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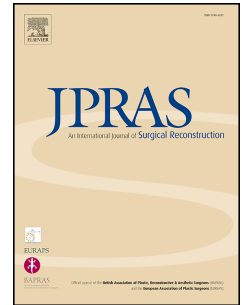
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“3D Bioprinting for Reconstructive Surgery: Principles, Applications and Challenges”

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few reports of successful translation into surgical practice. This review outlines the principles of 3D bioprinting including software and hardware processes, biocompatible technological platforms and suitable bioinks. The advantages of 3D bioprinting over traditional tissue engineering techniques in assembling cells, biomaterials and biomolecules in a spatially controlled manner to reproduce native tissue macro-, micro- and nano-architecture is discussed, together with an overview of current progress in bioprinting tissue types relevant for plastic and reconstructive surgery. If successful, this platform technology has the potential to biomanufacture autologous tissue for reconstruction obviating the need for donor sites or immunosuppression. The biological, technological and regulatory challenges are highlighted, with strategies to overcome these using an integrated approach from the fields of engineering, biomaterial science, cell biology and reconstructive microsurgery.

Keywords: 3D bioprinting, biomaterials, bioinks, biofabrication

century have revolutionized the specialty. Despite the developments in microsurgery and transplantation, we are still confronted with shortcomings relating to the availability and morbidity of donor sites. Three-dimensional (3D) biomanufacture of tissue would remove the morbidity associated with the use of autologous tissue or long-term immunosuppression. The surgical community worldwide is becoming increasingly aware of this research landscape and The American Society of Plastic surgeons recently highlighted the importance of translating bench research in tissue engineering into clinical practice¹. As a clinical specialty, plastic surgeons are well placed to be leaders in the developing field of 3D biomanufacture. A growing cohort of research active plastic surgeons, skilled in vascularisation, tissue viability/transfer and the manipulation of cells, will be well placed in the future to transplant tissue engineered constructs to treat a broad range of reconstructive challenges².

Traditional 3D printing

3D printing, also known as “rapid prototyping”, “solid free form fabrication”, “additive manufacturing” and “layered manufacturing” was first described by Charles Hull in 1986³, using imaging data to design and print 3D objects layer by layer. This technique allows mass customization, which undermines economies of scale and is predicted to revolutionize every sector of society⁴. This technology has already begun to influence the field of plastic and reconstructive surgery; from creating 3D models for training and surgical planning to manufacture of implants and personalized prostheses⁵.

printing, has resulted in the birth of the new bioprinting research field⁶. The global 3D bioprinting market was estimated to be \$487 million in 2014 and this is predicted to reach \$1.82 billion by 2022⁷. A bioprinter is used to dispense 'bioinks', consisting of cells, scaffolds and biomolecules, in a spatially controlled manner rather than traditional tissue-engineering methods of assembly, consisting of non-specific cell seeding of scaffolds⁸. 3D bioprinting, by controlling the nano-, micro- and macrostructure, may replicate complex native-like tissue architecture more faithfully in the laboratory⁹. This would allow biomanufacture of physiologically relevant multicellular tissue constructs on demand with direct translational potential obviating the need for autologous tissue harvest¹⁰ which could transform reconstructive surgery¹¹.

The success of this platform technology ultimately depends on not only on the process itself, but answers to the fundamental scientific questions regarding the correct blend of cell source, suitable scaffold and ideal microenvironment². Given the future potential and synergistic goals of bioprinting and reconstructive surgery in restoring 'form and function'¹², we propose that plastic surgeons should be well versed in the principles and intimately involved in the future developments of 3D bioprinting to ensure it maintains clinical applicability.

A literature review of publications in English was performed on Pubmed, Medline and Embase using the terms (3D printing OR bioprinting OR additive manufacturing OR biomanufacture) AND (reconstructive surgery OR surgery OR tissue biomanufacture). Publications from January 1970 to December 2016 and in English were included in the review. In the first screening, the abstracts from the literature search were scrutinized against the inclusion and exclusion criteria. Only those articles relating to 3D bioprinting of tissues directly relevant to plastic and reconstructive surgery were included (bone, cartilage, muscle, nerve, fat, and skin) and those relating to solid organ bioprinting were excluded. Articles relating to traditional 3D printing of non-organic material were also excluded. The articles whose abstracts fulfilled the inclusion criteria were then retrieved and the references in the relevant articles were also screened.

Data Extraction and Analysis

We assessed the types of 3D printing technologies that are suitable for printing biological materials (**Table 1**) as well as the principles for using 3D bioprinting over more traditional tissue assembly techniques (**Table 2**). Of the 50 publications of relevant bioprinted tissue we identified the cell type, biomaterial and bioprinting technique used, whether the studies were in vivo or in vitro as well as final outcome.

3D bioprinting is an automated, computer-aided deposition of cells, biomaterials and biomolecules⁶ which has been made possible by recent advances in engineering, material science, computer science and cell biology. Printer hardware is controlled by software to precisely deposit biological materials in a layer by layer fashion (**Figure 1**). In its simplest form, a 3D bioprinter uses a syringe to deposit biomaterial and cells (between 2 and 20 million cells per mL) in the correct xyz coordinates by computer controlled stepper motors to create the structure required (**Figure 2**). As the material is deposited, the speed and volume can be altered to ensure that object resolution is maintained. Bioprinted structures are then cultured in a bioreactor under specific conditions in order to produce physiologically relevant engineered tissue.

