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Regulation of Vascular Smooth Muscle Cells and Mesenchymal Stem Cells by Mechanical Strain

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Abstract: Vascular smooth muscle cells (SMCs) populate in the media of the blood vessel, and play an important role in the control of vasoactivity and the remodeling of the vessel wall. Blood vessels are constantly subjected to hemodynamic stresses, and the pulsatile nature of the blood flow results in a cyclic mechanical strain in the vessel walls. Accumulating evidence in the past two decades indicates that mechanical strain regulates vascular SMC phenotype, function and matrix remodeling. Bone marrow mesenchymal stem cell (MSC) is a potential cell source for vascular regeneration therapy, and may be used to generate SMCs to construct tissue-engineered vascular grafts for blood vessel replacements. In this review, we will focus on the effects of mechanical strain on SMCs and MSCs, e.g., cell phenotype, cell morphology, cytoskeleton organization, gene expression, signal transduction and receptor activation. We will compare the responses of SMCs and MSCs to equiaxial strain, uniaxial strain and mechanical strain in three-dimensional culture. Understanding the hemodynamic regulation of SMC and MSC functions will provide a basis for the development of new vascular therapies and for the construction of tissue-engineered vascular grafts.

keyword: Mechanical strain, Smooth muscle cell, Mesenchymal stem cell, Equiaxial strain, Uniaxial strain, Three-dimensional culture

1 Introduction

Vascular smooth muscle cells (SMCs) populate in the media of blood vessel play an important role in the control of vasoactivity and the remodeling of the vessel wall. Under physiological and pathological conditions, SMCs do not always stay terminally differentiated. For example, during atherosclerosis and restenosis, SMCs in the intima de-differentiate into a proliferative and synthetic phenotype. Increased expression of smooth muscle (SM) markers such as h-caldesmon, calponin, SM myosin heavy chain (MHC) and SM α-actin typically corresponds to a more differentiated state with a contractile phenotype (Owens, 1995; Thyberg, 1996). Blood vessels are constantly subjected to hemodynamic stresses, and the pulsatile nature of the blood flow results in cyclic mechanical strain in the vessel walls. Accumulating evidence in the past two decades indicate that mechanical strain regulates vascular SMC phenotype, functions and matrix remodeling.

Recent studies suggest that bone marrow mesenchymal stem cells (MSCs) can be expanded and stimulated to differentiate into a variety of cell types, including vascular SMCs (Caplan and Bruder, 2001; Ferrari et al., 1998; Galmiche et al., 1993; Jiang et al., 2002; Pittenger et al., 1999; Prockop, 1997; Wakitani et al., 1995). Thus, MSCs can be a potential cell source for vascular regeneration, and may be used to generate SMCs to construct tissue-engineered vascular grafts for blood vessel replacements. Since vascular grafts are subjected to vascular microenvironmental factors such as mechanical strain in bioreactors and *in vivo*, it is important to understand how mechanical strain regulates MSC differentiation and functions. In this review, we will focus on the effects of mechanical strain on SMCs and MSCs, e.g., cell phenotype, cell morphology, cytoskeleton organization, gene expression, signal transduction and receptor activation.

Different types of mechanical strains are present in blood vessel walls at different locations. For example, in the straight part of the blood vessels, the cyclic mechanical strain is mainly in the circumferential direction, while at the branch points and aneurysm areas, cyclic mechanical strain can be relatively isotropic. To determine the effects and mechanisms of how mechanical strain regulates cell functions, several types of mechanical stretch devices have been used in the past. In two-dimensional

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(2-D) culture, cells are cultured on deformable substrates (e.g., silicone membranes), and mechanical strain at various magnitudes and frequencies can be applied to the cells through the deformation of the substrates. Equiaxial (biaxial) strain and uniaxial strain are most commonly used to investigate the effects of mechanical strain *in vitro*. To simulate the extracellular matrix (ECM) environment *in vivo*, three-dimensional (3-D) culture systems have been used to study the effect of mechanical strain on SMCs and MSCs. Different types (e.g., isotropic vs. anisotropic, 2-D vs. 3-D) of mechanical strain may have different effects on cell functions, and these important differential effects have not been appreciated until recently. Here we will review the literature and discuss the effects of each type of mechanical strain on cellular functions.

