, which consisted of N, N-dimethylacrylamide or N-isopropylacrylamide (NIPAM), synthetic hectorite clay, and nanofibrillated cellulose. During the printing process, the cellulose fibrils undergo shear-induced alignment in the longitudinal direction as the ink flowed through the deposition nozzle (Figure 1h), resulting in hydrogel filaments with 4-fold higher swelling strains in the transverse compared to the longitudinal direction (Figure 1i). By controlling the anisotropic swelling behaviour of the construct, this allowed the team to control the curvature of bilayered structures. Structures with uniform cylindrical curvatures were obtained when bilayers were printed with orthogonal ($90^\circ/0^\circ$) layer alignment, whereas $-45^\circ/45^\circ$ alignments yielded twisted bilayered strips. Furthermore, spatially inhomogeneous curvatures were obtained by varying the interfilament spacing, and, when various folding mechanisms were combined, more complex shape transformations could be achieved. For example, mimicking the morphology and nastic motion of the orchid *Dendrobium helix* (Figure 1j, k).

Taken together, these examples demonstrate that the passive, osmotically-driven process of hydrogel swelling can be used to induce temporal shape-transformation for 4D printing in a biomechanically non-challenging environment. Therefore, a direct biomedical application of this concept as proposed by the authors, for example to mimic complex movements of native tissues such as the heart, is currently restricted by material limitations including slow and low-force actuation, as well as the limited reversibility of shape-transformation events. Furthermore, the concept remains to be translated to more cyto- and biocompatible materials with cell-instructive bioactivity to induce a desired cellular behaviour and construct deformation in response to physiological stimuli.

Figure 1: Shape transformation of 4D-printed structures induced by osmotic swelling. (a) Schematic illustrating the fabrication of PEG bilayers of distinct molecular weight and swelling-induced curvature. PEG bilayers can be programmed to form (b) spherical capsules, (c) helices, and (d) cylinders with microtopographical features on their surfaces (scale bars: 200 μm , adapted with permission from [7]). (e) Schematic rendering of a printed joint folding upon water-uptake and swelling of the actuating hydrogel components (red). The final angle of folding is controlled by disk-shaped angle limiters. Shape-transformation of a

planar grid into a (f) sinusoidal wave and (g) double-curvature surface (adapted and reprinted with permission from [8]). (h) Shear-induced alignment of bioink-embedded cellulose fibres during extrusion through a deposition nozzle and (i) resulting anisotropy of perpendicular ($\alpha_{\perp}$) and longitudinal ($\alpha_{\parallel}$) swelling strains. (j) Swelling-induced shape-transformation mimicking the nastic movement of (k) the orchid *Dendrobium helix* (adapted and reprinted with permission from [9]).

Thermal actuation of smart materials

In addition to hydrogel-based soft programmable matter, the shape memory properties of thermoplastics can be used to impart the fourth dimension in 3D-printed structures. Similar to osmotically-driven shape change in composite hydrogel bioinks, shape memory thermoplastics are typically polymers with temperature-mediated behaviour predicated on the material's intrinsic transition temperature(s) (introduced and reviewed in [10]). Complex and intricate origami-type folding transitions have been demonstrated by utilizing the conventional properties of shape memory polymers as illustrated in Figure 2. Here a cube structure has been printed in its folded, permanent shape using a 3-layered laminate of two commercially (Stratasys®, Minnesota, United States) available polymers – one layer of VeroWhite sandwiched between two layers of TangoBlack. Upon completion of printing, the structure was unfolded at a temperature above the glass transition temperature of the hinge, then cooled while in the flattened state. When the temperature is raised above the transition temperature, the structure reverts to its permanent, folded shape. This approach has been further developed to demonstrate the potential for 4D printing of more complex structures, such as an interlocking clasp and a replica UPS mailing box [11]. These more complicated structures require the hinges to fold in sequence, rather than simultaneously, to avoid spatial collisions during folding. By using hinges with a range of transition temperatures, the sequence in which they fold during heating can be controlled – with the hinge exhibiting the lowest transition temperature folding first and the hinge with the highest transition temperature folding last.

