Editorial

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Regenerative Medicine

Controlled mechanotransduction in therapeutic MSCs: can remotely controlled magnetic nanoparticles regenerate bones?

"Using magnetic nanoparticles, loading can be applied directly to mechanoreceptors without deforming or loading the scaffold."

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'If we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have found the safest way to health.'

Hippocrates.

Mechanotransduction, the conversion of the external, environmental forces acting on a cell into internal biochemical signaling pathway, is now becoming a recognized route for delivering tissue-forming stimuli to therapeutic cells. In the body, complex loading forces transduced through tissue serve to inform cells of their physical environment and can therefore have profound effects on tissue homeostasis, regulating many aspects of cell behavior including proliferation, differentiation and ECM composition [1–4]. With this growing awareness of the role of cell biomechanics, several researchers have now started to focus their attention on exploiting these biomechanical pathways to resolve long-standing challenges in regenerative medicine – the optimal construction, repair and integration of tissues in the body.

Are mechanical forces clinically important?

The importance of mechanical loading has been known for many years – physiotherapy and rehabilitation exercise are routinely used to improve patient outcomes and speed up return to normal function. Recent work has shown how the mechanical interaction between stem cells and substrates of varying stiffness can result in their differentiation down alternative pathways, and so there is a growing appreciation that the mechanical properties of cells and their physical interactions at the tissue implant interface can have dramatic effects on the success or failure of therapies [5].

The complex nature of mechanosensing in bone has been intensively researched and a variety of force transduction mechanisms have been described, including the integrin-cytoskeleton-nuclear matrix structure, stretch-activated membrane ion channels, G-protein-dependent pathways and links between the cytoskeleton and the PLC or PLA pathways [2,6–8]. For regenerative medicine, our challenge is to develop methods for selectively activating these mechanosensing pathways so that we can translate research in cell mechanotransduction into actual clinical therapies.

How can we apply mechanical forces to specific cellular targets?

Magnetic nanoparticles provide a unique approach to directing mechanotransduction in cells, and have several features which make them highly translatable to clinical situations. By surface coating a (superparamagnetic) iron oxide nanoparticle with biomolecules such as receptor ligands or mechanosensor-binding antibodies the nanoparticles can be very precisely targeted, allowing researchers to take control over individual mechanotransduction pathways by simply tailoring the size and coating of the particle. Once bound to the target receptor on the cell, applying a variable magnetic field (from either a moving array of permanent magnets

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or an electromagnet) results in an equivalent force on the particle, which is transduced into a force acting directly on the target mechanoreceptor [9,10].

In our research group, we have shown that we can activate a wide variety of different targets in this way, ranging from the traditional and well-studied force transduction pathways which are regulated by extracellular matrix-integrin-cytoskeletal deformation (by using the integrin-binding RGD tripeptide motif as a surface coating for the nanoparticles), to stretch-activated ion channels (through a detailed molecular understanding of how the ion channel operates and binding the nanoparticle-antibody complex to exactly the right epitope – e.g., an intracellular gate-activating 'lever') [10–12]. It has also been shown that certain ligands can be used as nanoparticle coating molecules, as the movement of the ligand within the receptor may regulate its activity – this has recently been demonstrated as a workable approach for targeting the Wnt signaling pathway (in press).

Can we translate magnetic activation to the clinic?

This technology is particularly advantageous as a translatable therapy since magnetic nanoparticles have been used for several years as contrast agents for magnetic resonance imaging, and so many manufacturers have regulatory approval for these applications. With this in mind, targeted magnetic nanoparticles are currently being trialed in preclinical models to demonstrate their effectiveness, generating advances in understanding and technology at each stage from the original 2D cell culture methods, through 3D hydrogels, microinjection, small and ultimately large animal models [11,13].

One particular advantage of this route toward translatable mechanotransduction is that it does not rely on the mechanical properties of the biomaterial scaffold. The medical devices and implants sector has survived a damaging early history of implant failure due to mismatching mechanical properties. Originally observed in the necrotic failure of metal orthopedic fixation devices and articular prostheses, avoiding stress shielding and matching tissue mechanics to materials became a justified mantra for biomaterials research. For many tissues and particularly for orthopedics this is still a crucial tissue engineering maxim, and for long term or permanent implants these remain essential requirements.