2 Effects of Equiaxial Strain

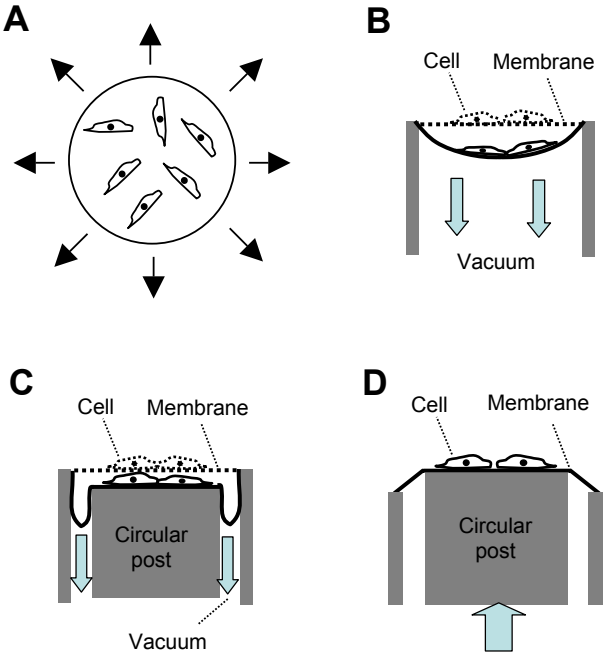

Figure 1 : Mechanical devices used to apply equiaxial strain. (A) Equiaxial strain (Top view). (B) A device to apply equiaxial strain using vacuum (sideview). Note non-uniform deformation across the membrane. (C) An improved device with circular post under each well to achieve uniform mechanical strain (sideview). (D) A device with the post under wells moving up and down (sideview).

A simple and most widely used *in vitro* model of mechanical strain is equiaxial strain, in which cells are strained uniformly in all directions (Figure 1A). In 1985, Banes *et al.* created a system where cells were seeded onto a deformable substrate (current studies typically use a flexible elastomeric membrane) and were subjected to cyclic deformations though the application of negative vacuum pressure underneath the substrate, thereby creating relatively uniform strain in all directions (Banes et al., 1985). However, the variation of mechanical strain from the center to the edge of the loading wells and the difference of radial strain (E_{rr}) and circumferential strain ($E_{\theta\theta}$) result in inhomogeneous mechanical strain across the surface (Gilbert et al., 1994) (Figure 1B). This problem has been solved by adding a circular post under each well so that the mechanical strain in the central area is uniform (Vande Geest et al., 2004) (Figure 1C). This mechanical stretch system has been improved and is now commercially available under the name "Flexercell" (Flexcell International Corp.). In the mean time, several other types of mechanical devices have been developed to apply equiaxial strain. For example, a device with the circular post moving up and down has been used to apply equiaxial strain to cells (Sotoudeh et al., 1998) (Figure 1D). The biaxial stretch of cruciform elastic substrate also gives well-defined equiaxial strain (Waters et al., 2001). Some early studies utilized custom-built devices to impart equiaxial strain to cells (Gutierrez and H.A., 1999; Lee et al., 1996; Lee et al., 2001; Schaffer et al., 1994), but the majority of recent studies used the Flexercell apparatus. By adjusting the frequency and magnitude of the strain, researchers can utilize this type of system to apply cyclic strain roughly approximating *in vivo* physiological conditions. Typical experiments simulating vascular conditions use ∼5-30% strain at frequencies of ∼30-90 cycles per minute (cpm).

In one of the early papers studying mechanical stimulation of SMCs, Birukov *et al.* reported that equiaxial cyclic strain caused a serum-independent increase in the expression of h-caldesmon (Birukov et al., 1995). Shortly thereafter, Reusch *et al.* demonstrated that cyclic strain in neonatal rat SMCs increased SM MHC isoforms SM-1 and SM-2, while causing a concomitant decrease in non-muscle MHC (Reusch et al., 1996). However, the authors also noted that the increase in SM-1 was dependent on the ECM. Cells cultured on either laminin or collagen type I showed cyclic strain-induced increases in

SM-1, while those cultured on fibronectin underwent no change in SM-1 expression (Reusch *et al.*, 1996). Furthermore, Reusch *et al.* examined adult rat SMCs rather than neonatal rat SMCs, and showed that similar conditions of cyclic strain did not induce a corresponding SM-1 increase in the adult cells (Reusch *et al.*, 1997). These varying results suggest that the changes in differentiation depend on both the underlying matrix composition and the state/age of SMCs. The reasons for these differences in strain-induced differentiation are currently unknown, but they suggest that both the cell-matrix interaction and the initial cell phenotype may significantly affect the transduction of strain-induced events. The importance of the relationship between cyclic strain and cell-substrate attachment was further substantiated in a study by Cunningham *et al.*, which demonstrated that cyclic strain rapidly increased focal contact-associated vinculin and paxillin levels, both of which are involved in cell-matrix attachment (Cunningham et al., 2002). Additional studies on SMC differentiation have used SM α-actin as a marker of SM differentiation, such as that by Tock *et al.*, which showed that cyclic equiaxial strain increased both SM α-actin protein expression and promoter activity (Tock *et al.*, 2003).

