WORKSHOP

Updates in Multi-Disciplinary Approaches for Stem-Cell Based Cartilage Regeneration

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Physical modulation to divert stem cells to chondrogenic cell fate

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Abstract

Mechanical forces experienced by cells contained within musculoskeletal tissues have a major influence on tissue homeostasis, pathogenesis and regeneration. These forces are often lacking during static *in vitro* or *ex vivo* culture and yet the interplay between mechanical forces and other endogenous or exogenous signals is likely to result in novel or synergistic effects. Mechanical load has long been recognised as a requirement for cartilaginous tissue development. This has led to the development of various bioreactors that are able to apply one or more stimuli to the cultured tissue to further investigate effect of various loading regimes. Using such systems, it is increasingly apparent that the physical stimuli that maintains chondrocyte phenotype and the load required to trigger chondrogenesis of mesenchymal stem cells may be different. Within this presentation the influence of mechanical stimulation on the initiation of chondrogenic differentiation will be highlighted. The mechanism of action of chondrogenic load will be discussed.

Introduction

During regular articulation cells experience localised mechanical stimuli. Maintenance of tissue homeostasis requires a basal level of mechanical stimulation to maintain the health of the tissue, with long term unloaded conditions such as bed rest known to have a detrimental effect. Novel therapies for cartilage repair are frequently investigated in the absence of these stimuli, potentially making clinically relevant conclusions difficult. Incorporation of an *in vitro* bioreactor system into the study allows the composite effect of physical and soluble stimuli to be established. It also allows for detailed investigations into the mechanisms underlying the process. *In vitro* studies frequently rely on the application of exogenous growth factors. While this is a highly successful approach for mechanistic studies, it does not investigate the regulation of the endogenous source of these factors during normal healing.

Bone marrow derived mesenchymal stem cells (BMSCs) are frequently used as a source material for cell-based cartilage repair strategies and they would also be naturally present during marrow stimulation techniques,

such as microfracture. Whereas the articulating joint provides a unique, multiaxial load environment, *in vitro* studies are classically performed under static conditions, or using uniaxial load alone. Using a complex, multiaxial load bioreactor (Figure 1), we have demonstrated that superficial shear, superimposed over uniaxial load, can provide a chondrogenic signal in the absence of exogenous growth factors, namely TGF-β (Kupcsik *et al.*, 2010; Li *et al.*, 2010). This response is due to an increase in the production of endogenous TGF-β by the mechanically stimulated cells. Crucially, shear is major driver of the response observed (Schatti *et al.*, 2011) as no chondrogenic induction was observed using compression alone under these conditions.

As the mechanical load applied has a direct influence on the chondrogenic differentiation, it becomes a logical next step that the location of a cell within a 3D implant would vary the load experienced in a localized manner. We have demonstrated that asymmetrical seeding of the construct, with a greater percent of the total cells being deposited in the superficial zone, leads to increased cartilage matrix deposition compared to even cell distribution, while keeping the cell number constant (Gardner *et al.*, 2017b). Deposition of both glycosaminoglycan and collagen II are increased in asymmetrically seeded scaffolds when compared to homogenously seeded scaffolds. This induced anisotropy is an interesting example of naturally occurring changes induced by physical loads. Load leads to an increase in the production of the latent form of TGF-β. Multiaxial load is capable of activating latent TGF-β, even in the absence of cells, by removing the non-covalently bound latency associated peptide, a critical step in the functional activity of endogenous TGF-β (Gardner *et al.*, 2017a). This provides new insight into mechanically induced chondrogenesis and offers an experimental test bed for clinical therapies. It allows for the identification of novel markers and clinically relevant targets that are only modified during articulation (Gardner *et al.*, 2016). Comparing static chondrogenesis to that induced by mechanical load has led to the identification of targets that are differentially regulated when load is applied. In addition, the therapy in its entirety, including the effect of the rehabilitation protocol, can be investigated using human derived cells. This is contributing to a new field of regenerative rehabilitation (Perez-Terzic and Childers, 2014). The use of human cells and a more physiologically relevant loading environment leads to more clinically relevant studies being performed which should increase the potential translation into the clinic.

