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Directing bone marrow-derived stromal cell function with mechanics

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Abstract: Because bone marrow-derived stromal cells (BMSCs) are able to generate many cell types, they are envisioned as source of regenerative cells to repair numerous tissues, including bone, cartilage, and ligaments. Success of BMSC-based therapies, however, relies on a number of methodological improvements, among which better understanding and control of the BMSC differentiation pathways. Since many years, the biochemical environment is known to govern BMSC differentiation, but more recent evidences show that the biomechanical environment is also directing cell functions. Using in vitro systems that aim to reproduce selected components of the in vivo mechanical environment, it was demonstrated that mechanical loadings can affect BMSC proliferation and improve the osteogenic, chondrogenic, or myogenic phenotype of BMSCs. These effects, however, seem to be modulated by parameters other than mechanics, such as substrate nature or soluble biochemical environment. This paper reviews and discusses recent experimental data showing that despite some knowledge limitation, mechanical stimulation already constitutes an additional and efficient tool to drive BMSC differentiation.

Introduction

Over the last decades, tissue engineering has been proposed as a method to repair or regenerate a growing number of tissues and organs (skin, bone, heart, pancreas, etc.). This therapeutic strategy consists of transplantation of ex-vivo expanded regenerative cells, sometimes associated with a biomaterial scaffold and/or molecular agents for tissue reconstruction. Many cell types are envisioned as source for those regenerative cells: from organ/tissue specific cells (chondrocytes, skin fibroblast, etc.) to totipotent embryonic stem cells (Muschler et al., 2004). One of the most promising sources of regenerative cells, however, might be the bone marrow. Evidence that bone marrow contains cells having an important proliferation capacity and being able to form bone and cartilage was first showed by Friedenstein et al. (1970) in the late 60s. Since then, many studies have established that these cells can differentiate in vitro into chondrocytes, osteoblasts, adipocytes, and myoblasts (Chamberlain et al., 2007; Krampera et al., 2006) (Fig. 1A). They are currently referred either as bone marrow-derived mesenchymal stem cells (as they can give rise to different mesenchymal tissues) or bone marrow-derived stromal cells (as they are isolated from the supportive structure (stroma) of the bone marrow, BMSCs). Multipotent BMSCs constitute one of the most promising sources of regenerative cells, because, as adult cells, they (i) can be harvested from the patient, minimizing risks of rejection and pathogen transmission; (ii) possess high proliferate rates, allowing the implantation of a large number of regenerative cells; and (iii)

are able to give rise to several differentiated cell types, expanding the number of potential applications.

As a consequence, they have been used in numerous animal models to repair bone defects (Kadiyala et al., 1997; Kon et al., 2000; Petite et al., 2000), cartilage lesions (Guo et al., 2004; Uematsu et al., 2005), and infarcted hearts (Dai et al., 2005; Fazel et al., 2005; Tomita et al., 1999). Few clinical studies, however, have been conducted to date: BMSC transplantation has been shown to be capable of increasing bone mass in children with osteogenesis imperfecta (Horwitz et al., 2001), repairing large bone defect (Quarto et al., 2001), reconstructing a distal phalanx (Vacanti et al., 2001), improving cardiac function after acute myocardial infarction (Chen et al., 2004), and repairing cartilage defects in osteoarthritis patients (Wakitani et al., 2002). Although these results are promising, they were not able to establish that tissue engineering approaches were superior to standard treatments. But, it is the general belief that with further advancement, tissue engineering protocols will eventually be helpful in clinics.

To improve such protocols, it is important to understand and control the pathways guiding uncommitted BMSCs to become differentiated and functional cells. Since many years the differentiation of BMSCs has been induced by various cocktails of biochemical agents and growth factors, such as dexamethasone, β -glycerophosphate and ascorbic acid for osteogenesis (Jaiswal et al., 1997; Pittenger et al., 1999) or serum free medium and

transforming growth factor β (TGF β) for chondrogenesis (Johnstone et al., 1998; Pittenger et al., 1999). More recently several studies have established that the cell mechanical environment can also play a role in cell function and that it is possible to control BMSC proliferation and differentiation with mechanical loadings. This paper reviews recent published works addressing the mechanical regulation and stimulation of BMSC proliferation and differentiation. Parameters eventually affecting cell response to mechanical loadings are also discussed.

