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Cover story

Bioink-guided spatio-temporal gene delivery for tissue engineering

The essence of tissue engineering is to combine biomaterial scaffolds, cells, and appropriate biochemical and/or physicochemical factors to replace or regenerate tissues or organs damaged by trauma or disease. The great premise that kick-started the field, however, remains elusive. Despite almost four decades of research, there are relatively few examples of tissue engineered products in clinical use. In orthopedic medicine, the use of autologous cultured chondrocytes on a porcine collagen membrane to treat symptomatic cartilage defects of the knee is a rare example of a clinically successful tissue engineered product [1]. Engineering more complex tissues, such as the interface between hard and soft tissues (*e.g.*, bone–articular cartilage, bone-ligament, bone-tendon), remains a significant challenge. Traditional tissue engineering strategies have proven inept for specifically recapitulating the spatially defined structure, composition and biomechanical properties of such musculoskeletal interfaces. This has motivated increased interest in the use of three-dimensional (3D) bioprinting to deposit cell, protein and/or gene laden bioinks, in a controlled manner to engineer spatially defined implants capable of regenerating complex tissues.

The work of Daniel Kelly and Fergal O'Brien and their teams in this issue describes a hydrogel bioink that can be used to spatially and temporally control the presentation of therapeutic genes to stem cells within 3D printed constructs [2]. By blending fast and slow degrading hydrogels, they were able to produce bioinks whose porosity increased with time following printing. They next incorporated nanoparticle-pDNA complexes [3,4] into these bioinks and found that their rate of release was higher in pore-forming bioinks compared to solid bioinks. When stem cells were co-encapsulated with pDNA complexes encoding for reporter genes within the bioinks, the transgene expression was increased in the pore-forming bioinks. Modulating the porosity of these bioinks made it possible to direct either rapid and transient, or slower and more sustained transfection of host or transplanted cells *in vivo*.

To demonstrate the utility of these bioinks, the Kelly-O'Brien team delivered either osteogenic (BMP2) or chondrogenic (combination of TGF-β3, BMP2 and SOX9) genes to mesenchymal stem cells *in vitro* and *in vivo*. Specific combinations of bioinks, delivery vectors and plasmids allowed engineering of stable cartilage or bone-like tissues. These bioinks were used to engineer spatially complex tissues such as osteochondral unit. Stem cells and plasmids encoding for either osteogenic or chondrogenic genes were zonally positioned within a 3D printed scaffold to produce spatially-defined, gene activated implants. *In vivo*, these bioprinted tissues supported the development of a vascularized and mineralized tissue overlaid by a layer of stable cartilage.

The regeneration of spatially complex tissues *in vivo* has proven to be highly challenging. By combining nanoparticle-mediated gene delivery and a new class of pore-forming hydrogel bioink, the Kelly-O'Brien team has developed a new approach to locally control the differentiation of stem cells *in vivo*. Testing such gene activated constructs in a more clinically relevant large animal model is needed to better determine the efficacy of this approach. Furthermore, a route to cost-effectively scale the production of such cell and gene-laden biomaterials is required. The importance of this study is that it shows how plasmid DNA uptake over time can be controlled by tuning the porosity of a hydrogel, and furthermore how such biomaterials can be processed into cell-laden bioinks capable of locally producing therapeutic proteins within 3D bioprinted implant to recapitulate the native complexity of biological interfaces. The tissue engineering field has come a long way, and eventually it will reach the goal of producing tissue-engineered human parts. It will, however, require better understanding of complex body responses against artificial implants. In the meantime, the tissue-engineered constructs can be used as representative organs mimicking *in vivo* conditions for testing the efficacy of various drugs. The Kelly-O'Brien approach moves the field in the right direction, one step closer to the goal of clinical applications.

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