Advancing biomaterials towards biological complexity

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3D printing has the potential for on-demand, patient-specific devices with high structural complexity. However, the tools and biomaterials most widely available for 3D printing are largely limited to fused deposition modelling (FDM) with commodity plastics that lack cell-scale resolution and biological cues. The lack of biologically active materials, also called 'bioinks,' is a key issue that limits the ability to produce engineered tissues, tissue interfaces and functional organs. While advances are being made in the field of biomaterials, current technologies can be combined into hybrid systems that overcome the limitations of a single technique or material, as described in this article.

Biomaterial limitations in current 3D printing practice

Biomedical 3D printing and other biofabrication techniques rely heavily on polymers, which are repeating chains of synthetic or natural building blocks. Polymers can range from synthetic commodity plastics, such as polyethylene used to make shopping bags, to customized materials with tunable properties, such as some of the tyrosine-derived polymers described below. The medical applications of 3D printing today are largely in the field of dentistry, prosthetics and surgical guides that use predominantly non-degradable polymers as printer inks.

One of most common methods for 3D printing is FDM, where molten polymer is extruded through a nozzle in a programmable pattern. This technique is a fast, versatile and accurate way to build complex shapes from one or more materials. It is by far the most widely used 3D printing technique and dozens of inexpensive printers are available for beginners and professional users alike. However, the currently available polymers greatly reduce the practically achievable resolution. While motors are typically accurate down to a few microns, the extruded polymer fails to regularly obtain resolutions better than 50 μm.

Although increased resolution is necessary to engineer functional tissues and organs, it is also important to pair this technology with the development of better bioinks. Commonly used polymer inks for FDM printing have a sharp melt transition, which means that by shifting the temperature by only a few degrees, they can transition from a solid to a honey-like state, allowing them to be printed and then quickly harden. The polymers used most frequently in FDM are poly(lactic acid) (PLA), poly(butyl styrene) (ABS) and poly(caprolactone) (PCL). These three polymers cover a large range of properties, where PLA is the stiffest, PCL is the most flexible and ABS is the strongest. However, ABS does not degrade, PLA degrades with an associated burst of acidic by-products and PCL degrades too slowly for many applications. When designing the next generation of 3D printable polymers, the rate of degradation, associated by-products and mechanical properties must be taken into account.

The transition to degradable bioinks

The medical field is transitioning towards materials that will degrade and ultimately be resorbed while actively participating in the healing process. Ideally, polymeric implants will interact dynamically with the body, degrading at the same rate as they are replaced with the patient's own tissue. Polymers can be modified to degrade at defined rates in the presence of water. One approach to designing degradable polymers is to use only natural building blocks that are neither toxic nor acidic and to make sure that upon degradation, the very same building blocks are recreated. In this way, possible toxicity concerns of the polymer degradation products can be largely alleviated. We have used the natural amino acid L-tyrosine to develop a library of degradable polymers with widely tunable mechanical properties, biological activity and degradation rates ranging from minutes to years. These polymers release the same tyrosine-based building blocks upon degradation, a feature that was helpful in the commercialization of several medical implants.

Two promising applications of our family of tyrosine-derived polymers are a 3D printed meniscus (Figure 1) and an implantable brain electrode (Figure 2A). The electrode was coated with a thin film of stiff, degradable polymer to allow it to be inserted into the brain. Once the electrode is inserted, the polymer

is exposed to brain tissue and degrades within two hours, allowing the very fine and flexible electrode to interact directly with the brain tissue while limiting scarring. To ensure the correct mechanical properties of these devices, we have used two different polymers. For the brain electrode, we selected an exceptionally stiff but ultra-fast degrading polymer that allowed the rapid exposure of the electrode to the brain. In contrast, for the meniscus replacement device we selected a polymer that is strong, resilient for repeated loading in the knee and relatively slowly degrading to provide sufficient time for the patient's own cells to infiltrate into the device and recreate a functional meniscus. These two applications illustrate the critical importance of selecting bioinks with appropriate mechanical properties for the desired application and confirm the need to develop bioinks with a wide range of mechanical properties.