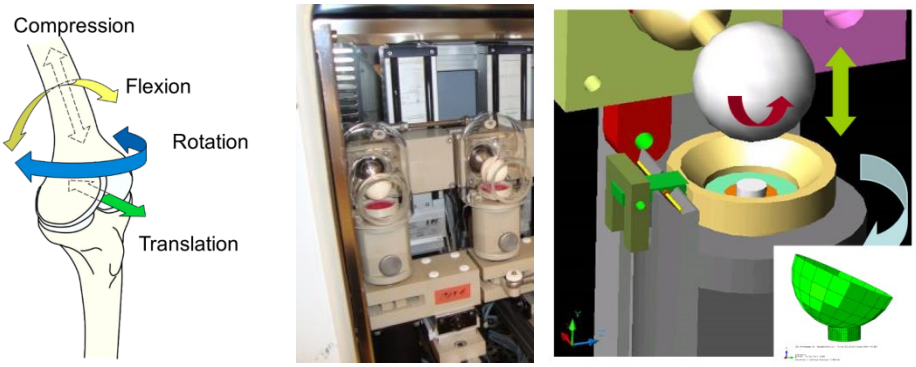

Figure 1. Articular motion is a combination of complex multiaxial load (Left). This can be mimicked using a ceramic hip ball bioreactor system (middle) that is able to recapitulate the articulating motion (right).

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Gene-enhanced cartilage regeneration

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Gene transfer has been used experimentally to promote cartilage regeneration and treat osteoarthritis (OA). While it is controversial to apply gene therapy for nonlethal conditions, there is a possibility that the transfer of therapeutic transgenes may dramatically increase the effectiveness of cell therapy. Single or combination of growth factors and transcription factors has been transferred to expanded stem cells or chondrocytes using both nonviral and viral approaches. Recently, an injection cell therapeutics based on TGF-β1-transduced chondrocytes (Invossa ®, Kolon Life Science) was approved to treat OA in Korea.[1)]

The current challenge for the clinical applications of genetically modified cells is ensuring the safety of gene therapy while guaranteeing effectiveness. Viral gene delivery methods have been mainstays so far with enhanced safety features being recently refined. On the other hand, nonviral delivery which is inherently safer than viral delivery has been greatly improved in transfection efficiency. Our group has been using microporation of SOX trio genes to enhance chondrogenesis from stem cells.[2-3)] We have recently developed *SOX-6, 9*-transfected human adipose stem cells ($^{SOX\text{-}6,\ 9}$ASCs) to treat OA and tested their effectiveness in arresting OA progression when injected intra-articularly (IA) in a surgically-induced OA caprine model. *SOX-6, 9*-transfection led to *in vitro* chondrogenesis of ASCs comparable to that achieved by growth factor treatment. IA injection of $^{SOX\text{-}6,\ 9}$ASCs arrested the progression of surgically-induced OA in goats.[4)]

Although major therapeutic effects of a gene-cell therapeutics come from transient paracrine actions, in vivo survival and engraftment possibly would greatly improve clinical outcomes. This can be more appealing in OA in which the presence of durable tissue regenerated from cell engraftment and differentiation is quite pertinent and desirable for long-term good results. The results from the author's or other groups shows that IA-injected cells rapidly disappear from joint cavity when used in suspension form. On the other hand, implanted cells survive longer and engraft to the chondral defect when immobilized on the site. Use of hydrogel carrier or administration in spheroid form may also increase the cell survival and promote engraftment.[5)]

While the gene transfer has been purposed to transform cells by endowing functional characteristics, the direct reprogramming of somatic cells into chondrocytes provides a new approach. Tsumaki's group in Japan reported in vitro direct conversion of dermal fibroblast into chondrogenic cells without undergoing intermediate pluripotent state by forced expression of SOX49, KLF4, and c-Myc using retroviral vectors.[6-7)] Considering that the in vivo direct conversion technique has greatly advanced recently in other tissues using safer methods such as nonviral gene transfer, the technique may also be investigated for cartilage regeneration and treat OA in future studies

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