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3D Printing for the Clinic: Examining Contemporary Polymeric Biomaterials and their Clinical Utility

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Abstract: The advent of additive manufacturing offered the potential to revolutionize clinical medicine, particularly with patient specific implants across a range of tissue types. However, to date, there are very few examples of polymers being used for additive processes in clinical settings. The state of the art with regards to 3D printable polymeric materials being exploited to produce novel clinically relevant implants is discussed here. We focus on the recent advances in the development of implantable, polymeric medical devices and tissue scaffolds, without diverging extensively into bioprinting. By introducing the major 3D printing techniques along with current advancements in biomaterials, we hope to provide insight into how these fields may continue to advance while simultaneously reviewing the ongoing work in the field.

Keywords: Additive manufacturing, biomaterials, degradable polymers, 3D printing, clinic.

Overview

3D printing, additive manufacturing (AM), or rapid prototyping have gained significant attention in both industry and academic applications, such as automotive parts, consumer electronics, bio(medical) devices/therapies, and even food. In the biomedical space, 3D printing offers clinicians the opportunity to rapidly design and produce patient-specific devices, therapies, and even organs. Even more importantly, AM can be used to produce patient-specific or personalized implants fitted exactly to their physiology. Despite this major motivation for examining clinical applications of 3D printing, there is still a distinct lack of such technology anywhere near patients or clinics, particularly for polymeric materials. The reason for this is simple: the materials that are necessary for producing polymeric implants using 3D printing (which would include properties such as tissue-material matched thermomechanical properties, biocompatibility, degradability/resorbability, tissue-conductivity and guidance, *etc.*) have not been sufficiently developed and understood to be translated into the clinical setting. To continue on the route towards the development of 3D printing in the clinic, a better understanding of polymeric materials is crucial. Here, methods of 3D printing polymeric biomaterials are briefly introduced with regards to the major processing the material undergoes and how it pertains to recent advances specific to cardiovascular, orthopedic, neural, and similar applications that require tissue engineering scaffolds. These advances are critically reviewed with regard to their clinical potential as it relates to the material properties and biological behaviors. The materials' routes to the clinic are also discussed.

Overview of 3D Printing Techniques

Fused Filament Fabrication (FFF)

Fused filament fabrication (FFF, also known as fused filament deposition [FFD] or fused deposition modeling [FDM]) is perhaps the most common 3D printing method. Typically, a print head extrudes a partially melted filament of thermoplastic polymer onto the substrate or part. The movement of the print head defines the part geometry; parts are produced in layers of partially integrated filament, with each

layer completed prior to a change in the z-position.[1, 2] This allows for several advantages relative to other printing methods, namely that no solvents or curing agents are required to prototype a final part. However, , FFF parts typically possess limited quality final products with partially integrated layers that result in poor mechanical stability. Moreover, the final device may require long print times in solid parts or suffer from poorly filled solid sections during faster productions, as well as limited resolution which is dependent upon the extrusion dye on the print head (resolutions typically achieved between 100-200 μm).[3] Additionally, large print volumes typically require support material, particularly for parts with irregular or over-hanging geometries. While the support material may be an advantage for building complex features, its removal may be difficult, and ultimately the use of filament limits the utility of the final parts outside of prototyping in many cases.[2, 4] Finally, while no additives are required for FFF printing, most polymer filaments include plasticizers which may leach from the material. It should be noted that despite the limitations of this method, FFF has been the foundation for a host of other polymeric 3D printing techniques including bio-printing (not covered in great detail here), direct ink writing (DIW), and multimaterial printing. This means that FFF can be used to process ceramics, composites, powders (polymeric and otherwise), metals, and combinations as composite matrices, thus opening avenues in new material sources, such as recycled or bio-sourced materials, *i.e.* cellulose.[1, 2, 5-9] Moreover, since the spectrum of biofabrication technologies is continuously expanding, FFF continues to progress beyond its primary use of design prototyping towards more mainstream manufacturing, and certain material filaments such as polylactic acid and other polyesters (unspecified by manufacturers) are being labeled as medical grade materials intended for short-term skin contacting applications such as prosthetics, described in greater detail later.

Direct Ink Write

DIW, also sometimes referred to as robocasting, deposits material in a line-drawn layer-by-layer manner, similar to FDM, but utilizes ink rather than solid polymer filament as the material source.[10, 11] The solid final product is typically the result of high viscosity thermoplastics, such as cellulosic materials

and polyethylene glycols, siloxanes or filled polymers, where fillers may be ceramic particles, fibers, or other additives.[12-19] Alternatively, crosslinking reactions taking place in the ink, such as with acrylates or thiol-ene crosslinking, as a result of UV-crosslinking or acid/base catalyzed reactions result in a final, crosslinked structure (supramolecular hydrogen bonds are leveraged in the same manner). The part maintains its shape during printing as a consequence of the rheological properties of the ink while the crosslinking takes place.[20] Ideally, DIW materials are able to maintain their shape provided that no external shear is applied; only during the extrusion process itself is the material able to flow.[11] The major advantage of DIW in polymers relies on the highly precise print features (below 100 μm) that are possible as a result of the use of automated print heads coupled with small diameter nozzles which control material flow, as well as the wide array of compatible materials that can be used, which include thermoplastic polymers and composites, thermosetting materials, hydrogels, graphene, ceramics, and even metals in specialized cases.[21-28] The limitation of this method, therefore, is the viscosity of the material during and after printing.

Selective Laser Melting and Selective Laser Sintering

Laser-based polymerization systems are typically more expensive compared with FFF, but provide substantial improvements regarding resolution and print quality.[16, 29, 30] A range of laser-based systems have been developed for photopolymerizations, as well as powder sintering. Metallic additive manufacturing uses high energy methods for typically subtractive manufacturing, including electron beam-based production of metallic implants, such as personalized orthopedic titanium implants. For polymers, selective laser melting and selective laser sintering (SLM and SLS) are similar methods used to produce structures, with porous materials and other complex parts requiring multiple manipulations of the material during the printing process. Traditionally, this method was utilized for sintering polyamides, such as nylons, where polymer powder in a layer is melted or sintered into the desired shape. Powder is then rolled into another layer for subsequent sintering.

The advantages of SLM/SLS currently cover the following aspects: the range of performance polymers that can be used, which now include poly(aryl ether ketone)s (PAEKs), polystyrenes, elastomers, and semi-crystalline thermoplastics; the speed at which manufacturing can occur; and the complex geometries which may be produced.[31, 32] Interestingly, it is the semi-crystalline nature of the polymer that ensures success in SLM/SLS manufacturing, as amorphous polymers, such as polycarbonates, typically do not process as successfully or yield inferior mechanical properties.[33] Another benefit of this method is based on the fact that performance polymers are first processed in their traditional manner, prior to printing. For instance, PAEKs, which are exceptionally insoluble, only melt at high temperatures and hence, the polymer is powderized to be laser sintered or melted. This is a distinct difference from other methods, where polymers are processed as liquids or must include plasticizers and stabilizers to drive processing. For PAEKs, SLS/SLM is distinctly advantageous for medical applications compared with FFF (the only other technique capable of processing such materials readily), as the resulting scaffolds are slightly porous and, more importantly, do not have structural defects from filament extrusion.[34, 35] However, the obvious disadvantage of using powders is that all printed parts are partially porous at the surface, requiring more extensive post-AM processing in cases where a high degree of surface finish is desired, and resolutions of 50 μm are typically achievable, well above many other printing methods.

Stereolithography, 2-Photon Lithography, and Digital Light Processing

For photopolymers, considered to be the fasting growing field for materials research, stereolithography (SL), digital light processing (DLP) and 2-photon lithography (2PL) are three of the most common methods, although numerous other methods are beginning to make use of UV- or visible-light curing techniques.[2, 36-39] In SL, a photomask is used to define the exposure area for a narrow slice of the material (approaching a 2D section when assuming that the slice thickness approaches a negligible size). The exposed areas are crosslinked in the presence of a photoinitiator, while the areas not exposed remain as a low viscosity resin. After a selected exposure time, the entire printing area is covered in a photomask. A baseplate (picker, plate, *etc.*) is then used to pull the solid component off of the tray-mask interface (or

lowered below the polymer surface in certain setups) before resetting at a slightly increased height in preparation for the next exposure period. In this way, the crosslinked polymer, traditionally an acrylate and/or epoxide containing polymer or oligomer, may be "pulled" out of the liquid resin. SL is dependent upon the spatial resolution of the exposed image (defined by pixels) and, therefore, many resin components must account for viscosity, as well as photoinhibitors for increasing spatial control. Issues with resin mixtures include: overcuring, which may be as a consequence of high viscosity, photopolymerization heating, prolonged exposure times, photoinitiator-diluent compatibility, and mechanical integrity during the printing process.

DLP is very similar to SL, as is the more recently developed continuous liquid interface production (CLIP) method.[40] DLP uses projector screens rather than lasers to photocure polymer resins, where a single image is projected for each slice (SL uses a mobile laser array to produce the features within each layer). As a result of this difference, several effects may be achieved, particularly regarding surface features and resolution and very recently, printed structures with dimensions exceeding 1 m in the print axis with resolutions down to 1 μm for SL, while features of approximately 10 μm are achievable with DLP.[41] In DLP, every feature is based upon pixels and, consequently, the printing resolution is limited by the screen resolution and the pixels every feature is assembled from, which ultimately limits the print resolution for DLP-based processing, where volume is traded for resolution. Liquid crystal display (LCD) based printing is a slight variation on DLP printing but provides essentially the same end result. CLIP differs from other methods with regards to the location of the photopolymerization unit, and the end result of this is that the part may be constantly polymerized and "pulled from the resin," thereby increasing the integration of different layers.[40]

2PL is currently being used to achieve very precise surface features or surface patterning, with resolutions below 80 nm currently being achieved.[42] Unlike other photopolymerizations, 2PL utilizes two-photon photoinitiators, which enhances the precision with which crosslinking may be obtained in 3D space/on 2D surfaces. Typically, 2PL can offer excellent voxel (3D pixel) resolution for printing, but with severe limitations on the printing area/volume. This means that while 2PL may yield very precise

structures, macroscopic structures which might be produced *via* SL (stent, bone plate or screw) are too large for the build volume, particularly in the z-axis; large printed scaffolds are typically a single millimeter in any given dimension or less.[43-45] In terms of voxel control, the use of dual-light behavior with photoinitiators and photoinhibitors has recently been demonstrated for production of inhibition volumes used for localized volume printing in single exposures, on size scales more similar to SL than 2PL.[46]

Inkjet and Polyjet Printing

Polyjet printing relies on very similar technology to traditional, 2D ink jetting printers, and broadly may be thought to include the techniques inkjetting, polyjet, and binder (jet) printing. During an inkjet process, very fine polymer droplets are deposited on a surface to slowly build up structures.[2, 47-49] In certain cases, the polymers are solvated as part of the ink formulation and will rapidly undergo evaporation to leave behind the solid polymer film. Other techniques are beginning to make use of a UV-curing component (often referred to as polyjet photopolymerization [printing] (PPP)) in order to utilize material formulations in which oligomers and crosslinkers are viscous fluids until photocrosslinking occurs.[2, 47, 48] Other methods are beginning to make use of reactive inks that undergo crosslinking or chain extension during or prior to droplet formation, and upon deposition are of sufficient molecular weight or crosslink density that the shear stress required to induce flow is achievable under static conditions; subsequent reactions or post processing can then be used to finalize the part geometry and molecular structure.[50-52] This provides better layer-to-layer integration and reduces the possibility of nozzle clogging because of solvent evaporation.[2] Inkjet printing, while a versatile method, is limited by the time required to produce monolithic structures, as well as difficulties in producing complex structures which do not have supporting design features or additional material. All of this stated, inkjet printing is one of the most promising methods for conductive devices and materials due to the method's versatility and the ease with which additives may be utilized including metals and ceramics may be utilized during the multimaterial printing process.[2, 48, 49, 53]