Traditional 3D printing is relatively simple and can be done from a home computer with the correct software. Digital image data can be acquired from pre-existing templates or designed manually using open source Autodesk Inventor software (**Figure 3**). For more complex shapes or custom made implants 3D digital data is acquired from computed tomography (CT), magnetic resonance imaging (MRI) or laser scanning (**Figure 1**). This volumetric data can be manipulated by computer-aided design, computer-aided manufacturing (CAD-CAM), Mimic or open source Tinkercard software and then converted into standard tessellation language (STL)¹³. The STL file must be further processed by “slicer” software (e.g. Cura, Slic3r) that converts the model into layers and produces a G-code file. Following these G-code instructions, various commercially available, numerically controlled printers e.g. Ultimaker 2, lay down specific volumes of material required in

investigating. In our laboratory, we use extrusion-based bioprinters (**Figure 2**), custom designed and manufactured by our in-house engineering team running Cura software but other commercially available 3D bioprinters such as The 3D Bioplotter (EnvisionTEC, Gladbeck, Germany) and NovoGen MMX (Organovo and Invetech) also exist.

Bioprinting technologies

3D bioprinting technologies are classified depending on whether they use direct-write i.e. biomaterials are transferred by direct contact (e.g. extrusion based, laser guided direct writing, dip pen nanolithography), or resist-write technology i.e. where the structure is formed remotely by selective polymerization which traps the desired biomaterials (e.g. ink jet based, stereolithography).

Currently there are five main 3D bioprinting techniques and the advantages and disadvantages are summarized in **Table 1**;

- 1) Stereolithography^{14,15}
- 2) Extrusion based^{16,17}
- 3) Laser assisted^{18,19}
- 4) Ink jet based^{20,21}
- 5) Nanobioprinting^{22,23}

1) Stereolithography, which uses a laser beam to polymerise photocurable resin layer-by-layer, is regarded as the first 3D printing technique¹⁴. It was initially developed to create

and indirect moulds²⁴. Continuing improvements in biocompatibility and biodegradation of resins as well as cell encapsulation during processing makes stereolithography a promising bioprinting technology of the future^{25,26}.

2) Extrusion based bioprinting (e.g. bioplotting or fused deposition modelling) involves dispensing viscous bioink (biomaterials, biomolecules, cells) through a nozzle or syringe^{16,17,27}. After printing the constructs can be solidified (i.e. gelled) either physically or chemically layer-by-layer which makes this technique slower than others e.g. laser assisted or ink jet based^{17,27,28}. Despite relatively high shear and extensional forces or higher temperatures, cell viability in the tissue constructs is reported to be as high as 90%²⁹. One of the main drawbacks of extrusion based bioprinting is its reliance on optimal material viscosity, which when not achieved, can lead to leaks and affect resolution of the final tissue construct.

3) Laser assisted bioprinting involves either trapping and depositing cells in a laser beam (i.e. laser-guided direct writing¹⁹) or inducing the transfer of material from a source film by a pulsed laser onto a nearby receptor substrate in the form of a microdroplet (i.e. laser-induced forward transfer³⁰). Laser assisted bioprinting is nozzle-free and therefore compatible with a wide range of biomaterial viscosities and avoids the clogging problems of extrusion based techniques^{31,32}. Despite suggestions of lower cell viability than other techniques, laser assisted bioprinting has been shown to print mammalian cells without affecting function or causing DNA damage^{33,34}.

aggregation²¹ leading to low cell density within the tissue construct³⁶. The ability of ink-jet printing to combine multiple cell types, its high resolution and ongoing research into increasing cell concentrations make this a promising technology for complex tissue printing³⁷.

5) The emerging technology of nanobioprinting uses either nanoscale surface modifications of scaffolds to increase cell-matrix interactions or incorporates nanoparticles into bioinks e.g. superparamagnetic iron oxide to non-invasively manipulate and track cells within tissue engineered structures e.g. using an external magnet^{22,23}.

The selection of 3D bioprinting technique depends on the size of the tissue construct, sensitivity of cell placement, biomaterials and biomolecules as well as the desired resolution of print. The first commercial 3D bioprinter and market leader is the Organovo Novogen MMX BioprinterTM, which is an extrusion-based technique that uses cellular spheroids as tissue building blocks (ivetech website). While each of the 3D bioprinting techniques demonstrates specific properties such as high resolution, low price, high safety profile and high throughput, it is clear that achieving more advanced applications in tissue engineering will require a combination of these processes. Our group routinely uses extrusion based 3D bioprinting due to a combination of in house expertise and familiarity with the process.

