Tissue Engineering Scaled-Up, Anatomically Accurate, Osteochondral Constructs for Joint Resurfacing

Tariq Mesallati, Eamon Sheehy, Tatiana Vinardell, Conor T. Buckley, Daniel J. Kelly.
Trinity Centre for Bioengineering, Trinity College Dublin, Dublin, Ireland.

Disclosures:

Introduction: Partial and total joint replacements are the only surgical procedures currently available to treat articular cartilage degeneration associated with diseases such as osteoarthritis (OA). An alternative to this procedure would be to tissue engineer an anatomically accurate osteochondral construct and use it to replace the diseased joint. However, the generation of tissues of sufficient functionality and scale to resurface an entire joint remains a significant scientific and engineering challenge. This challenge is compounded by the fact that there are a limited number of therapeutically useful chondrocytes (CC’s) available in OA cartilage. Mesenchymal stem cells (MSC’s) can potentially be used as an alternative to CC’s; however, cartilage formed using MSC’s has a tendency to become hypertrophic and undergo endochondral ossification [1, 2]. The challenge of treating OA is further compounded by the fact that it affects multiple tissues of the joint including the articular cartilage and underlying bone. There is a need, therefore, to regenerate both these tissue types to treat an OA joint. The global objective of this study was to tissue engineer a scaled-up, anatomically shaped, osteochondral construct suitable for partial or total joint resurfacing. We first demonstrate that it is possible to engineer an osteochondral construct by promoting endochondral ossification in an MSC-seeded alginate hydrogel, where an overlaying layer of stable cartilage tissue is generated by self-assembly of chondrocytes. Given that scaling up such an approach to resurface an entire joint would require excessive numbers of chondrocytes, we hypothesized that it was possible to tissue engineer phenotypically stable cartilaginous tissue within the cartilage layer of the osteochondral construct using a co-culture of CC’s and MSC’s. Finally, we hypothesized that we could scale-up this approach to tissue engineer an anatomically accurate osteochondral construct which could potentially replace an entire diseased joint.

Methods: We first sought to determine the combination of cell and scaffold type that could be used to tissue engineer phenotypically stable cartilage overlaying functional bone. Articular cartilage, bone marrow (BM) and infrapatellar fat pad (IFP) were harvested from the femoropatellar joints of 4-month old porcine donors. The chondral layer of the osteochondral constructs were formed by combining co-cultures of CC’s and MSC’s in either agarose hydrogels (5mm diameter; 1.5mm thick; 2% conc) or self-assembled tissues (5mm diameter), while the underlying osseous layer was formed by combining BM-MSC’s in alginate hydrogels (5mm diameter; 2 mm thick; 2% conc). Agarose hydrogels and self-assembled constructs were seeded with the following cell types: CC only, BM-MSC only, IFP-MSC only, BM-MSC & CC (4:1 ratio), and IFP-MSC & CC (4:1 ratio). These bi-layered constructs were maintained in chondrogenic medium supplemented with 10ng/mL TGF-B3 for 6 weeks, after which they were implanted subcutaneously into nude mice for a further 6 weeks. In the second phase of the study, anatomically accurate MSC-seeded alginate constructs (~2cm diameter) mimicking the geometry of the medial femorotibial joint were generated from moulds fabricated by rapid prototyping (Fig. 2A). These constructs were covered by a self-assembled layer of engineered cartilaginous tissue (BM-MSC & CC co-culture). After 6 weeks of in vitro culture, the scaled-up constructs were implanted subcutaneously into nude mice for a further 8 weeks. The biochemical content of all constructs was assessed pre- and post-implantation. Calcium content of samples was identified through a commercial calcium assay kit (Sentinel Diagnostics). Histology samples were stained with alcian blue and aldehyde fuchsin for sulphated glycosaminoglycan (sGAG) accumulation, picro-sirius red for collagen deposition, and H&E for nuclei, cytoplasm and extracellular matrix presence. Deposition of collagen types I, II and X was identified by immunohistochemical analysis, as was the presence of CD31. Groups were analysed for significant differences using a general linear model for analysis of variance. Tukey’s test for multiple comparisons was used to compare conditions (p<0.05).

Results: A more cartilage-like tissue was generated in vitro in the chondral layer of the osteochondral constructs using self-assembly rather than agarose encapsulation (data not shown). Hence all data presented is for osteochondral constructs where the chondral layer is engineered using a self-assembly approach. Mineralization was detected in the osseous alginate layer of all osteochondral implants in vivo following subcutaneous implantation into nude mice (Fig. 1I-L), with evidence of early bone development formed via endochondral ossification (Fig. 1N). An in vivo phenotypically stable top cartilage layer could not be engineered within the osteochondral constructs using either BM- or IFP-derived MSC’s, as evident by a thinning of the chondral layer and reduced sGAG and collagen type II staining (Fig. 1A, C, E, G). In addition, BM-MSC constructs were found to contain high levels of mineral through μCT analysis (Fig. 1I), while both BM- and IFP-MSC’s accumulated relatively high levels of calcium (Fig. 1M). In contrast, using a co-culture of CC’s and either BM- or IFP-derived MSC’s to engineer the chondral layer was found to enhance the in vitro development (data not shown) and in vivo phenotypic stability of tissue engineered cartilage. This co-culture led to greater cartilage thickness and morphological stability in the self-assembled chondral layers (Fig. 1B, D, F, H). Co-
culture was also found to dramatically reduce mineralisation within the cartilage layer of CC-BM-MSC constructs (Fig. 1I, J), and significantly reduce levels of calcium compared to corresponding MSC-only groups (Fig. 1M).

After 8 weeks in vivo culture (Fig. 2B), a layer of phenotypically stable cartilage remained on the surface of the scaled-up anatomically accurate engineered implant (Fig. 2E, F), bearing a resemblance to native articular cartilage. There was also evidence of mineralisation and immature bone development in the underlying osseous alginate layer (Fig. 2C, D).

Discussion: We found that MSC seeded alginate hydrogels supported the development of endochondral bone in the osseous region of bi-layered hydrogels. Co-culture of CC’s and MSC’s was found to lead to the development of phenotypically stable cartilage when implanted in vivo. Furthermore it is possible to scale-up these approaches to tissue engineer biological implants of a clinically relevant size. These findings open up the possibility of a tissue engineered treatment option for OA.

Significance: The lack of cell or tissue engineering based therapies to treat joint degeneration due to diseases such as osteoarthritis remains one of the greatest challenges facing modern orthopaedic medicine. This study aims to directly address this problem by tissue engineering anatomically accurate osteochondral constructs of a clinically relevant size, paving the way to a clinical application of this strategy to repair diseased joints.

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2. Farrell et al. (2011) In-vivo generation of bone via endochondral ossification by in-vitro chondrogenic priming of adult human and rat mesenchymal stem cells. BMC Musculoskeletal Disorders
Figure 1. (A-D) Aldehyde Fuchsin staining for sGAG production. Main image is magnified shot of top half of inset construct. (E-H) Collagen type II immunohistochemistry staining of constructs. (I-L) Micro-CT scans of constructs. (M) Calcium accumulation of chondral layer of constructs. (N) H&E stain of alginate.
Figure 2. (A) ABS mould of tibial plateau. (B) Macroscale image of scaled-up osteochondral construct. (C) Micro-CT scan of construct. (D) H&E stain of alginate. (E) Aldehyde Fuchsin staining for sGAG production. (F) Collagen type II immunohistochemistry staining of construct.