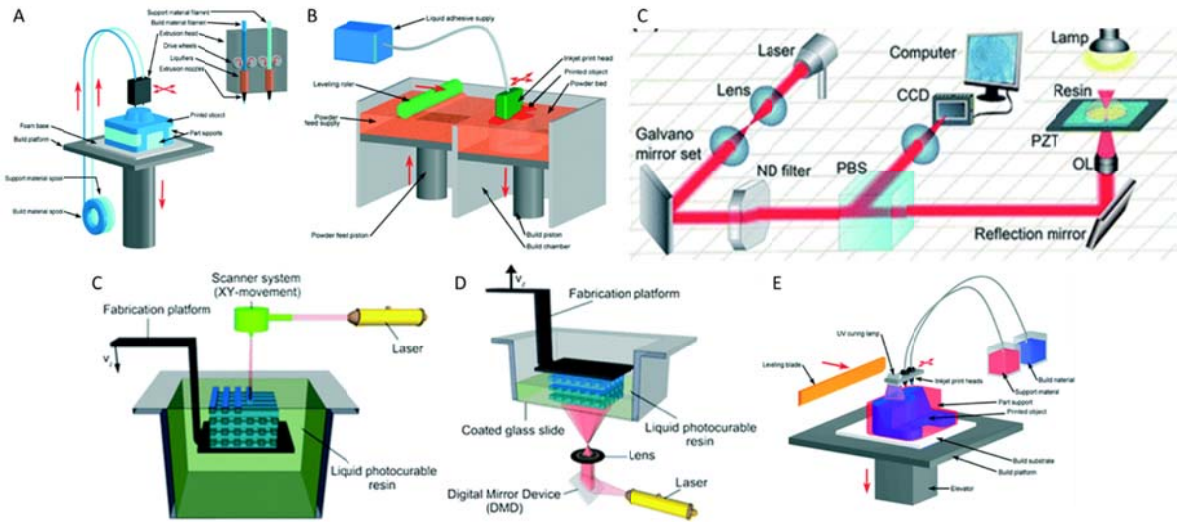

Figure 1. Models of the primary 3D printing techniques, which may vary slightly in practice displaying different orientations or configurations. General diagrams of fused filament fabrication (A), selected laser sintering (B), 2-photon lithography (C), stereolithography (D), digital light processing (E), and inkjet printing (F). Reproduced/Adapted[54] from S. Waheed, J.M. Cabot, N.P. Macdonald, T. Lewis, R.M. Guijt, B. Paull, M.C. Breadmore. 3D printed microfluidic devices: enablers and barriers. *Lab Chip*, 2016, 16, 1993-2013 with permission from The Royal Society of Chemistry.

3D Printing Materials Overview

The majority of 3D printing methods, which display varying processing times and attributes (Table 1 and Figure 1), utilize only a few materials to produce 3D printed devices. Furthermore, different printing methods allow for varying levels of part complexity and size, as well as resolution and final features (Figure 2). Commercial 3D printing materials include polycarbonate (BPA-derived) (PC), polylactic acid (PLA), polycaprolactone (PCL) (typically poly(ε-caprolactone)), polyglycolic acid (PGA), polypropylene (PP), acrylonitrile butadiene styrene (ABS), polystyrene (PS), polyethylene terephthalate (PET),

polyurethane (PU, derived from aromatic diisocyanates), and poly(ethyl ether ketone) (PEEK) or PEAK.[2] These commodity polymers are known for their use in a variety of different applications, although there are a number of limitations in their performance. Typically, the limitation for medical applications is not necessarily a result of the polymers' thermomechanical considerations (Table 2) but rather a suitable body of working supporting biocompatibility. Interestingly, literature abounds with examples and reviews focusing on PLA, PCL, PC, PEEK, PET, Nylon, PS, and PGA being used in medical-related applications; however, few commercial examples of 3D printed implantable medical devices can be found.[55] Focusing on the leachable components briefly, a number of methods have been utilized to produce resins, inks, and processable polymers for various 3D printing techniques. Most of these methods utilize additives to ease the processing requirements or to achieve certain design criteria, such as diluents in resins and inks that must be below certain viscosity thresholds or plasticizers in polymer filament.[2] While these methods are very effective, concerns will arise due to the extractable components and the rate at which they will leach from the material. Photoinitiators based on lithium phenylphosphates, which were demonstrated to be both water soluble and highly biocompatible, have been used along with PEG diacrylates and are currently being examined.[56, 57] Such photoinitiators may be suitable for implantable biomaterials because of their cytocompatibility.

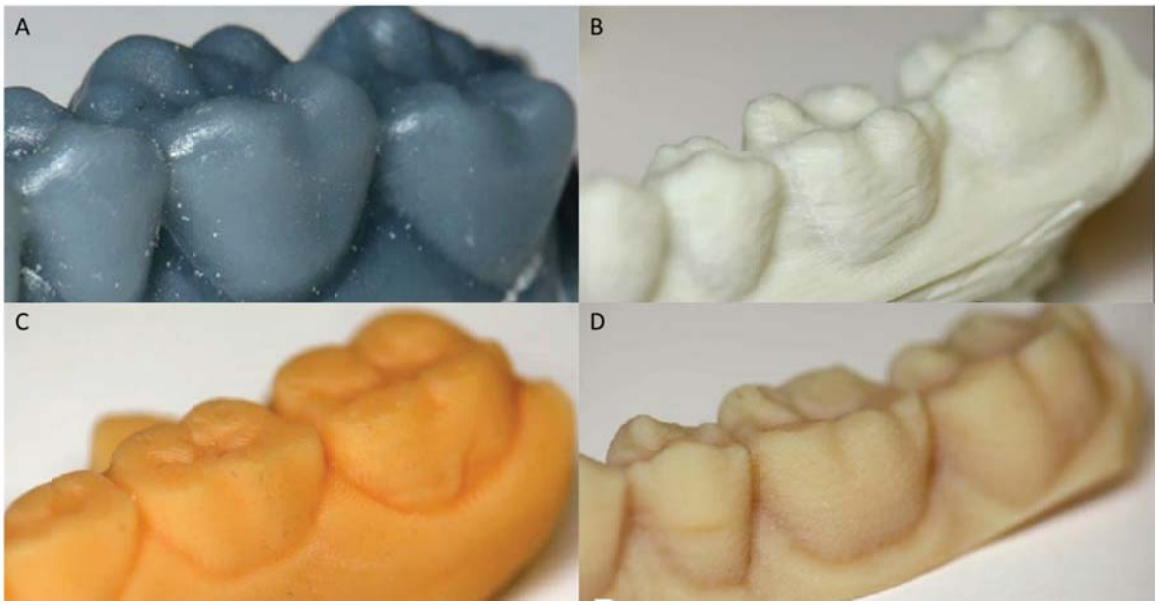

Figure 2. Comparison of layer-by-layer 3D printing methods, demonstrating the layer integration during the printing process for stereolithography (A), fused filament fabrication (B), digital light processing (C), and polyjet printing (D). Reprinted by permission from the Journal of Clinical Orthodontics[58]: Groth, C. K., N.D.; Jones, P.E.; Graham, J.W.; Redmond, W.R., Three-Dimensional Printing Technology. *Journal of Clinical Orthodontics* 2014, 45, (8), 475-485.

Table 1. Qualitative comparisons of major 3D printing methods and their main positive/negative attributes.

Technique Class	**Technique**	**Advantages**	**Disadvantages**	**Materials[2]**	**Resolution/Build Volume[2]**
Extrusions	Fused Filament Fabrication (FFF)	Rapid production of parts, inexpensive prototypes and equipment	Poor resolution with rough surfaces, high temperatures are required to process materials	Commodity polymers (PLA, ABS, PC, HIPS, polyurethanes, PS)	0.01-1 cm/Up to 1 m^3
	Direct Ink Write	Broad range of	Limited by	Thermoplastic	0.1 to 1 mm/150

	(DIW)	materials, access to multimaterials and hydrogel printing	rheological properties of the materials	polymers and composites, *in situ* crosslinking materials, hydrogels	x 150 x 140 mm^3
Powders	Selective Laser Sintering (SLS)	Excellent mechanical property control	Rough, porous surfaces which may require post-processing; limited secondary use of powders (resuse of unused material from print bed)	PEEK and derivatives, polyamides	50-100 µm/ 250 x 250 x 250 mm^3
Photopolymers	Stereolithography (SLA)	High surface quality and finish, high precision	Limited material selection (mechanical properties), print geometry limited by green strength of printing part (during printing)	Acrylates and epoxides	25 – 100 µm/ 250 x 250 x 250 mm^3
	Digital Light Processing (DLP)	Good surface quality and finish, good precision, low equipment costs	Limited material selection (mechanical properties), print geometry limited by green strength of printing part (during printing)	Acrylates and epoxides	50 – 100 µm/ 250 x 250 x 250 mm^3
	2-Photon Lithography (2PP)	Extremely high surface resolution and control	Slow process, with limited build volumes and high equipment costs	Acrylates and epoxides	0.1 – 5 µm/ 5 x 5 x 1 mm^3
Inks	Inkjet and Polyjet Printing	Access to multimaterial and composite printing, high speed printing	Limited processing abilities (limited to low viscosity inks)	Photopolymers, acrylates, composites	10-100 µm/ 200 x 300 x 300 mm^3

Table 2. Common polymeric materials for 3D printing and their general properties.

Material	T_g **(°C)**	T_m **(°C)**	**Process Temp (°C)**	E **(MPa)**	**Strain to failure (%)**	**Ultimate tensile strength (MPa)**
Polycarbonate	145-150	288-316	150-180	59-2600	100-300	52-62
Polylactic acid	55-63	150-175	180-220	1170-3100	10	15-40
Polycaprolactone	-60	60	55	380	1200	15
Polypropylene		130-160	220-250	1100-1700	20	32
ABS	105-110	NA	220-240	2207-2988	13-38	30-45
Nylon	47-60	220-260	240-270	1700-3200	1.9-24	48
PET	70-78	260	230-255	2000-27000	8 -17	55
Polyurethane	-35	NA	210-260	720-940	275	20-360
Polystyrene	100	210-249	120-260	3000-3500	0.1-300	30-100
PEEK	416-472	above 500	450	3760-3950	2	70.3-103

Part of the reason for the lack of suitable biomaterials is their poor performance over time, excluding biocompatibility. Indeed, biomaterials tend to display limited post-polymerization functionality, poor material-tissue cueing, tissue-material mechanical property mismatching, and inappropriate degradation behaviors. Therefore, more advanced materials are needed to address these limitations, including designing new polymer systems for specific tissue applications/fields (orthopedics, cardiovascular and soft tissues, neural and nervous tissue). Ultimately, the lack of clinically relevant 3D printing polymers derives from a combination of poor understanding of industrial potential, as well as regulatory hurdles associated with novel manufacturing and material systems in medical applications. An important note to make here is that while factors such as elastic modulus are often discussed for tissue guiding cues, mechanical property matching is by no means a deciding factor for devices as noted by the frequent use of metals in cardiovascular stents, for example.