Figure 2: Sequential folding of shape memory polymer structures. In the upper panel, the clasp structure is printed in its 3D form, then flattened to a temporary state. The hinges have different transition temperatures, so upon exposure to a uniform temperature stimulus, the hinges with the lower transition temperatures fold first. The sequential folding is critical to the structure taking the desired shape. In the lower panel, a structure inspired by a UPS mailbox self-assembles by sequential folding in a water bath (adapted and reprinted with permission from [11]).

An alternative method of exploiting the shape memory properties of thermoplastics is to fabricate layered multi-material self-assembling 2D structures which upon heating readily transform into a predefined three-dimensional shape (Figure 3). This has been demonstrated by printing laminate structures termed printed active composites (PACs) in which shape memory polymer fibres are deposited within an elastomeric matrix [12]. The main advantage of this method is the simplicity of printing a 2D sheet compared to a 3D structure – printing a 2D sheet is less time-consuming and requires much less raw material as well as printable by less sophisticated commoditized 3D printers. The primary disadvantages are the lack of complexity and accuracy of the 3D shape as well as the requirement of stress-loading which is necessary to program shape-transformation in these materials.

Figure 3: Self-assembly of printed structures into defined three-dimensional forms. On the left, the schematic shows the arrangement of shape-memory polymer fibres (pink) in the lower section of the laminate structure, with an elastomeric matrix in the upper layer (yellow). To induce self-assembly, the 2D sheet is heated above the glass transition temperature of the shape-memory polymer, stretched, and then cooled under stress. When the sheet is unloaded, the laminate structure bends in a predictable way and takes the desired 3D form. Figure from [12]. When the folded structure is heated above the transition temperature the 3D structure reverts to its flattened state.

Expanding on this concept, Wu *et al.* combined two shape memory polymers exhibiting different glass transition temperatures and printed structures with multiple shape transitions. A proof-of-principle was demonstrated by printing layered structures in which fibres of two shape memory polymers with glass transition temperatures of 38 °C and 57 °C were deposited in each layer [13]. To program the shape memory effect, the printed structure was heated to ~ 70 °C and stretched to 10% strain. The strain was maintained while the structure was cooled to ~ 0 °C then released. The different glass transition temperatures allowed the structure to form multiple shapes depending on the temperature applied. Figure 4 illustrates this concept through a simple linear strip, the structure is almost flat at 0 °C, with a slight bend towards the side which contains the fibres with the lower transition temperature. At 15 °C, the strip assumes a clear bend, and at 30 °C the angle increases further. At 60 °C, which is above the transition temperature of both fibres, the structure returns to its flat state. A more complex example is shown with an insect-like structure, which is first programmed by biaxial stretching, then heated to in 30 °C water. After 37 s, the insect structure has assumed its standing form, and upon further heating to 60 °C, the structure reverts to its permanent flat shape.

Figure 4: Multi-shape printed structures containing two shape memory polymers with different transition temperatures. The top panel shows a schematic of the structure, with fibres of each shape memory polymer deposited in layers within an elastomeric matrix. The strip is programmed by stretching to $\sim 10\%$ strain at 70°C , then cooling to 0°C and releasing the strain. The strip is able to bend to multiple forms which are temperature dependent. At 60°C the strip returns to its original flat state. In the lower panel, this principle is demonstrated in a more complex insect-like structure. From [13].

