REGENERATIVE MEDICINE IN 2016

Important milestones on the way to clinical translation

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Regenerative medicine can be viewed as 'tissue engineering V2.0'. Discoveries and novel applications of technology advanced the field considerably in 2016, with the use of new biomaterials, stem cells and biologically active molecules.

This year saw strides forward in all three arms of the tissue engineering triad — cells, scaffolds and signalling molecules. Developments in 2016 included the identification of a new stem cell population in fibrocartilage and its signalling pathway, a refined method for 3D bioprinting of collagenous structures with micro-heterogenous domains, and whole-joint tissue resurfacing technology with tunable properties to thwart the effects of an inflammatory environment.

There is no doubt that the field of regenerative medicine has enthusiastically embraced the paradigm-shifting technology of 3D bioprinting. Although there have been important developments in printing geometrically complex shapes in the past 3 years, the ability to reproduce the heterogeneous spatial arrangement of biologically complex structures has eluded investigators to date. In 2016, Rhee and co-workers demonstrated a bioprinting technique to generate constructs with discrete microdomains that exhibit distinct material properties[1]. The authors developed a novel 'bioink' with a high-density collagen biogel (10-fold higher concentration of collagen compared with previously studied hydrogels[2]) and used a modified build plate that was heated to 37 °C to enable rapid polymerization of the hydrogel, resulting in improved fidelity of the printing process. This novel bioink preparation displayed excellent cell viability with cells starting to align along the collagen fibres in an organized fashion via integrin receptors[1]. Using this methodology, cells and collagen fibres can be oriented in specific directions to resist the tensile and circumferential forces found in the meniscus. This technology marks a step closer to biomimetic replication of the micro-cytoarchitecture of complex collagen structures.

Importantly, the mechanical properties of the bioink produced by Rhee *et al.*[1] are superior to those of other hydrogel bioinks currently available for printing soft tissues such as cartilage[3], thus marking progress towards the development of constructs capable of load-bearing. This report signals the first demonstration of successful bioprinting of constructs that replicate the complex microarchitecture of the knee meniscus, which has organized collagen bundles in load-oriented geometries. Ultimately, heterogeneous 3D-printed constructs could have applications in a number of other musculoskeletal tissues such as articular cartilage, which contains discrete layered zones with differing material properties and Benninghoff arcades (the unique cytoarchitecture of oriented collagen).

Tissue engineering strategies have traditionally relied on the use of cells, whether fully differentiated or stem cells, that are expanded in culture and then used alone, seeded onto porous scaffolds or delivered by hydrogel. An alternative strategy is to recruit endogenous stem cells, which obviates the need for stem cell isolation, culture and delivery. A 2016 article by Embree and co-workers[4] details the discovery of a new population of fibrocartilage stem cells (FCSCs) located in the superficial zone of the condyles of the temporomandibular joint (TMJ). The authors convincingly demonstrated the generation of cartilage, bone and haematopoietic marrow from a single FCSC. This finding suggests the potential use of culture-expanded FCSCs for cell-based therapies for fibrocartilage structures. Embree *et al.*[4] further showed that the maintenance and phenotypic status of FCSCs is regulated through the canonical Wnt signalling pathway, and that the Wnt signalling inhibitor sclerostin maintain the FCSC population and joint homeostasis. They demonstrated in another experiment the ability of FCSCs to be chondroprotective in a rabbit model of TMJ degeneration[4].

Fibrocartilage structures in the body include not only the TMJ but also the meniscus, vertebral discs, and tendon-to-bone enthesis; options for the treatment of injury to these structures are limited. The study by Embree *et al.*[4] suggests sclerostin might have use as a potential therapeutic in fibrocartilage degeneration through its effects on resident FCSCs. Whether the resident population of stem cells in the superficial zone of hyaline cartilage, which makes up other joints such as the knee and hip, will respond to a therapeutic such as sclerostin remains to be seen[5]. If so, this approach would be broadly applicable to degenerative joint diseases such as osteoarthritis.

The recruitment of endogenous stem cells is an attractive strategy for the treatment of cartilage injuries and the characterization of FCSCs by Embree and colleagues[4] is an important next step in developing this

Key advances

- 3D bioprinting can produce biomimetic constructs that replicate the complex microarchitecture of the knee meniscus[1]
- A resident population of fibrocartilage stem cells could be exploited for cartilage regeneration and repair via manipulation of the Wnt signaling pathway[4]
- The combination of gene therapy and tissue engineering can produce both cell-based and acellular engineered constructs with tunable and inducible anticytokine effects[9]