Herein, we examine how the healthcare-related 3D printing field is promoting materials development, with a focus on the state-of-the-art and what is necessary to further translate these material concepts into clinically relevant technologies. The focus has been broken into application-specific materials, which are classified according to the tissue they are designed for. We then turn to examples of materials and printing techniques for which few biomedical applications have been proposed, but that we feel may be suitable with certain additional experimentation.

Clinical Uses of 3D Printed Polymers

The success of an implant depends on the type of biomaterial used for its fabrication, which should ideally be biocompatible, biodegradable, mechanically durable, and easily moldable.[59-64] Despite these multiple benefits, the main areas of commercialization for 3D printed medical devices are limited to hearing aids, dental implants, some prosthetics, and surgical guides/anatomical models for surgical planning. Today, more than 90% of hearing aids are manufactured using SLA, which has eliminated the traditional handcrafting process for custom hearing aids and has transformed the manual, labor-intensive industry into an automated, patient-oriented fast process.[65] The other main market for clinical 3D printing is dentistry, which includes dental wax-ups, orthodontic patterns, and crown and bridge molds produced more quickly and with significant increased production capacity with local control of the model processing using a 3D scan rather than uncomfortable impressions.[66] Models of mandibles and other complex tissue structure can be realized rapidly using digital scanning techniques including laser scanning, computerized tomography (CT) and magnetic resonance imaging (MRI).[67-69]

One of the major considerations for 3D printing of medical devices is the need to pass the device through regulatory approvals, with ABS, PLA, PCL, and PU being the main polymers associated with clinical use and 3D printing.[70-72] The limitations here include obvious considerations such as material toxicity and compatibility, as well as immune response, mechanical behaviors, and scalability. For example, acrylates, which are used in almost all lithographic methods (SL, Polyjet) are cytotoxic but can be replaced by less reactive methacrylates, thiol-ene systems, and other photoreactive monomers. PLA, which is one of the

most commonly used polymeric materials for implants, has poor mechanical properties for many applications. While the incorporation of degradable sections from PLA (or other polyesters such as PCL) have been explored, the resultant materials are limited in their resorbability, the degradation products are inflammatory and acidic, and the products themselves may lead to failure in bulk materials where their presence can catalyze hydrolysis. These and other 3D printing-compatible, degradable biomaterials have also not been utilized in US Food and Drug Administration (FDA)-approved implantable devices and applications, despite their use in traditionally manufactured biodegradable implants.[2, 33, 59, 73, 74] Less commonly considered design characteristics are the methods of sterilization (gamma beam, electron beam, x-ray, steam and auto clave, ethylene oxide), the environment of use (low pH, hemocompatibility, water content), and property migration (how will degradation or polymer plasticization alter material properties). Such deficiency highlights the serious shortcoming that exists in the field. Indeed, few materials are available to meet the wide array of clinical needs that physicians and patients have, and one of the major goals in biomaterials and 3D printing sciences should be the development of a library of truly translatable materials for various additive manufacturing processes. For this reason, only a few select cases of a "one-off" with a 3D printed device have been reported, such as a tracheal stent; however, the focus within the field has been primarily on the use of models and guides rather than the development of implantable devices directly from printing.[75] In this singular case, three pediatric patients with otherwise fatal airway collapses were treated with personalized tracheal airway stents, produced using SLS of PCL.[76, 77] Since this study, approximately 50 other pediatric patients have been treated in a single center treatment program that is allowed by the FDA under its Emergency Use Exemption, where the patient can only be treated with an unapproved technology.[75, 76] Although such remarkable achievement should be highly lauded, more work is needed to translate similarly promising results into the clinic for other devices and applications, as well. To that end, it is of utmost importance to shift our focus from those applications in the clinic to recent work in 3D printing materials development and provide a top-down assessment of materials development avenues, with the goal of providing insight into translating these materials to the clinic.

Orthopedic polymers

Additive manufacturing technologies are well-suited to create well-defined porosity and patient-specific biomimetic structures, and while most tissue scaffolds would obviously benefit from such feature control, orthopedic applications almost necessitate it. Structural support, typically provided by the orientation and complexity of hard tissue that is defined by a lifetime of bone loading and remodeling, is compromised by trauma or deformities and any replacement or therapy needs to account for the bone function in addition to cosmesis and healing over time. 3D printing enables researchers to produce parts to meet these needs, however, the majority of clinical work in orthopedics is focused on metals, and nearly all commercial representation, is focused on metal-related methods. Polymers and polymeric composites are of great interest in orthopedic engineering on account of their thermomechanical properties more closely matching those of tissue, in addition to their degradability and biocompatibility. Bioceramics, especially calcium-phosphate based ceramics, are composite that have been used to treat bone defects due to their low density and compositional similarities to natural bone.[78, 79] These composite materials generally make use of making use of the more commonly available polymers as matrices for the composites, including PCL and PLA, although there are a few interesting exceptions where the polymeric design is being leveraged for achieving novel material properties. One of the most common additives is hydroxyapatite (HA, primary inorganic component of bone), which is used in bone cement for craniofacial defect repair and coatings for femoral components of hip replacements because of its strong mechanical properties and osteoconductivity.[80] In vivo studies have shown that bone displays greater affinity for implants containing higher quantities of HA over those with only trace amounts, and that by designing porous scaffolds, osteogenesis and vascularization can be induced individually from each other.[81] Up to 40% hydroxyapatite has been loaded in SLA resins for printing methacrylate-capped 3-arm poly(trimethylcarbonate) orthopedic scaffolds, and no difference was reported in the cellular adhesion or viability with varying concentrations of HA.[82] However, commercially available bone substitutes, including allografts and their derivatives including demineralized bone matrix, or xenograft derivatives,

such as Bio-Oss, have better ability to drive osteo-induction and increase mineral deposition when PCL is processed via SLS into scaffolds compared to synthetic additives, such as HA or tricalcium phosphate.[83] Calcium silicate ($CaSiO_3$)-based porous scaffolds were fabricated using SLS, with various surface features, specifically rod-like and sheet-like hydroxyapatite nanostructures, and were examined for cellular response. Nanopatterning the surface was linked with increased cellular adhesion and migration, as well as reduced degradation, that the authors proposed as being due to the presence of the HA surface layer.[84]

A PLGA-based scaffold was composited with calcium phosphate and icaritin, a phytomolecule claimed by the authors to provide structure and mechanical support as well as facilitate bone regeneration, and was tested for osteonecrosis applications *in vivo* using rabbits. The study demonstrated fibroblast infiltration as a result of the icaritin presence, as well as regulated osteoblast differentiation. Within 4 weeks of implantation, bone growth was seen in the macropores of the scaffold, with minimal inflammation; at 8 weeks, the icaritin-containing scaffold was reported to have retained greater structure integrity compared with only PLGA or PLGA-calcium phosphate scaffolds. While the bone formation was not found to be statistically different between the scaffolds, this study does further demonstrate the potential of 3D printing polymeric materials for rapid bone growth.[85] Comparison of icaritin, a similar flavonoid, with bone morphogenetic protein-2 was performed using both a surface coating and an incorporation method during printing. Bone morphogenic protein coated to the surface was found to be the best of the examined methods.[86, 87]

PLA-based 3D printed scaffolds (using FFF), surface modified with HA, exhibited a constant release of calcium ions in aqueous medium for 10 days and a 50% higher adhesion and proliferation of human mesenchymal stem cells (hMSCs) compared to traditional PLA scaffolds. Differentiation studies also showed that hMSCs osteogenesis on PLA-HA scaffolds, measured as expression of osteogenic genes and alkaline phosphatase activity, was twofold compared to PLA scaffolds without HA coating.[88] Moreover, two biologically-inspired nanomaterials have been synthesized that consist of osteoconductive nanocrystalline HA and core-shell poly(lactic-co-glycolic acid) (PLGA) nanospheres encapsulated with

chondrogenic transforming growth-factor β1 (TGF-β1) for sustained delivery.[89] Then, a novel table-top stereolithography 3D printer was employed to fabricate a porous and highly interconnected osteochondral scaffold with hierarchical nano-to-micro structure and spatiotemporal bioactive factor gradients. This study concluded that human bone marrow-derived mesenchymal stem cell adhesion, proliferation, and osteochondral differentiation were greatly improved in the biomimetic graded 3D printed osteochondral construct in vitro compared to the non-graded scaffold.[89] Low-temperature extrusion of PLGA (75:25) in dioxane with 50-80 μm magnesium particles was used to produce porous scaffolds containing tricalcium phosphate and magnesium of up to 4 wt% composite loading. MRI and micro-CT imaging were used to determine blood perfusion increased along with neovascularization at 8 weeks compared with the controls, and that at 12 weeks 56% greater bone growth was found with the composite materials compared to the materials without magnesium in rabbit ulnar bone defect model.[90] As a follow-up study, residual dioxane was analyzed in the scaffolds using mass spectrometry (similar to protocols which would be required for FDA guidelines such as ISO 10993), and was used to develop an optimized processing technique for further translation of this technology.[91]

It has been reported that a novel synthetic osteoregenerative biomaterial, called hyperelastic “bone”, can avoid the technical, surgical, and manufacturing limitations of current bone graft materials (Figure 3B).[92] The hyperelastic bone can be synthesized using particle-laden liquid 3D inks by combining ceramic powder (HA) and PCL or PLGA to produce hydroxyapatite-polycaprolactone or hydroxyapatite-poly(lactic-co-glycolic acid) for the delivery of osteogenic proteins, such as recombinant human bone morphogenetic protein-2 (rhBMP-2), resulting in bone formation in critical-sized rabbit segmental diaphyseal defect.[92] To control the delivery of the rhBMP-2, collagen (for long term delivery up to 28 days) and gelatin (for short term delivery within a week) solutions encapsulating rhBMP-2 were dispensed into a hollow cylindrical type of PCL/PLGA scaffold.[92]