Stimulating cells

In vivo Mechanobiology

Starting from studies of the late 19th century on bone internal structure (Meyer, 1867), the idea that body, organ, or tissue structure is associated with its mechanical environment or function has become widely accepted. More recently, investigations have also explored *in vivo* mechanical adaptation of soft connective tissues, such as articular cartilage (King et al., 2005; Saadat et al., 2006; Wong and Carter, 2003), intervertebral disc (Brickley-Parson and Glimcher, 1984; MacLean et al., 2008; Stokes et al., 2006), circulatory system tissues (ben Driss et al., 1997; Opitz et al., 2007; van Gieson et al., 2003; Lehoux, 2006), and tendons and ligaments (Hayashi, 1996). Many of these investigations have suggested an association of cell shape, tissue composition and micro-structure to different types of mechanical environments. Although *in vivo* mechanical environments result from geometry- and muscle activity-dependent external loads that are not definitively known, some general patterns have been identified. Much experimental evidences suggest that, among other extracellular matrix (ECM) components, production of proteoglycans by chondrocytes is mostly stimulated by cyclic hydrostatic pressures, collagen type I produced by fibroblasts, chondrocytes, or smooth muscle cells usually appears in direct, shear-induced, or pressure-induced (vessels) tensile environments, and collagen type II production by chondrocytes or fibroblasts is rather affected by compressive loads.

In vivo remodeling of tendons and ligaments has been extensively investigated and illustrates perfectly the natural changes occurring in soft tissues and associated cells in response to different loadings. Under *in vivo* altered mechanical loadings, complete or partial phenotype changes from ligament to bone have been observed (Tsukamoto et al., 2006). Under normal physiological loadings, entheses or wrap-around tendons are a good illustration of how gradients in cell and tissue phenotypes, i.e. from fibrous to fibro-cartilaginous, can be closely related to gradients in local mechanical environments, i.e. from tensile to compressive (Matyas et al., 1995; Benjamin and Ralphs, 1998). Entheses link purely fibrous to calcified tissues and are believed to act as dissipaters to progressively reduce the strain energy density from the strong but deformable tendons/ligaments to the stiff but less deformable adjacent bone (Shaw and Benjamin, 2007). At given points of this transition, cell phenotype and ECM were shown to be partly controlled by the local mechanical environment (Kotani et al., 1998; Benjamin et al., 2006). Although the origin of enthesis cells is still a matter for discussion, progenitor cells contained in the endotenon (connective tissue between the strands in a tendon) and/or in the adjacent bone marrow have been proposed as precursors (Benjamin et al., 2006). Note that such assumption has been partly raised by the improved healing of chondral entheses after implantation of tendon grafts covered with BMSCs (Lim et al., 2004).

Healing of cartilage defects in knee joints, including subchondral bone disruption, is also believed to involve progenitor cells (Appleton et al., 2007; Metsäranta et al., 1996; Buckwalter and Brown, 2004). In such cases, tissue mechanical environment was shown to significantly influence the type of produced ECM. An *in vivo* study on skeletally mature monkeys showed that production of collagen type II and hyaline tissue repair were higher with imposed intermittent passive flexion of the knee than with cast immobilization (Buckwalter et al., 2003). However, repaired cartilage obtained under motion had lower aggregate modulus and higher permeability than undamaged articular cartilage. Actually, with cast immobilization, muscles are still tonic and maintain the tissue under compression, while passive flexion motion increases shear deformations in the damaged tissue. Thus, at short term, cast immobilization most likely preserved a higher relative amount of hydrostatic pressure, known to favor glycosaminoglycan (GAG) synthesis, largely responsible for the properties of normal articular cartilage (Katta et al., 2008; Wong and Carter, 2003).

In bone healing or distraction osteogenesis, progenitor cells are also suspected to play a key role, through successive formation of fibrous, cartilaginous, and osseous tissues, controlled by the local mechanical environment. Such hypothesis has been successfully used in different theoretical models that have been able to predict, more or less phenomenologically, *in vivo* bone fracture healing (Isaksson et al., 2008; Lacroix and Prendergast, 2002), load-dependent tissue growth and remodeling (García-Aznar et al., 2007; Isaksson et al., 2009), and cartilage repair (Kelly and Prendergast, 2005). In all these models, predicted phenotypes were mainly controlled by deviatoric strains and parameters associated with volumetric strains, e.g. fluid flow.

