Mesenchymal stem cell responses to mechanical stimuli

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Summary

Mesenchymal stem cells (MSCs) have the potential to replace or restore the function of damaged tissues and offer much promise in the successful application of tissue engineering and regenerative medicine strategies. Optimising culture conditions for the pre-differentiation of MSCs is a key goal for the research community, and this has included a number of different approaches, one of which is the use of mechanical stimuli. Mesenchymal tissues are subjected to mechanical stimuli *in vivo* and terminally differentiated cells from the mesenchymal lineage respond to mechanical stimulation *in vivo* and *in vitro*. MSCs have also been shown to be highly mechanosensitive and this may present an ideal method for controlling MSC differentiation. Here we present an overview of the response of MSCs to various mechanical stimuli, focusing on their differentiation towards the mesenchymal tissue lineages including bone, cartilage, tendon/ligament, muscle and adipose tissue. More research is needed to elucidate the complex interactions between biochemically and mechanically stimulated differentiation pathways.

Key words: mechanical stimuli, mesenchymal stem cell, osteogenesis, tenogenesis.

Introduction

Mesenchymal stem cells (MSCs) are a promising cell source for tissue engineering and regenerative medicine strategies and offer an alternative to fully differentiated cells that are often in limited supply due to tissue damage or disease. MSCs have multipotent differentiation potential, self-renewing ability, and apparent immunosuppressive properties[1]. Typically MSCs are isolated from the stroma of adult bone marrow (BMSCs), but cells with similar phenotypic characteristics and differentiation capabilities have also been isolated from a range of other mesenchymal tissues including adipose[2] (ADMSCs), tendon, muscle and skin. MSCs cultured *in vitro* can be chemically-induced to differentiate into cell types of the mesoderm, including bone, cartilage, tendon/ligament and fat (for a recent review see Vater et al.[3]). Common biochemical agents and growth factors include: dexamethansone (dex) and bone morphogenetic proteins (BMPs) for osteogenesis, serum free medium and transforming growth factor ß (TGF-ß) for chondrogenesis, dex, insulin, and 3-isobutyl-1-methylxanthine (IBMX) for adipogenesis[4](Fig. 1). It has also been claimed that MSCs are able to differentiate into other tissue types such as smooth muscle, endothelial and nervous tissue[5].

A key task for tissue engineers is to identify the appropriate culture conditions for development of a tissue engineered construct *in vitro* ready for implantation *in vivo* that forms the target tissue and reduces subsequent healing time. It is well known that biochemical cues, such as cytokines, growth factors and signalling events[6], can control the function of stem cells, as well as environmental factors (e.g. surface chemistry and topography)[7,8] but it is also becoming clear that mechanical forces can greatly influence stem cell behaviour.

Tissues and cells in the human body are exposed to a range of different external forces, which influence their development and maintenance[9]. For example, it is well documented that excercise increases bone and muscle mass[10] and inadequate loading as occurs during space flight, prolonged periods of bed rest or spinal cord injury[11] results in decreased muscle and bone mass. MSCs *in vivo* reside in the stem cell niche, which contains many biochemical factors that function to regulate their behaviour. In the bone marrow, mechanical forces in the form of tension, compression, and fluid-induced shear are all present[12] but the nature of these forces is not well understood, neither is what effect they have on stem cell mobilization and function.

Many different cell types have been demonstrated to be highly mechanosensitive in vitro[13] and recent TE strategies for MSC differentiation have included modifying intrinsic (via substrate stiffness[8,14], and external stresses to simulate the physiologically relevant mechanical environment. Mechanical stimulation of MSCs *in vitro* has shown that tensile strain enhances osteogenesis and tenogenesis but inhibits adipogenesis[15,16], hydrostatic pressure and compressive loading induces chondrogenesis[17], and fluid flow induced shear stress upregulates genes associated with osteogenesis[18,19]. In this review we will describe some of the outcomes seen when these

stimuli have been applied to MSCs. However, due to the wide variety of mechanical stimuli available, the enormous array of possible loading conditions, and the addition of different chemical stimulants, the optimum conditions for controlling lineage specific MSC differentiation remain unspecified.

Mechanical Regulation of MSCs

The most extensively studied differentiation pathways of MSCs are osteoblastic, chondrogenic, and adipogenic, other pathways such as tenogenesis and myogenesis have also been investigated to a lesser extent. The optimum durations, magnitudes and frequencies of mechanical loading for lineage specific differentiation of MSCs is not known due to the difficulty of undertaking multiple loading regimens within one set of experiments. The response of MSCs to loading are likely to be age-specific, may be specific to site of origin, and appear to depend on how differentiated the cells are at the time of loading, as well as whether loading is performed in conjunction with biochemical supplements[20,21].