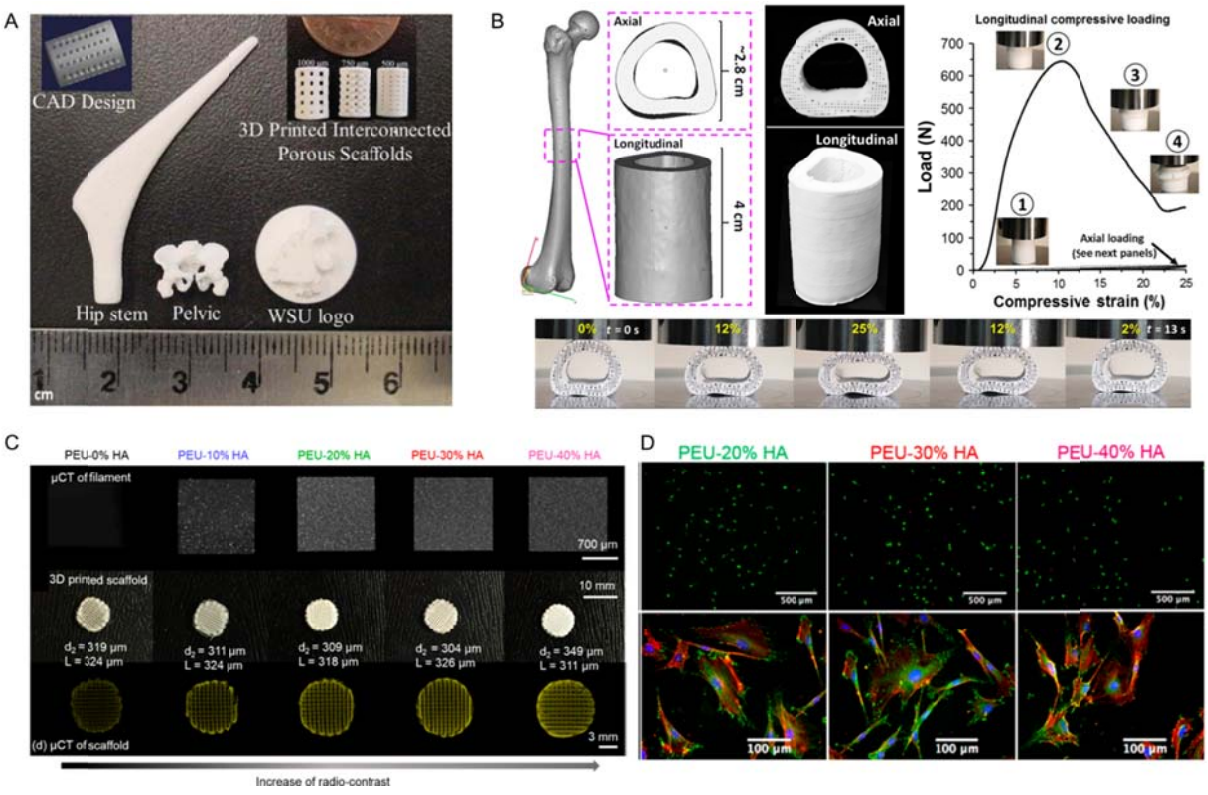

Figure 3: (A) 3D printed parts (fabricated at Washington State University) showing the versatility of 3D printing technology for ceramic scaffolds fabrication with complex architectural features. Reprinted from Acta Biomater., 8, Bose, S. *et al.*, Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: a review, 1401-1421, Copyright 2012, with permission from Elsevier. ; (B) Digital representation of average adult human femur and corresponding femoral midshaft section longitudinal and axial views, including axial and longitudinal views of natural and 3D-printed femur, accompanied by longitudinal compressive loading profile of 3D printed replacement and corresponding single axial compression. From Sci. Transl. Med., 8, Jakus, A. E. R. *et al.*, Hyperelastic "bone": A highly versatile, growth factor–free, osteoregenerative, scalable, and surgically friendly biomaterial, Reprinted with permission from AAAS; (C) Fabrication and characterization of 3D printed porous composite scaffolds. The μCT images of the filaments proved that the HA were homogeneously distributed within the

filament. There was no significant aggregation but an enhancement of radio contrast when increasing the HA content. The μCT images of the scaffolds showed porous structure with a porosity of 75%. The pore size was ~320 μm, and the diameter of the strut was ~300 μm; (D) In vitro cell viability of MC3T3-E1 cells after 1 day of cell culture on the composite scaffolds was studied by live/dead assay (scale bar 500 μm). Live cells were stained green, and dead cells were stained red. In vitro cell attachment and spreading after 3 days on the composite scaffolds was studied by immunohistochemistry (scale bar 100 μm). Reproduced with permission from ref 95, Copyright 2017 American Chemical Society.

Several studies have successfully leveraged the previous works with orthopedic composites and used alternative polymer compositions for bone repair. Polydopamine has been used as a glue for FFF-type printing of HA and thermoplastic polyurethanes, demonstrating a method of enhancing the integration of different layers to improve mechanical properties.[93] While the authors discussed the potential of this technology for tissue engineering, the study was primarily limited to materials characterization, and thus further demonstration is needed to validate their claims of increased cellular adhesion and performance. Salvianoic acid B was incorporated into PLGA and tricalcium phosphate scaffolds produced with FDM-type techniques in high, medium and low doses.[94] Osteogenic differentiation of murine primary mesenchymal stem cells was used to confirm osteoconductive scaffolds, and at 8 weeks after implantation to examine spinal vertebrae fusion osteogenesis, which was confirmed to increase with increasing salvianoic acid B dosing. However, the authors also report that little difference was displayed in the fusion rate compared with the control scaffolds, indicating that perhaps this method requires additional optimization prior to its application being fully realized.[94] L-Phenylalanine-based poly(ester

urea)s (PEU) have been formulated with HA to achieve highly porous scaffolds 3D printed by FDM, which stimulate cell proliferation and differentiation of MC3T3-E1 (murine pre-osteoblasts). The scaffolds possess increased mechanical properties, as well as osteoinductivity and osteoconductivity with the addition of HA.[95] Piperazine-based polyurethane-urea scaffolds were printed in direct ink write-like method, allowing for gradient piperazine distribution and control throughout the scaffold, with the highest achieved elastic modulus of approximately 155 MPa. Scaffold degradation was tuned through chain extension using PDLLA containing piperazine; PDLLA were first extended with hexamethylene diisocyanate to produce triblock soft segment diols, after which additional piperazine and HDI were added to yield a thermoplastic polyurethaneurea dissolved in dioxane for printing (optimized to 60 wt% TPU). After implantation in a rat tibia over 8 weeks, greater bone growth was found with piperazine-TPUs compared with PDLLA controls as determined by fibrous growth in the defect region, and no toxicity was seen in the examined heart, liver, and kidneys. The degradation of the piperazine resulted in increased pH, and while this has been reported as being conducive for osteogenesis, the osteoblast activity was found to decrease; the authors believe that this is a dose-dependent behavior and that these are ideal, alternative materials for orthopedic engineering.[6, 96]

Poly(3-hydroxybutyrate) (PHB) was printed into porous scaffolds by SLS and functionalized with osteogenic growth factor (OGF) as a post-printing process. OGF was released from the scaffold over a 72-hour period, and image analysis indicated bone marrow derived stem cells underwent morphological differentiation indicative of osteogenesis.[97] In a different study, PHBV, a copolymer of PHB and 3-hydroxyvalerate, was processed into porous scaffold composites containing calcium sulfate hemihydrate via FDM, after which chitosan-based hydrogels were coated to the surfaces. Beyond the performance of PHBV and PBHV with

calcium sulfate hemihydrate, the hydrogel-coated scaffolds displayed increased adhesion and proliferation of bone marrow stromal cells, along with increased expression of osteogenesis-related genes including runt-related transcription factor 2, osteocalcin, osteoprotein, and bone morphogenetic protein-2 compared with glyceraldehyde-3-phosphatedehydrogenase as a house keeping gene. Murine intramuscular implantation studies were performed using cylindrical printed scaffolds with ~400 μm pores and displayed similar trends, with increased bone formation with the hydrogel-coated scaffold, while the PBHV and composite scaffolds did display bone growth or reduced growth, respectively.[98] Similar studies focusing on PBHV, calcium phosphate, HA and PLLA have demonstrated similar results using PLLA/HA microsphere-based nanocomposite and calcium phosphate/PBHV nanoparticle-based nanocomposite porous scaffolds manufactured using SLS. Alkaline phosphatase activity, as well as cellular proliferation, osteoblast attachment, and differentiation were enhanced with the calcium phosphate nanoparticles, while PLLA/HA scaffolds were comparable to bulk PLLA.[99]

Poly(propylene fumarate) polymers crosslinked with diethyl fumarate are also of interest for designing scaffolds for bone regeneration by SLA, providing suitable mechanical properties for human bone regeneration and a range of degradation rates depending on the crosslinking content. In vivo degradation and tissue regeneration studies have still to be determined in this polymer scaffold.[100, 101] This material has recently been commercialized as well, further demonstrating the translational potential from material development from basic synthesis studies to clinical applications.

While excellent progress in orthopedic-focused 3D printing has been made, there are still some important gaps in materials development. For instance, the research community currently tends to focus on commodity polymers for the majority of research, which will have two opposing

outcomes. On the one hand, by using better understood and widely used, regulatory considerations surrounding the therapeutic or part design will be reduced somewhat, and the path to the clinic may be eased. On the other hand, as a community, while we are well aware of the limitations of these commodity polymers, there is a tendency to continue to use the materials even in applications where the materials do not behave optimally. For this reason, further work is required to broaden the library of orthopedic-intended polymers and match the polymer chemistry with biological studies.

Polymers for soft tissue regeneration

Soft tissue refers to any tissue that is not hardened or calcified, such as vasculature, skin, tendon, muscle, cartilage, fat, brain, or fascia, and connects, supports, or surrounds bone and internal organs. When soft tissue is damaged because of pathogenesis, resection of tumors, trauma, degeneration, or congenital deformities, its restoration can be promoted by engineered soft tissue substitutes that impart mechanical support during the reconstruction, as well as suitable volume and shape. Depending on their chemistry and manufacturing process, polymers display enormous variability in their features, and thus 3D printed polymer scaffolds have the potential to revolutionize regenerative medicine if designed with the physicochemical properties and biological functions of the damaged soft tissue we aim to restore. Indeed, polymeric 3D structures can display elastic moduli in the range of 10-100 kPa (hydrogel-like materials) to > 10 GPa (rigid epoxy composites) depending on the nature of the biomaterials used.[102, 103] Hence, materials must be carefully chosen based on both the mechanical and structural requirements of the final construct. Not only do polymers allow us to access a wide range of mechanical properties that we can tune to match those of interest, but also predictable degradation rates and enhanced cytocompatibility. In addition to that, 3D printing results in polymeric scaffolds with

finely detailed, uniform porous geometry and interconnectivity, as well as controlled surface area. Specifically, when applying extrusion-based additive manufacturing, the balance between shear rate and viscosity is crucial as the polymeric resin needs to easily flow out from the printing nozzle while the 3D printed retains its integrity and stability. Hence, printing materials, such as hydrogel-like and polymeric resins, should not gel under manufacturing conditions, which can be overcome by a fine temperature control, but still produce mechanically relevant soft constructs.[104] Several excellent reviews already cover the discussion of the recent advances of additive manufacturing and 3D biofabrication for tissue engineering in general, as well as for soft tissue in particular.[105, 106]

In this section, we highlight noteworthy examples with some of the greatest impact regarding 3D printed constructs for soft tissue biomedical applications based on degradable polymers while sharing our perspective on the directions that should be taken in the next years in order to achieve clinical translation. We have mainly focused on biomaterial inks, which result in cell-free scaffolds, as opposed to bioinks (*i.e.* resins that contain cell suspensions, cell-laden hydrogels, microcarriers, cell/tissue spheroids and/or decellularized matrix components), which produce in general softer hydrogel-like constructs. Thus, we direct the reader to the other recently published reviews for more details on the advances in bioinks for 3D printing.[107]

Vasculature

Manufacturing 3D printed devices for soft tissue applications is regarded as a challenging task in comparison to hard tissue engineering applications, such as a bone regeneration, as a consequence of the complications that arise from material processing limitations in addition to biological and mechanical performance. For instance, for hard tissue, PLA, PCL, PPF, and PEUs have been explored as resorbable materials able to undergo 3D additive manufacturing because

of the high melting points and adequate viscosity of the resin, which yields high stability during the 3D printing process. [16, 29, 36, 108-112] In contrast, only relatively recently have bioresorbable materials been used with additive manufacturing for soft tissue medical applications, such as the previously mentioned patient-specific non-cellular implants for treating tracheomalacia (*i.e.* flaccidity of the supporting tracheal cartilage).[76, 77, 113, 114] For this specific purpose, 3D printed devices are engineered to adapt and change with tissue growth based on biomechanical and degradation properties over a specified period, such as in 3D printed splints that respond to airway geometry changes and are scalable to mass-manufacturing levels.[75] Indeed, in two reported cases, custom-designed resorbable airway splints were implanted to patients with successful results regarding treatment efficacy, adverse reaction and biodegradation (Figure 4A).[77, 115] However, the safety of the scaffolds needs to be tested with more patients, while considering other parameters, such as age.