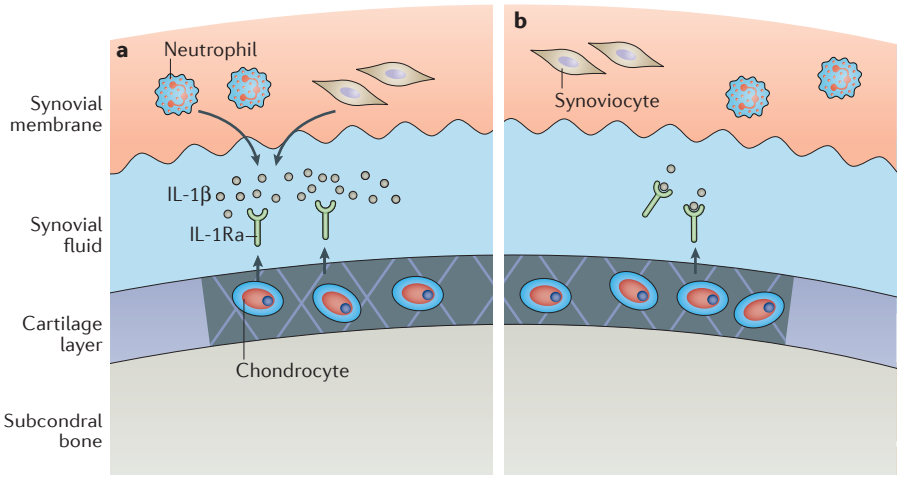

Figure 1 | **Gene therapy combined with tissue engineering produces engineered constructs with therapeutic anticytokine effects. a** | Chondrocytes or chondro-differentiated adipose-derived stem cells are genetically modified to express IL-1 receptor antagonist (IL-1Ra) when treated with doxycycline. **b** | IL-1β level in the synovial fluid is reduced after induction of IL-1Ra release.

treatment strategy. This group had previously demonstrated the recruitment of endogenous stem cells using an anatomically shaped scaffold loaded with transforming growth factor (TGF) β3 to induce chondrogenesis and chemoattract mesenchymal stem cells (MSCs), resulting in hyaline cartilage resurfacing[6]. The new data published in 2016 suggest a similar population of FCSCs might exist in the meniscus[4], which is one of the more difficult structures to repair with orthopaedic surgery.

The applications for tissue engineering technologies have now progressed beyond the treatment of focal cartilage defects to that of entire joint surfaces using novel woven scaffolds seeded with cells (either differentiated chondrocytes or stem cells). In almost all cartilage repair studies to date, however, little attention has been given to the environment into which an anabolic treatment to restore cartilage is placed. Following damage to the joint there is an increase in inflammatory mediators such as IL-1β and TNF, which could compromise the integrity of a tissue-engineered construct as well as the rest of the joint surface. Additionally, IL-1β, which is a key player in the aetiology and progression of OA, has been shown to inhibit MSC-based repair of articular cartilage[7,8]. Gene therapy using IL-1 receptor antagonist (IL-1Ra) is promising as a potential therapeutic to counter the effect of IL-1β. In 2016, Moutos *et al.*[9] combined IL-1Ra gene therapy with tissue engineering to produce engineered constructs with therapeutic anticytokine effects (FIG. 1).

Moutos and co-workers expanded upon their initial work in developing anatomically designed whole-joint resurfacing techniques[10] by adding the capability to provide anti-inflammatory properties, thus protecting the engineered construct in an inflammatory environment. An advanced textile manufacturing approach was used to produce orthogonally oriented fibres that provide mechanical functionality immediately upon implantation with patient-specific geometries; the gene therapy approach used a scaffold-mediated lentivirus transduction technique to enable controlled delivery of anticytokine therapy[9]. The study characterized the ability of the IL-Ra-expressing constructs to inhibit the effects of IL-1β on the development of cartilage *in vitro*.

Such a scaffold-mediated lentivirus transduction technique could be used as an acellular approach utilizing endogenous stem cells in other joint-resurfacing applications. In addition, this approach could be adapted for other growth factors, such as TGFβ, which has been shown to be an important morphogen in the recruitment of stem cells and their differentiation toward cartilage in other acellular models of scaffold-based cartilage repair[6]. This method of transduction results in high titres of anticytokine therapy in an exogenously tunable and inducible manner. The combination of textile manufacturing and scaffold-mediated lentiviral transduction provides a tissue engineering strategy for total-joint resurfacing. The ability of the gene construct to self-regulate the production of IL-1Ra in response to variations in IL-1β concentration within the synovial environment — that is, acting as a feedback mechanism — as well as the use of multiple morphogens in combination to simultaneously provide vigorous support to the cartilage layer would be substantial enhancements of this system. Key challenges to the clinical translation of this technology include determining how well this resurfacing approach will perform *in vivo*, whether this construct will integrate with the subchondral bone to form a solid base for joint motion, and how complex such a surgery will be to perform.

Progress made in 2016 moves the field of regenerative medicine closer to clinical translation. Improved resolution in 3D bioprinting foretells the use of off-the-shelf meniscus, obviating the need for allografts. Harnessing the power of FCSCs, for use as allogeneic cell-based therapies or by exploiting the Wnt pathway with sclerostin or a small-molecule analogue, could present promising therapies for difficult-to-treat fibrocartilage injury. Finally, the concept of providing a whole-joint tissue-engineering solution, rather than treating only cartilage injury, reaches a new level of sophistication with constructs capable of thwarting the effects of an inflammatory environment. Challenges remain but can be met by careful and continued refinement of these developments.

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Competing interests statement
The author declares no competing interests.