Interestingly, while the studies mentioned above reported cyclic-strain induced SMC differentiation, other studies have also noted increased SMC proliferation in response to cyclic strain (Sudhir *et al.*, 2001; Yang *et al.*, 1993), and at least one study has demonstrated a concurrent increase in SM differentiation in parallel with this strain-induced proliferation (Birukov *et al.*, 1995). Predel *et al.* showed that mechanical strain increased venous but not arterial SMC proliferation (Predel et al., 1992), suggesting SMCs from different vascular beds may respond differently. Further investigation by Kim *et al.* demonstrated that proliferation of SMCs increased with cyclic strain on fibronectin- and vitronectin-coated membranes, but had no effect on collagen type I- or laminin-coated membranes, again suggesting a link between ECM substrate and the response to cyclic strain (Kim et al., 1999). Combining these results with those discussed previously by Reusch *et al.*, it appears that cyclic strain of SMCs on laminin- or collagen type I-coated membranes may increase differentiation without altering proliferation, while cyclic strain on fibronectin-coated membranes may lead to increased proliferation with no change in SM differentiation (Kim et al., 1999; Reusch et al., 1996). There is evidence that equiaxial strain-induced SMC proliferation is regulated by platelet-derived growth factor (PDGF), epidermal growth factor receptor (EGFR), basic fibroblast growth factor (bFGF), PI-3 kinase/Akt pathway, NF-κB pathway and p27Kip1 (Hishikawa et al., 1997; Iwasaki et al., 2000; Sedding et al., 2003; Sudhir et al., 2001; Wilson et al., 1993; Zhou et al., 2003). Still, another study by Morrow *et al.* linked cyclic strain to a significant decrease in SMC proliferation and increase in apoptosis (though the type of ECM-coating was not explicitly stated) (Mayr et al., 2002; Morrow et al., 2005b; Sotoudeh et al., 2002; Wernig et al., 2003), suggesting that several other variables, including the magnitude of the strain, frequency of the strain, confluency of the cells, etc., may be involved in the proliferative response of SMCs to this mechanical stimulation.

Several studies have also linked the increases in SM differentiation with activation of members of the mitogen-activated protein kinase (MAPK) family, which are known to be activated by changes in stress-promoting agents such as osmolarity, heat shock, and hypoxia (Cano and Mahadevan, 1995; Davis, 1993). In several different studies with rat SMCs, cyclic equiaxial strain was shown to activate all three members of the MAPK family (ERKs, JNKs, and p38 MAPK), though some studies only found the activation of one or two members of MAPKs (Hamada et al., 1998; Hu et al., 1998; Li et al., 1999; Reusch et al., 1997; Tock et al., 2003). Furthermore, Tock *et al.* demonstrated that inhibition of either JNKs or p38 MAP kinase effectively blocked the strain-induced increase in α-actin promoter activity in SMCs, while constitutively active forms of JNK or MKK6 (a p38 MAPK kinase) increased promoter activity further, implying a direct link between cyclic strain and MAPK activation (Tock et al., 2003). At upstream, strain-activation of p38 MAPK is mediated by the Ras/Rac pathway (Li et al., 2000). Again, Reusch *et al.* have suggested that cyclic strain-mediated activation of the various MAPK pathways may be dependent on both ECM coating and the state of the cell, noting that in neonatal rat SMCs, cyclic strain induced activation of JNK/SAPK on either laminin or pronectin, but of ERK only on pronectin. However, both pathways were stimulated by cyclic strain in adult rat SMCs independently of matrix composition (Reusch et al., 1997). Furthermore, a study by Li *et al.* demonstrated that MAPK activation occurs in both

a time-and magnitude-dependent response to this mechanical stimulation (Li et al., 1999). Li *et al.* further showed that cyclic strain increases expression of MAPK phosphatase-1 (MKP-1), which may act as a negative feedback regulator of MAPK signaling (Li et al., 1999).

MAPK signaling is known to be regulated at least in part by PDGF activity through the phosphorylation/activation of PDGF receptors (PDGFRs), and a study by Hu *et al.* showed that cyclic strain induced PDGFRα phosphorylation similarly to activation with exogenous PDGF-AB (Hu et al., 1998). This PDGFRα activation was dependent on the magnitude of the strain and was concomitant with ERK activation, but was not inhibited by antibodies against all PDGFs, suggesting that the growth factor receptor activation may actually be caused by mechanical stresses directly perturbing the cell surface and/or altering receptor conformation, rather than by strain-induced secretion of PDGF (Hu et al., 1998). Two additional studies have corroborated this idea by showing that the cyclic strain-induced mitogenic (i.e. proliferative) response in SMCs is not inhibited with antagonists against PDGF-AB (Sudhir et al., 2001; Yang et al., 1993). However, Sudhir *et al.* went on to show that the strain-induced mitogenic response was diminished with blocking-antibodies against bFGF, suggesting that several factors may play a role in the strain-induced pro-proliferative response in SMCs (Sudhir et al., 2001). Interestingly, Reusch *et al.* reported that neutralizing antibodies against PDGF-AB actually enhanced the strain-induced increase in SM-1, though this study used neonatal rat SMCs and this effect might be cell-type dependent (Reusch et al., 1996).