There are many ways in which researchers have stimulated cells with mechanical forces *in vitro* which can generally be categorized into the primary type of stress they induce[22]. These include stretching (tensile stress)[23] hydrostatic pressure or platen abutment (compressive stress), fluid flow (shear stress)[24,25], ultrasound[26,27], high frequency, low magnitude displacement (vibration)[28,29], and direct cell membrane magnetic stimuli[30]. For each stimulation mode the stimulus can be applied in 2D (monolayer culture) or 3D (multilayer culture) and differences between cells cultured in 2D and 3D have been observed in terms of cellular morphology and migration strategies, matrix adhesion, gene and protein expression and responses to fluid flow[31].

It is important to note that in many loading systems there will be secondary effects along with the main mechanical stimulus. For example, in 3D tensile and compression loading systems, there will be fluid drawn in and out of the scaffold causing shear stress at the cell membrane and improved nutrient transfer to the cells[32]. Also, the cells will most likely be subjected to additional compressive or tensile forces caused by substrate bending or the Poisson's effect. Scaffold architecture will also regulate how much of the applied force is received by the cells, for example in a cell-seeded gel the loading received will be relatively homogeneous throughout the scaffold whereas a porous scaffold will have an uneven strain transfer. In-

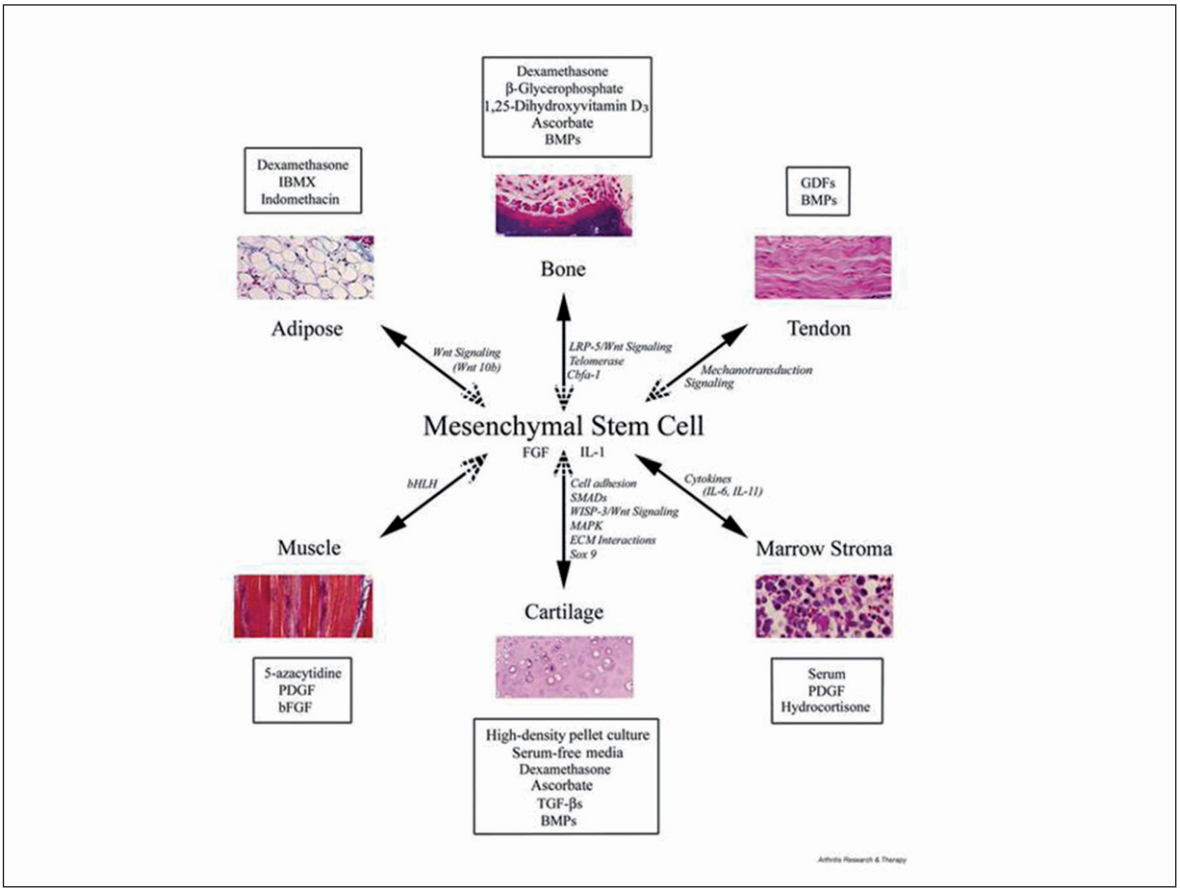

Figure 1. Diagram summarising the lineage potential of adult human MSC. The figure depicts *the in vitro* culture conditions (boxed) used to promote the differentiation into the lineage indicated and some of the signalling pathways and transcription factors involved in the process (italics). Reprinted from Arthritis Research and Therapy (4), BioMed Central, with kind permission of Professor Tuan.

creased nutrient transfer from fluid flow can result in better cell infiltration and matrix distribution, as well as enhancement of cell differentiation due to mass transport effects[33]. Therefore, in order to optimise stimulation regimens it is important to identify the effects of individual stimuli.

