

WORKSHOP

Regenerative Rehabilitation: The Role of Mechanotherapies Used to Optimize Regenerative Medicine Outcomes

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Regenerative Rehabilitation: Background and Introduction

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Abstract

Both regenerative medicine and rehabilitation medicine seek to restore tissue and function when these have been lost through aging, injury, disease or congenital processes. These two approaches to therapy emerged as separate disciplines and have tended to remain this way. At best they are applied sequentially, with the regenerative component preceding subsequent physical therapy. During the last decade, it has become apparent that this dichotomy is inappropriate. The clinical outcomes are likely to be far better if the regenerative and rehabilitation medicine aspects are combined at an early stage. This integration of disciplines recognizes the importance components other than the traditional mix of cells, scaffolds and growth factors that we normally think of in the context of tissue regeneration. Relevant stimuli delivered by rehabilitation vary by discipline. For example, for orthopaedics important stimuli include load and other types of mechanical stimulation; for neurology, electrical signals are also important. Promising clinical and pre-clinical examples are to be found in the areas of spinal cord injury, muscle injury, bone healing and cartilage repair. Presentations on the last two of these are given in this workshop. The regenerative rehabilitation community has organized an Alliance for Regenerative Rehabilitation Research and Training (AR³T; <http://www.ar3t.pitt.edu/>), funded by a R24 grant from NIH, and holds an annual symposium.

Key references

1. Ambrosio F, Russell A: Regenerative rehabilitation: A call to action *J Rehabil Res Dev* 47: xi-xv, 2010
2. Sicari BM et al: An acellular biologic scaffold promotes skeletal muscle formation in mice and humans with volumetric muscle loss. *Sci Trans Med* 6: 234ra58, 2014
3. Perez-Terzic C, Childers MK: Regenerative rehabilitation – a new future? *Am J Phys Med Rehabil* 93: S73-S78, 2014
4. Dolgin E: Cellular rehab *The Scientist* 12/1/2015
5. Grahn PJ et al: Enabling task-specific volitional motor functions via spinal cord neuromodulation in a human with paraplegia *Mayo Clinic Proc* 92: 544–554, 2017

Stimulation of bone healing through mechanical loading

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Abstract

Regeneration of bone requires a coordinated network of molecular signals where the local mechanical environment plays a major role in the success of the healing process. The initial hypothesis was that the healing of large bone defects and fractures can be accelerated by the imposition of an appropriate mechanical environment. Within this presentation is an overview of the progress made in this research area on how the amount of rhBMP-2 needed could be reduced, and its effectiveness increased, by providing an optimized mechanical environment to achieve bone union of large bone defects. Additionally, the latest findings of improved fracture healing through the manipulation of fixation stability introducing a potential clinical strategy to improve the healing outcome of unstable fractures, particularly for non-unions through increased stabilization, will be discussed. These findings are of significant importance as it could provide new treatment strategies and rehabilitation protocols to optimize the healing of bones.

Introduction

The management of bone defects and impaired fracture healing remain among the most challenging clinical problems. Several treatments exist to aid in the healing of such complications, including biologics such as recombinant human bone morphogenetic protein-2 (rhBMP-2), yet all have met with limited success. In order to achieve consistent bone healing, a broad spectrum of growth factors are required to interact with one another in a highly organized response. Critically important, the mechanical environment around the fracture site will significantly influence the way bone heals, or if it heals at all (Glatt et al., 2017). The mechanical environment itself is determined by the stiffness of the implant used to stabilize the fracture and weight-bearing, and if fixation is either too flexible or too rigid the healing might fail. The cellular response from mechanical loading is heavily dependent upon the magnitude of interfragmentary movement (IFM), the type of loading conditions, and on the differentiation stage of the progenitor cells, which will all determine the size and quality of the callus formed (Kenwright and Gardner 1998). Accordingly, stiff fixation that minimizes IFMs will result in limited callus formation, whereas flexible fixation that increases IFMs will result in the formation of a larger callus. Moreover, shear load is detrimental to fracture-healing, whereas the same amount of axial load is beneficial. Based on these observations, many authors have suggested that the delayed introduction of controlled motion (“dynamization”) as healing progresses may lead to faster maturation of bone (De Bastiani et al 1984), but this procedure remained controversial and has not greatly influenced clinical practice.