The bioprinting field spans from promoting endogenous self-repair to creating biomimetic tissues for use in reconstructive surgery³⁸. The traditional approach to tissue assembly has been to seed solid but porous scaffolds with a cell suspension (**Table 2**), with the porosity often achieved by particulate leaching or electrospinning^{39,40}. This results in variable control over pore size, shape and interconnectivity, which are key factors influencing cell migration and proliferation⁴¹. Other methods of tissue assembly have included layering or rolling^{42,43}, cell encapsulation within hydrogels⁴⁴ and scaffold free cell patterning for tissue self-assembly^{45,46} (**Table 2**). The major limitations to all these approaches are a lack of control over cell-to-cell contact and microarchitecture, which are the key determinants of cell function⁴⁷ as well as supply of nutrients and removal of waste products due to a lack of vascularity, restricting the final size of the constructs^{48,49}.

3D bioprinting offers the potential of biofabricating biological structures with a prescribed macro- (overall patient-specific shape), micro- (composition and arrangement of extracellular matrix affecting pore size and shape) and nanostructure (nanotopography and biomolecule attachments for optimal cellular interaction) to more closely replicate native tissue anisotropy^{6,10} (**Figure 4**). 3D microenvironments which provide optimal cell-cell and cell-matrix interactions are critical for cell adhesion, proliferation, and differentiation and hence tissue regeneration⁵⁰ (**Figure 4**). The potential benefits of bioprinting over other types of tissue assembly include repeatability, customization (personalised medicine), vascularization, high-resolution manufacture, automation and

Bioprinting may be used to pattern cells, scaffolds and biomolecules but there are different approaches on how to form the final tissue construct¹¹. The tissue micro and macroarchitecture is either matched completely to the functional native tissue from the start, known as biomimicry^{12,53} or the patterning of cells and biomolecules on supporting bioactive structures is used to drive the cells towards tissue formation based on embryonic tissue self-assembly⁵⁴. A third approach is to use biopatterning to reproduce the smallest structural and functional component i.e. mini-tissue building blocks with larger constructs assembled by either biomimicry or self-assembly^{52,55} (**Table 3**). These strategies are currently being used singly or in combination to bioprint a variety of tissue types for potential future use in reconstructive surgery (**Table 4**).

Most research has focused on bioprinting bone using three main techniques (ink jet based, stereolithography and fused deposition modelling), often using biomaterials alone without cells^{56,57}. Choosing medical grade materials allows swift clinical translation to demonstrate integration and bony consolidation, bypassing the need to extensive in vitro and animal work⁵⁸, but can limit development of the field by excluding favourable candidates which are not yet medical grade. Other tissue types in the early stages of research include cartilage^{59,60,61}, muscle^{62,63}, nerve^{21,64}, fat^{34,65} and skin^{66,67} (**Table 4**). The panacea would be to one day bioprint composite vascularized flaps with their own internal microvasculature that could be directly anastomosed to the recipient's blood vessels using microsurgical techniques, obviating the need for donor sites (**Figure 5**). It is perhaps this

Challenges

Biological

Simply depositing cells, scaffolds and biomolecules in a spatially controlled manner is not sufficient to create durable native-like tissue for transplantation. The transition of mechanically weak 3D bioprinted neo-tissue constructs to native-like surgically relevant tissue is a vital step in the post-printing process and is the main factor limiting successful clinical translation^{52,68}. This transition into functional tissue can either be undertaken *in vitro* during several months of bioreactor-based culture using a variety of physiological conditions and growth factor combinations⁶⁹ or *in vivo* through implantation of the construct allowing in situ growth that supersedes the natural tendency for degradation⁷⁰. Identification of optimal “maturogenic factors” for different tissue types will be pivotal in driving progress in this field⁶⁸ (**Figure 6**).

One of the biggest challenges to the size and complexity of tissue engineered constructs is believed to be due to a lack of vasculature, relying instead on the porous structure of scaffolds to allow flow of nutrients and waste until extrinsic neovascularization develops from the host⁷¹. Preliminary evidence has shown that increasing pore size or adding angiogenic growth factors e.g. VEGF promotes natural angiogenesis and inosculation but this is too slow to allow any meaningful increase in the size and complexity of the tissue construct⁴⁶. Although there has been some progress in bioprinting isolated cell lined

Printing complex composite tissue has other unique challenges, such as long biomanufacture times with resulting reduction in cell viability⁷², cellular dedifferentiation with loss of regenerative potential⁷³, release of acidic by-products from degradation of biomaterials⁷⁴ as well as non-homogenous matrix synthesis, lacking post-printing tissue remodelling and hence poor long-term maintenance of mechanical strength⁷⁵.

Technological

Ensuring high resolution or 'fidelity' of bioprinted constructs and finding the optimal printable support material remain the major hurdles for printing complex biological structures⁷⁶. Higher resolutions not only allow better replication of native architecture but can also control pore size and interconnectivity which is important when considering that diffusion distances of over 400-500µm may limit oxygen transport and hence cell viability⁹. Currently stereolithography and ink jet based techniques provide some of the best available resolution but are limited by the lack of appropriate biomaterials, lower cell viabilities and poor mechanical strength. Laser-assisted bioprinters are able to print at a microscale resolution but preparation of individual ribbons for deposition can be time consuming and not cost effective³⁰. Mathematical modelling may help with increasing resolution whilst optimising the printability of tissue constructs by adjusting for a variety of variables simultaneously e.g. cell seeding density, porosity⁵⁴. Automated robotic systems may provide cost-effective and potentially scalable means of automating the bioprinting process for commercial biofabrication⁷⁷.

respect to controlling growth potential and practicalities such as availability of stem cell banks, upscaling, sterility and storage of tissue engineered constructs².