In vitro systems

Most of the studies looking at BMSC phenotype modulation by mechanical means have aimed to reproduce *in vitro* one component of the *in vivo* mechanical environments under highly controlled conditions. To improve chondrogenic differentiation, for instance, BMSCs have been exposed to hydraulic pressure, trying to reproduce the hydrostatic stress experienced by cartilage. To this end, BMSCs have been put under hydrostatic pressure (Angele et al., 2003; Finger et al., 2007; Miyashita et al., 2006; Scherer et al., 2004). They have also been seeded into hydrogels (agarose (Campbell et al., 2006; Huang et al., 2004; Mauck et al., 2007; Mouw et al., 2007), fibrin (Pelaez et al., 2008), hyaluronan/gelatin (Angele et al., 2004), etc.) and exposed to carrier compression (Angele et al., 2004; Campbell et al., 2006; Huang et al., 2004; Mauck et al., 2007; Mouw et al., 2007; Pelaez et al., 2008; Terraciano et al., 2007) (Fig. 2B). It is difficult to compare these two types of mechanical stimulation. Hydrostatic pressure is a single component of the compressive environment in cartilage. In contrast, carrier compression involves a mixture of codependent pressure, strain, fluid flow, and gradients, and is more similar to the *in vivo* mechanical environment of loaded articular cartilage. They both, however, led to equivalent improvement of chondrogenic differentiation of BMSCs, with increased expression of early markers (Finger et al., 2007; Huang et al., 2005; Miyashita et al., 2006; Terraciano et al., 2007) and enhanced deposition of cartilaginous ECM (Angele et al., 2003; Angele et al., 2004; Mauck et al., 2007; Miyashita et al., 2006; Mouw et al., 2007; Terraciano et al., 2007).

To drive osteogenesis, BMSCs have been exposed to substrate strain, mimicking bone matrix deformation during loading (gait,

motions, etc.) by scaffold stretching resulting in uniaxial (Fig. 2C) (Byrne et al., 2008; Diederichs et al., 2009; Haasper et al., 2008; Jagodzinski et al., 2004) or bi- or equi-axial strain (Fig. 2D) (Friedl et al., 2007; Mauney et al., 2004; Qi et al., 2008; Simmons et al., 2003; Sumanasinghe et al., 2006; Thomas and el Haj, 1996; Ward Jr. et al., 2007; Yoshikawa et al., 1997). Scaffold strains resulted in up-regulation of early markers of osteogenic differentiation (Friedl et al., 2007; Haasper et al., 2008; Qi et al., 2008; Ward Jr. et al., 2007), as well in improved deposition of bone proteins and calcification of the ECM (Diederichs et al., 2009; Mauney et al., 2004; Simmons et al., 2003; Ward Jr. et al., 2007; Yoshikawa et al., 1997).

Some studies have also explored the effects of shear stress on osteoblastic differentiation of BMSCs (Kreke and Goldstein, 2004; Kreke et al., 2008; Li et al., 2004) (Fig. 2A). Current hypotheses propose that bone matrix deformation during gait results in the oscillatory movement of interstitial fluid inside the canalicular network, resulting in application of shear stress to bone cell processes. Finally, flow perfusion of 3D porous scaffolds has also been used to induce osteogenesis. Though the primary reason to use such systems was to improve nutrient supply and waste removal in thick scaffolds, flow perfusion results in BMSC exposure to (uncontrolled and heterogenous) shear stress (Bancroft et al., 2002; Goldstein et al., 2001; Holtorf et al., 2005a; Meinel et al., 2004; Sikavitsas et al., 2002; Sikavitsas et al., 2003; Stiehler et al., 2009; Zhao et al., 2007) (Fig. 2A). This shear stress has been showed to have an effect by itself. Increased viscosity of the perfused medium, resulting in larger shear stress at the same solute flow rates, enhanced calcium deposition by rat BMSCs (Sikavitsas et al., 2003). Applying shear stress to BMSCs, either controlled (fluid flow) or uncontrolled (flow perfusion), led to increased expression of early markers (Jagodzinski et al., 2004; Jagodzinski et al., 2008; Scaglione et al., 2008; Stiehler et al., 2009) and improved osteogenic maturation of the ECM (Bancroft et al., 2002; Holtorf et al., 2005a; Jagodzinski et al., 2008; Kreke et al., 2008; Kreke et al., 2005; Kreke and Goldstein, 2004; Meinel et al., 2004; Sikavitsas et al., 2003; Zhao et al., 2007).

Finally, substrate strain (uniaxial (Kurpinski et al., 2006; Park et al., 2004; Engelmayr Jr. et al., 2006; Gong and Niklason, 2008; Hamilton et al., 2004; Nieponice et al., 2007; Park et al., 2004); Figs. 2C and D) has also been used to improve BMSC differentiation into vascular smooth muscle cells (VSMCs) for vascular tissue repair (Engelmayr Jr. et al., 2006; Hamilton et al., 2004; Nieponice et al., 2007; Park et al., 2004), as these tissues mainly experience circumferential traction.

Affecting orientation

One of the first cell functions affected by mechanical loading is cell spreading. Substrate strain, indeed, has been shown to induce elongation and alignment of BMSCs (Hamilton et al., 2004; Kurpinski et al., 2006; Nieponice et al., 2007; Park et al., 2004). The orientation of such alignment, however, was reported to be perpendicular (Chen et al., 2008; Hamilton et al., 2004; Kurpinski et al., 2006; Park et al., 2004) as well as parallel (Nieponice et al., 2007) to the direction of the applied strain. These differences may be explained by the different substrates used: 2D collagen type I coated substrates (Hamilton et al., 2004; Kurpinski et al., 2006; Park et al., 2004) versus 3D fibrin gels (Nieponice et al., 2007). In 2D systems, BMSCs are hypothesized to re-orientate to minimize stretch forces applied to the cell bodies (Park et al., 2004). In

stretched 3D gels, elongation and orientation of the pores in the strain direction may impede BMSC re-orientation perpendicular to this strain.