Tensile Loading

Tenogenesis: when targeting tenogenesis, MSCs are often seeded on collagen-based or collagen coated scaffolds and then cultured in standard media as there is currently no defined medium for inducing tenogenesis of MSCs. Chen et al.[34] subjected hBMSCs to 3% and 10% global strain and observed an increase in Collagen type I (Col I), Col III, and tenascin-C mRNA at 10%, whereas 3% strain favoured osteogenic differentiation. Farng et al.[35] subjected mouse BMSC-seeded poly(caprolactone) scaffolds to 10% strain, which also increased tenogenic gene production of Col I, Col III, and the tendon transcription factor scleraxis. Zhang et al.[36] observed the effects of varying the time period (3-36 h) rat BMSCs were subjected to 10% cyclic strain and saw that Col I, Col III, and tenascin-C mRNA were upregulated after 24 h. 10% strain at a low frequency (0.0167 Hz) on human and bovine BMSCs in collagen gels also resulted in an upregulation of Col I, Col III, and tenascin-C mRNA, but this took 14 days of culture[37]. Strains lower than 10% have also been used in an attempt to induce tenogenesis. 1% strain was applied to human BMSC-seeded collagen gels resulting in an upregulation of Col III and maintaining the level of scleraxis mRNA, whereas in static controls the expression reduced over time[38]. The effect of a 6.7N static strain was observed by van Eijk et al.[39] on goat BMSC-seeded PLGA scaffolds. Initially, collagen content was highest in scaffolds strained during seeding, but after 21 days, unloaded scaffolds had the highest collagen content suggesting that a constant strain inhibits differentiation.

Myogenesis: the proportion of MSCs that have exhibit a myogenic phenotype is low using defined or conditioned culture medium[40]. Some studies have suggested that myogenic differentiation of MSCs can be influenced by mechanical tension. Increased gene and protein expression associated with myogenesis have been seen when bovine MSCs were subjected to stretching including the calcium binding protein calponin[41], the calcponin related protein SM22 [42] and smooth muscle actin (SMA)[43,44]. However, Park et al.[42] found that while uniaxial strain increased SM22 expression, equiaxial strain reduced its expression highlighting the different regulatory roles of these two stimuli. Ku et al.[45] subjected human BMSCs to strains of 7-20% over 4 days and observed the highest collagen production at 14% strain and an increase in lysyl oxidase (Lox), however, there were no increases in Col II mRNA (the collagen found in cartilage) or Alkaline Phosphatase (ALP) activity. ALP is involved in bone mineralisation and high ALP activity is used to indicate osteogenic differentiation. Colazzo et al.[46] subjected human BMSCs and ADMCSs to 14% strain for 3 days causing increases in collagen production and Col IV mRNA, and upregulation of Col III and elastin cross-linking in ADMSCs. Huang et al.[40] tested a range a loading regimens and showed that 10% stretch at 1 Hz for 24 hours was optimal for inducing cardimyocyte gene upregulation in rat MSCs compared to lower or higher strains and frequencies, interestingly the stretch stimulus was much more effective than a 1 Pa unidirectional shear stress stimulus. In agreement, Maul et al.[47] compared the effects of cyclic tension, compression, and fluid flow induced shear stress on the expression of smooth muscle related proteins and only stretch (1-10% at 1or 2.75 Hz) upregulated SMA and calponin.

Osteogenesis: application of cyclic tensile loading to MSCs has resulted in increased expression of early osteogenic markers as well as increased mineralised matrix deposition, both in the presence and absence of osteogenic media. Bone morphogenic protein 2 (BMP-2) expression was upregulated following cyclic loading of human BMSC-seeded gels[48,49] and rat ADMSCs on 2D substrates[50]. Alkaline phosphatase activity (ALP) increased with 1 week of cyclic loading in human BMSCs. It appears that dexamethasone can have a synergistic effect or an inhibitory effect on mechanically induced osteogenesis, depending on the concentration used and the marker investigated. For example Jagodzinski et al.[51] applied tensile strain to human BMSCs for 6 hours/day, on the first 3 days of culture, at two different strain rates (2% and 8%). Cyclic tensile strain upregulated COLI mRNA and ALP activity, but only at the 8% strain rate. Stretching alone was seen to be as effective as dex treatment alone and there was a synergistic effect of the combination of dex and cyclic tensile strain. Muaney et al.[52] investigated the effect of the concentration of dex (0, 10 or 100nM) on the osteogenic enhancing potential of loading (bending). Without dex, loading alone was able to upregulate ALP activity and expression, but it had no effect on the bone matrix proteins osteopontin (OPN) and oscteoclacin (OCN). The addition of 10nM dex seemed to cause a synergistic response but at higher dex levels (100nM) the effect of loading was suppressed.