Biomanufacture, as with other tissue engineered products, will need to comply with current Good Manufacturing Practice regulations and gain The Food and Drug Administration (FDA) or European Medicine Agency (EMA) approval⁷⁸. Bioprinted constructs that contain cells are classified as combination products by the FDA and advanced therapy medicinal products by the EMA and require rigorous clinical trials testing prior to approval for routine use^{78,79}. The main hurdle will be to standardize, validate and continuously monitor 3D bioprinting manufacture from the design to production stage, which is extremely difficult for a customizable and hence intrinsically variable process⁴¹.

Conclusion

The 3D bioprinting field is a rapidly expanding area of worldwide research intimately linked with tissue engineering. Successful clinical translation would have a significant impact on the morbidity and mortality of patients and the healthcare economy. The ultimate success of this platform technology depends on answers to the fundamental scientific questions regarding the correct blend of cell source, suitable printable scaffold and ideal microenvironment to mimic native tissue anisotropy; if in fact these can be answered at all. 3D bioprinting is still in its infancy, highlighted by the fact that most current studies have been in vitro proof of concept only, with no widely available 3D bioprinted tissues on the market. Given the future potential and synergistic goals of bioprinting and reconstructive surgery in restoring 'form and function', we propose that plastic surgeons should be well

challenges to ensure successful clinical translation will only be possible via an integrated approach with a combination of technologies from the fields of engineering, biomaterial science, cell biology and reconstructive microsurgery.

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scanning). 3. Image post-processing (3D model converted to STL; standard tessellation language). 4. 3D bioprinting (e.g. bioplotting or nozzle-based).

Figure 2. 3D Bioprinter Hardware; a) Swansea University experimental 3Dynamic Workstation Omega dual extrusion 3D bioprinter (extrusion based), b) 3D bioprinting of geometrical shapes, and c) auricular cartilage containing human nasoseptal chondrocytes.

Figure 3. 3D Bioprinter Software; a) Autodesk Inventor software to design 3D model based on patient photographs, b) STL file of auricular cartilage on Cura software, which is used to create G-code instructions for the 3D printer.

Figure 4. The advantages of 3D bioprinting of scaffold, cellular and biomolecule components for tissue engineering.

Figure 5. Progress towards panacea of bioprinting composite pre-vascularized flaps for reconstructive microsurgical implantation.

Figure 6. Current technological, biological and regulatory challenges in 3D bioprinting research

drawbacks.

3D bioprinter	Summary of technique	Advantages	Disadvantages	References
Stereolithography e.g. Photosolidification, resin printing	Polymerisation of photocurable resin layer by layer	<ul style="list-style-type: none"> - Extremely high resolution with ability to create complex shapes and microarchitecture 	<ul style="list-style-type: none"> - Few suitable biomaterials are stable during polymerisation - Significant non-degradable components - Poor mechanical properties 	3,14,15
Laser assisted e.g. Laser guided direct writing, laser induced forward transfer	Deposition of cells either in a laser beam or using pulsed laser for transfer	<ul style="list-style-type: none"> - High resolution - Avoids problems of clogging - Compatible with wide range of biomaterial viscosities - High cell density - Mesenchymal stem cells retain trilineage potential - Medium-fast 	<ul style="list-style-type: none"> - Lower cell viability - Limited construct size - Not cost effective 	3,18,19,30,31,32,
Extrusion based e.g. Bioplotting, fused deposition modelling, pneumatic vs. biomechanical	Viscous liquid or molten material extruded through nozzle or syringe as a continuous strand of individual dots	<ul style="list-style-type: none"> - Deposit clusters of cells - Scaffolds for soft tissue engineering - Possible to control pore size, morphology and interconnectivity - Material flexibility 	<ul style="list-style-type: none"> - Material viscosity and potential for leaks can affect resolution - Limitation on complexity of shapes with overhang - Problems with cell viability with sheer or high temperature - Limited mechanical stiffness - Slow 	16,17,27
Ink jet based e.g. thermal 3D ink jet bioprinting, piezoelectric dispensing	Photopolymer-based bio-ink is jetted by an inkjet and cured with UV light	<ul style="list-style-type: none"> - High resolution - Multi – “colour” printing, each ink (cellular, ECM, biomolecule) positioned in precise location i.e. introduce gradients - Complex scaffolds with microstructure control (internal channels/overhangs) - Fast 	<ul style="list-style-type: none"> - Potential for cell death at higher temperatures and pressures - Smaller nozzles prone to clogging - Organic solvents as binders can dissolve polymers - Limited available pore sizes 	20,35