Controlling proliferation

Mechanical loadings can also affect cell proliferation, although their exact effects are still unclear. Many types of mechanical stimulations have been, indeed, shown to improve (Fig. 3) (Bancroft et al., 2002; Holtorf et al., 2005a; Jagodzinski et al., 2008; Nieponice et al., 2007; Pelaez et al., 2008; Qi et al., 2008; Riddle et al., 2008; Sikavitsas et al., 2003; Song et al., 2007; Yoshikawa et al., 1997) as well as to decrease (Diederichs et al., 2009; Gong and Niklason, 2008; Hamilton et al., 2004; Simmons et al., 2003; Zhao et al., 2007) BMSC growth. Mechanical loadings have also been reported to have no effects on cell proliferation (Angele et al., 2003; Goldstein et al., 2001; Huang et al., 2004; Kreke and Goldstein, 2004; Terraciano et al., 2007; Thomas and el Haj, 1996). These findings seem independent from the type of stimulation (shear stress, compressive load, or substrate stretch), the cell species (human, rat, or goat), or the cell culture medium (containing FBS, dexamethasone, osteogenic supplement, or TGFβ) used. Nevertheless, the diversity of the *in vitro* systems (2D, 3D, substrate nature, cell species, medium, etc.) and of the loading protocols (duration, frequency, amplitude, time in static conditions before loading, etc.) may explain this wide variety in responses.

Driving differentiation

Beyond the question of proliferation, one of the main goals of mechanobiology applied to BMSCs is to drive and to improve their differentiation in mature cells in order to ultimately enhance the mechanical and the regenerative properties of engineered tissues.

Osteogenic pathway (Fig. 1B)

The application of flow perfusion (Jagodzinski et al., 2008; Stiehler et al., 2009), fluid flow (Scaglione et al., 2008), and scaffold stretching (Ward Jr. et al., 2007; Haasper et al., 2008; Jagodzinski et al., 2004; Qi et al., 2008) stimulates the gene expression levels of two early osteogenic markers: osterix (Osx) and runt-related transcription factor 2 (Runx2). Both are transcription factors required for bone formation (Ducy et al., 1999; Nakashima et al., 2002) and regulating commitment of undifferentiated BMSCs to the osteogenic lineage. Runx2 expression seems to be highly sensitive to loading amplitude with inhibited expression for low flow perfusion rates (0.1 vs 10 ml/min) (Bjerre et al., 2008; Jagodzinski et al., 2008) and increased expression for high substrate strain (2% vs 8%) (Haasper et al., 2008; Jagodzinski et al., 2004). This inductive effect, however, can be abolished by application of high shear stress (0.001 vs 1 Pa) (Li et al., 2004; Scaglione et al., 2008). Fluid flow, flow perfusion, hydrostatic pressure, and substrate stretching enhance the formation of an ECM through the regulation of gene (Friedl et al., 2007; Haasper et al., 2008; Jagodzinski et al., 2004; Kim et al., 2007; Kreke et al., 2008; Scaglione et al., 2008; Stiehler et al., 2009) and protein (Diederichs et al., 2009) expression of collagen type I. Such loadings also improved the differentiation of BMSCs into osteoprogenitors by increasing their alkaline phosphatase (ALP) activity (Bancroft et al., 2002; Bjerre et al., 2008; Friedl et al., 2007; Goldstein et al., 2001; Holtorf et al., 2005b; Holtorf et al., 2005a; Jagodzinski et al., 2004; Kim et al., 2007; Mauney et al., 2004; Meinel et al., 2004; Sikavitsas et al., 2003; Stiehler et al., 2009; Zhao et al., 2002). This differentiation process can be

observed as early as after 3 days but may require up to 20 days, with the fastest maturation observed under daily stretching conditions. Fluid flow, flow perfusion, scaffold stretching, and hydrostatic pressure also contribute to further maturation of BMSCs and ECM, by inducing gene (Bjerre et al., 2008; Jagodzinski et al., 2004; Kim et al., 2007; Kreke and Goldstein, 2004; Li et al., 2004; Scaglione et al., 2008) and protein (Bancroft et al., 2002; Holtorf et al., 2005a; Holtorf et al., 2005b; Jagodzinski et al., 2008; Kim et al., 2007; Kreke et al., 2008; Yoshikawa et al., 1997) expression of osteocalcin (OCN), osteopontin (OPN), and bone sialoprotein (BSP) (non collagenous bone proteins, playing roles in matrix mineralization and osteoblast adhesion processes). Finally, flow perfusion (Fig. 4) (Bancroft et al., 2002; Holtorf et al., 2005a; Holtorf et al., 2005b; Meinel et al., 2004; Sikavitsas et al., 2003; Stiehler et al., 2009; Zhao et al., 2002) and scaffold stretching (Fig. 5) (Mauney et al., 2004; Simmons et al., 2003; Ward Jr. et al., 2007) improve the late stage of osteogenic differentiation increasing calcium deposition in the ECM. This process seems sensitive to loading duration as it is mainly observed for continuous stimulations.