In another example, 3D printed PCL-based scaffolds coated with fibrin/mesenchymal stem cells (MSCs) have been used for the reconstruction of a partial tracheal defect in an animal model, both the shape and function of the trachea being completely recovered 8 weeks after the operation (significant neo-cartilage regenerated) (Figure 4A).[116] In this case, the mechanical strength and favorable environment provided by PCL for cells to survive and function was enhanced by the biological coating. More recently, for the same application, poly(glycerol sebacate fumarate) gadodiamide-poly(ethylene glycol) diacrylate yielded 3D printed scaffolds by a combination of *in situ* photopolymerization and extrusion 3D printing;[117] the focus was placed on modulating the mechanical and thermal properties of the material by altering the process parameters. Targeting the regeneration of blood vessels, PLA/PCL scaffolds releasing heparin were prepared by electrospinning in a tubular shape, and then fused deposition modelling was

used to print PCL on the outer layer to improve its mechanical properties.[118] Similarly, mimics of soft vascular tissue have also been prepared by an integrative method of 3D printing, dip coating, and salt leaching combining polyvinyl alcohol (PVA), PCL, and TPU.[119] In another example, a porous PCL scaffold which was prepared with SLS for cardiac tissue engineering applications displayed a porosity value of 85%, pores ranging from 40 to 100 μm, and a compressive stiffness of 345 kPa. C2C12 myoblast cells were cultured on the polymeric construct for 21 days, differentiating within 6 days of culture.[30]

Overall, although it is going to take some more time and research efforts, additive manufacturing in combination with 3D, clinical imaging proves the potential of such constructs to mimic living tissue and thus be translated into clinical and commercial settings, where tissue engineering applications, such as pharmaceutical testing and regenerative therapies, will clearly benefit from such advances. For instance, the main drawbacks of perfusion, which is the traditional method to induce blood vessels formation, can be avoided by applying 3D printing technology in combination with CT scanning or MRI imaging, which appears as a feasible alternative that reduces cost and time consumption. However, despite these advantages, the personalized nature of 3D printed splints, which are individually designed for each patient, brings some concerns regarding FDA approval, which is a general problem all 3D printed products face.

The smooth process from imaging to 3D prototype is a multidisciplinary task that only by close collaboration between all the elements involved (radiologists, clinicians, computer scientists, and material scientists together) can continue to evolve.[120] However, the integration of clinical routine in this workflow has not advanced to the necessary level for clinical work, although there are concerted efforts to combine advances in printing technology and materials

with patient specific clinical imaging to achieve flexible, tough vascular graft materials and other applications (Figure 4).[119] In the future, and more specifically for soft tissue applications, at least long-term *in vivo* testing with larger animals (not only mice or rabbits) must be considered as a priority, while standardized protocols for the preparation of the 3D printed structures, as well as the quantification of the output need to be put in place.

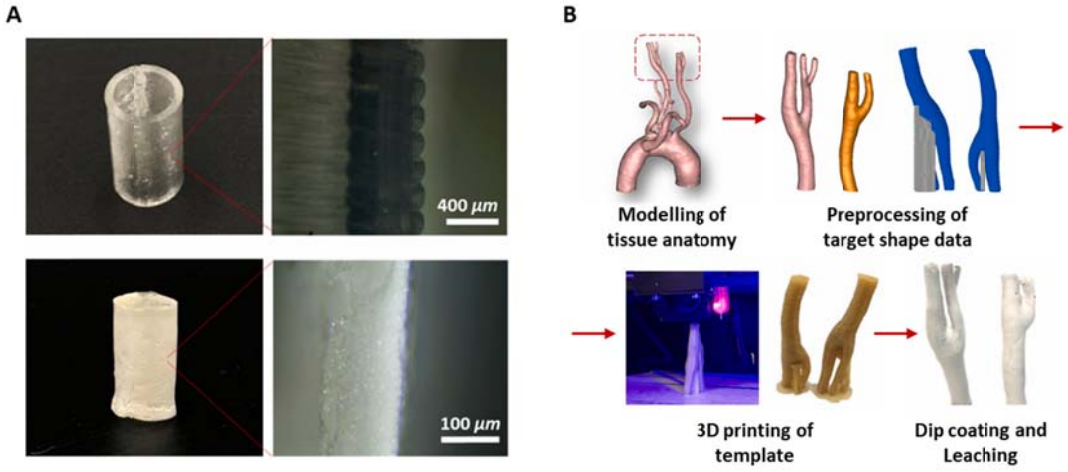

Figure 4. (A) Cylindrical tubes and cross-sections of their walls fabricated by FDM-based 3D printing (top row) and the dip-coating process (bottom row) for vascular tubes; (B) schematic of the procedures for their fabrication. Reprinted from J. Mech. Behav. Biomed. Mater., 91, Lee, J. E. *et al.*, Fabrication of 3D freeform porous tubular constructs with mechanical flexibility mimicking that of soft vascular tissue, 193-201, Copyright 2019, with permission from Elsevier.

Cartilage

Articular cartilage and intervertebral disc (IVD) tissue degenerate with time, which directly contributes to joint and back pain. In addition, the low self-healing capability of cartilage limits the recovery of injuries caused by articular disorders or sport damage. Cartilage is a heterogeneous tissue with a lamellar structure that includes several layers, each one with

different chondrocyte morphology and density, collagen organization and matrix composition.[121] Therefore, bearing this in mind, 3D printing fabrication methods represent a unique option for preparing scaffolds for cartilage regeneration with fine-tuning of the mechanical and the spatial features. For instance, the surface morphology of a 6-month-old rabbit joint was captured with multi-slice laser scanning and reconstructed by computer-aided design to produce an anatomically correct acellular scaffold. The construct, which was obtained by 3D layer-by-layer printing, was made of hydroxyapatite (HA) powder and PCL, and later infused with TGFβ3-adsorbed collagen gel. The superior portion of the scaffold displayed internal microchannels designed for the regeneration of cartilage (diameters of 400 μm), while the inferior portion was designed for the regeneration of bone (diameters of 200 μm).[122] In this case, after implantation into rabbits, the regeneration of the entire articular surface was attributed to the modularisation of a large tissue scaffold with repeating and interconnecting microchannels, which triggered cellular responses, such as cell homing, diffusion, histogenesis, and angiogenesis. Most importantly, the histological and mechanical properties of the regrown cartilage matched those of native rabbit cartilage. The same system (*i.e*. PCL/HA scaffolds) was designed as a multiphase scaffold with three distinctive region-specific microstructures (readily adjustable pore/channel sizes while maintaining physical integration) for the spatiotemporal delivery of BMP2, CTGF, and amelogenin to promote the regeneration of multiple periodontal tissues.[123]

It is clear thus that the challenge is to create scaffolds that mimic as close as possible the *in vivo* cellular environment of articular cartilage. Consequently, the success of the scaffold, in that cells colonize, proliferate and, finally, differentiate into chondrocytes, depends on several parameters, such as total porosity, pore size, interconnectivity, mechanical, and physico-chemical properties. With this in mind, a series of 3D printed scaffolds based on PCL were

prepared with different geometry topologies and porosities to elucidate the role played by scaffold micro- and macroarchitecture in cell response.[124]

It is important to note that most of the examples cited so far exploit the benefits of PCL. Indeed, this synthetic polymer, which can be 3D printed in a variety of methods, including DIW and FFF, without using toxic solvents, has been widely used for soft tissue engineering applications.[125] Not only does it display good biodegradability by hydrolysis, but also has excellent mechanical properties. Some recent works report the printing of cell-laden hydrogels together with synthetic biodegradable polymers, such as PCL,[126, 127] to impart mechanical strength to the bioinks, and thus produce stable scaffolds for cartilage regeneration. Hence, this approach, which overcomes previous limitations on the size, shape, structural integrity and vascularization of bioprinted tissue constructs, opens the door to the design of complex tissue and organs. Based on this principle, Kang *et al.* presented in an extensive work an integrated tissue-organ printer able to fabricate stable, human-scale tissue constructs of any shape, which included mandible and calvarial bone, cartilage and skeletal muscle.[128] In another example, combining hydrogels with 3D printed, highly porous PCL scaffolds resulted in biomechanically functional constructs with mechanical features similar to those of native articular cartilage that can be modulated by the porosity of the printed mesh.[129] In comparison to the hydrogel components, which will degrade within months, the long duration of PCL in vivo, with degradation times of years, is proposed to enable it to act as a more permanent reinforcement for the new tissue, which conflicts with the idea of sufficient mechanical support only while the tissue is healing.[125]

Similarly, and considering the lamellar structure of cartilage, several scaffold designs were constructed by printing hybrid materials in layers that integrated mechanically competent

synthetic polymers, such as PCL or poly(ethylene glycol) dimethacrylate (PEGDMA), with biomaterials, such as alginate, gelatin, or fibrin-collagen.[130, 131] In those examples, cell-laden hydrogels were deposited within a robust framework following microextrusion- or inkjet-based technologies. This approach was exploited to fabricate a complex porous framework (for ear regeneration) by co-printing PCL with cell-laden alginate hydrogel and using PEG as a sacrificial layer to support the main structure (Figure 4B).[132] Both cartilage and fat tissue were induced to regenerate from separately printed chondrocytes and adipocytes.

Following this work, other examples also demonstrated the feasibility of additive manufacturing technologies to produce 3D structures that simulate tissue and organs with higher shape complexity and multiple cell types for cartilage regeneration. For instance, Kesti *et al.* developed a bioink for printing cartilage grafts based on two unmodified polysaccharides, gellan and alginate, combined with cartilage extracellular matrix particles and a cation-loaded transient support polymer (*i.e.* Pluronic F127) to stabilize overhanging structures, while a bioink composed of nanofibrillated cellulose and alginate resulted in a suitable hydrogel that enhanced the growth of cartilage tissue. [133, 134] In another example, although lacking biodegradability, sodium alginate and PEG were arranged in an interpenetrating network to produce a biocompatible 3D printed hydrogel scaffold as tough as native cartilage.[135]

Other biodegradable polymer-based resins have been used to fabricate 3D printed scaffolds with the proper structure, function, and cellularity necessary for potential cartilage (Figure 5) and disc tissue repair *in vivo*.[62] 3D printed ABS and PLA thermoplastic scaffolds obtained by extrusion with large pore dimensions (*ca.* 700 μm) promoted the cell adhesion and proliferation of primary bovine chondrocytes, which generated a neo-matrix based on aggrecan proteoglycan and collagen type II after a three-week culture period *in vitro*.[136] However, despite such

successful result, the biodegradable scaffolds were too stiff in comparison to native cartilage tissue. Taking into account that cells are able to produce a biological matrix, the authors stated that a more flexible implant would be adequate for an *in vivo* setting. Aware of the importance of optimizing the mechanical and geometrical features of the scaffold for soft tissue regeneration, Hung *et al.* chose biodegradable polyurethane (PU) elastic nanoparticles, combined with hyaluronan and bioactive molecules, as a water-based printing resin to produce a more flexible biologically relevant design using a self-developed low temperature fused deposition manufacturing (LFDM) system.[62] Specifically, after optimization of the printing parameters, which included low temperature, the disk-like scaffolds showed highly compliant nature and elastic recovery. Most importantly, MSCs, which were seeded on the multi-component scaffolds, self-assembled and underwent effective chondrogenesis *in vivo* using a rabbit knee defect model.