aphy	relying on capillary action or electrostatic interactions	adhesion, proliferation, and differentiation) - Nanobioprinting can manipulate and track bioactive factors and cells within tissue engineered constructs	nanoparticle effect on cell behaviour	22,23
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technique				
Cell seeding i.e. top-down approach	Cell seeding of scaffolds (synthetic, natural or decellularised), followed by maturation in bioreactor	<ul style="list-style-type: none"> • Feasible for thin or avascular tissues e.g. skin, cartilage • Control macrostructure 	<ul style="list-style-type: none"> • Lack of precision over cell placement • Limited vasculogenesis • Problems with mass transfer (i.e. nutrients and waste products) 	47
Self-assembly i.e. bottom-up approach	Pattern cells to promote cellular self-sorting, self-assembly and ECM production i.e. mimic postnatal tissue development	<ul style="list-style-type: none"> • Control microstructure • Cell driven process 	<ul style="list-style-type: none"> • In depth understanding of embryological tissue/organ development • Size of construct dependent on angiogenesis • Slow to scale up 	45,46
Layering/rolling	Cell sheets stacked to form a thicker tissue or rolled to form hollow tubes	<ul style="list-style-type: none"> • Pattern multiple cell types 	<ul style="list-style-type: none"> • Cohesiveness depends on ECM production by cells 	42,43
Cell-laden hydrogels/microgels	Hydrogels used as artificial ECM to encapsulate cells	<ul style="list-style-type: none"> • Provide scaffold for cell proliferation, differentiation and ECM production 	<ul style="list-style-type: none"> • Size constructs limited by mass transfer of metabolites 	44
Electrical cell guiding	Electromagnetic forces to pattern cells	<ul style="list-style-type: none"> • Position with single-cell resolution 	<ul style="list-style-type: none"> • Lack control over ECM deposition 	19
3D Bioprinting	Cells, scaffold and biomolecules deposited in a precise 3D structure	<ul style="list-style-type: none"> • Simultaneous control of micro and macrostructure • Micro- and nano-structuring topography and biomolecules can influence cell differentiation • Potential to create complex tissue and vascularity • Automated high resolution manufacture with scalability 	<ul style="list-style-type: none"> • In depth understanding of native microarchitecture required • Need biocompatible bioinks 	51,52

Biomimcry	Identical reproduction of tissue microarchitecture	<ul style="list-style-type: none"> • Cells, ECM, biomolecules • High printing resolution • In depth understanding of microenvironment 	11,12,53
Autonomous self-assembly	Based on embryonic tissue development, cell as the primary driver of tissue formation	<ul style="list-style-type: none"> • Cells, biomolecules • Knowledge of embryonic tissue development 	54,55
Mini-tissues	Reproduction of the smallest structural and functional component, larger construct assembly by biomimicry or self-assembly	<ul style="list-style-type: none"> • High printing resolution of mini-tissues • Methods of macro assembly 	52,55

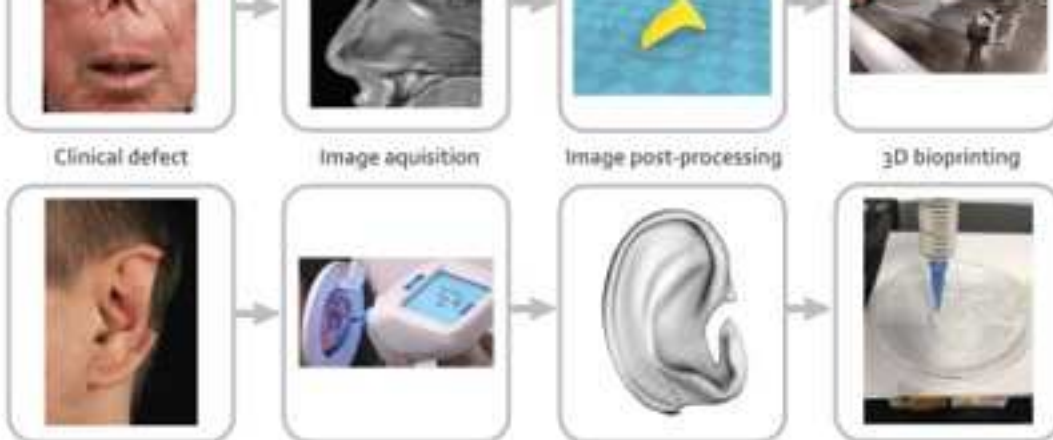
neurotrophic factor; VEGF, vascular endothelial growth factor; BMP-2, bone morphogenic protein 2; TGFb1, transforming growth factor beta 1; FGF2, fibroblast growth factor 2.