Chondrogenic pathway (Fig. 1B)

The application of hydrostatic pressure (Finger et al., 2007; Miyanishi et al., 2006), scaffold compression (Huang et al., 2005; Terraciano et al., 2007), and stretching (Friedl et al., 2007) significantly stimulates the expression of the early chondrogenic marker *sox9*, a transcription factor involved all along the chondrogenic pathway (Lefebvre and Smits, 2005). Compressive loads (hydrostatic pressure and scaffold compression) improved the formation of unspecific ECM with increased collagen type I gene expression (Angele et al., 2004; Finger et al., 2007; Mouw et al., 2007; Wagner et al., 2008), but also enhance the cartilaginous differentiation of BMSCs through gene expression of collagen type II and aggrecan (Angele et al., 2004; Huang et al., 2004; Huang et al., 2005; Mauck et al., 2007; Miyanishi et al., 2006; Mouw et al., 2007; Pelaez et al., 2008; Terraciano et al., 2007; Wagner et al., 2008). Scaffold compression (Fig. 6), hydrostatic pressure, and scaffold stretching enhance, not only short term transcriptional activity of chondrogenic markers but also long term GAG deposition (Angele et al., 2003; Angele et al., 2004; Mauck et al., 2007; McMahon et al., 2008; Miyanishi et al., 2006; Terraciano et al., 2007). This ECM maturation (and cell differentiation), however, requires prolonged exposure to mechanical loads (with 5–14 days of stimulation).

Finally, mechanical stimulations can affect chondrogenic terminal differentiation by inducing chondrocyte hypertrophy. In vivo, this stage of differentiation precedes vascular invasion of cartilage, chondrocyte apoptosis, and subsequent replacement by osteoblasts. This terminal differentiation, however, should be avoided for cartilage tissue engineering, as it may lead to BMSC death. Several studies reported no effects of mechanical loadings on collagen type X (characterizing this stage of differentiation) (Angele et al., 2004; Friedl et al., 2007; Huang et al., 2004), though scaffold compression can enhance its gene expression (Campbell et al., 2006).

Myogenic pathway (Fig. 1B)

So far few studies have concentrated on mechanical stimulations driving vascular smooth muscle cell (VSMC) differentiation of BMSCs when compared to the extensive work done on bone and cartilage phenotype, though myogenic differentiation is also influenced by mechanical loading. The

application of scaffold stretching, indeed, induces increased gene and protein expression of calponin (Kurpinski et al., 2006; Nieponice et al., 2007), SM22 α (Park et al., 2004), α smooth muscle actin (α SMA) (Engelmayr Jr. et al., 2006; Hamilton et al., 2004; Kobayashi et al., 2004; Nieponice et al., 2007), and smooth muscle-myosin heavy chain (SM-MHC) (Kobayashi et al., 2004), all contractile apparatus proteins involved in VSMC cytoskeleton. SM22 α regulation, however, seems to be dependent on the type of stimulation as equiaxial strain is reported to decrease its expression when uniaxial strain increases it (Park et al., 2004).

All these studies provide evidence that BMSCs can adapt their phenotype in response to their mechanical environment. It is, however, difficult to determine which type of mechanical loading specifically enhanced osteogenesis, chondrogenesis, or myogenesis, as similar mechanical stimulations (e.g. 10% strain by scaffold stretching) can improve all three phenotypes. Moreover, mechanical loading has been reported to have no or negative effects on BMSC differentiation. Shear stresses (induced by fluid flow or flow perfusion) and strains (caused by scaffold stretching), for example, have been shown to decrease Runx2, OCN, and BSP expression (Bjerre et al., 2008; Byrne et al., 2008; Scaglione et al., 2008; Stiehler et al., 2009) as well as calcium deposition (Sikavitsas et al., 2002). Compressive (by scaffold compression or hydrostatic pressure) loads have also been reported to have no effects on collagen type II and/or aggrecan gene expression (Campbell et al., 2006; Finger et al., 2007; Scherer et al., 2004). These chondrogenic markers can also be inhibited by hydrostatic pressure (Scherer et al., 2004) or scaffold bending (Ward Jr. et al., 2007). As for myogenic phenotype (α SMA, calponin, and SM22 α protein expression), it can be inhibited by substrate strain (Park et al., 2004). These discrepancies might be explained by the broad range of loading patterns, substrates, and biochemical environments, rather selected from empirical knowledge, than from a thorough understanding of the mechanotransduction phenomena. Furthermore, the local mechanical conditions at the cell level have not been well controlled. In many prior investigations, the loading has been applied to the boundary of the construct. This is especially true for cells in 3D or scaffold environments. Often the mechanical conditions have been calculated considering homogenous linear continuum mechanics principals. And, although even more sophisticated constitutive models have been employed in some cases, they have not been rigorously validated. Hence, the actual mechanical stimulation of the cell may not be that which we can assume from the applied macroscopic loading conditions.