Polymer properties and fabrication techniques can be used in combination to match the stiffness and elasticity of a tissue regeneration scaffold to the target tissue. Compared with conventional scaffold fabrication techniques (such as porogen leaching), 3D printing can be used to generate large macroscopic pores in a more reproducible manner. Because of the current resolution limitation of 3D printing, this technique cannot be used to generate reproducible arrangements of micropores with dimensions less than 50 μm. Hence, additional fabrication techniques are still needed to produce the smaller micropores required for cells. Scientists have been trying to address the challenge of creating reproducible and highly regular pore structures within tissue regeneration scaffolds with a myriad of porogens and materials. Salt crystals are often used as a porogen, as they can be processed with the polymer and then washed out of the final product, leaving behind open macropores resembling a sponge. Porogen leaching can be combined with freeze drying to incorporate additional micropores on the cell scale to create pore structures that almost look as if they were created by living organisms (Figure 2C). We have shown that such complex scaffolds comprised of macropores (about 200–400 μm in diameter) and micropores (about 5–20 μm) are beneficial for bone regeneration (Figure 4). An additional level of complexity can be achieved by incorporating hydrogels into the design of a tissue regeneration scaffold. Hybrid scaffolds containing hydrogels can be used for the regeneration of soft tissues. We are currently exploring these types of scaffolds as a growth substrate for dental pulp stem cells in an attempt to regenerate living teeth. Figure 2D illustrates how these hydrogels are filled into a well within a porous bone regeneration scaffold. Ultimately, these scaffolds are designed for implantation within the mandible to allow the regrowth of living teeth.

Figure 1: Applications of a degradable bioink. Meniscus repair scaffold fabricated at the New Jersey Center for Biomaterials in collaboration with the Rutgers Orthopaedic Research Lab. This 3D printed implant is currently under commercial development by NovoPedics, Inc.

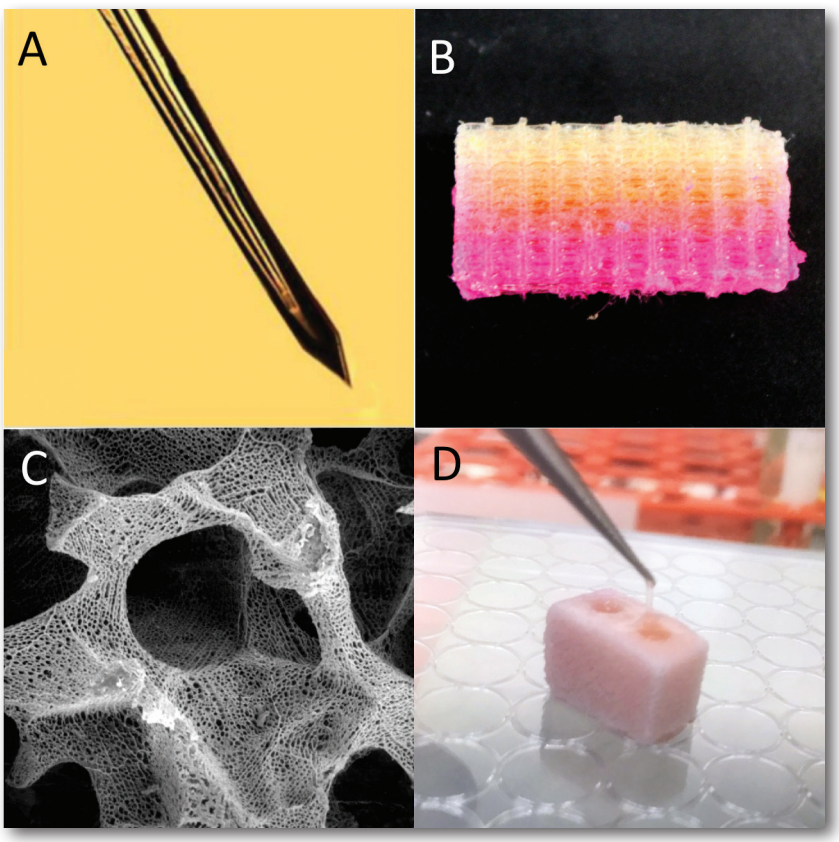

Figure 2: Synergistic combinations of fabrication techniques create fascinating new implants and devices. **(A)** Combining photolithography with solvent casting provides a reinforced neural probe for insertion into brain tissue. **(B)** 3D printing combined with airbrushing provides a complex scaffold with a gradient of properties. To illustrate this capability the 3D printed scaffold is filled with airbrushed fibres loaded with a red or yellow pigment. Changing the ratio of yellow and red fibres creates a gradient of colours. **(C)** The combination of porogen leaching and freeze drying provides a bone regeneration scaffold with a bi-modal pore distribution. Such scaffolds have been shown to be particularly effective in regenerating bone (see also Figure 4). **(D)** A porous scaffold with hydrogel inserts is used in an attempt to regenerate living teeth.