In a similar example, a FDM printer was used to fabricate an anatomical 3D printed lumbar cage based on polyhedral oligomeric silsesquioxane poly(carbonate-urea) urethane.[137] The geometry of the design resembled the contour of the vertebrae, and four different filling densities were considered to mimic the complex structure of the spinal cord and ensure its compatibility for load bearing applications. The filling density, which was reduced to increase the printing resolution and time, influenced the mechanical properties of the final device. Indeed, the lower the filling density, the poorer interconnection between the printed layers, which can lead to failure under mechanical loads. Adipose-derived stems cells (ADSCs) attached and proliferated on such devices *in vitro*, which evidences the cytocompatibility of the scaffolds and their potential to regenerate surrounding tissue.

We consider of major importance the need to expand the library of biodegradable polymers that are suitable for 3D printing while displaying suitable thermomechanical properties,

hydrolytic degradation profile and biological response. Currently, as described, only a few of them, mainly PCL, have been exploited to produce 3D printed intricate objects. Recently, PPF was synthesized with different block length ratios using PEG macroinitiators of various molecular mass, and was photochemically printed from an aqueous solution to yield hydrogels with high cytocompatibility *in vitro* across a number of engineered cell lines.[138] Overall, we consider that the development of sustainable materials for 3D printing applications is of major importance. In this line of research, anisotropic cellulose nanocrystals (CNC) were used as the main component of a viscoelastic ink that enabled the patterning of 3D objects by direct ink writing.[139]

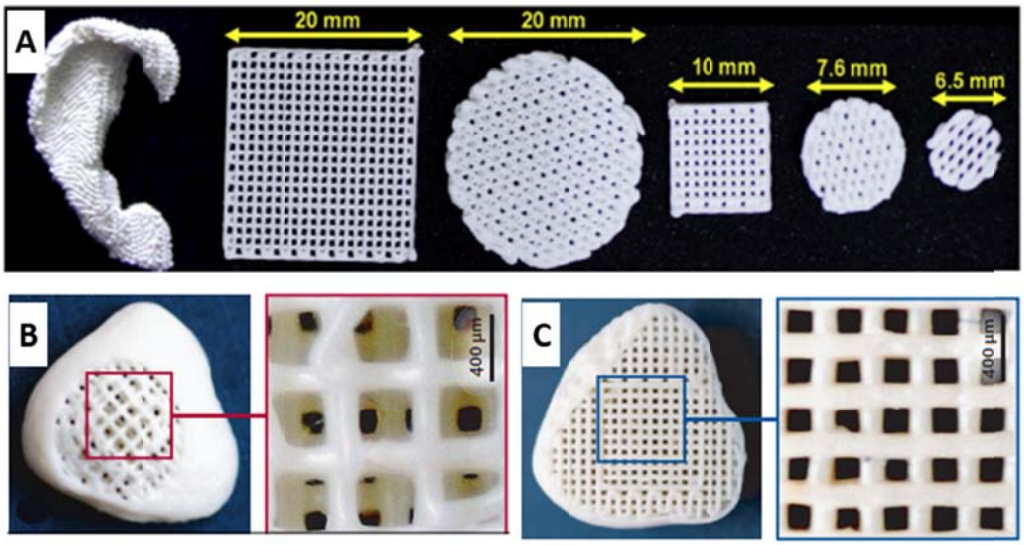

Figure 5. (A) Compliant 3D printed scaffolds with various shapes and dimensions with biodegradable polyurethane nanoparticles as the major component for water-based 3D printing. Reprinted from Biomaterials, 83, Hung, K. C. *et al.*, Water-based polyurethane 3D printed scaffolds with controlled release function for customized cartilage tissue engineering, 156-168, Copyright 2016, with permission from Elsevier; (B-C) Anatomically correct bioscaffold made of PCL-HA with internal microchannels opening to the synovium cavity (B) and bone marrow (C).

Reprinted from The Lancet, 376, Lee, C. H. *et al.*, Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study, 440-448, Copyright 2010, with permission from Elsevier.

Tendons and ligaments.

Scaffold-based regenerative approaches are especially relevant for tendon and ligaments because of the limited number of biological grafts and artificial prostheses.[140] However, matching the native features of this tissue, which include low vascularity, unusual flexibility, robustness and hierarchical fibrous structure, is a challenging task when designing the construct and choosing the biomaterial. Despite this, a single integrated muscle–tendon unit construct was produced by co-printing thermoplastic PU and PCL with cell-laden hydrogels (bioinks containing C2C12 myoblasts and NIH/3T3 fibroblasts).[141] Interestingly, this set up, which included multiple printing nozzles and combined inkjet- and microextrusion-based technologies, yielded a mechanically heterogeneous biomimetic polymeric scaffold that was elastic on the muscle side and relatively stiff on the tendon side, which induced a tissue-specific distribution of cells with myoblasts and fibroblasts, respectively. Hence, the supportive role of the polymers facilitated high cell viability and tissue development. Furthermore, recent studies have dealt with the regeneration of anterior cruciate ligament (ACL), which is conceived as a more complex task in comparison to other tendons and ligaments.[142] Specifically, PEG-PPF was chosen as a biocompatible and biodegradable polymer to obtain a complex porous scaffold to increase the stability of an ACL graft.[143] The 3D printed scaffold was loaded with growth factors and coated with HA before implantation simultaneously with the grafts into an *in vivo* model (rabbit with ACL defect).

Skin, adipose tissue and other organs.

The fabrication of scaffolds to support regeneration of other soft tissue, such as skin, has also attracted wide attention.[144, 145] However, in this case, most of the published works deal with biofabrication methods and 3D constructs produced with bioinks that encapsulate stem cells for skin regeneration.[146] For this application, and considering the lamellar structure of skin, 3D architecture and arrangement of cells play an important role in aiding skin regeneration and wound healing. We just mention briefly an example where a finger-shaped porous scaffold was successfully fabricated by an indirect 3D printing of PCL, which provided high elasticity to the final construct that was coated with a self-assembling peptide. Overall, the systems accelerated angiogenesis and promoted tissue regeneration.[147] Similarly, adipose tissue regeneration is highly centered in autologous fat transfer, or the use of cell-laden synthetic substitutes, such as biodegradable polymers (*i.e.* poly(a-hydroxyacids).[148] For this application, 3D biofabrication has been exploited to produce anatomically relevant tissue constructs by delivering suitable matrix materials (printing decellularized adipose tissue or PLA nanofiber-alginate hydrogels) in combination with living cells.[149, 150]

From the above examples, additive manufacturing technology is regarded as a feasible option to prepare personalized scaffolds and implants at reduced cost with biomaterials that fulfil all the requirements of the target soft tissue to be regenerated. However, current advances are driving the tissue engineering field towards the 3D bioprinting of organ-like structures and organ-on-a-chip platforms to develop *in vitro* models of the brain, heart, kidney and mostly liver, among others.[37, 151-159] For such organs, cell-laden hydrogels based on gellan gum, gelatin methacryloyl, silicone elastomers, alginate, gelatin/PEG, or gelatin/collagen are generally chosen as the bioink. Lee and Cho introduced a novel 3D bioprinting method to produce a liver-on-a-chip where cells

and biomaterials were spatially positioned to mimic the natural conditions and function of the liver.[160] In this example, PCL was exploited as the biodegradable mechanically platform to support the delivery of the cell-laden gelatin/collagen bioink.

When 3D printing materials for soft tissue applications, some practical issues play a critical role in the successful outcome of the process, such as the speed at which the constructs are printed, which affects their reliability, as well as the printing resolution. Moreover, in most of the examples cited, part of the design included support material, which later on needs to be removed or at least degrade faster than the actual functional biomaterial, which complicates the manufacturing of the object. Hence, future goals should include the development of self-supporting 3D printed systems, as well as maximizing the volume of printed material per unit of time, while retaining local composition and high resolution. As mentioned earlier, manufacturing multi-material objects, displaying different spatial mechanical properties (*i.e.* soft and hard materials with integrated functionality), is another challenge to accomplish, especially if we consider stereolithography. Similarly, the range of materials available is very limited and compromises functionality in favor of shape/structure; therefore, developing new materials for 4D printing will pave the way to the next generation of biomaterial constructs. Finally, although outside of the material science field, simulation tools that actually predict how soft material will behave under printing conditions will avoid trial-and error design, which is a time-consuming task.

Polymers for nerve tissue regeneration

A major clinical need also exists for designing graft materials for peripheral nerve injury repair, with the final aim of overcoming the limitations of the current gold standard for nerve repair, *i.e.* autograft and allograft implantation can result in donor site morbidity, neuroma

formation, and often require long-term immunosuppressant therapy with high risk of infection. To that end, nerve guidance conduits (NGC), which are fabricated from natural or synthetic materials, have undergone extensive research.[161, 162] The ideal NGCs construct should comply with a set of very specific requirements that result in a biocompatible, bioresorbable and porous scaffold that mechanically supports outgrowing axons, while minimizing the interactions between the myofibroblasts and axon growth. Among the different fabrication methods used to prepare NGCs, 3D printing has emerged as a promising technology because of the possibility to mimic closely the features of the native peripheral nerve tissue with micron-scale resolution.[163] Indeed, 3D printing approaches are now regarded as valuable tools for the preparation of polymer-based scaffolds for neural tissue regeneration. Despite the fact that the plasticity of neural tissue allows for some degree of flexibility regarding structural features during the design of 3D printed conduits, the final conduit is expected to display enough detail and adequate mechanical properties to facilitate and promote nerve regeneration while integrating the new tissue into the native peripheral nervous system. Recently, direct ink writing and projection micro-stereolithography (PμSL) were reported to offer unique advantages (*i.e.* high vertical aspect ratios, overhanging parts, and flexibility of printing both elastic and viscoelastic materials) to design defined 3D artificial axons.[164] Using a library of biocompatible polymers, such as PDMS, Poly-HEMA, or poly(HDDA-co-starPEG), which spanned a wide range of mechanical properties, they produced axon-like microstructures with diameters < 10 μm in both vertical and horizontal orientations with high aspect ratio values.

From a general point of view, the design of scaffolds for repairing damaged nerve tissue is a complex task since an adequate balance between a series of parameters needs to be met, which includes structural, mechanical, and biochemical features. Specifically, aspects such as

permeability, flexibility, and degradation can determine the success of the nerve regeneration. In that regard, the selection of the biomaterial, which has a direct impact on the overall performance of the final conduit, has always attracted attention.[165] In most cases, neural healing devices lack the ability to bridge large gaps (size > 3 cm) and their biological performance is inferior to that of other reconstructive surgeries (*i.e.* autografts and allografts). [166] In this context, 3D printing can advance this field of research by producing innovative NGCs based on biodegradable polymers that display improved mechanical properties by tailoring their material composition with precise spatial resolution, as well as including into the composition bioactive molecules. For decades, degradable polymers, such as PLLA and PLGA, have been studied as suitable biomaterials for the productions of NGCs for peripheral nerve regeneration although the manufacturing process used was melt extrusion.[167, 168] By a modified piezoelectric inkjet system, a copolymer made by PLA/PCL was 3D printed to render cylindrical nerve conduits with potential application as cell-regulated nerve growth factor delivery systems.[169]

More recently, stereolithography has been combined with other fabrication techniques, such as electrospinning and electrospraying, to produce structurally complex composite 3D printed scaffolds able to support nerve regeneration. In particular, PEG-based inks were printed onto highly aligned electrospun PCL fibres or a mixture of PCL/gelatin nanofibers,[38] and PLGA core-shell nanoparticles.[170] Some noteworthy studies, although few in number, highlight the potential of poly(glycerol sebacatemethacrylate) as biodegradable biomaterial for peripheral nerve repair, only recently has it been specifically applied to obtain NGC by additive manufacturing.[171]