Tissue Type	Cell Type	Biomaterial +/- Biomolecules	Bioprinting Technique	Outcome	In vivo/ vitro	Ref
CARTILAGE						
Articular	Chondrocytes (bovine)	Calcium polyphosphate	Ink jet based	Good compressive strength and supported cartilage formation	In vitro	59
Articular	Chondrocytes (bovine)	Poly (trimethylene carbonate)	Stereolithography	Constructs supported differentiated chondrocyte phenotype	In vitro	80
Articular	Chondrocytes (porcine)	PLGA and type II collagen	Extrusion based (fused deposition modelling)	Good chondrocyte distribution and neocartilage formation	In vitro	60
Articular	Mesenchymal stem cells (human)	Scaffold free	Laser assisted (laser induced forward transfer)	MSC viability and bone and cartilage differentiation	In vitro	63
Articular	Chondrocytes (human)	PEG dimethacrylate	Ink jet based	Osteochondral plugs with good compressive strength	In vitro	81
Fibro-cartilage	Mesenchymal stem cells (human)	Methacrylated gelatin BMP2 and TGFb1	Extrusion based (nozzle extrusion of micro droplets)	Anisotropic fibrocartilage, chondrogenic and osteogenic differentiation	In vitro	82
BONE						
Calvarial	None	Monolithic monetite	Ink jet based	Good integration between the implant and the calvarial bone	In vivo (rabbit)	56
Calvarial	None	PCL/PLGA bounded with TCP	Extrusion based (fused deposition modelling)	Good bone formation	In vivo (rabbit)	57
Calvarial	C2C12 progenitor cells (mouse)	DermaMatrix BMP2 with noggin	Ink jet based	Spatial control of bone formation	In vitro and in vivo (mouse)	83
Calvarial	None	PCL-TCP biodegradable scaffold	Extrusion based (fused deposition modelling)	After 6 months the implant was well integrated with bone consolidation on CT	In vivo (human)	58
Craniofacial / Bone spacers	None	Polymethylmethacrylate filament	Extrusion based (fused deposition modelling)	Feasibility of biofabrication of customised 3D structures with variable porosity	In vitro	84
Tibial and Maxillary	C2C12 pre-myoblastic cell line	Ceramic BMP2	Ink jet based	Customisable, biocompatible, osteoinductive scaffold	In vitro and in vivo (rabbit and porcine)	85

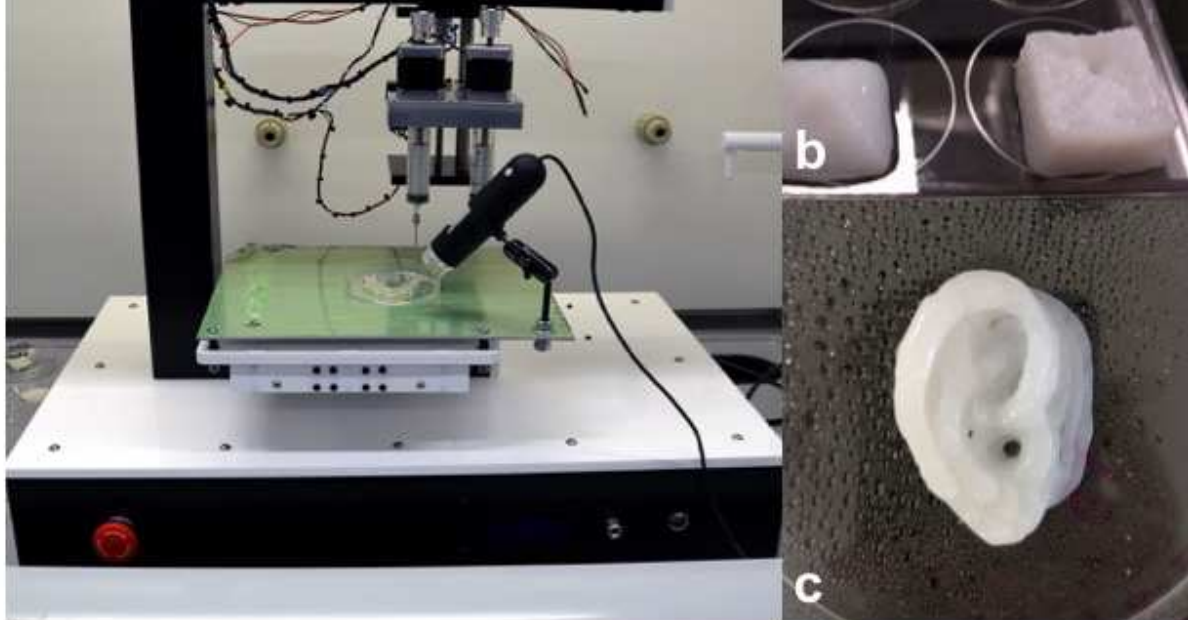
Non-specific	Osteosarcoma MG63 cells (human)	Electrospun PCL as substrate	Laser assisted	Supported cell survival and enhanced cell proliferation	In vitro and in vivo (mouse)	88
Non-specific	None	Calcium phosphate granules	Stereolithography	Mechanical properties can be adjusted via inner channel structure and hydroxyapatite and tri-calcium phosphate ratios	In vitro	89
Non-specific	None	HA and TCP	Stereolithography	Good biocompatibility and osteinductivity	In vivo (rat)	90
Non-specific	None	Polylactic acid and a bioactive CaP glass	Extrusion based	Compression strength is dependent on scaffold geometry and the presence of glass	In vitro	91
Non-specific	Osteoblasts (human)	PLGA	Stereolithography	Compatible with osteoblast proliferation, mechanically similar to trabecular bone	In vitro	92
Non-specific	Mesenchymal stem cells (goat)	BMP2 loaded gelatin microparticles	Extrusion based (pneumatic)	Osteogenic differentiation and bone formations, increased by slow release of BMP2	In vitro and in vivo (mouse and rat)	93
Non-specific	Mesenchymal stem cells (human)	Acrylated PEG hydrogel with bioactive glass and HA	Inkjet based	Osteogenic and chondrogenic differentiation with minimal printhead clogging	In vitro	94
FAT						
	Adipose derived stem cells (human)	Alginate hydrogel spheroids	Extrusion based	Uniform dimensions of bioprinted spheroids	In vitro	65
	Adipose derived stem cells and endothelial colony- forming cells (human)	Fibrinogen and hyaluronic acid solution	Laser assisted (laser induced forward transfer)	3D cell arrays generated as proof of concept, resulting in formation of vascular-like network	In vitro	34
NERVE						
	Primary embryonic hippocampal and cortical neurons (rat)	Fibrin gels	Inkjet based	Healthy neuronal phenotypes and electrophysiological characteristics post-printing	In vitro	21
	Neural stem cell (rat)	Poly-acrylamide-based hydrogel with FGF2 or CNTF	Inkjet based	Printed gradient of increasing levels of CNTF showed a linear increase astrocytic differentiation	In vitro	64

