Mechanical regulation of matrix metalloproteinases

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1. ABSTRACT

Matrix metalloproteinases can degrade and modify almost all components of the extracellular matrix hence their enzymatic activity is tightly regulated under physiological conditions. Primary modes of enzyme regulation include transcriptional control, zymogen activation and dynamic inhibition by tissue inhibitors of matrix metalloproteinases. Recent studies have demonstrated that mechanical regulation of matrix metalloproteinases largely operate through these regulatory pathways. Over the last decade a large cohort of studies have been conducted on many tissue/cell types using diverse loading parameters in vivo and in vitro suggesting that mechanical load is essential in maintaining normal tissue function via the matrix metalloproteinases. However there may be a mechanically-regulated homeostasis, with cells responding to and interpreting growth factors and other biochemical signals within the context of mechanical forces to provide a suitable cellular matrix metalloproteinase response. On the contrary, mechanical overload can result in unrestricted matrix metalloproteinase activities eventually leading to matrix degradation, mechanical dysfunction and failure of the tissue. In this chapter, the effect of mechanical load on matrix metalloproteinase expression will be reviewed, and the signal transduction pathways involved in modulating the metabolic homeostasis of various tissues including blood vessels, intervertebral disc and components of the synovial joint with emphasis on articular cartilage discussed. Both mechanically-induced stimulation and inhibition of matrix metalloproteinases will be discussed and placed into context with their potential relevance to disease.
**Mechanical Deformation of Tissue**

- Dynamic compression
- Static compression

**Figure 1.** A schematic representation of how mechanical deformation, acting on a tissue, can alter both the intra- and extracellular environment of the cells. Exposure of cells to mechanical stimulation disseminates further effects across the extracellular matrix which can induce changes in gene and protein expression.

### 2. Mechanical Load/Mechnotransduction

All cells and tissues of an organism are continually subjected to mechanical forces. “Mechanical load” is defined as the force exerted on the tissue and subsequently perceived by the cells; these forces arise from several different origins and are often influenced by the tissue’s function. Not surprisingly, most studies to date have focused on cells of tissues with primary structural functions such as the cardiovascular system, components of the synovial joint and skin. These tissues are subjected to a wide range of mechanical loads from pressure induced forces generated through gravitational effects on body weight (components of the synovial joint) to physiological, hemodynamic forces derived from pulsatile circulating flow (in soft tissues such as the heart, blood vessel walls, bladder etc). Such mechanical forces have been measured *in vivo* and range from 0 – 100 dyn/cm² in arterial and venous vascular walls (1) to 5-10 MPa in hip articlar cartilage (2) and 500 N in intervertebral disc (3). The effect of mechanical load on a tissue alters the intra- and extracellular environment of the cells. Application of an extracellular mechanical stimulus can be “dynamic” *i.e.* periods of loading followed by unloading/cessation of load, or “static” whereby a constant force is applied for the duration. Exposure of cells to these mechanical stimuli disseminate further effects including elevated hydrostatic pressure, streaming potentials due to movement of ions, extracellular pH changes and loss of fluid through expulsion, which in itself generates shear stresses and tensional forces (Figure 1).

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Table 1. A. comparison of the effect of different mechanical loads on MMP regulation in blood vessels, skin, intervertebral disc, Achilles tendon and tissues of the synovial joint

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cyclic compression</th>
<th>Static compression</th>
<th>Shear stress</th>
<th>Tensile stretch</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12, 16, 32, 34, 40</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11, 17, 21</td>
</tr>
<tr>
<td>Dermal fibroblast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>41, 42, 44</td>
</tr>
<tr>
<td>Nucleus pulposus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>41, 42, 44</td>
</tr>
<tr>
<td>Achilles tenocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>55</td>
</tr>
<tr>
<td>Synovial fibroblast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>72, 73</td>
</tr>
</tbody>
</table>

A. comparison of the effect of different mechanical loads on MMP regulation in blood vessels, skin, intervertebral disc, Achilles tendon and tissues of the synovial joint. Clearly, the cell types respond uniquely to alterations in mechanical stimulation and synthesise MMPs according to environmental and cellular demands.