Even though we have focused our discussion of thermal actuation towards low glass transition temperature thermoplastics, one should not be misled to believe that thermal actuation is limited to these types of materials. Many hydrogel materials exhibit temperature-sensitive properties which can be leveraged and employed in pre-design strategies to produce smart or multifunctional 3D objects. Spinks *et al.* have demonstrated a 3D-printed hydrogel valve capable of controlling flow by using a thermally sensitive alginate-poly(N-isopropylacrylamide) (PNIPAAm) hydrogel [14]. The dual network-type hydrogel consisted of a tightly crosslinked alginate matrix and a loosely crosslinked network of PNIPAAm. The PNIPAAm network was progressively photocrosslinked during printing at 10°C , and the completed printed structure was immersed in calcium chloride to crosslink the alginate matrix. At 20°C the alginate-PNIPAAm hydrogel is highly swollen (swelling ratio ~ 9) with the valve assembly in the open position. At 60°C the hydrogel water content is significantly reduced (swelling ratio ~ 1) and the actuating arms of the valve assembly contract, closing the flow path (Figure 5).

Figure 5: 3D printed alginate-(PNIPAAm) valve assembly that is in the open position when swollen (at $\sim 20^\circ\text{C}$) and in the closed position at 60°C . Above $\sim 32^\circ\text{C}$, the temperature-sensitive (PNIPAAm) expels water, which functions to contract the actuating arms and close the flow path. Figure from [14].

In a similar study, folding of hydrogel structures was demonstrated by printing composites containing two different hydrogel formulations [15]. The first was a mixture of polyether-based polyurethane (PEO-PU) and 2-hydroxyethyl methacrylate (HEMA) as a monomer. The second formulation was a mixture of polyether-based polyurethane (PEO-PU) and N-isopropylacrylamide (NIPAM) as the monomer. In both cases, the mass ratio of PEO-PU was 1:1, and the PEO-PU component provided the viscosity required to print the inks. To prevent phase separation of NIPAM during polymerisation, ethanol was used as the solvent for both formulations. 3D printing was used to deposit the two hydrogels in spatially defined regions, allowing 'hinges' to be fabricated. The differential swelling between the two hydrogel formulations caused the hinges to bend when swollen at ambient temperature, and revert to a flattened form when heated to 60°C (Figure 6).

Figure 6: Printed hydrogel constructs with hinges that bend or fold based on temperature. The hinge structures are made from hydrogels with differential swelling characteristics – the NIPAM component, shown as the lighter-coloured material, swells significantly at low temperatures to bend the hinge. At 60 °C the NIPAM component expels water, and reverts to a flatter state. Image from Naficy et al. 2017.

The highlighted works including the previous examples of alginate-poly(NIPAAm) actuating valves and hinged structures composed of mixtures of PEO-PU, poly(HEMA) and poly(NIPAAm) show the importance of 3D printing technologies for the fabrication of translatable smart materials. In the discussed works, the constituent materials are not inherently ‘smart’ – on their own they have been widely used in many different applications, and without the functions demonstrated in these examples. Yet, by using 3D printing to combine these materials into spatially-defined structures, smart hydrogel constructs with actuating or folding functions can be produced. Although these proof-of-principle studies demonstrate some of the possible functionalities that can be integrated into 3D printed hydrogel constructs, they are not immediately applicable to typical medical applications as the materials used are commoditized polymers where clinical applications require medical grade polymers. For example, both the actuated valve and the hinge structures undergo shape transformation when heated to 60 °C – far above physiological temperatures, and to prevent phase separation of the poly(NIPAAm) during polymerisation, the valve was printed at 10 °C, while ethanol was used as the solvent for the hinges. Nevertheless, overcoming these challenges for medical applications is the next research frontier for these temperature sensitive constructs and should be the focus of targeted follow-up studies with clinical application.

As illustrated through the course of our discussion, recent advances in material science and 3D printing have produced a plethora of designs that can respond to local endogenous and exogenous stimuli such as changes in temperature, ionic strength, or other environmental factors, to reversibly and/or irreversibly alter the state or shape of a material. While the term ‘smart’ has been generally adapted by the scientific community to describe such materials, it should be kept in mind that these responses do not necessarily qualify such

systems as ‘intelligent’ or ‘smart’. As David W. Grainger outlined in his eclectic commentary [16], all chemical and physical systems do continuously adapt to local and global stimuli through thermodynamically-driven processes to achieve the lowest possible energy state at a new equilibrium position; yet, materials do not use a logic algorithm or intellectual capacity to decide to change from one state to another.