In addition to the MAPK and PDGFR signaling pathways, some recent studies have implicated transforming growth factor-β1 (TGF-β1), bFGF and vascular endothelial growth factor (VEGF) in the SMC response to cyclic strain (O'Callaghan and Williams, 2000; Quinn et al., 2002). O'Callaghan and Williams found that cyclic equiaxial strain transiently increased mRNA levels of TGF-β1 in human SMCs (O'Callaghan and Williams, 2000), while Li *et al.* discovered elevated levels of TGF-β1 in the media of rabbit SMCs after cyclic strain (Li et al., 1998). Furthermore, this increase in TGF-β1 appears to be linked to strain-induced matrix remodeling. It has been hypothesized that *in vivo* mechanical stimulation of blood vessels may cause specific changes in ECM composition leading to more effective overall stability of the vessel wall (Lee et al., 2001). Multiple studies have demonstrated that equiaxial cyclic strain leads to increases in collagen (Li et al., 1998; O'Callaghan and Williams, 2000; Sumpio et al., 1988), fibronectin (O'Callaghan and Williams, 2000; Tamura et al., 2000), and the proteoglycans versican, biglycan, and perlecan (Lee et al., 2001). Equiaxial strain increased matrix metalloproteinase-2 (MMP-2) expression but suppressed MMP-1 expression (Grote et al., 2003; O'Callaghan and Williams, 2000; Yang et al., 1998), suggesting that the increase in ECM proteins is not simply due to inhibition of ECM degradation. Several of these studies also noted that blocking TGF-β1 resulted in attenuation of strain-induced collagen synthesis (Joki et al., 2000; Li et al., 1998; O'Callaghan and Williams, 2000), suggesting that TGF-β1 signaling may play a critical role in cyclic strain-induced matrix remodeling by SMCs. Li *et al.* have further shown that this mechanism may be regulated by cyclic-strain induced secretion of angiotensin II (Li et al., 1998).

More recently, in two separate studies, the Notch signaling pathway has been implicated in the SMC response to equiaxial cyclic strain (Morrow et al., 2005a; Morrow et al., 2005b). Morrow *et al.* reported a strain-induced decrease in Notch 3 signaling in rat SMCs with a concurrent decrease in proliferation and increase in apoptosis (Morrow et al., 2005b). These effects were inhibited by overexpression of Notch 3 signaling (Morrow et al., 2005b). A second study by Morrow *et al.* found that equiaxial strain downregulated Notch 1 and 3 signaling in human SMCs while causing increases in several SM markers. Expression of these markers was enhanced further when combined with selective inhibition of Notch signaling (Morrow et al., 2005a).

Mechanical strain can modulate many other aspects of vascular SMCs as well. Equiaxial strain, together with angiotensin II, increased the expression of parathyroid hormone-related peptide, which can relax the blood vessel wall (Noda et al., 1994). Inflammatory cytokines such as IL-6 could be induced by mechanical strain via the Ras/Rac-p38MAPK-NF-kB pathway (Zampetaki et al., 2005). Equiaxial strain has also been shown to increase the expression of connexin 43 (Cx-43), a cell gap junction protein (Cowan et al., 1998). Interestingly, hypoxia-inducible factor α, which has a significant effect on vascular remodeling, was induced by mechanical strain under normoxic conditions (Chang et al., 2003).

Stem cells represent unique cell populations that have the capacity for both self-renewal and differentiation into other cell lineages. We hypothesize that cyclic strain may provide a suitable mechanical stimulus to encourage bone marrow MSC differentiation into a SMC phenotype. This may be a possibility for embryonic stem cells (ESCs) as well. Currently, only a few articles have been published concerning the effects of equiaxial strain on stem cells. Our lab has shown that cyclic equiaxial strain (10%, 60 cpm) reduces expression of the SM markers α-actin and SM22 in human bone marrow MSCs (Park et al., 2004). This effect is reversed when using uniaxial cyclic strain, which will be discussed in the next section. In contrast, Simmons *et al.* have also studied the effect of equiaxial cyclic strain on MSCs, but under less intense loading conditions (3%, 15 cpm) and with osteogenic differentiation media. This study reported that cyclic strain led to increased matrix mineralization via activation of the ERK1/2 pathway (Simmons et al., 2003), implying possible differentiation toward an osteogenic phenotype. Additionally, Saha et al. demonstrated that cyclic strain actually promotes self-renewal of human ESCs rather than differentiation (Saha et al., 2005).

Overall, research into the effects of equiaxial cyclic strain on various cell types has revealed several important aspects of mechanotransduction on vascular cells and stem cells. In general, cyclic strain in SMCs leads to increases in SM markers while causing various changes in proliferation and matrix remodeling. These effects appear to be regulated in part by various signaling molecules including the MAPK family, PDGF, TGF-β1, and the Notch pathway. Additionally, studies have revealed that cyclic equiaxial strain may play a role in guiding stem cell fate between differentiation and self-renewal. While the response to this mechanical stimulus appears to be partially dependent on cell type, species, age of cells, ECM substrate, chemical components in media and strain conditions, etc., the results of these studies have advanced our understanding of cardiovascular development, disease, and repair in response to cyclic strain.