Modulating the effects

The effects of mechanical loading can be, indeed, modulated by the loading pattern applied: stimulation amplitude, duration, and frequency can all alter cell response. For example, osteogenic differentiation (calcium deposition) is shear stress-dependent in fluid flow systems (from 0.3 to 3 ml/min (Bancroft et al., 2002)) and is improved by increased viscosity of cell culture media (Sikavitsas et al., 2003). Chondrogenic differentiation (GAG and collagen content, aggrecan gene expression) is also reported to depend on load magnitude, in hydrostatic pressure systems (from 0.1 to 10 MPa (Miyanishi et al., 2006)). Regarding duration of loading, several studies demonstrate improved differentiation (calcium deposition and runx2 expression for osteogenesis; GAG content and aggrecan and collagen type II expression for chondrogenesis) for longer duration of mechanical stimulations (from 1 to 8 h/day of stretching (Diederichs et al., 2009; Haasper et

al., 2008), scaffold compression (Huang et al., 2005; Mauck et al., 2007; Terraciano et al., 2007), or hydrostatic pressure (Angele et al., 2003)). Even if most of the studies use frequencies of 0.5 or 1 Hz (considered as physiological loading frequencies during gait), frequency can also influence cell response as demonstrated by higher aggrecan and collagen type II gene expression for 1 Hz than for 0.1 Hz scaffold compression (Pelaez et al., 2008).

Another factor influencing BMSC response to mechanical loading is the substrate on/in which cells are seeded. First, the mechanical properties (elasticity) of the matrix itself can direct BMSC proliferation, with higher proliferation for stiffer (7500 Pa) than for softer (250 Pa) matrices (Winer et al., 2009), and differentiation, with more rigid (34 kPa) matrices favoring osteogenesis and softer substrates (11 kPa) favoring myogenesis (Engler et al., 2006) (Fig. 7).

Second, the response of BMSC to mechanical loading is also dependent on the biochemical nature of the scaffold. For example, increased gene expression of OPN and BSP induced by fluid flow is abolished by calcium phosphate coating, due to higher expression in static control for this type of substrate (Scaglione et al., 2008). Fibronectin coating, on the other hand, improves protein expression of calponin by BMSCs exposed to strain, when collagen type I coating or control substrate do not (Gong and Niklason, 2008). These differences might be explained by the involvement of different integrins in the BMSC adhesion to these substrates.

Integrins are transmembrane receptors thought to act as mechanosensors. They are composed of an α and a β sub-unit and bind to ECM proteins on one side of the membrane and to cytoskeletal proteins on the other side (Fig. 8). With 18 α and 8 β sub-units, 24 heterodimers are known, each of which can bind to a definite ligand. Integrins form focal adhesions, insuring cell adhesion as well as signal transduction informing the cell about the ECM. Applying external pulling forces on these focal adhesions can activate, through the cytoskeletal proteins, different signal transduction pathways, all leading to the phosphorylation of ERK1/2, which, in turn, can up/down regulate different transcription factors (Iqbal and Zaidi, 2005). Different integrins, however, will bind to, and signal through, different transduction pathways, resulting in different tuning of ERK1/2 activation. It appears, then, that mechanosensing might be matrix dependent, as shown with a mouse myoblast cell line (Grossi et al., 2007). Adhesion to fibronectin and to collagen, for example, involve different sets of integrins, with $\alpha 5 \beta 1$ and $\alpha 4 \beta 1$ for fibronectin and $\alpha 1 \beta 1$ and $\alpha 2 \beta 1$ for collagen (Docheva et al., 2007; Hynes, 2002), potentially explaining the different cell responses observed with BMSCs seeded on these substrates in Gong and Niklason's study (Gong and Niklason, 2008).