Mechanical Environment and Healing of Large Bone Defects

This knowledge led us to hypothesis that the mechanical environment can be optimized by providing higher IFMs in the first weeks of healing (larger callus formation), followed by the increase in fixation

rigidity, which allows angiogenesis to take place, thus removing calcified cartilage and replacing it with woven bone. This final period of higher rigidity enhances bone formation and maturation in the most timely and efficient manner. We were the first in the field to suggest this mechanical regimen, called Reverse Dynamization (Glatt et al., 2012a; Glatt et al., 2016a), which is counter intuitive to what had been done in prior experimental and clinical studies. We used a rat bone model to investigate the effects of the fixator stiffness on the healing of large bone defects treated with BMP-2. The results of this study confirmed that the healing of large bone defects in response to BMP-2 treatment is strongly influenced by the local mechanical environment, but more importantly, that it can be improved by changing the stiffness of fixation stability using reverse dynamization (Glatt et al. 2012a,b). Based on these observations, a subsequent study determined whether the dose of BMP-2 could be reduced without compromising the healing process when using this enhanced mechanical environment (Glatt et al., 2016a). This study showed that while the initial healing was slightly delayed, forming a smaller callus throughout the healing period, the quality of healing bone was similar or slightly superior to that treated with the higher dose of BMP-2. This finding is of clinical importance since extremely large amounts of rhBMP-2 are regularly used, which raises costs and contributes to adverse side effects, several of which are severe, but could be reduced by optimizing the mechanical environment.

Mechanical Environment and Fracture Healing

In a different set of experiments, we wanted to assess whether reverse dynamization has the same effect on spontaneously healing fractures. To confirm this we used a 1 mm osteotomy rat model, and the results from this study showed that when reverse dynamization, from flexible to rigid fixation, was applied at 3 weeks the healing outcome at the end of the treatment period was the same as the dynamization regimen (Claes et al., 2011; Bartnikowski et al., 2016). This is not surprising considering that fracture movement arises from the combined flexibility of the fixation devices and the compliance of tissue material in the fracture gap, and is a consequence of weight-bearing and any loads applied (Glatt et al., 2012b). For dynamization implemented at the later stages of healing, accelerated bone healing is more likely a consequence of bone adaptation following Wolff's law rather than fixator dynamization itself. However, when reverse dynamization was applied at 1 week the healing outcome was far superior to that of the dynamized group, rigid and flexible fixation groups (Bartnikowski et al., 2016). On the contrary, the dynamization regimen at 1 week was detrimental to bone healing when compared to any of the other groups tested (Claes et al., 2009). This data confirmed that reverse dynamization has the same effect on fracture healing as it does on the healing of large bone defects. Furthermore, there is an optimal time to alter the stiffness of the fixator to enhance and accelerate healing in the most efficient manner.

Clinical Translation

The data from these studies appear to suggest there is no single set of mechanical circumstances that suits all stages of fracture repair, and that healing might be enhanced by systematically manipulating fixation stiffness during different phases of healing. Selecting the specific mechanical conditions, as well as determining the most appropriate time to modulate this environment will be critical to achieve the optimal conditions for bone repair. This is particularly true for reverse dynamization, where initially flexible conditions are followed by more rigid fixation, as this new concept requires further investigation

to optimize the dynamization regimen better define its role clinically (Glatt et al., 2016b; Glatt et al., 2016a,b; Glatt et al., 2017). It will also require the development of new orthopaedic implants, because contemporary internal fixation does not allow for any modulation of the mechanical properties, such as stiffness, without a secondary intervention. Lastly, the local mechanical environment could also be manipulated through a regimen of rehabilitation, where patients would initially apply loads by weight bearing for a period of time (micro-stimulation), and then off-loading for a period of time (no stimulation), which would theoretically further accelerate the healing process.