Summary of tensile stimuli: the literature agrees in general that osteogenesis of MSCs tends to occur at strain magnitudes lower than that for tenogenesis and that cardiomyogenesis is stimulated by using larger strains. In the absence of osteogenic media, upregulation of early (ALP activity) and late (mineralized matrix deposition) osteogenic markers have been observed[53]. Tenogenesis is also induced in the absence of any chemical inducers, but as for osteogenesis, it appears that static stretching or long term continuous loading has a negative effect on matrix production[39,54]. Stretching inhibits adipogenesis even in the presence of adipogenic media[55] and stretching does not appear to be favourable for chondrogenesis, although tensile strains induced in a more biomimetic loading regimen, that of sliding contact loading does slightly enhance chondrogenesis of MSCs[56]. These findings indicate that intermittent, cyclic stretching of MSCs is beneficial for the osteogenic, tenogenenic, and myogenic lineages and the production of a fibrous matrix.

Compressive Loading

Compressive loading of MSCs has mainly been investigated for its potential in promoting chondrogeneic differentiation. The chondrogenic response of MSCs to loading is highly complex and the various loading regimens used and the effects that the time at which loading is applied to the cultures has on the outcome is reviewed in more detail elsewhere[21].

Osteogenesis: a small number of studies have investigated the effect that global compressive loading[20,57] or hydrostatic compression[58] of cell-seeded scaffolds may have on osteogenic induction. Hydrostatic compression at loads lower than those usually used for chondrogenesis upregulated ALP activity and the bone transcription factor RUNX2/cbfa1 in MSCs[58]. Interestingly early markers of either osteogenesis or chondrogenesis can be induced by dynamic compression in the same batch of human MSCs under the same conditions, in an alginate gel-filled collagen sponge, just by varying the strain magnitude (10% strain induced osteogenic genes 15% induced both osteo and chondrogenic genes) but it is not clear what type of tissue would be formed by these cells[59]. In our studies[20] scaffold compression of a polymer scaffold seeded with human MSCs upregulated genes associated with bone matrix formation (Fig. 2) and enhanced the formation of mineralised bone-like matrix. Continuous loading was not necessary to induce MSC differentiation in that study, just 2 hours of loading every 5 days upregulated calcium deposition by more than 50%. However as discussed previously in this type of porous scaffold the individual cells are unlikely to be subjected to compression but to secondary effects such as tension and bending of the scaffolds struts and fluid flow of media into and out of the scaffold[20,21]. Overall, true compressive loading of MSCs seems to be beneficial for the production of a non-fibrous, cartilage-like matrix, in contrast to tensile loading.

Fluid Flow Induced Shear Stress

The most commonly used method for inducing shear stresses over a cell monolayer is the parallel-plate flow chamber[60,61] although simple lab equipment such as rockers and orbital shakers can also be used to apply a characterised, though less homogenous flow stimulus to cells[62.64] (Brennan 2012). In 3D cultures flow is applied us-