3 Effects of Uniaxial Mechanical Strain

Although equiaxial strain has been widely used to study strain effects on SMCs, uniaxial strain more appropriately mimics the mechanical strain experienced by SMCs in the straight portion of blood vessels. Uniaxial strain can best be described as the application of a strain field along one axis. Investigating uniaxial strain effects can be helpful to create a suitable model as it captures the dominant strain field experienced by vascular cells in the blood vessel. Arterial SMCs align in the circumferential direction in blood vessels. In this environment, SMCs are elongated in their contractile phenotype. Once the cells are taken from their native blood vessel and cultured *in vitro*, SMCs take on a more spread morphology characteristic of the proliferative phenotype (Owens, 1995; Thyberg, 1996a). This phenomenon has been the inspiration for many research projects.

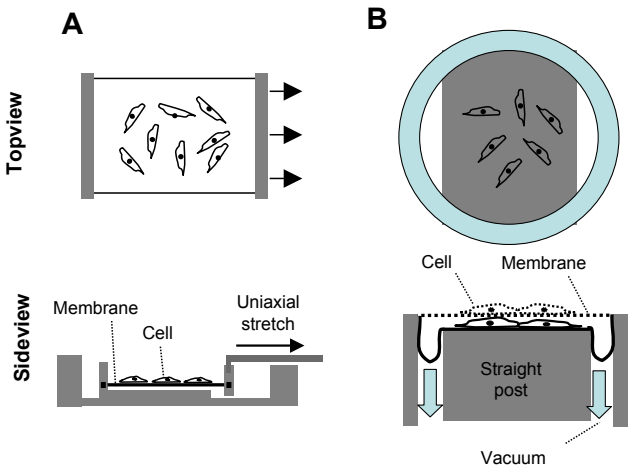

Figure 2 : Two types of mechanical devices to apply uniaxial strain. (A) Uniaxial stretch of the membrane. (B) Using vacuum to obtain relatively uniform strain in the central area.

Several types of mechanical stretch devices have been used to apply uniaxial strain to cells (Figure 2). For example, one end of the elastic substrate can be attached, and the other end is stretched uniaxially (Park et al., 2004) (Figure 2A). In this device, the uniaxial strain is quite uniform in the central area except for slight compression at the orthogonal edge. To obtain pure uniaxial strain across the elastic substrate, the orthogonal edges are controlled so that there is no deformation in that direction (Wang et al., 2001). Another device uses vacuum to induce the deformation of the elastic substrate as in Figure 1C except that a straight loading post is used (Figure 2B), which results in well-defined uniaxial strain in the central rectangular region of the elastic substrate on the post (Vande Geest et al., 2004).

One of the early studies regarding uniaxial stretching of

vascular cells was preformed by Kanda et al. This study clearly demonstrated the effect of uniaxial strain on vascular cell orientation (Kanda and Matsuda, 1993). Effects of cyclic uniaxial strain, between 5% and 20% at 15 cpm – 120 cpm, were observed on vascular SMCs, endothelial cells (ECs) and fibroblasts. Stress-loaded cells tended to align perpendicularly to the direction of applied strain. Higher amplitudes and frequencies yielded more pronounced orientation effects on all three cells types. Although the end orientation was quite similar, the time to re-align was cell type dependent as SMC and fibroblast response was notably more rapid than that of ECs. The applied strain did not lead to an observable change in cell morphology as compared to non-strained samples. Hayakawa *et al*. further showed that Ca^{2+} chelator did not inhibit the reorientation of stress fibers in response to strain, but the cell orientation was less dramatic. This study leads to the conclusion that rearrangement of stress fibers is necessary but not sufficient for cell orientation (Hayakawa et al., 2001). The alignment of cells perpendicular to the direction of strain suggest that cells may try to minimize the stress applied to them, and the different cell orientation may result in different cellular responses to the mechanical strain.

Cellular responses to uniaxial strain may be very different from that to equiaxial strain. Asanuma *et al*. studied effects of uniaxial strain on matrix-degrading enzymes in SMCs (Asanuma et al., 2003). Cyclic strain decreased MMP-2 and MMP-9 after 48 hours of cyclical strain, though static strain increased MMP-2 and MMP-9 mRNA levels at all time points. Interestingly, cyclic equiaxial strain and 3-D strain increased MMP-2 production (O'Callaghan and Williams, 2000; Seliktar et al., 2001). These results suggest that SMCs exhibit a selective response to different strain types.