	Neural stem cell (murine)	Collagen and VEGF- releasing fibrin gel scaffolds	Extrusion based (pneumatic)	Sustained release of VEGF promoted cell migration	In vitro	97
VASCULATURE						
	Microvascular endothelial cells (human)	Fibrin	Inkjet based (thermal)	Cells aligned themselves inside the channels and proliferated to form microvasculature channels	In vitro	36
	Mesenchymal stem cells and umbilical vein endothelial cells (human)	Polyester urethane urea support	Laser assisted (laser induced forward transfer)	Enhanced capillary density and integration of human cells into murine vasculature	In vitro and in vivo (rat)	98
	Umbilical vein smooth muscle cells and fibroblasts (human)	Agarose rods supports	Extrusion based	Engineered tubes of multiple layers and complex branching geometry	In vitro	46
	Umbilical vein endothelial cells and umbilical vein smooth muscle cells (human)	Matrigel support	Laser assisted	Self-assembling lumen networks	In vitro	99
	Umbilical vein endothelial cells and fibroblasts (human)	Aqueous plutonic F127 support and gelatin methacrylate cell carrier	Extrusion based (nozzle)	Fesibility of multinozzle printing, scalable with multiple cell types	In vitro	100
	Mesenchymal stem cells (human)	Alginate, gelatin and hydroxyapatite hydrogel	Extrusion based (nozzle)	Hollow channels generated, orientation influenced size and shape of channels	In vitro	101
	Umbilical vein endothelial cells (human)	Carbohydrate glass support	Extrusion based (nozzle)	Creation of rigid filament networks	In vitro	102
	Hepatoma cells and intestinal epithelial (human), fibroblasts (murine)	Hyaluronic acid metacrylate and gelatin ethanalamide methacrylate	Extrusion based (nozzle)	Viable cells that remodelled vessel to a naturally secreted extracellular matrix	In vitro and In vivo (mouse)	103
	Umbilical vein smooth muscle cells (human)	Sodium alginate	Extrusion based (nozzle)	Good proliferation and deposition of smooth muscle matrix and collagen	In vitro	104

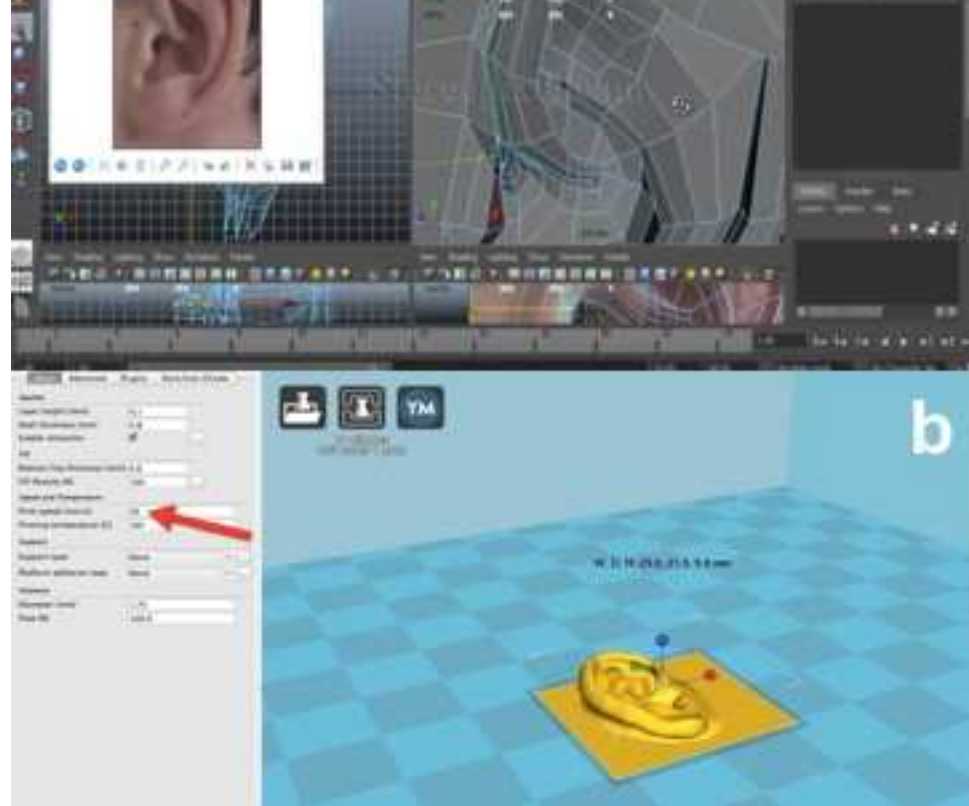
	Keratinocytes and fibroblasts (human)	Type I collagen hydrogel	Extrusion based (pneumatic)	Dermal and epidermal layers, >95% cell viability	In vitro	66,67
	Keratinocytes and fibroblasts (human)	Fibrin and type I collagen hydrogel	Ink jet based (in situ)	Improved healing and less contracture than controls	In vivo (mouse)	107
	Amniotic fluid derived and mesenchymal stem cells	Fibrin-collagen gel	Ink jet based (pneumatic)	Improved wound healing with bioprinted amniotic derived stem cells	In vivo (mouse)	108
	Fibroblasts and keratinocytes (human)	Matrigel support	Laser assisted	Multilayered epidermis with vascularisation from wound bed	In vitro and vivo (mouse)	109
	Fibroblasts, keratinocytes, mesenchymal stem cells (human)	Collagen hydrogels	Laser assisted (laser induced forward transfer)	Multilayered skin, cellular DNA, differentiation and proliferation not affected	In vitro and in vivo (mouse)	110,111
MUSCLE						
	Muscle derived stem cells (murine)	Fibrin scaffolds, BMP2	Inkjet based	Formation of myotubes, BMP2 controlled myogenic and osteogenic micro-patterning	In vitro	62
	Myoblasts and fibroblasts (human)	PU (with myoplasts) or PCL (with fibroblasts)	Extrusion based	High cell viability, differentiation and initial developments of muscle tendon unit	In vitro	63
	Smooth muscle cells (rat)	Collagen	Extrusion based	Long term cell viability in culture	In vitro	112
	Myoblasts (murine)	Alginate and gelatin	Extrusion based	Mechanical properties altered by structure design and culture	In vitro	113