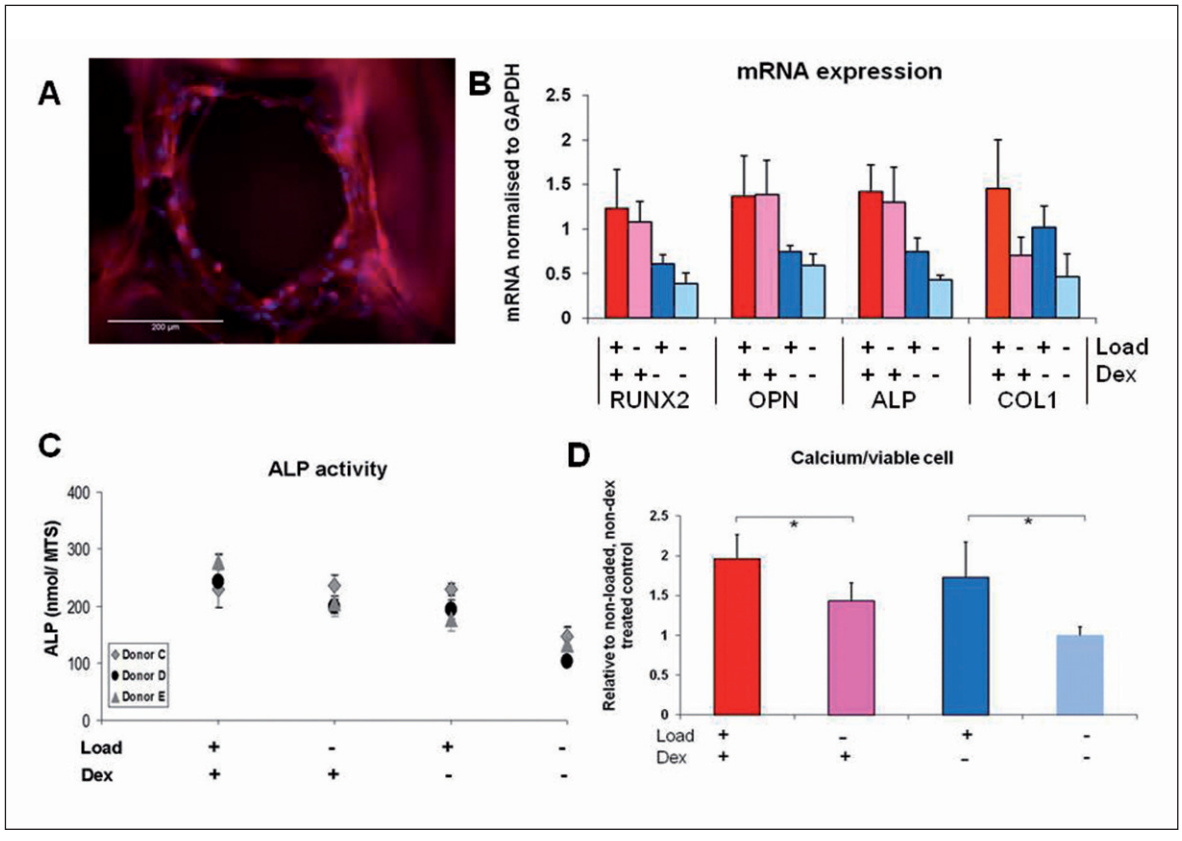

Figure 2. Compression loading of human MSCs in polyurethane foam scaffolds. **A**: Fluorescent micrograph of a pore of the scaffold with MSCs attached (blue = cell nucleus stained with DAPI, red = cell cytoskeleton stained with TRITC-phaloidin). **B**: PCR analysis of mRNA for RUNX2, OPN and ALP showed that these genes were only slightly upregulated by the short (2 hour) loading period and not as much as by continuous dex treatment. However Col 1 was upregulated by loading and inhibited by dex which was also reflected in collagen analysis by Sirius red at a later time-point (data not shown). ALP activity was stimulated by loading to levels seen in dex treated cells as was calcium secretion which was highest with a combination of dex and loading. Adapted from (20) reproduced with kind permission from eCM journal (www.ecmjournal.org).

ing perfusion bioreactors[65,66] with a steady, pulsatile and uni-directional flow having all been investigated. The majority of studies have focused on osteogenic differentiation of MSCs. Given that osteoblasts (bone forming cells) and osteocytes (the terminally differentiated cell that reside in bone matrix) have been repeatedly shown to respond to fluid forces *in vitro*[67-69], there is an natural assumption that fluid shear stress will influence osteogensis of MSCs. MSCs seeded on 2D substrates are usually subjected to levels of shear stress around (0.1-2 Pa) whereas the shear stresses experienced in 3D constructs are often much lower than in 2D experiments (0.1 mPa-0.03 Pa) as summarised in McCoy and O'Brien[70].

Overall, fluid flow appears to either induce or enhance osteogenesis in MSCs. Osteogenic human MSCs were subjected to 1.2 Pa shear forces for either 30 or 90 mins and an increase in ALP activity was seen, with 30mins of stimulation showing the highest levels[71]. However, there was no increase in cbfa1expression and interestingly Col I gene expression was lower after flow. This enhancement of ALP activity was also seen in other 2D systems[61] including our own[62]. In our laboratory only 1 hour per day of oscillating flow at 1Hz, beginning on day 5 of culture was sufficient to upregulate ALP, collagen and calcium production (Fig. 3). However, some studies have noticed an inhibition of ALP activity by flow[72]. Interestingly, Yourek et al.[19] noted that although cellular ALP in human MSCs was lower after exposure to 24 hours of continuous fluid flow compared to static controls there was more ALP release into the media in the cells exposed to flow, which is not usually measured in most experimental set-ups, suggesting that flow causes ALP mobilisation, rather than inhibiting osteogenic differentiation. In those experiments there was little additional effect of fluid flow induced sear stress when dex was already present but shear stress upregulated the bone matrix proteins OPN and bone sialoprotein (BSP) and the growth factor BMP-2 to induce osteogenesis when dex was not present.

Steady perfusion of 3D cell-seeded constructs is almost always reported to stimulate ALP activity, with the greatest effects occurring at the earlier time points of 4-8 days[65,66,73] and then often levelling off, although in some studies, significant increases have been seen up to 14-16 days of culture[74,75]. Increasing the flow rate can increase calcium production up to a point suggesting that increasing the fluid flow induced shear stress affects later stages of differentiation more than earlier stages or is more important for matrix formation than stimulation of differentiation[66,74]. The aim of continuous fluid flow through a scaffold is usually to improve nutrient perfusion with any mechanobiological effects of fluid flow induced shear stresses being a positive side-effect. We have shown that continuous perfusion is not necessary to upregulate ALP activity or mineralisation of human MSCs both of which can be upregulated by short bouts of oscillatory fluid flow in a variety of scaffold types including the