We have performed studies to demonstrate the differential effects of uniaxial and equiaxial strains on MSCs (Park et al., 2004). Cyclic equiaxial strain decreased while cyclic uniaxial strain transiently increased the expression of SM α-actin and SM-22α. Hamilton et al., also showed that cyclic strain increased SM α-actin and h-calponin but decreased proliferation in rat bone marrow progenitor cells (Hamilton et al., 2004). Uniaxial but not equiaxial strain gave a transient increase of collagen I expression (Park et al., 2004). Furthermore, a DNA microarray analysis showed cyclic uniaxial strain increased SMC markers and regulated matrix molecule expression without altering the expression of cartilage and bone differentiation markers such as collagen II and alkaline phosphatase. Another study comparing equiaxial strain versus uniaxial strain was performed by Hornberger, *et al.* (Hornberger *et al.*, 2005). Hornberger applied both types of cyclic strains to C2C12 myotubes. Both strains were applied at 15% strain for 45 seconds followed by a 15 second rest period. This stimulation lasted for 10 minutes. Both strain modes gave an increase of ERK and Akt phosphorylation, but equiaxial strain showed the phosphorylation of ribosomal S6 kinase ($p70^{SK6}$) while uniaxial strain did not.

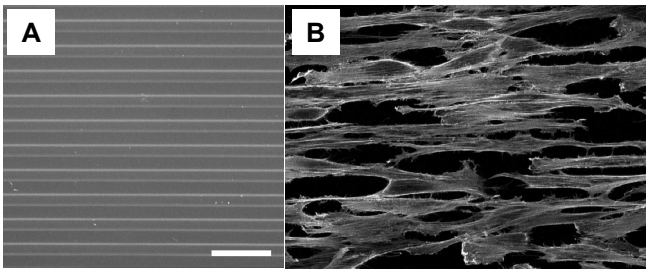

Figure 3 : Controlling cell orientation using micropatterned surfaces. (A) Scanning electron microscopy image of silicone membrane surface patterned with microgrooves. (B) Actin cytoskeleton in MSCs cultured on a silicone membrane with microgrooves (in horizontal direction) under static condition. Bar=50 μm.

SMCs in native blood vessel walls have an elongated morphology and align in the circumferential direction with well-organized structure (Canham and Mullin, 1978; Peters *et al.*, 1983; Walmsley *et al.*), which is important for the efficient contraction of vessel wall and may mediate the responses of SMC to passive mechanical strain due to the pulsatile pressure. To keep cells aligned in the stretch direction, aligned collagen fibrils and microgrooves have been used. McKnight *et al*. applied uniaxial strain to SMCs on a thin gel of polymerized collagen with aligned fibrils (McKnight and Frangos, 2003). Although SMC markers did not show a change between aligned and nonaligned cultures, the phosphorylation of ERK1/2 decreased in aligned SMCs but increased in non-aligned SMCs at low strain rates ($1\%s^{-1}$). However, high strain rates ($10\%s^{-1}$) increased phosphorylation of ERK1/2 in both aligned and non-aligned SMCs in a G protein-dependent manner. Simpson *et al.* and Gopalan *et al.* performed studies on

how anisotropic strain modulated contractile proteins in aligned myocytes (Gopalan *et al.*, ; Simpson *et al.*, 1999). Parallel collagen fibrils or collagen strips were used to align myocytes. It was observed that the principal strain parallel to the myocytes did not alter myofibril accumulation or expression of atrial natriuretic factor (ANF), Cx-43, or N-cadherin compared against the unstrained controls. Transverse principal strain, though, increased myofibrils and the expression of ANF, Cx-43, and N-cadherin (Gopalan et al., 2003). By controlling the cell alignment via microfluidic patterning, transverse strain was found to have greater effects on myocytes than parallel or longitudinal strain. Aligned microgrooves have been fabricated by soft lithography and used to culture cells for uniaxial strain experiments (Thakar et al., 2003; Wang and Grood, 2000) (Figure 3). Studies using SMCs showed that cell shape and orientation were affected by culturing cells on micropatterned surfaces (Thakar et al., 2003). We showed that application of uniaxial strain to SMCs and MSCs with different orientation (parallel or perpendicular) induced different responses in cell proliferation and gene expression (unpublished data).

Uniaxial strain regulates many important genes involved in vascular remodeling and inflammatory responses. For example, Nguyen, *et al.* showed that cyclic uniaxial strain (20%, 1-Hz) increased protease-activated receptor-1 (PAR-1) expression, which was further enhanced by bFGF but not PDGF-AB (Nguyen et al., 2001).

In summary, uniaxial strain induces cells to align in the direction perpendicular to the strain. This is present across all the cell types mentioned in this review. Importantly, equiaxial and uniaxial strain produce very different results. Therefore, it is important to take into account the *in vivo* environment of a cell when designing an experiment involving strain. Finally, cell orientation may change cellular responses to uniaxial strain.

