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EDITORIAL

Three-Dimensional Bioprinting in Regenerative Medicine: Reality, Hype, and Future

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Regenerative medicine aims to repair or restore function to tissues and organs. The field has seen major advances recently even if serious challenges still remain, stemming primarily from the inherent complexity of the human body. The organs in our body are results of millions of years of evolution in the course of which nature experimented until it "got it right." This experimentation was not without victims: many of the shapes and forms that appeared eventually were eliminated by natural selection, favoring those with the most relevant function and compatible intricate structure. As a consequence, present day tissues and organs are all complex, and their complexity varies depending on the cell type and organization, architecture, and function. Flat tissues (e.g., skin) are the least complex, tubular structures (e.g., blood vessels) more complex, hollow nontubular organs (e.g., bladder) even more complex, and solid organs (e.g., liver or heart) the most complex.

Just three decades ago, most human cell types could not be grown and expanded outside the body, human stem cells had not yet been identified, and technologies such as cloning had not yet been developed. Today, with advances in cell and molecular biology, most types of cells can be grown in vitro, multiple types of human stem cells have been established and organs have been engineered in the laboratory. Engineered organs such as skin, urethra, blood vessel, bladder, and vagina have been implanted in patients in limited numbers in clinical trials. Several tissue-engineered products are advancing through the regulatory pathway so they can be eventually commercialized and disseminated widely. However, most of the engineered tissues now in the more advanced stages of the regulatory pathway were made by hand.

The engineering of a living tissue and its fabrication by hand require several steps, each with its own hurdles. The undertaking starts with the sourcing of the seed population of cells. For this, typically, a tissue biopsy is performed. The resulting cells then have to be grown and expanded in vitro. This may sound easy. However, reliably obtaining cells with similar release criteria that will perform with the expected level of function requires extensive knowledge of cell and molecular biology. Harvesting and cell expansion techniques, culture media protocols, growth factor additives, environmental conditions, and sterility are just a few of the many details necessary to have the right cells as a starting material. Furthermore, an adult liver for example contains approximately 100 billion cells, and is composed of multiple cell types, including hepatocytes, stellate cells, and Kupffer cells. In order to create such an organ, the various cells need to be expanded at the same time, to large numbers and thus divided many times outside of the body. Extreme care needs to be exercised to make sure that the resulting cells do not become transformed and remain functional consistent with their intended use.

Equally complex are the biomaterials that are necessary to make normal tissues. They need to have the right properties to support cells in vitro but also characteristics to make them biocompatible and suitable for impantation into the patient. Finally, adding the cells to the biomaterials with the interactions necessary to have the right environment for tissue formation in bioreactors and incubators adds yet another level of complexity to the process.

In parallel to the development of regenerative medicine, the field of three-dimensional (3D) printing has also advanced. Three-dimensional printing has been used in the manufacturing industry for decades. It was first applied to make plastic samples to be used as prototypes for more complex parts that would typically be later mass produced. This was a laborious and costly process. The printers initially were cumbersome and expensive pieces of machinery, some with a cost of more than a million dollars, and technically difficult to operate. Technological advances have led to the current state-of-the-art printers that are being used to manufacture a wide range of products, from complex tools to automotive parts and entire buildings. The design and operation of 3D printers have been markedly simplified. Desktop 3D printers can now be used even by children and are being sold in toy stores at a low cost, some for less than $100.

Three-dimensional printing, now also a major component of additive manufacturing, led to the more recent advent of bioprinting, the specific

application of the technology in the life sciences and medicine. Just like 3D printing can assemble different inorganic materials together into actual products, the process of bioprinting can assemble cells, proteins, and hydrogels into living structures. Although scientists in regenerative medicine initially had been constructing tissues manually to bring the technologies of the field to patients, it soon became obvious that the process needed to be automated, as the by-hand approach was arduous, time-consuming, technically demanding, and expensive. Bioprinting had the potential to offer several advantages, such as reproducibility, precision, automation, scalability, and lower costs; features that could eventually allow the delivery of tissues on demand.

The modern era of bioprinting commenced in the early years of the 21st century with the appearance of the inkjet bioprinter. Progress since has been remarkable, as can be judged by the steadily increasing number of publications and professional meetings devoted to the topic or by the number of Google-search entries on the subject. Inkjet bioprinting was soon followed by other specialized formulations of the process (i.e., extrusion, acoustic, laser, and liquid bioprinting). With the appearance of commercial entities, the field has matured beyond basic academic research.

Unfortunately, as with any new technology, a number of forward-looking statements were made and erroneously interpreted as the then prevailing reality. By the time the general public became aware of bioprinting, 3D printing with inanimate materials had already produced a myriad of useful products expeditiously. Thus, many could expect that bioprinting, being a specific application of the more general technology, would deliver equally versatile and easy-to-manufacture living biological products; the field of 3D bioprinting quickly captured the public's imagination. Just like toys could be printed with the push of a button, many thought, tissues and organs would be printed in a similar manner. However, nothing could have been further from the truth.

The fabrication of tissues by hand is complex, but the conversion of the technology to 3D bioprinting compounds the complexity. The printing of biological products implies a number of challenges specific to living matter. For example, whereas printing nonliving matter results in a useful product as soon as printing is complete, this is not the case when cells are involved. Bioprinters deliver bioink units, the analogues of the inorganic ink drops. These delicate structures, prepared in advance, are composed of cells and cell-supporting materials. The latter are typically some sort of biocompatible hydrogel, a novel material, specifically developed for the cell types to be printed and playing the role of a temporary extracellular matrix during printing. The cells within the bioink units have to survive the process of going through fine nozzles. This imposes stringent requirements on the biomechanical and architectural properties of the bioink units. The discrete bioink units need to self-assemble into useful biological constructs postprinting. This process, referred to as maturation, is governed by biology and requires time. During maturation the printed construct must be maintained in bioreactors that provide the environment to keep it alive. Numerous conditions and parameters need to be fine-tuned to eventually produce a tissue or organ that has the needed compositional integrity, 3D organization, and adequate properties for the desired function and implantation into the patient. If the correct conditions for maturation (e.g., temperature) are not provided, no useful product emerges. In short, 3D bioprinting is intricate and multifaceted.

The above discussion may suggest that we might in vain hope for the anticipated benefits to be delivered by bioprinting in regenerative medicine, replacement organs, and other domains. This, however, is not necessarily the case; it all depends on one's realistic expectations. In our view, bioprinting will bring about innovation in several areas, some already under way.

As bioprinting allows for the construction of anatomically and physiologically accurate 3D biological structures, it is poised to advance the drug development process. Tissue models, such as liver or cardiac organoids fabricated with human cells may precede animal trials to test for efficacy and toxicity. Bioprinted tissues can be interconnected (e.g., liver, heart, kidney, etc.) to test drugs on a body-on-a-chip model before the start of human clinical trials. With improvement of such models, eventually animal trials can be reduced and, many of us hope, perhaps even eliminated. On the therapeutic side, bioprinting will likely follow the same sequence as with hand-made organs, achieving clinical success first with the least complex tissues, such as skin, that are already being delivered, even if not yet shelf-ready.

In the more distant future, with further progress in large-scale cell culture, bioprocess engineering, and genetic strategies, it is possible that we will be able to design specific printable living structures that are not even conceivable today. Tissues printed with gene-edited cells from the diseased patient to achieve a normal endpoint or combination of extended bioprinted tissue units functionally interconnected similarly to that in the human body are examples that could lead to unforeseen progress in regenerative medicine. Bioprinting offers many promising opportunities. However, patience and perseverance are needed to realize the full potential of the technology.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.