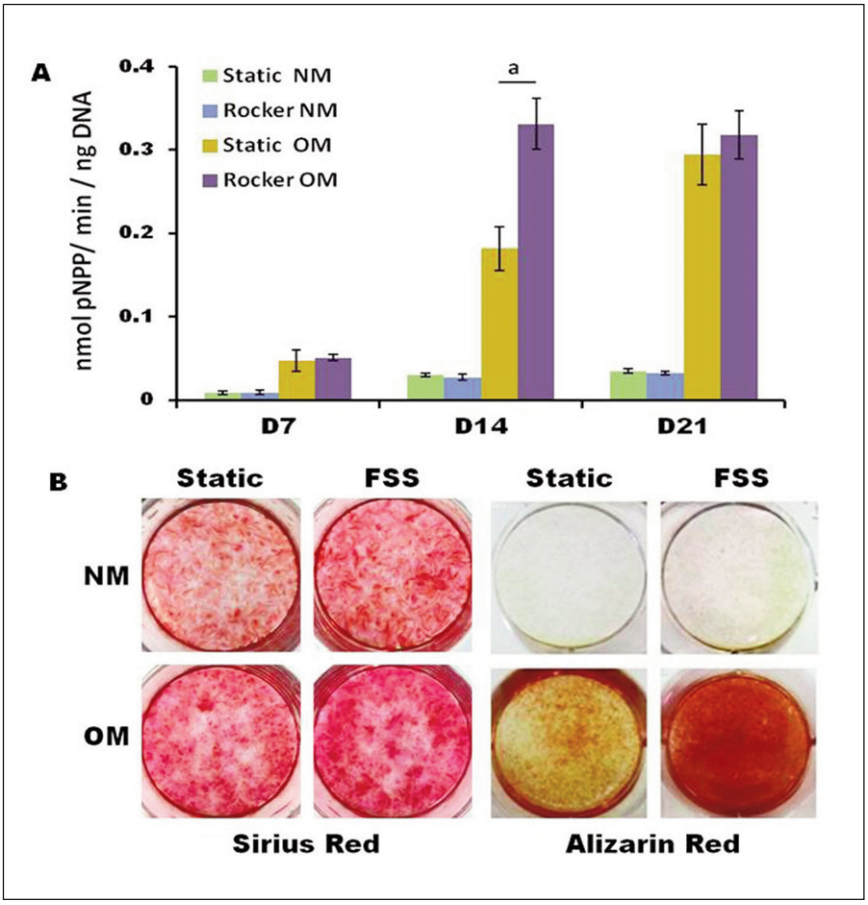

Figure 3. Osteogenic progenitor cells of the hES-MP line were subjected to oscillatory fluid flow induced shear stress (FFSS) using a simple rocking platform. ALP activity **(A)** was significantly increased at day 14 with FSS for cells cultured in osteogenic media containing dex (OM). Matrix deposition at day 21 **(B)** was highest in both FFSS groups for Sirius Red (collagen) and in FSS + OM group for Alizarin Red (calcium). Adapted from (62) reproduced with kind permission from eCM journal (www.ecmjournal.org).

polyurethane foam described in figure 2 and a non degradable borosilicate glass scaffold[76,77].

Low Magnitude High Frequency Loading

Other methods of mechanically stimulating MSCs *in vitro* have included direct straining of cell-bound integrins by magnetic force for osteochondrogenesis[78,79] and low-intensity pulsed ultrasound (LIPUS) for promoting osteogenesis[80-82] or chondrogenesis[81,83,84] (reviewed in 21). While these techniques have so far had limited use in the mechanical stimulation of MSCs, the studies performed suggest that they may be useful tools for non-invasive stimulation of MSC differentiation.

An intriguing recently advocated stimulus for MSC differentiation is low magnitude, high frequency (LMHF) loading (or vibration) for osteogenesis[29,85]. This is based on the findings that whole body vibration in animals enhances bone formation[86] and decreases the formation of adipose tissue[87,88]. It was also shown by Luu et al.[87] that more MSCs within a population showed commitment towards the osteogenic lineage compared to the adipogenic lineage after the animals were subjected to vibration. LMHF vibration could be a way to stimulate cells in 3D scaffolds without needing a specific bioreactor tailored to the shape of the construct and making it much easier to maintain sterile conditions. In our laboratory whole plate vibration (15-60 Hz) was performed on a human MSC cell line (hES-MP from Cellartis) resulting in increased ALP activity in the presence of dex at 60 Hz after 45 min of stimulation[29]. However, we found that the outcome was highly dependent on the precise combination of acceleration rate, frequency and even the serum lot that the cells were cultured in, ALP was not upregulated at any other frequency tested and we did not find effects on extracellular matrix deposition. Similarly Lau et al.[89] found no effects of a 1h per day 60Hz LMHF loading regimen on rat MSCs. In contrast Sen et al.[85] performed LMHF loading on a mouse MSC cell line in multipotential media and observed a down regulation in adiponectin and PPAR (adipogenic) gene expression, whereby the mRNA for the bone matrix protein osteocalcin was upregulated. Preliminary experiments in our laboratory subjecting MSCs to LMHF loading in a range of 3D scaffolds have also not yet provided any evidence of a positive effect of LMHF on osteogenesis. However Zhou et al.[90] seeded MSC on demineralised bone scaffolds and found upregulation for the mRNA of a cbfa1 and range of osteogenic matrix proteins. As very little is understood about how cells sense these LMHF movements these different results could be explained by different orientation and arrangements of cells relative to the substrate movement causing the vibration to initiate very different mechanosensory effects.