BMSC sensitivity to mechanical stimulation seems also dependent on the soluble biochemical environment. In several studies, mechanical loading alone is enough to improve the expression of different osteogenic markers at the gene level (Runx2, osteocalcin, osteopontin, etc. (Chen et al., 2008; Friedl et al., 2007; Kreke et al., 2005; Li et al., 2004; Qi et al., 2008)), though very few report terminal differentiation of BMSCs (calcium deposition) with mechanical stimuli only (Ward Jr. et al., 2007). The addition of dexamethasone, a glucocorticoid often added into differentiation media for BMSCs, seems to enhance the sensitivity of BMSCs with higher calcium deposition and OPN expression by BMSCs exposed to strain or shear (Holtorf et al., 2005a; Mauney et al., 2004). As for the chondrogenic pathway, effects of

mechanical loading in absence of TGF β (inducer of BMSC chondrogenesis) are unclear. Some studies show this growth factor as not necessary for improvement of chondrogenic phenotype and cartilaginous matrix deposition by mechanical stimulation (Campbell et al., 2006; Huang et al., 2004; Mauck et al., 2007), when others demonstrate that TGF β is required (Finger et al., 2007; Mouw et al., 2007; Scherer et al., 2004).

The influence of these biochemical substances on BMSC responses to mechanical loads might be explained by synergistic effects of both biochemical and mechanical stimulations reciprocally needed to stimulate specific signaling pathways. It is also possible that BMSCs need to reach a threshold state of differentiation (improved by dexamethasone and TGF β) and/or to start to deposit matrix to efficiently relay the mechanical stimuli. Studies reporting enhanced induction of osteogenesis (Thomas and el Haj, 1996; Zhao et al., 2007), chondrogenesis (Mouw et al., 2007), and myogenesis (Kobayashi et al., 2004) by mechanical stimulations of BMSCs previously differentiated in static conditions support this hypothesis.

Conclusion

BMSCs represent one of the most promising sources of regenerative cells for the repair of numerous tissues. Though several animal and clinical studies have demonstrated their potential, a better understanding and control of their proliferation and differentiation is required to fulfill their potential. One of the critical point is to encourage and maintain the appropriate cell metabolism and phenotype. Most of the studies conducted so far have focused on biochemical stimulation and gene transfer to do so, but more recent data report that BMSC proliferation and differentiation are also affected by their mechanical environment. Mechanical stimulation, therefore, represents an additional and an efficient tool to improve and maintain BMSC differentiation. Providing correct mechanical environments both in vitro and in situ will lead to improved engineered tissues and to a greater eventual success in the clinics.

Conflict of interest statement

None of the authors of the submitted review paper, E. Potier, J. Noailly, and K. Ito "Directing mesenchymal stromal cell function with mechanics", have any financial and/or personal relationships with other people or organisations that could inappropriately influence (bias) this work.

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Figure 1. Differentiation potential of bone marrow-derived stromal cells.

(A) Under the appropriate induction BMSCs are able to give rise to adipocytes, osteoblasts, chondroblasts and myocytes. (B) Differentiation pathways of BMSCs: (a) osteogenesis is characterized by the involvement of osterix (Osx, aka SP7) and runt-related transcription factor 2 (Runx2, aka cbfa-1), increased alkaline phosphatase (ALP) activity, collagen type I (COL1), osteopontin (OPN, aka SPP1), osteocalcin (OCN, aka BGLAP), and bone sialoprotein (BSP) gene and protein expression, and calcification of the extra-cellular matrix (ECM) (Aubin and Triffitt, 2002); (b) chondrogenesis is characterized by the involvement of the SRY (sex determining region Y)-box 9 (Sox-9) transcription factor, collagen type II (COL2), tenascin (Tnc), aggrecan (Acan), and cartilage oligomeric matrix protein (COMP) isoform gene and protein expression, and glycosaminoglycan (GAG) deposition (Lefebvre and Smits, 2005); (c) myogenesis (for vascular smooth muscle cells, VSMCs) is characterized by collagen type I (COL1), matrix metallo-proteinases (MMPs), α smooth muscle actin (aSMA), smooth muscle-myosin heavy chain (SM-MHC), calponin (Cnn1), and SM22 α (aka Tagln) gene and protein expression (Rensen et al., 2007).

Figure 2. Common techniques for mechanical stimulation of cells (Brown, 2000).

(A) Application of fluid-induced shear stresses: cells are seeded on a 2D substrate (such as coated glass slides) and exposed to laminar fluid flow (a), are seeded onto a 3D porous scaffold (such as titanium fiber mesh) and exposed to medium perfusion through the scaffold (b), or to turbulent flow by medium steering (c). (B) Application of compressive loads: cells are seeded into a hydrogel (such as agarose) and exposed to unconfined (eventually confined) compression between platens (a) or to hydrostatic pressure (b). (C) Scaffold stretching (uniaxial): cells are seeded onto 2D or into 3D scaffolds (such as coated silicon or collagen I gel) hold between a fixe and a moving grip (a), or exposed to four point bending (b). (D) Scaffold stretching (bi- or equi-axial): cells are seeded on a 2D substrate (such as collagen coated silicon membranes) deformed by applied vacuum or fluid pressurization (a) or by prong or platen displacement (b).