While the recent developments highlighted in this opinion paper are certainly exciting and potentially applicable for biomedical applications, follow-up studies employing and characterizing the newly introduced concepts in depth and equally important by using medical grade materials [17] are generally not pursued. Despite claims that such concepts will profoundly impact applications like drug delivery or TE&RM, research efforts often do not reach beyond the initial proof-of-principle phase. This may partially be the result of the dilemma caused by the highly competitive environment in academic science [18]. In order to secure grant funding, scientists are increasingly required to publish in high-impact journals. However, papers following-up on a previously published concept are not held in the same regard as highly innovative enough to be published in such journals, which, in turn, forces scientists to concentrate on new, innovative ideas, rather than the logical development of their original idea towards a translatable biomedical application.

Yet, in order to translate research findings and truly improve current clinical standards, fundamental concepts need to be further developed in detailed and intricate *in vitro* and *in vivo* studies assessing applications, efficacy and safety. A good example for a material that is currently being developed collaboratively by numerous research groups around the globe is gelatin methacryloyl (GelMA), a chemical derivative of gelatin which can be crosslinked via free radical polymerisation. Originally synthesized and rheologically characterised by Van Den Bulcke *et al.* in the year 2000 [19], the material has later been identified as cytocompatible and cell-instructive, initiating the rapid development of three-dimensional cell culture models and scaffolds [20, 21]. Although still in the research state, to date, the applications of GelMA and GelMA-composites are extremely versatile, ranging from organ-on-a-chip [22] to tissue engineering of functional vascular networks [23], articular cartilage [24], and skin [25], and a large number of translation research studies are published with increasing regularity. Hence, we and others are working on up scaling medical grade GelMA and GelMA-composites to translate another material from bench to bedside.

So far, we have not found any report in the literature which illustrates the performance of a 4D printed implant. The targeted tissue and its environment need to be a focal point in the design of intended constructs. Unfortunately, current studies often do not reflect on this aspect. A key challenge in designing smart biomaterials is to capture the degree of complexity needed to mimic the extracellular matrix (ECM) of natural tissue. We are still a long way from creating a one-to-one molecular analog of the ECM and the dynamic mechanisms by which information is revealed in the ECM proteins in response to challenges within the host environment. Programmable matter is not just a mere inert three-dimensional structure, but rather a highly complex vehicle for multifaceted dynamic (chemical, biological and mechanical) extracellular signalling promoting a developmentally conducive niche through at the defect site progenitor cell recruitment, growth and differentiation. [26, 27] [25, 26]. 4D designs need to be developed that promote step-wise remodelling of the surrounding tissue(s) including the newly formed ECM. The ECM with its dynamic composition acts as a constant reservoir for soluble signalling molecules and

mediates signals from other sources to migrating, proliferating, and differentiating cells during all phases of the healing process. The challenge now will be to create programmable matter that guide the regeneration the natural ECM generating downstream bioactive molecules during the remodelling processes while providing sufficient mechanical stability over time.

Conclusion

The development and translation of multifunctional smart materials for 3D/4D bioprinting into TE&RM should be based on studying the relevant cross-disciplinary literature in depth and breadth as well as implementing the key design parameters into the print of an architecture and morphology which allows cell migration, proliferation and subsequently vascularized tissue formation and remodelling.