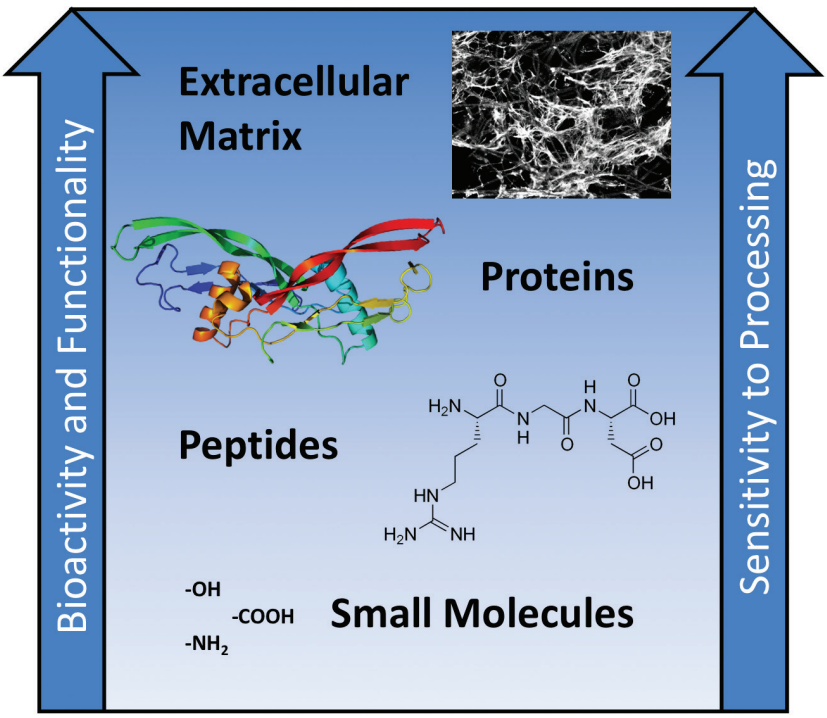

Figure 3: Modifying materials can improve functionality. The balance between functionality and sensitivity to processing conditions must be considered when designing a biomaterial. In general, the more complex the structure the more difficult it is to identify suitable processing and fabrication conditions.

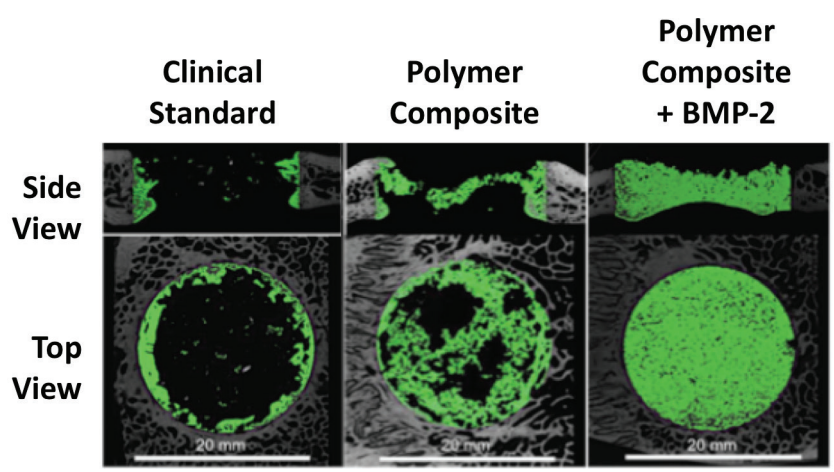

Figure 4: Functionalization of tyrosine-derived polymer composite scaffolds with BMP-2 promotes bone regeneration. Scaffolds were implanted in a goat calvarial critical-sized defect with or without and to make sure that upon degradation for 16 weeks. Green represents new bone as assessed by microCT. One of the products used clinically in human patients supports only marginal bone regeneration (left panel). Significantly better results are obtained with the experimental, state-of-the-art bone regeneration scaffold shown in Figure 2B. The best results are obtained when the same scaffold is augmented with the specific biological function of BMP-2.

The transition to functional bioinks

The correct physical properties and geometry are necessary, but not sufficient, to create a truly bioactive scaffold. The base bioink often requires surface functionalization with increasingly complex cues (Figure 3). Altering surface charge and chemical functionality are the simplest ways to promote non-specific cell attachment. Modifying the surface with chemically reactive functional groups can be achieved by means as simple as surface treatment with plasma or chemical etching. While effective, more complex surface chemistry patterns are required for the attachment of particular cell types in specific locations. This is important because when a device is implanted only a subset of cell types within the body will aid in tissue regeneration, while others will actively interfere with functional recovery. Specifically, the tendency of fibroblasts to migrate into tissue scaffolds and form scar tissue is an example of an undesirable outcome.