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Bibliography

Bartnikowski N, Claes LE, Koval L, Glatt V, Bindl R, Steck R, Ignatius A, Schuetz MA, Epari DR. (2017) Modulation of fixation stiffness from flexible to stiff in a rat model of bone healing. *Acta Orthop* 88(2): 217-222.

DeBastiani G, Aldegheri R, Renzi Brivio L. (1984) The treatment of fractures with a dynamic axial fixator. *J Bone Joint Sur Br* 66: 538–545.

Claes L, Blakytyn R, Gockelmann M, Schoen M, Ignatius A, Willie B. (2009) Early dynamization by reduced fixation stiffness does not improve fracture healing in a rat femoral osteotomy model. *J Orthop Res* 27: 22–27.

Claes L, Blakytyn R, Besse J, Bausewein C, Ignatius A, Willie B. (2011) Late dynamization by reduced fixation stiffness enhances fracture healing in a rat femoral osteotomy model. *J Orthop Trauma* 25: 169–174.

Glatt V, Miller M, Ivkovic A, Liu F, Parry N, Griffin D, Vrahas MS, Evans CH (2012a) Improved healing of large segmental defects in the rat femur by reversed dynamization in the presence of bone morphogenetic protein-2. *J Bone Joint Surg Am* 94(22): 2063-73.

Glatt V, Evans CH, Matthys R. (2012b) Design and Characterization and in vivo testing of a new, adjustable stiffness, external fixator for the Rat Femur. *Eur Cell Mater* 23:289-99.

Glatt V, Bartinkowski N, Quirk N, Schuetz M, Evans CH (2016a) Reverse Dynamization: Influence of Fixator Stiffness on the Mode and Efficiency of Large-Bone-Defect Healing at Different Doses of rhBMP-2. *J Bone Joint Surg Am.* 98(8): 677-87.

Glatt V, Tepic S, Evans CH. (2016b) Reverse Dynamization: A Novel Approach to Bone Healing. *J Am Acad Orthop Surg* 24(7): e60-1.

Glatt V, Evans CH, Tetsworth KA (2017) Concert between Biology and Biomechanics: The Influence of the Mechanical Environment on Bone Healing *Front Physiol* 7: 678.

Kenwright J, Gardner T. (1998) Mechanical influences on tibial fracture healing. *Clin Orthop Relat Res* 355(Suppl.): S179–S190.

Chondrogenesis in response to mechanical load for cartilage repair

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Abstract

Mechanical load has long been recognised as a benefit for cartilaginous tissue development. However, 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. 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. 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. While the use of load to induce chondrogenic differentiation has been the focus of much work, the additional benefits of bioreactors, such as novel biomarker identification and material design aspects, will also be highlighted. Data obtained could provide useful insights into the role of rehabilitation protocols after cartilage repair procedures.

Introduction

Bone marrow derived mesenchymal stem cells (BMSCs) are frequently used as a source material for cell based cartilage repair strategies. 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).

In vitro regenerative medicine studies are frequently performed in the absence of these stimuli, potentially making clinically relevant conclusions difficult. Incorporation of a bioreactor system into the study allows the composite effect of physical and soluble stimuli to be established. *In vitro* studies frequently rely on the application of exogenous growth factors. While this is a highly successful approach for mechanistic studies and tissue engineering, it does not investigate the endogenous source of these factors during normal healing.