Figure 3. Effects of flow perfusion (and compressive loading) on BMSC proliferation (Jagodzinski et al., 2008).

(Reprinted from Journal of Biomechanics, 41, Jagodzinski, M., Breitbart, A., Wehmeier, M., Hesse, E., Haasper, C., Krettek, C., Zeichen, J., Hankemeier, S., Influence of perfusion and cyclic compression on proliferation and differentiation of bone marrow stromal cells in 3-dimensional culture, 1885–1891, copyright (2008) with permission from Elsevier.) Human BMSCs were seeded into bovine bone matrix and subjected, either to flow perfusion (10 ml/min; solid circles), or to flow perfusion and scaffold compression (10 ml/min, 10% strain, 0.5 Hz, hollow circles), or left in static culture (solid triangles). n=7; *: p<0.03.

Figure 4. Effects of strain on osteogenic differentiation of BMSCs (Simmons et al., 2003).

(Reprinted from Journal of Biomechanics, 36, Simmons, C. A., Matlis, S., Thornton, A. J., Chen, S., Wang, C. Y., Mooney, D. J., Cyclic strain enhances matrix mineralization by adult human mesenchymal stem cells via the extracellular signal-regulated kinase (ERK1/2) signaling pathway, 1087–1096, copyright (2003) with permission from Elsevier.) Mechanical strain was applied to human BMSCs through bending of a collagen coated silicon membrane (3% strain; 0.25 Hz). Osteogenic differentiation (calcium deposition) was evaluated with von Kossa staining and biochemical assay. Values presented are means $\pm$ SD; n=3; *: p<0.05 vs unstrained control.

Figure 5. Effects of flow perfusion on osteogenic differentiation of BMSCs (Simmons et al., 2003).

(From Bancroft, G. N., Sikavitsas, V. I., van den, D. J., Sheffield, T. L., Ambrose, C. G., Jansen, J. A., Mikos, A. G. (2002) Fluid flow increases mineralized matrix deposition in 3D perfusion culture of marrow stromal osteoblasts in a dose-dependent manner. Proc Natl Acad Sci USA 99: 12600–12605, copyright (2002) National Academy of Sciences, U.S.A.) Rat BMSCs were seeded into a titanium fiber mesh and exposed to different flow rates (0.3, 1, or 3 ml/min) for 16 days. Calcium content of cultured scaffold, with *: highest, **: second highest, ***: third highest calcium content. Each group statistically different ($p < 0.05$) from all other groups.

Figure 6. Effects of scaffold compression on chondrogenic differentiation of BMSCs (Mauck et al., 2007).

(Reprinted from Biomechanics and Modeling in Mechanobiology, 6, Mauck, R. L., Byers, B. A., Yuan, X., Tuan, R. S., Regulation of cartilaginous ECM gene transcription by chondrocytes and MSCs in 3D culture in response to dynamic loading, 113–125, copyright (2007) with permission from Springer.) Bovine BMSCs were seeded into agarose scaffolds and exposed either to unconfined compression (10% deformation, 180 min/day, 1 Hz) or to free-swelling conditions for 5 days. Both groups were then maintained in free-swelling conditions for 23 more days. After 3 days of culture, GAG deposition was evaluated with Alcian Blue staining in free-swelling (A) and dynamically loaded (B) samples. Scale bar=50 μ m. GAG content was determined by biochemical assay (C). n=3; *: p<0.05.

Figure 7. Effects of matrix elasticity on BMSC differentiation (Engler et al., 2006).

(Reprinted from Cell, 126, Engler, A. J., Sen, S., Sweeney, H. L., Discher, D. E., Matrix elasticity directs stem cell lineage specification, 677–689, copyright (2006) with permission from Elsevier.) Human BMSC aggregates were seeded on variably compliant polyacrylamide gels and cultured in identical media conditions. On the softest substrate (0.1–1 kPa) BMSCs displayed neurogenic marker ($\beta 3$ tubulin). On moderately stiff substrate (8–17 kPa), BMSCs up-regulated myogenic transiption factor expression (MyoD1). On stiffer substrate (25–40 kPa) BMSCs expressed osteogenic transcription factor (cbfa1 aka Runx2). Scale bar=5 μ m.

Figure 8. Integrins receptors. Integrins are transmembrane receptors composed of an α and a β sub-unit.

They bind to extra-cellular matrix proteins (such as collagen, fibronectin, etc.) on one side of the cell membrane and to cytoskeletal proteins (such as actin, talin, etc.) on the other side.