Low-temperature deposition manufacturing (LDM) is a rapid prototyping manufacturing technology, similar to FMD, that allows for the preparation of 3D-printed scaffolds under controlled temperature conditions, thus preventing the heat-related loss of activity if bioactive

molecules are used. The robustness and application of this fabrication method was applied to several synthetic biodegradable polymers, which included PLGA, PU, PDLLA, and PLLA, with promising results. [172] Specifically targeting nerve regeneration, this technique has been applied to PU-collagen mixtures, including a double-layer PU-collagen NGC via a double-nozzle, low-temperature, deposition manufacturing system where both the porosity and mechanical strength of the construct were studied in relation to the polymer concentration.[173, 174] Also, thermo-responsive water-based biodegradable polyurethane dispersions were synthesized as bioinks and 3D bioprinted to encapsulate neural stem cells (NSCs).[175] Finally, even though lacking biodegradability, multiple additive manufacturing methods utilizing polymer inks based on polydimethylsiloxane (PDMS) and pHEMA with diverse architectures have been used to demonstrate that artificial axons will display biological-relevant stiffness, diameter and spacing in engineered environments that reflect key physiological (and pathological) mechanical, geometric, and biochemical components.[164]

Several studies exploit the advantages of gelatin-methacryloyl (GelMA) to 3D engineer hydrogels as NGCs.[176] This photocrosslinkable collagen-modified biopolymer retains its natural cell-binding motifs after modifications; however, the poor mechanical properties of GelMA hydrogels may yield to soft and fragile 3D scaffolds. To solve that problem, poly(ethylene glycol) diacrylate (PEGDA) has been introduced into the ink composition and 3D printed customizable NGCs (*i.e.* the mechanical properties of PEGDA-based hydrogels can be fine-tuned by the molecular weight and concentration of PEGDA, as well as the light-exposure intensity and time) with unprecedented resolution, speed, flexibility, and scalability.[177] In another example, GelMa hydrogels were rapidly fabricated by a continuous 3D printing process incorporating drug loaded PEG-PCL nanoparticles. Overall, by exploiting natural and synthetic

biopolymers, the authors designed biofunctional, nanoparticle-enhanced physical microenvironments that were suitable for axonal elongation. Indeed, the encapsulated drug, which facilitated nerve regeneration, experienced a sustained released from the PCL-based nanoparticles.[178] A novel biofabrication technology, cryo-polymerization, was developed to prepare GelMA gels as bio-conduits for clinical use in peripheral nerve regeneration.[179] Indeed, the resulting scaffolds, cryo-gels, showed enhanced interconnected pores with great potential for tissue engineering applications. 3D printed nerve guidance channels were produced by stereolithography using PCL resins that were afterwards filled with aligned cryo-matrix supplemented with nerve growth factors, seemingly the first study to apply such approach.[39]

So far, the versatile strategies brought by 3D printing have made a significant impact in the production and bio-application of NGCs, thus evidencing the immense potential of additive manufacturing in this field of tissue regeneration. However, the complexity of the central nervous system (CNS) architecture has hindered the design of 3D printed scaffolds for CNS structures, such as the spinal cord. Hence, efforts have been focused recently on developing 3D biomimetic, hydrogel-based scaffolds able to model the complex CNS tissue as new clinical approaches to treat neurological diseases.[180, 181]

Recent publications report the use of conductive polymers, such as polyaniline (PANI), poly(3,4-ethylenedioxythiophene) (PEDOT), or poly(pyrrole) (PPY), in NGC to enable electrical stimulation locally and promote tissue growth.[161] However, the brittleness and poor solubility of such polymers hinders their processing, especially as 3D constructs by additive manufacturing technologies. In a breakthrough example, an integrated 3D printing and layer-by-layer casting method has been used to produce single- or multi-layered porous scaffolds that combine graphene, polydopamine, arginylglycylaspartic acid (RGA) and PCL.[182] The technique,

which avoided many of the complications associated with traditional procedures (poor quality control, weak mechanical strength, random gaps, and uneven drug delivery), yielded nerve conduits that ensured peripheral nerve regrowth *in vitro* and *in vivo* by combining electrical and biological signals and using a biodegradable material for the first time. Indeed, PCL successfully supported the tubular structure for a long period, while displaying appropriate stiffness and mechanical performance. Similarly, the conductive properties of graphene were exploited to produce gelatin-based conduits for nerve regeneration.[183] Specifically, a conductive graphene PLA filament was used to 3D print an interdigitated circuit with the desired dimensions. The resulting scaffolds, which were tailored with adequate biodegradability and microstructural/mechanical properties, provided spatial and local control on cellular processes by electrical stimulation, and thus were envisioned as potential platforms to develop alternative peripheral nerve regeneration strategies. Indeed, the development of conductive inks that can then be 3D printed is becoming a topic of interest. The ultimate goal is to produce scaffolds that not only support cells, but also act as conducting guidelines for the cells to attach, proliferate and differentiate. This has been demonstrated using a conductive ink that includes cellulose nanofibrils and carbon nanotubes as 3D guidelines with a specific nanotopography which, in combination with electrical conductivity, promoted neural cell development.[184] Within this context, silk fibroin protein-based materials have great potential to revolutionize the field of bioelectronics, with applications as futuristic as e-skins, e-bandages, biosensors, wearable displays, implantable devices, or artificial muscles using inkjet and FFF techniques.[49, 53, 185-192]

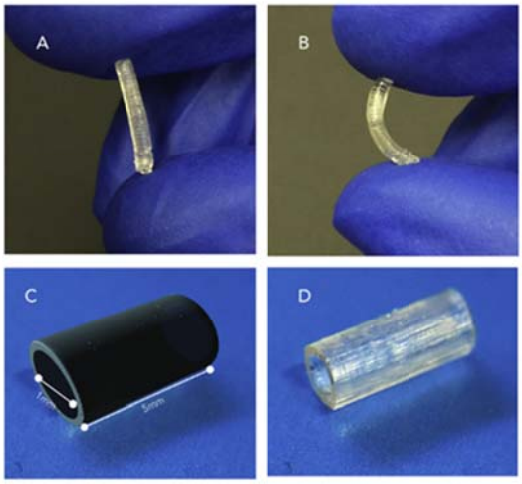

Figure 6. Digital photographs highlight the elastic properties of poly(glycerol sebacate methacrylate) 3D printed nerve guidance conduits (A) when compressed (B); computer model image of the ideal 3D printed nerve annotated with dimensions (C) and the final scaffold ready for implantation (D). Reprinted from Acta Biomater., 78, Singh, D. *et al.*, Additive manufactured biodegradable poly(glycerol sebacate methacrylate) nerve guidance conduits, 48-63, Copyright 2018, with permission from Elsevier.

Materials without specific applications

One of the most appealing aspects of material science may be found in developing a material or a formulation without a specific application in mind. Silane chain transfer techniques have been demonstrated in photoinitiated silane-ene chemistry.[193] In this method, a difunctional bis(trimethylsilyl)silane unit possessing abstractable hydrogens was examined in homopolymerizations with acrylates as well as in crosslinking reactions that ultimately yielded gels, albeit at slower rates compared with traditional thiol-acrylate reactions. As with more traditional resin systems, material property tuning is achieved by varying silane and acrylate ratios, such as strains at break ranging from *ca.* 5 to 25%. While no specific applications were

proposed or demonstrated, one could envision the translation of this technology towards siloxane printing for soft tissue applications, anti-fouling surfaces for catheters, or even lower friction surfaces in completely polymeric total joint replacements.

PDMS-derived materials are of high importance for medical technologies, as traditionally manufactured siloxanes are used for prostheses, soft tissue implants, and blood contacting devices, but processing using additive manufacturing has been difficult. Progress has been made in processing, such as with reactive inkjet printing using a commercially available two-part silicone (Polytek PlatSil 71-Silliglass), where the PDMS crosslinking took place in the presence of a platinum catalyst and siloxane oligomers.[51] PDMS-resin (Dow Corning SE 1700) was further used for direct ink write examinations of nanosilica-filled materials containing 40% (volume) microballoon shells of poly(acrylonitrile-co-vinylidene chloride-co-methyl methacrylate) (AzkoNobel Expancel 551 DE 40 d42) or formaldehyde resin microballoons (Asia Pacifici Microballoons BJO-0930), after which materials were thermally cured up to 150 °C to induce crosslinking. These materials displayed shape memory and a multi-regime mechanical response related to the presence of different fillers; the obvious limitation of such materials is the unknown bio- and cytocompatibility of most components, as well as the lack of specific medical application. However, these and follow-on control PDMS studies further elucidate the level of control possible for designing the next generation of biomaterials.[194, 195] A possible future candidate for surface contacting materials, such as those found in prosthetics or replacement skin/digits, could be found in polysiloxane photopolymers developed in the Long group, with thermal stabilities exceeding 200 °C in addition to preliminary photopolymerizations using SL.[196] While this work did not target biological applications, there still exists a need for personalized prosthetic inserts and sleeves in order to reduce discomfort and abrasions in patients

due to rubbing. 3D printable siloxanes, which have been notoriously difficult to realize, offer a promising solution to this problem by providing avenues to personalized prosthetic sleeves and pads, not just the sockets currently made using FFF.[197, 198]

Semi-crystalline polyurethanes are finding use in FFF due to their excellent thermomechanical behavior, including the distinct melt and flow conditions required for precise printing. The majority of work currently being done with new materials is focused on development of printable compositions and characterizing and application-specific properties, as opposed to examining biomaterial-tissue interactions. However, a number of these systems, including green-source polyurethanes made from 5-membered cyclic carbonates without isocyanates, are quite promising due to both their material properties as well as their safety; non-isocyanate polyurethanes do not have the same synthetic and processing risks as traditional polyurethanes.[199] Poly(carbonate urethanes), while not specifically being used for 3D printing, are used to produce scaffolding materials to better treat bladder tissue engineering, on the grounds that large deformations are not well compensated for using traditional biomaterials, a trend repeated with contemporary 3D printing materials as well.[200] With such materials produced, the range of applications would then match those already existing for polyurethane materials.