4 Effects of Mechanical Strain in 3-D culture

Although 2-D mechanical strain experiments give us valuable information on SMC responses, the application of mechanical strain to SMCs in 3-D culture can better mimic vascular environment *in vivo*, and can be used to construct tissue-engineered vascular grafts in bioreactors. SMCs behave very differently in 3-D as opposed to 2-D, expressing less SM markers and displaying reduced proliferation and migration (Li et al., 2002; Stegemann and Nerem, 2003a). In addition, SMCs align parallel to the direction of stretch in 3-D, along organized collagen fibers, unlike in 2-D where contact guidance channels fabricated on the substrate are necessary for cells to remain aligned with the strain direction. Kanda and Matsuda studied bovine aortic SMCs in a 3-D collagen gel ring with both static strain and dynamic 5% strain at a frequency of 60 cpm for four weeks (Kanda and Matsuda, 1994). Cells that received either static or dynamic strain had an elongated, spindle-shaped morphology organized parallel to the direction of strain compared to static cells in unstrained gels which were rounded and distributed randomly throughout the gel. Collagen fibers in the strained gels were also aligned in the direction of strain, whereas the collagen fibers in static gels were randomly distributed. Other groups have confirmed that SMCs (Jeong et al., 2005) and/or collagen fibers (Isenberg and Tranquillo, 2003; Seliktar et al., 2000) align parallel to the direction of strain compared to a disorganized static construct.

Mechanical strain promotes matrix synthesis and SMC proliferation in native arteries and vein (Kolpakov et al., 1995; Zeidan et al., 2000). In constructed vessels, the cells need to secrete their own matrix of dense collagen with cross-linked elastin fibers. Not only does this matrix contribute to the appropriate biophysical environment for alignment, but also provides the strength and elasticity that the blood vessel needs to withstand the strains of blood pressure. Niklason *et al.* showed promising strengths of tissue-engineered blood vessels with mechanical stimulation, which were implanted in swine and remained patent for 24 days (Niklason et al., 1999). The study used a biodegradable poly (glycolic acid) (PGA) scaffold around which bovine aortic SMCs were seeded. After 8 weeks of culture, those vessels exposed to 5% pulsatile strain at 165 cpm had rupture strengths of 2000 mm Hg and had produced 40% more collagen than vessels in static culture. Suture retention strengths were 91 grams, more than 3 times those of static vessels. The vessel mechanical properties came solely from the matrix the cells produced since the PGA had become fragmented and almost completely degraded after 8 weeks. Seliktar *et al.* also found that 8 weeks of mechanical stimulation increased the strength of 3-D SMC-seeded collagen gels, with ultimate stress and material modulus 58 and 142kPa, respectively, compared to 16 and 68kPa for static gels (Seliktar et al., 2000). MMP-2 was also upregulated with 3-D strain and was neces-

sary for this increase in mechanical properties (Seliktar et al., 2001). Isenberg *et al.* reported even higher ultimate tensile stress and modulus values of 400kPa and 3200 kPa for dynamically conditioned collagen vessels cultured for 5 weeks at 5% strain, 30 cpm, compared to 250 and 2000 kPa for static vessels, resulting in a 1.6-fold increase in both ultimate stress and modulus (Isenberg and Tranquillo, 2003). Seliktar's mechanical strain system induced a higher fold change in mechanical properties with its higher strain, frequency and time course, but Isenberg's constructs started out with higher mechanical properties, most likely due to the glycation via ribose to cross-link collagen in order to reduce creep. Contrary to Niklason's study as well as others (Jeong et al., 2005; Kim et al., 1999), in which SMC collagen production was enhanced by mechanical strain, Isenberg did not see an increase in collagen content with 2.5, 5 or 10% mechanical strain. This discrepancy may be due to the shorter time course (5 weeks compared to 8 weeks) or the different ECM used to make the 3-D construct. Although an increase in collagen content was not seen, Isenberg did observe an increase in elastin in mechanically conditioned constructs compared to almost none in static controls. The elastin was organized, aligned circumferentially, and appeared to be cross-linked.

Isenberg's study also differs from others in the field concerning stretch-induced increase of proliferation. No change was found in cell number after 5 weeks of culture, however other studies have reported an increase in cell proliferation on collagen scaffolds when serum is present (Jeong et al., 2005; Kim et al., 1999; Stegemann and Nerem, 2003b). Niklason et al. did not observe an increase in proliferation with strain either; however, this is probably due to the ECs seeded in the lumen of the construct, which are known to inhibit SMC proliferation to prevent hyperplasia. Other studies that did not show proliferation with strain were those that added a growth factor such as VEGF or TGF-β1 (Stegemann and Nerem, 2003b), or under serum-free conditions. Interestingly, Kim et al. reported an increase in proliferation with strain on fibronectin scaffolds, however no change in proliferation on collagen sponges with strain under serum-free conditions (Kim et al., 1999). Adding serum increased proliferation via strain for both fibronectin and collagen scaffolds. It was found that fibronectin and vitronectin from the serum were adsorbed onto both of these scaffolds, raising the question that these adsorbed proteins may be responsible for the increase in proliferation due to mechanical stimulation.