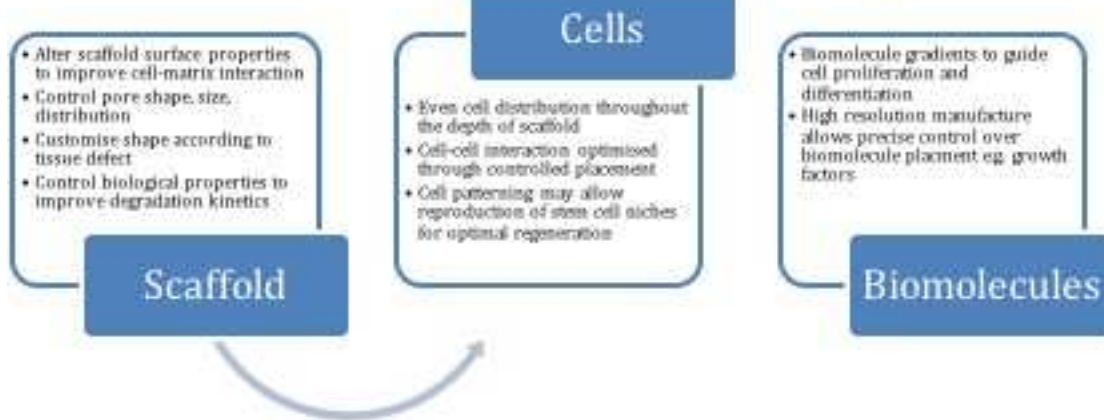


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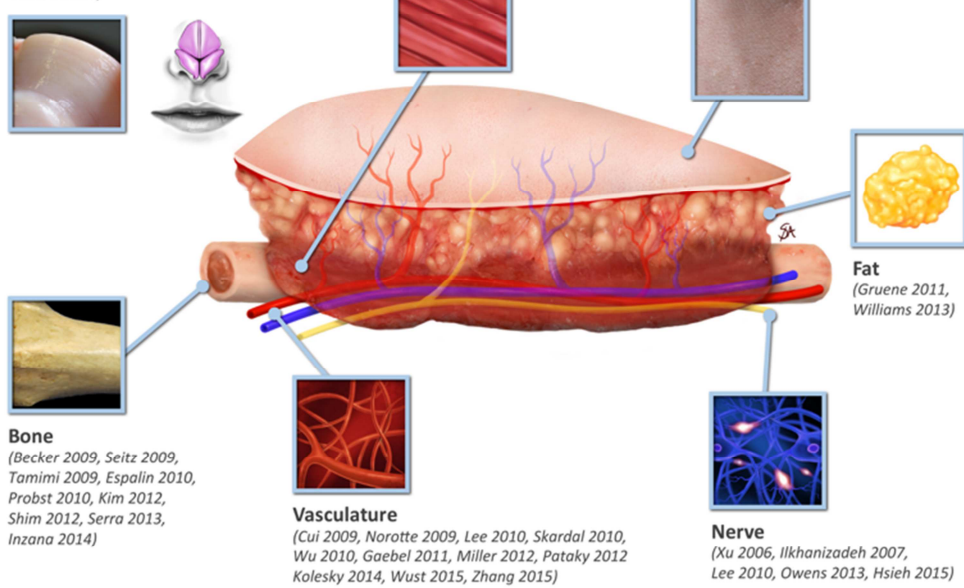


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