References

1. Tibbits, S., *The emergence of "4D printing"*. TED talk http://www.ted.com/talks/skylar_tibbits_the_emergence_of_4d_printing/transcript?language=en, 2013.
2. Gladman, A.S., et al., *Biomimetic 4D printing*. Nature materials, 2016.
3. Truby, R.L. and J.A. Lewis, *Printing soft matter in three dimensions*. Nature, 2016. **540**(7633): p. 371-378.
4. Thomas A. Campbell, S.T., Banning Garrett, *The next wave: 4D printing programing the material world*. The Atlantic Council of the United States, 2014.
5. Tibbits, S., et al., *4D Printing and universal transformation*. 2014.
6. Tibbits, S., *Design to Self-Assembly*. Architectural Design, 2012. **82**(2): p. 68-73.
7. Jamal, M., et al., *Bio-Origami Hydrogel Scaffolds Composed of Photocrosslinked PEG Bilayers*. Adv Healthc Mater, 2013. **2**(8): p. 1142-1150.
8. Raviv, D., et al., *Active printed materials for complex self-evolving deformations*. Sci Rep, 2014. **4**: p. 7422.
9. Gladman, A.S., et al., *Biomimetic 4D printing*. Nat Mater, 2016. **15**(4): p. 413-8.
10. Lendlein, A. and S. Kelch, *Shape-Memory Polymers*. Angewandte Chemie, 2002. **41**(12): p. 2034-2057.
11. Mao, Y., et al., *Sequential self-folding structures by 3D printed digital shape memory polymers*. Scientific Reports, 2015. **5**: p. 13616.
12. Ge, Q., et al., *Active origami by 4D printing*. Smart Materials and Structures, 2014. **23**(9).
13. Wu, J., et al., *Multi-shape active composites by 3D printing of digital shape memory polymers*. Scientific Reports, 2016. **6**: p. 24224.
14. Bakarich, S.E., et al., *4D Printing with Mechanically Robust, Thermally Actuating Hydrogels*. Macromolecular Rapid Communications, 2015. **36**(12): p. 1211-1217.
15. Naficy, S., et al., *4D Printing of Reversible Shape Morphing Hydrogel Structures*. Macromolecular Materials and Engineering, 2017. **302**(1): p. 1600212.
16. Grainger, D.W., *Connecting drug delivery reality to smart materials design*. Int J Pharm, 2013. **454**(1): p. 521-4.
17. Poh, P.S., et al., *Poly lactides in additive biomanufacturing*. Adv Drug Deliv Rev, 2016. **107**: p. 228-246.
18. Pasterkamp, G., I. Hoefer, and B. Prakken, *Lost in the citation valley*. Nat Biotechnol, 2016. **34**(10): p. 1016-1018.
19. Van Den Bulcke, A.I., et al., *Structural and rheological properties of methacrylamide modified gelatin hydrogels*. Biomacromolecules, 2000. **1**(1): p. 31-8.
20. Loessner, D., et al., *Functionalization, preparation and use of cell-laden gelatin methacryloyl-based hydrogels as modular tissue culture platforms*. Nat Protoc, 2016. **11**(4): p. 727-46.
21. Yue, K., et al., *Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels*. Biomaterials, 2015. **73**: p. 254-71.
22. Bhise, N.S., et al., *A liver-on-a-chip platform with bioprinted hepatic spheroids*. Biofabrication, 2016. **8**(1): p. 014101.
23. Chen, Y.C., et al., *Functional Human Vascular Network Generated in Photocrosslinkable Gelatin Methacrylate Hydrogels*. Adv Funct Mater, 2012. **22**(10): p. 2027-2039.
24. Levett, P.A., et al., *A biomimetic extracellular matrix for cartilage tissue engineering centered on photocurable gelatin, hyaluronic acid and chondroitin sulfate*. Acta Biomater, 2014. **10**(1): p. 214-23.
25. Zhao, X., et al., *Photocrosslinkable Gelatin Hydrogel for Epidermal Tissue Engineering*. Adv Healthc Mater, 2016. **5**(1): p. 108-18.

26. Badylak, S.F., *The extracellular matrix as a biologic scaffold material*. *Biomaterials*, 2007. **28**(25): p. 3587-93.
27. Dawson, J.I. and R.O.C. Oreffo, *Bridging the regeneration gap: Stem cells, biomaterials and clinical translation in bone tissue engineering*. *Archives of Biochemistry and Biophysics*, 2008. **473**(2): p. 124-131.