Summary of interactions between loading and biochemical supplements

There are a number of studies that have shown mechanical loading alone is able to induce expression of osteogenic genes (Runx2, osteopontin, osteocalcin)[19,61] and a few that report calcium deposition by MSCs can be stimulated as a result of mechanical stimulus alone[91]. In most cases, the addition of dex appears to enhance the sensitivity of MSCs to shear stress or strain including enabling mechanoregulated-increased calcium deposition[62]. However collagen production by MSCs can be inhibited by dex, including in our studies[20,62] and tenogenesis in terms of improved Col1 production seems to occur best with no additional supplements[36,92]. Smooth muscle differentiation stimulated either by fluid flow induced shear stress or cyclic tensile strain was enhanced synergistically with the addition of 5-aza[40,60]. It may be that biochemical factors and mechanical forces need to work synergistically to stimulate specific pathways or that MSCs need to be at a certain level of maturity (along the specific differentiation pathway) before they sense the load.

Mechanical regulation of MSC proliferation and migration

When Li et al.[72] subjected hMSCs to oscillatory fluid flow, they noticed an increase in intracellular calcium mobilization as well as cell proliferation and in another study by Riddle et al.[93], fluid shear stresses were seen to enhance hMSC proliferation in part due to calcium signalling. Cyclic tensile strain can also increase MSC proliferation as demonstrated by Ghazanfari et al.[94]. In contrast, there are many studies that have shown mechanical stimulation to have no effect on cell proliferation[95-97] as well as reducing MSC proliferation[16,43,98]. These mixed findings can most likely be explained by the diversity of conditions including mechanical stimulation, MSC species, and culture media used, as well as the wide range of loading parameters used. However, high strains and flow rates can be also detrimental to cell viability for instance Kearney et al.[99] subjected rat MSCs to uniaxial cyclic strain and observed that 7.5% strain or greater lead to cell apoptosis.

In MSC-seeded tissue engineered constructs, flow perfusion, even for short bouts, appears to cause cells to spread evenly through the entirety of the construct, compared with poor spreading under static conditions, indicating that flow improves cell mobility[100]. However Ode et al.[101] showed that MSCs in a fibrin clot subjected to high stains of 20% (aimed at mimicking a fracture healing environment) had a lower ability to migrate compared to non strained cells, an effect mediated by the surface proteins CD73 and integrin ß 1.

Mechanical loading has been shown to affect cell orientation and spreading, and in particular substrate strain can cause elongation and alignment of MSCs[41,43,102]. Differences in direction of alignment relative to the load direction have been observed with MSCs seeded on 2D substrates orientating perpendicular[41,43] and MSCs seeded in 3D gels orientating parallel[102]. It is thought that on 2D substrates, cells re-orientate to minimize the stretch forces felt by the cell body[42] while in 3D matrices, elongation of scaffold pores and struts caused by the strain may dictate cellular orientation. Fluid flow in a parallel plate flow chamber can also induce cell morphological changes and orientation usually in parallel to the flow direction, when it is unidirectional. Interestingly even when rat BM-

SCs from the same batch, grown in the same bioreactor are subjected to fluid flow or tensile strain they align parallel to the flow direction but perpendicular to the tensile strain direction[47].

Mechanotransduction mechanisms

Targeting the mechanisms responsible for the conversion of extracellular mechanical stimuli into biochemical signals will aid with future stem cell strategies. There have been a number of possible cell membrane mechanoreceptors identified including integrins (transmembrane proteins), stretch activated ion channels and g-protein coupled receptors, the pericellular glycocalyx, and the non-motile primary cilia.

Integrins couple the cytoskeleton to the ECM and cluster at focal adhesion points on the cell surface forming an integrin-ligand bond with the ECM. Application of an external force pulls on the integrin-ligand bond, which transfers across the cell membrane and can result in cytoskeletal deformation. Another mechanism involves deformation of the plasma membrane causing ion flux into/out of the cell via stretch activated ion channels or g-protein coupled receptors[103]. The third proposed mechanism, the glycocalyx, is a pericelllular GAG-proteoglycan rich layer surrounding the cell membrane that creates a drag force when fluid flow passes over causing plasma membrane deformation[104-106]. More recently, the primary cilium, an immotile microtubule-based organelle, that protrudes like an antenna from the apical cell surface, has been implicated as a mechanosensor in a variety of cell types including MSCs[107]. Primary cilia have been shown to bend under fluid flow[107], adjust their length in response to load in tendon cells[108] and contain receptors that participate in numerous signalling events including stretch activated ion channels[109] and integrins[110]. Recently it has been shown that the primary cilia of human MSCs are required for the modulation of osteogenic and adipogenic differentiation pathways in static conditions, opening up the possibility that they may also mediate mechanically activated differentiation pathways in these cells[111]. Mesenchymal progenitors from the cell line hES-MP that we have demonstrated is mechanosensitive to a range of fluid flow stimuli also exhibit primary cilia as visualised by acetylated tubulin staining (Fig. 4).