However, in the last several years the new generation of highly biocompatible, porous or hydrogel materials with active surfaces and structures has yielded a wealth of interesting, adaptable materials for applications in regenerative medicine which are handicapped only by their poor mechanical properties. When implanted into load bearing areas of the body these materials would rapidly fail, while in mechanically unstimulated defects the introduced cells to do not receive the stimulatory loading required to generate optimum bone matrix.

Using magnetic nanoparticles, loading can be applied directly to mechanoreceptors without deforming or loading the scaffold. This opens the possibility for fascinating collaborations with biomaterials researchers – creating cell-seeded materials that can be implanted, injected or melded into bone defects and which can now, using magnetic nanoparticles, be mechanically stimulated to provide the osteogenic cues cells need for optimum tissue formation. Our laboratory has demonstrated that both direct cell injection (into mineralizing cartilage) and tissue engineered collagen hydrogels form bone under these conditions – and the scope for testing other biomaterials-based approaches is almost limitless [14].

One other translatable strategy is to precondition tissue engineered scaffolds using a laboratory-based magnetic force bioreactor. This device, comprising a moving magnetic array allows the patient's stem cells to be cultured and predifferentiated down a specific lineage (e.g., either chondrogenic or osteogenic) depending on which receptor the nanoparticles are targeted to. This approach may have several advantages as it offers a level of drug-free control over differentiation and is amenable to inclusion in many existing cell expansion methods for cell therapy.

Can these technologies complement growth factor-based approaches?

It has also been shown by researchers that mechanotransduction and growth factor signaling are closely linked [15,16]. Working with collaborators in the UK, particularly those at Nottingham University's tissue engineering group led by Kevin Shakesheff, we have demonstrated that mechanotransduction via magnetic nanoparticles interacts synergistically with BMP2 released from poly(lactic-co-glycolic acid) (PLGA) microspheres, redirecting the 'ECM overproduction 'response often seen as a result of exogenously applied BMP2 into the production of denser, and presumably more effective bone matrix [14]. It is currently thought that many developmental and regenerative processes are strongly influenced by biomechanics, and so the potential exists for synergistic regenerative therapies which combine the best features of stem cell research, drug delivery and biomaterials into a fully optimized treatment which is orchestrated by appropriate mechanical cues delivered using nanoparticles.

Can we use these technologies to target cells to the site of injury?

Another exciting feature of the magnetic approach to mechanical activation is the potential for simultaneously targeting of stem cells to the site of injury. In collaboration with the Ludwig Boltzman Institute in Vienna, we have demonstrated how we can localize stem cells to a target site using remote magnetic attraction by labeling human mesenchymal stem cells with both a luciferase reporter and magnetic nanoparticles. Using an *in vivo* imaging system, we demonstrated we can inject these cells into the tail vein of SCID mice and then localize them to a dermal wound site using a rare earth magnet [17]. We have also demonstrated the principles of stem cell delivery using magnetic nanoparticles in *in vitro* circulation systems and identified the concentrations of particles and the field strengths necessary to enable us to attract the cells to a knee joint. Kobayashi *et al.* have shown the ability to deliver magnetically labelled mesenchymal stem cells (MSCs) to a location in the knee joint in cadaver rabbit and porcine models, independently demonstrating the ability to locate and hold MSCs in target sites in the joint [18].

Bottling exercise in a syringe may not be too far away from clinical practice

It is therefore highly possible that this nanoparticle-based approach can be used synergistically with research from across the spectrum of regenerative medicine, generating multi-stranded, translational approaches to deliver an optimized therapeutic pathway – and also one which comprises regulated 'off the shelf 'products at reduced cost to healthcare providers. Using external magnetic fields to generate the force on the nanoparticle conveniently also provides clinicians with the means to remotely regulate the internal healing processes electronically from the bedside without administering any further surgical or pharmaceutical intervention. Controlled mechanotransduction in therapeutic MSCs may therefore present a novel, drug-free method suitable for inclusion in many translational regenerative strategies for tissue repairs of the future.

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