EDITORIAL

# Tuning stem cells

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Orthopaedic surgeons, particularly trauma surgeons, spend a considerable amount of time trying to get bone to heal. While it is certainly a natural process, we aim to guide and manipulate the process to ensure that it is achieved as fast as possible, with the lowest chance of complications and the best long-term functional outcome for the patient. Although we have made huge strides in our understanding of bone healing, much remains to be discovered. And, as is the case in nature, the answer to some of our questions are often somewhat obscure. A case in point is the fact that we only discovered the reason for zebras having stripes in 2014.[1] White and black stripes are certainly not good camouflage in the African savannah. It turns out that tsetse flies and horse flies (the vector of various equid diseases) avoid black-and-white striped surfaces. While the answer seems obvious now, it eluded scientists for years. Similarly, there are aspects of bone healing which we have thought of as 'just the way things are'.

The diamond concept of bone healing, introduced by Giannoudis *et al.,* illustrates the complex interplay between growth factors, osteoinductive scaffolds, osteogenic cells and the mechanical environment.[2] The gaps in our knowledge, however, lie in the finer details and particularly at sub-microscopic level. Maybe more importantly, translating this knowledge into clinical practice remains a major challenge. The 'mechanical environment' element serves as good example of this. While orthopaedic surgeons have long recognised the interaction between physical stimuli (or lack thereof) and biology, the exact mechanisms involved were not well understood. Mechanobiology is an emerging scientific field that explores how physical factors, such as forces and mechanics, influence biological systems at the molecular, cellular and tissue level. The fundamental process which drives this is mechanotransduction, the ability of cells to convert mechanical stimuli into biochemical signals.

In the 1960s Pauwels recognised that compressive and deformation forces drive the differentiation of mesenchymal tissue.[3] Carter *et al*. expanded on the theory underlying the relationship between stress and strain and bone formation. They postulated that intramembranous bone formation dominates at low stress and strain levels.[4] Claes and Heigele took the theory one step further, quantifying the stress and strain levels required for the formation of different types of tissue.[5] For example, it was postulated that endochondral ossification predominates when local hydrostatic pressures are less than -0.15 mPA and strain in the region of -15 to 15%. Prendergast and colleagues encapsulated the concept with the description of the so-called 'mechano-regulatory' pathway which describes the differentiation of mesenchymal stem cells (MSC) where the emergence of a specific extracellular matrix can favour a divergence in phenotype.[6] Thus, the synthesis of extracellular matrix by differentiating MSCs is linked to the predominant mechanical and perfusion characteristics of the local environment. The authors also recognised the temporal and reciprocal nature of this relationship with change occurring within the differentiation tissues (and resulting extracellular matrix) over time, which in turn also impacts the mechanical environment during loading throughout the healing process.

The question then arose: How does the mechanical environment influence tissue generation? Basically, stem cells detect and respond to the stiffness of their environment. These external mechanical forces thus tune stem cell fate, driving differentiation towards a certain phenotype.[7] The major problem with stem cells is however the maintenance of this differentiation. This requires the creation of a 'mechano-niche', which is determined by the mechanical properties of the cells themselves, the extracellular matrix stiffness and finally external mechanical cues.[8] This process is mediated by the mechano-sensing apparatus of the stem cells, which is different from those found in the final differentiated cells.[9] Stem cells sense the nano-features of their dynamic scaffold (the surrounding extracellular matrix), including its so-called motion-tenso-elastic properties.[7] Within this context, scientists have demonstrated the ability of cells to express 'vibrational' (nano-mechanical) signatures of their health and differentiating potential.[10] Furthermore, we now have the ability to gather information about the nano-mechanical properties of cells with the aid of atomic force microscopy.[11]

This all sounds very theoretical, and it is, but we are starting to see some experimental work being done in the field. Glatt and co-workers, for example, have shown that reverse dynamisation, involving very low initial stiffness of the initial external fixator followed by an increase in stiffness, resulted in improved healing of osseous defects in a rat model.[12] The authors, however, cautioned that care is required in terms of the selection of stiffness parameters. The translation of mechanobiology into clinical practice is, however, in its infancy. We now have some evidence that it is possible to treat oligotrophic or atrophic non-unions without necessarily bone grafting them.[13] Furthermore, there seems to be growing support for the principle that non-unions will heal, if the optimal mechanical environment can be provided, without the need for biological augmentation (like autologous bonegraft).[14] It is thus thought that the biological potential to effect union always remains in the local MSCs. We just need to create and maintain an optimal 'mechano-niche' in order to tune stem cell fate towards the tissue we desire for healing.

Many questions remain and there are endless opportunities for experimental research in this area. How can we measure the stress and strain at a fracture/osteotomy site? How do we determine the optimal mechanical environment for individual fractures? Can

we develop an implant or external fixator that can allow accurate optimisation of the mechanical environment after application? Perhaps most exciting is the prospect of external stimulation of MSCs. Magneto-mechano stimulation of bone marrow mesenchymal stem cells, for example, through the manipulation of magnetic nanoparticles attached to cell membrane mechanoreceptors has been shown to possess the ability to upregulate gene expression involved in osteo- and chondrogenesis.[15] However, as we are now entering this 'nanosphere', we are left wondering if there is a sufficient scientific platform for the creation and development of these ideas, which may eventually have an enormous clinical impact.

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