As the mechanical load applied has a direct influence on the chondrogenic differentiation, it becomes a logical next step that cell location within a 3D implant would influence the load experienced by the cells in different locations. Using this device, 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.*, 2016c). Deposition of both glycosaminoglycan and collagen II are increased in asymmetrically seeded scaffolds when compared to homogeneously seeded scaffolds. This induced anisotropy is an interesting example of naturally occurring changes induced by physical loads. The increase in endogenous TGF- β leads to an increase in the latent form of the protein. Multiaxial load alone is capable of activating latent TGF- β by removing the non-covalently bound latency associated peptide, a

critical step in the functional activity of endogenous TGF- β (Gardner *et al.*, 2016b). This is possible even in the absence of cells. Such results are clinically relevant, and yet they could not be obtained under static conditions. This provides a 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.*, 2016a). Comparing static chondrogenesis to that induced by mechanical load has led to the identification of targets that are differentially regulated when load is applied. One of these is nitric oxide, a molecule normally associated with unwanted side effects in cartilage. Studies are underway to investigate if blocking nitric oxide synthase 2 (NOS2) enhances or inhibits MSC chondrogenesis.

A further example of the benefit of multi-axial load bioreactors is the ability to test new materials under complex loading, thus assessing whether the material can withstand the complex kinematic loads experienced during articulation. In addition, the therapy in its entirety, including the effect of the rehabilitation protocol, can be investigated using human derived cells. This is leading 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.

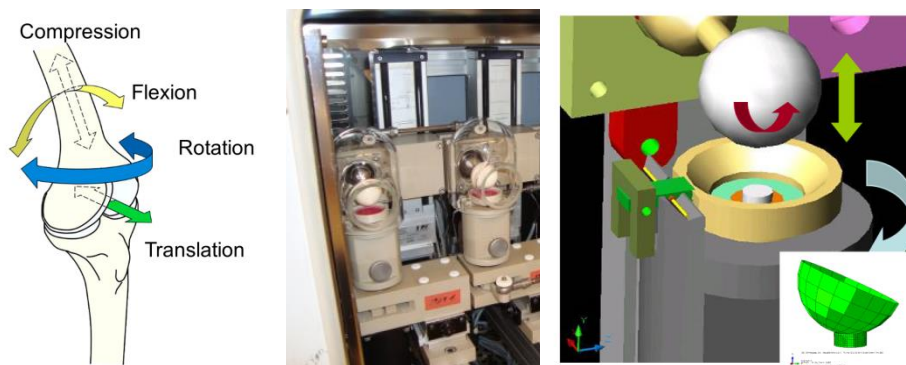


Figure 1. Articular motion is a combination of complex multi-axial load (Left). This can be mimicked using a ceramic hip ball bioreactor system (middle) that is able to recapitulate the articulating motion (right).

References

Gardner OF, Fahy N, Alini M, Stoddart MJ (2016a) Differences in human mesenchymal stem cell secretomes during chondrogenic induction. *Eur Cell Mater* **31**: 221-235.

Gardner OF, Fahy N, Alini M, Stoddart MJ (2016b) Joint mimicking mechanical load activates TGF β 1 in fibrin-poly(ester-urethane) scaffolds seeded with mesenchymal stem cells. *J Tissue Eng Regen Med*.

Gardner OFW, Musumeci G, Neumann AJ, Eglin D, Archer CW, Alini M, Stoddart MJ (2016c) Asymmetrical seeding of MSCs into fibrin-poly(ester-urethane) scaffolds and its effect on mechanically induced chondrogenesis. *J Tissue Eng Regen Med* **In Press**.

Kupcsik L, Stoddart MJ, Li Z, Benneker LM, Alini M (2010) Improving chondrogenesis: potential and limitations of SOX9 gene transfer and mechanical stimulation for cartilage tissue engineering. *Tissue Eng Part A* **16**: 1845-1855.

Li Z, Kupcsik L, Yao SJ, Alini M, Stoddart MJ (2010) Mechanical Load Modulates Chondrogenesis of Human Mesenchymal Stem Cells through the TGF-beta Pathway. *J Cell Mol Med* **14**: 1338-1346.

Perez-Terzic C, Childers MK (2014) Regenerative rehabilitation: a new future? *Am J Phys Med Rehabil* **93**: S73-78.

Schatti O, Grad S, Goldhahn J, Salzmann G, Li Z, Alini M, Stoddart MJ (2011) A combination of shear and dynamic compression leads to mechanically induced chondrogenesis of human mesenchymal stem cells. *Eur Cell Mater* **22**: 214-225.