Cell signalling peptides, which are short sequences of amino acids often derived from a full-sized protein, can be attached to the polymer to control cell attachment, migration, proliferation and ultimately also differentiation. The best-known example of such peptides is the tri-amino acid arginine-glycine-aspartate (RGD peptide). The chemical attachment of cell signalling peptides to a polymer is generally referred to as 'tethering'. We use surface-tethered peptides to direct cellular behaviour within tyrosine-derived polymeric scaffolds.

Whole proteins generally induce the strongest cellular responses in comparison to peptides, but are much more expensive and more difficult to work with. In spite of these drawbacks, proteins have been attached to the surface of biomaterials through a variety of methods, including physical adsorption, covalent bonding and non-covalent attachments. One example is the cytokine bone morphogenetic protein 2 (BMP-2), which is known to greatly improve the regeneration of bone. When used in combination with our porous bone scaffolds, BMP-2 increased bone ingrowth, facilitating the healing of very large defects (Figure 4).

The transition to hybrid scaffolds comprised of natural and synthetic components

The key limitations of 3D printing using FDM are a lack of resolution at the cellular level and a lack of bioactivity of the available bioinks. While it is possible that these limitations will be overcome by future technological advances, it seems that this process will take many years. Yet, in 2017 there were over 116,000 people in need of a life-saving organ transplant in the US alone,

but only 26,000 transplants were performed. There is a compelling and urgent need to make synthetic alternatives available to address the growing deficit of human organ donations. One innovative approach to address this goal is using FDM to lay down the reinforcing 3D structure of an engineered, artificial organ with synthetic bioinks and then use biologically derived bioinks to fill in the gaps with high-resolution and biospecific materials, forming a hybrid scaffold.

The most promising material for use in a hybrid scaffold is the extracellular matrix (ECM), a complex, tissue-specific and dynamic network of proteins as well as other components that is an ideal environment for tissue regeneration. The ECM contains the 3D architecture necessary to promote cell migration and differentiation. Starting from any tissue, cells are removed in a process termed 'decellularization'. Removing cells from the tissue leaves behind the ECM and reduces the immune response when the decellularized ECM is implanted into another patient. Decellularized skin, placenta and bone are examples of tissue-derived ECM that are already used clinically in reconstructive surgery to promote healing. Although tissue-derived ECM has been used as a source of biological scaffolds in tissue engineering and regenerative medicine, the decellularized ECM is often quite flimsy and lacks the strength and ability to retain a complex 3D structure.

This limitation can be addressed by an innovative approach: we use 3D printing to create a reinforcing structure that is then filled with natural ECM. This concept is similar to the use of steel beams that reinforce a layer of cement in modern buildings. This approach benefits from the fact that the 3D printed support structure does not have to be bioactive or have ultrafine resolution. Any 3D printable, degradable biomaterial can be used without further refinements in 3D printing technologies. The ultrafine resolution and bioactivity are provided by the natural ECM that is deposited within the 3D printed support structure. This hybrid approach can be used to combine the mechanical strength of synthetic scaffolds with the bioactivity of decellularized ECM to provide an ideal environment for 3D tissue regeneration and build large, complex structures with significant biological activity.

The future of 3D printable biomaterials

In order to produce synthetic tissues, the regenerative tissue scaffolds must be designed to recruit multiple cell types and induce the appropriate dynamic cellular behaviour. This will involve the use of multiple biomaterials with patterns and gradients of stiffness, porosity and functionality at the resolution of the cell. This is not currently possible with a single technology or material. One promising technique is hybrid scaffolds, which combine the speed and versatility of FDM with decellularized ECM to improve resolution, functionality and cellular response. Additionally, naturally derived polymers can be tailored and functionalized with a library of peptides, proteins and ECM to create a biomaterials toolbox for the design of complex tissues. Understanding how biomaterials can be synergistically combined to control the cellular response is an exciting area of current research. The ultimate goal of being able to print a functional liver, kidney or heart replacement is still many years away, but recent advances in the design and fabrication of complex regenerative tissue scaffolds are a promising beginning. ■

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Joachim Kohn is the Board of Governors Professor of Chemistry and Chemical Biology at Rutgers University and the Director of the New Jersey Center for Biomaterials. Dr Kohn is a leader in biomaterials science and widely known for the development of tyrosine-derived, resorbable polymers, which are now used in several FDA-approved medical devices. His current research efforts focus on the development of new discovery paradigms for revolutionary biomaterials using combinatorial and computational methods to optimize the composition, properties and cellular responses of biomaterials for specific applications, particularly tissue engineering and drug delivery. Email: kohn@dls.rutgers.edu.

Further Reading

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