Conclusions and Future Directions

Our understanding of the cues affecting MSC differentiation and development has advanced greatly over recent decades. Mechanical forces can greatly influence MSC differentiation and by harnessing its effects, it may be possible to improve pre-implantation culture methods of MSC-seeded constructs and also aid in the design of tissue stimulation/exercise regimens for a patient post-MSC implantation. External mechanical forces are able to induce or enhance MSC differentiation into a wide variety of tissue specific cells; however, precisely controlling the timing and outcome of MSC differentiation is still a large challenge.

Mechanical regulation of MSCs is a complex issue due to the wide range of external mechanical stimuli available (e.g. tension, compression, fluid shear) each accompanied by an almost unlimited choice of loading parameters. Further work is required to indentify which type of mechanical stimuli (or combinations) are the most appropri-

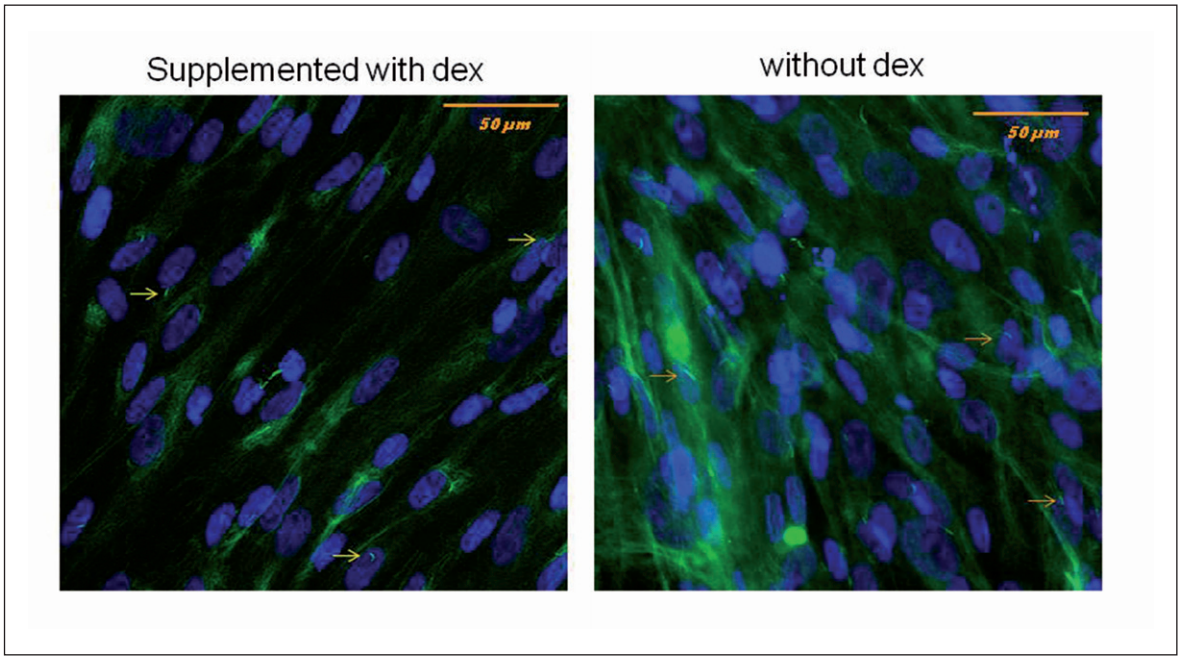

Figure 4. MSCs of the cell line hES-MP (embryonic derived mesenchymal progenitors) stained for acetylated alpha tubulin on day 7 of culture. The structures with a high aspect ratio that stain brightly are primary cilia (yellow arrows), the dispersed, background green staining is the cell microtubules. Nuclei are stained blue with DAPI.

ate as well as the load magnitude, duration and frequency, and when to initiate the loading during culture, in order to pinpoint the optimal strategies. Characterisation of the exact forces MSCs experience in loading systems is important to know what mechanisms are actually inducing the observed responses and to help simplify the design of future loading systems. While most bioreactor systems employ a common force type (e.g. tension or compression), there are also likely to be other mechanisms at work causing significant secondary effects which can cause a misinterpretation in the reason for the obtained results. Mathematical and computer modelling is important for characterising the forces that are being experienced by the cells and will subsequently provide a better understanding of what the cell is responding to[112].

Acknowledgements

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