Porous polyurethanes are well known for their potential in minimally invasive devices, leveraging shape memory behavior, although there are obvious limitations associated with the pore morphology and size distribution in such structures.[201-208] An exciting recent development in these materials is with reactive inkjet printing of PEG and several diisocyanate species to produce porous polyurethanes, although this method is still limited by uncontrolled porosity as a consequence of possible side reactions of the isocyanate groups that yield carbon dioxide leading

to pore formation.[50, 52] While this is a promising start to overcoming such limitations in printable materials with advanced properties, there is obviously extensive development still needed, and currently no specific medical applications have been proposed. However, we see that such materials could find use in the previously established applications for polyurethane foams, including aneurysm occlusion, soft tissue fillers, and orthopedic fillers.[201, 202, 209, 210] A series of work produced by Maiti *et al.* claims superiority of printed foam structures compared with traditionally foamed PDMS, however the stochastic foam used as a control is substantially different from the designed structure. While this demonstrates the potential of 3D printing in controlling production, it does not lend itself well to the argument for printing foam based upon the porous materials obtained during traditional foam blowing.[211]

However, one of the most exciting studies in porous "foam" materials was recently reported using FDM-processed poly(lactide), where gas-blown foams were used as templates for 3D printing (Figure 7).[212, 213] The presented work focused solely on mechanical behaviors, but the ability to mimic the fine features, as well as assemble specific unit cells in the structure, offers a promising avenue forward for controlled 3D printing of minimally invasive medical devices if a different material was utilized, such as the aforementioned polyurethanes. The issue with the PLA approach is its rigidity, and minimal strain achievable before failure.[214]

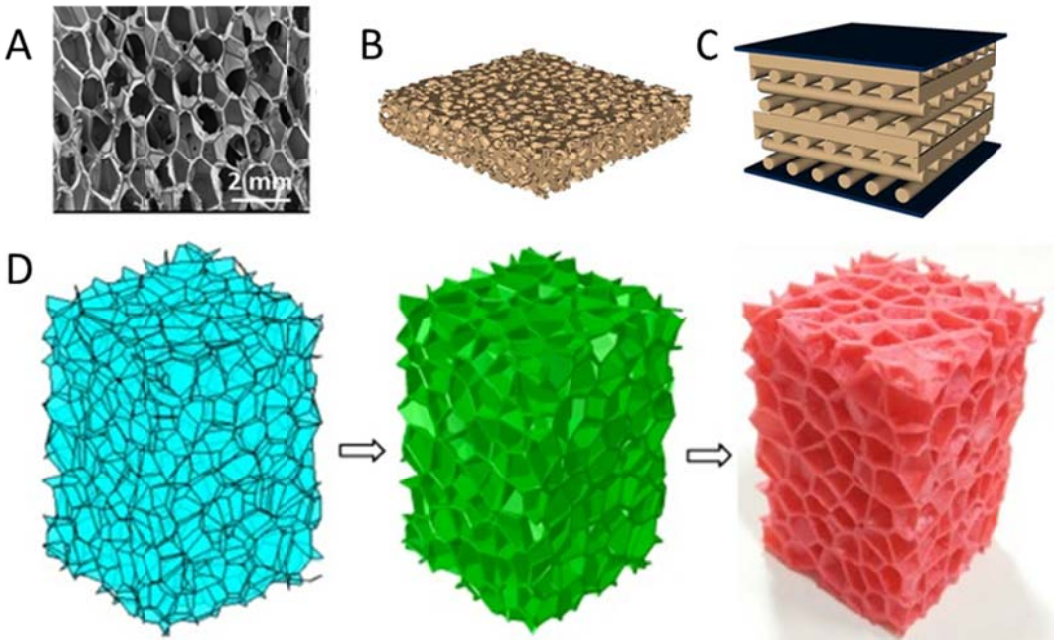

Figure 7. A porous polyurethane foam cross-section (A) and cartoon rendering (B) are compared with typical examples of printed porous structure (C). In comparison, a novel approach with developing printed foams based upon reassembled foam structures has been attempted, moving from wire-frame outline to a 3D rendering of the foam and pore membranes prior to production of the final 3D printed structure (D). (A) Reprinted from Acta Biomater., 59, Weems, A. C. W. *et al.*, Shape memory polyurethanes with oxidation-induced degradation. In vivo and in vitro correlations for endovascular material applications, 33-44, Copyright 2017, with permission from Elsevier. (B and C) Reprinted by permission from Macmillan Publishers Ltd: Sci. Rep., 6, Maiti, A. *et al.*, 3D printed cellular solid outperforms traditional stochastic foam in long-term mechanical response, 24871, Copyright 2016. (D) Reprinted from Mech. Mater., 127, Wang, S. *et al.*, Crushing and densification of rapid prototyping polylactide foam: Meso-structural effect and a statistical constitutive model, 65-76, Copyright 2018, with permission from Elsevier.

Conclusions and Future Perspectives on the Translation of Research Towards the Clinic

The field's general progress towards clinically relevant 3D printed biomaterials may be viewed in both positive and negative lights. A positive trend in the field has been the development of the printing technology, as seen by recent developments of CLIP and HARP for rapidly producing large structures.[40, 41] Negatively, we have not yet been able to achieve a wide variety of implantable devices through 3D printing. There are a number of reasons for this, including the typical regulatory difficulties that include: stringent testing of extractable and leachable components from the device; biocompatibility; consideration of performance in the tissue of interest; degradability; possible toxicity considerations; and ultimately the limited body of literature surrounding the material and application. The regulations surrounding medical devices are important to be fully considered, as such reviews are crucial to prevent patient harm due to negligence or improperly tested materials. However, sufficient testing and device design allow avenues to overcome these regulatory hurdles, as demonstrated with the Morrison study on pediatric 3D printed tracheal airway stents.[75]

One example for this would involve selecting polylactic acid or its derivatives for the production of 3D printed devices, such as bone plates or stents. Assuming optimal device design and performance, and taking into account the limitations that some stent studies have suffered due to poor material selection, PLA is a regulatory-friendly material that could streamline part of the regulatory process. Indeed, regulatory bodies do not regulate materials, but devices based on commonly used materials are better understood and may be more likely to gain approval as the long-term behavior and possible complications are already known in a larger sample population compared to unknown materials. The same battery of tests is necessary for accessing safety, but these can be conducted through a lens tempered with a body of literature.

A second example for overcoming this limitation is to focus on a relatively unknown material, for which more robust study designs are necessary to navigate the regulatory process. It is this example that we feel is more relevant for the next-generation biomaterials' 3D printing. The materials design component of the regulatory process here would need to include careful consideration and early adoption of a material platform that could meet the needs of a few specific clinical problems (*ie* a hydrogel for use in aneurysm occlusion as well as subcutaneous trauma repair). By establishing a firm hold over how to tune the materials properties and rigorously validating the bio- and cytocompatibility in statistically powered studies, early proof of concept may be obtained to justify support in future applications, which is essentially what has happened with PLA and PCL systems. We envision this step as crucial, as it is only with this proof of concept that translation may be achieved, and a material can find clinical usage.

In general, a number of early design factors may be considered to down-select the number of possible biomaterials for an application. These considerations include degradation products, possible adverse events in the specific application or device, and release profiles (of degradation products, leachables, and drug payloads) may be used as early screening methods to rapidly distinguish the truly promising translatable materials from those that are currently only of academic interest, which, although being equally important, deserve a different kind attention. Materials which produce known toxic, mutagenic, or carcinogenic moieties, or those which significantly alter cell culture media during *in vitro* tests, are most likely not going to be translatable. For materials that do possess one of these facets, for example a material which produces oxalic acid which has been problematic for renal function at sufficiently high concentrations, a series of degradation studies, cytocompatibility assays, and *in vivo* work may be used when viewed through the lens of toxicological risk. Further limitations are introduced by

materials which undergo thermal degradation, a major limitation for FFF methods, as well as materials which display poor integration within the entire part during printing. For example, FFF methods display varied layer integration as a function of temperature, which not only introduces anisotropy within the part but also between batches, which limits the reproducibility and would reduce the likelihood of regulatory approval. While research has focused on controlling the extrusion and plate temperatures to reduce this, in degradable biomaterials such as PLA these features also affect material properties including crystallinity and mechanical strength. Therapies that contain proteins as surface coatings, drugs with toxic side effects which may rapidly leach, or other biologically active components increase the regulatory burden prior to translation may be achieved. In these cases, the matrix material (the "carrier" of the active component), the active component, and the combination will all need to be examined using similar testing as with just the material itself prior to human use. Again, such considerations, when accounted for early in the design process, will reduce the number of "promising" biomaterials and potentially allow the community to focus resources on those with greater likelihood of translation.

From a more general point of view, we envisage the next generation of biomaterials for tissue engineering, therapeutic and diagnostic applications as multi-material, architected, shape-morphing and bio-inspired systems. Only by combining all these concepts will the printed construct mimic the unique features of complex human tissues and organs. Indeed, state-of-the-art biomaterials and additive manufacturing already enable the modelling of relevant physical and chemical factors for *in vitro* cancer models, for instance. [215]

However, 3D is not enough. Notably, cell-printing techniques, which allow for the precise spatial/temporal control of cell positioning, and 4D printing (*i.e.* fabrication of 3D objects based on "smart", stimuli-responsive polymers that change their chemical and physical properties in

response to specific environmental cues[216]) show high process flexibility and versatility.[217-219] Specifically, 4D printing, which uses similar technology than 3D printing,[220] allows for the fabrication of cell-instructive materials, also known as "smart" biomaterials,[221] with controlled structural features (size, geometry, and porosity), while functionality is triggered by external stimuli, such as temperature, light, magnetic force, pH, among others, forcing the cells to adapt and respond as it happens *in vivo*. For example, the mechanical stimulus imparted by the recovery of 3D shape memory polymers influenced the shape of cells and nuclei, which suggested that single mechanical stimulus is sufficient to initiate changes in the morphology of adherent cells.[217] In addition to shape change, with adequate polymers, instructions could also be printed to release a drug in a localized and sustained manner[222] or switch between bacteria-adhesive and bacteria-resistant forms in response to pH changes, allowing detection and removal of inactivated microorganisms.[223] Moreover, dynamic changes in the mechanical and structural features of the 4D printed objects can be exploited to understand in depth how these parameters influence cell adhesion, proliferation, and differentiation processes. Thus, as AM technologies continue to evolve, the library of polymers and useful material chemistries will need to do so as well to eventually transition from 3D to 4D printing for advanced functional materials.

Overall, the development of the next generation of biomaterials will be undoubtedly assisted by 3D printing technologies as they allow for simple and reproducible manufacturing, which ultimately results in better tissue regeneration and host integration. Not only that, but AM platforms will play a decisive role in producing materials with widely tuneable property gradients,[224, 225] as well as multi-material complex devices with a wide range of functionalities.[226] For instance, McCracken *et al.* used DIW to pattern ionotropic gradients in hydrogels.[227] Spatial gradients, when combined with geometry, generated 4D-printed structures that actuate in the

presence of local magnetic fields. All these works exemplify how 4D design complexity is accessible by 3D-printed gray-scale/gradient mechanics. As the potential of 4D printing in the biomaterial field is being understood, efforts will continue to design tools for modelling smart materials distributions, which facilitates the simulation of heterogeneous 4D objects made, among others, of shape changing smart materials.[228]

Finally, a great many of the presented studies here displayed very promising results, but the need to constantly find novelty and excitement in biomaterials is partially a hinderance towards translation. While innovation in the materials space will provide materials that are designed to 3D print, there is simultaneously a need for an extensive amount of testing that aims to establish greater control over material properties and provide the breadth of research that is already been performed for PLA. Only in this way can we as a community progress in developing alternative materials which may be more suitable for tissue engineering, implantable devices, and other bio-related 3D printing.

3D printing has the potential to revolutionize healthcare beyond just personalized implants, particularly as materials design and polymer chemistry advances. Over the course of this review, we have highlighted a number of potentially interested developments across a range of applications in 3D printing for clinically relevant materials. In summary, there are several trends which may be considered from the ongoing research across clinically translatable 3D printing. With regards to mechanical processing, composite materials may be expected to produce more mechanically robust scaffolds; however, the processing method may be more important to consider. For instance, FFF is known to result in layer integration issues, and so possible alternatives could be direct ink write or multi-material processing techniques to overcome this limitation. For better integration, photopolymers may be a better choice traditionally processed

as resins in vat polymerization, but DIW may offer a broader range of processing temperatures and 2PL may greatly enhance the possible surface feature resolutions compared with SL. All of this should be considered along with application-specific requirements, such as mechanical stability and material processability, as it is entirely possible that the more economic or more traditional method may still be more suitable for many applications. With regards to the material selection, the diversity of research being performed is both exciting and promising. Importantly, there does not seem to be a delineation of most materials into a limited number of tissue types; however, we also feel that more discretion may be needed for certain materials. While PCL and PLA are widely used in all fields, the long-term performance of these materials is questionable for some applications and more interesting candidates are being developed. It is our hope that such considerations may aid further the development of polymeric materials for clinically relevant therapies, scaffolds, and devices, to ultimately change how medicine is performed and improve the quality of life among many patients.

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