In addition to changes in matrix deposition and proliferation, cells change their phenotype in response to mechanical strain. Kanda and Matsuda showed that SMCs under dynamic strain had more myofilaments, dense bodies and extracellular filamentous materials compared to static strain or completely static cells which had more synthetic organelles (Kanda and Matsuda, 1994). Niklason *et al.* showed that mechanical strain induced an increase in late SMC differentiation marker MHC (Niklason et al., 1999). Although this study reported no difference in SM α-actin, Jeong et al. found a 2.5-fold increase in α-actin after 5 and 8 weeks of mechanical strain and did not find a significant increase in MHC (Jeong et al., 2005). This may be due to the EC layer which Niklason's study had and Jeong's study lacked, which could be responsible for releasing factors that along with mechanical strain, induces MHC expression in SMCs, promoting a more differentiated SMC phenotype. Growth factors along with mechanical strain can have a profound effect on regulation of phenotype. Stegemann *et al.* did not find a difference in α-actin expression with mechanical strain alone but strain and TGF-β1 together increased α-actin while strain and PDGF together decreased α-actin (Stegemann and Nerem, 2003b). This finding shows that chemical and mechanical factors work together to induce signal the cells differently than each stimulus alone. The interactions of signaling pathways induced by mechanical and chemical stimuli to modulate phenotype need to be studied, but first the signaling pathways induced by mechanical stress alone needs to be understood. Mechanical strain promoted SM differentiation by actin polymerization in mouse portal veins under static longitudinal stress (Albinsson et al., 2004; Zeidan et al., 2003). Inhibiting actin polymerization via ROCK blocked stretch-induced calponin and SM22α synthesis while inducing actin polymerization increased SM22α synthesis. In these longitudinally stretched veins, calponin and SM22α were found to be highly stretch-sensitive, followed by desmin, tropomyosin, and α-actin. Rho-associated kinase and cofilin were determined to be important in mediating this increase in contractile and cytoskeletal proteins. In addition to maintaining a SMC phenotype, mechanical strain has been shown to inhibit an osteoblast-like phenotype in SMCs (Nikolovski et al., 2003). Strained cells show decreased calcium deposition and down-regulated

osteoblastic genes, compared to unstrained cells.

Mechanical strain not only maintains the phenotype of SMCs but also is important for regulating processes in all cells exposed to a mechanical environment in the body, whether it is pressure, tension, or rotational force. Since mechanical strain is so important in phenotype regulation, our group is interested in studying its effects on stem cells. We have performed 3-D stretch experiments at 10% strain and 60 cpm with bone marrow MSCs cast in collagen-fibrin gels that have been shown to have a stronger ultimate tensile stress than collagen or fibrin gels alone (Cummings et al., 2004). With dynamic radial strain after 3 days, MSCs upregulated gene expression of SM markers and enhanced matrix remodeling, compared to static gels (unpublished data). These results indicate that vascular mechanical strain promotes a SM phenotype in MSCs. Another study that suggests the ability of mechanical forces to induce stem cell differentiation used translational and rotational strains on MSCs in a 3-D collagen gel (Altman et al., 2002). This study found that strain increased expression of ligament fibroblast markers collagen I and III and tenascin-C in the presence of ascorbate supplement. Bone or cartilage markers were not upregulated, indicating that this particular type of strain which ligament fibroblasts are exposed to is specific for signaling MSCs into this particular lineage.

Mechanical strain in 3-D is an ideal way to study cells *in vitro* in an environment that closely models *in vivo* conditions. Overall, studies show that dynamic strain on SMCs increases cell alignment, matrix synthesis, proliferation, and upregulates markers indicative of a contractile phenotype. 3-D strain is also a valuable tool for looking at MSC differentiation into various cell types, depending on the types of strain and chemical factors. Future studies need to focus on the mechanisms involved in stretch-induced cellular changes as well as to study the interaction of both strain and chemical stimuli.

5 Future Directions

The previous work has provided valuable information on the regulation of SMC and MSC functions by mechanical strain. Based on our discussion in this review, we propose the following directions for future research: (1) In-depth studies on the differential effects of equiaxial and uniaxial strain should be performed; (2) By controlling cell orientation using micropatterned surfaces, further studies are needed to unravel how cell orientation determines cell responses to anisotropic strains; (3) The regulation of MSCs and other stem cells by different types of mechanical strains should be further investigated; (4) Synergistic effects of mechanical strain and chemical factors on SMC and MSCs need to be understood; (5) Appropriate 3-D culture should be constructed for to investigate stem cell differentiation in 3-D ECM under mechanical loading. Understanding the hemodynamic regulation of SMC and MSC functions will provide a basis for the development of new vascular therapies and for the construction of tissue-engineered vascular grafts.

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