Mechanotransduction involves a series of discrete but common steps and is a “process of converting physical forces into biochemical signals and integrating these signals into (a) cellular response(s)” (4). Many reports have implicated mechanical load in modifying cellular responses affecting metabolism, cytokine and growth factor secretion and altered gene expression (5, 6). In conjunction with enzymatic activation by other proteases and cytokines (6), recent studies have indicated that mechanical load can regulate matrix metalloproteinase (MMP) expression and activation in vivo and in vitro. The MMPs, of which 26 have currently been identified, are a family of structurally related zinc endopeptidases with varied substrate specificity, ranging from multiple extracellular matrix components to cytokines, growth factors, and adhesion molecules (5). The MMP family comprises the collagenses (MMPs 1, 8 and 13) which degrade collagen, the stromelysins (MMPs 3, 7, 10 and 11) which have a broad substrate specificity (proteoglycans, laminin, fibronectin), the gelatinases (MMPs 2 and 9) which degrade denatured collagen, and the membrane-type MMPs (MT-MMP 1-4) which also have broad substrate specificity and reside at the cell membrane to activate other MMPs. The MMPs exist in a latent pro-form in the extracellular matrix (6); once activated they are extremely potent and if left unimpeded can eventually deplete the matrix of its components to cytokines, growth factors, and adhesion molecules (5). The MMP family comprises the collagenses (MMPs 1, 8 and 13) which degrade collagen, the stromelysins (MMPs 3, 7, 10 and 11) which have a broad substrate specificity (proteoglycans, laminin, fibronectin), the gelatinases (MMPs 2 and 9) which degrade denatured collagen, and the membrane-type MMPs (MT-MMP 1-4) which also have broad substrate specificity and reside at the cell membrane to activate other MMPs. The MMPs exist in a latent pro-form in the extracellular matrix (6); once activated they are extremely potent and if left unimpeded can eventually deplete the matrix of its macromolecules as demonstrated in pathological conditions. Thus the regulation of MMPs occurs at multiple levels including: (i) gene transcription, (ii) synthesis of inactive proenzymes, (iii) post-translational activation of proenzymes, or (iv) via the interaction of secreted MMPs with their inhibitors, the tissue inhibitors of MMPs (TIMPs).

Over the last decade a large cohort of studies have been conducted on many tissue/cell types using diverse loading parameters in vivo and in vitro suggesting that mechanical load is essential in maintaining normal tissue function via the MMPs. However there may be a mechanical homeostasis, with cells responding to growth factors and other biochemical signals within the context of mechanical forces to provide a suitable cellular MMP response. On the contrary, mechanical overload can result in unrestrained MMP activities eventually leading to mechanical dysfunction and failure of the tissue. This review will present the evidence linking mechanical stimulation with MMP modulation (Table 1), the hypothesised signal transduction pathways utilised (Table 2) and the relevance to tissue homeostasis and disease. Key findings that have given most insight into the field of MMP mechano-regulation have been illustrated of which one of the best-studied mechanotransduction paradigms investigated involves fluid shear regulation of MMPs by endothelial and smooth muscle cells, abnormal stresses and the correlation with atherosclerosis and angiogenesis. Additionally, evidence pertaining to the mechanical regulation of MMPs in connective tissues of the synovial joint will also be discussed with particular emphasis on mechanical stimulation of MMPs in articular cartilage and its relevance to the pathology of osteoarthritis. To maintain brevity MMP mechano-regulation of other tissues has not been addressed and lies outside the scope of this current review, as there is very little information pertaining to the intracellular mechanisms involved in mechanical regulation of MMPs in tissues such as bone and heart.

3. MECHANICAL STIMULATION OF MMP EXPRESSION

3.1. Atherosclerosis/Hyperplasia

Hemodynamic forces associated with blood flow fall into two categories, either cyclic circumferential strain
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Figure 2. A. schematic representation of how different mechanical loads acting on blood vessel smooth muscle cells, can regulate MMP transcription and synthesis. [A] Shear stress (12 dyn/cm², 15 hours) reduces PDGF-BB receptor expression diminishing the responsiveness of the cell to PDGF-BB; this results in a reduction in MT1-MMP synthesis and abrogation of pro-MMP 2 activation. B. Cyclic strain (4%, 1 hour) prevents PDGF-BB (5 ng/ml) mediated induction of MMP 1 by suppressing the complexing of Ets-1 with the AP-1 element in the MMP 1 promoter. C. High cyclical strain (10-16%, 72 hours) induces TGF-B expression, which upon binding to the TGF-B receptor promotes MMP 2 transcription and pro-MMP 2 synthesis and activation. D. Cyclical stretching (15% radial stretch) induces NOX-1 mRNA expression (an NAD(P)H oxidase subunit) and the rapid formation of reactive oxygen species. It is unclear whether oxygen radicals directly and/or indirectly mediate increased transcription and synthesis/activation of pro-MMP 2.

caused by a transmural force acting perpendicular to the vessel wall, or fluid shear stress generated by the frictional forces as blood drags against the endothelial cells. Both induce quite marked effects on MMP activities within the blood vessel walls. Adaptation of the blood vessel walls occurs through constant cellular migration, proliferation, death and extracellular matrix (ECM) synthesis and degradation by the vascular smooth muscle cells, and these are processes which accelerate in injured or repairing tissues. In any wound repair, the new tissue has to be sufficiently strong to withstand the mechanical forces it experiences which in the cardiovascular system involve large dynamic fluctuations in strain.

In diseased arteries in vivo, increased expression of MMP 1 has been observed at sites of mechanical overload (7, 8). Several groups have previously demonstrated that mechanical strain regulates MMP activity via stimulation of a mitogenic response in vascular smooth muscle cells (9, 10, 11) and endothelial cells (12) through the induction of platelet-derived growth factor-beta (PDGF-BB) (Figure 2). Modulation of endothelial cell-
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derived PDGF expression by shear stress has been reported both in vitro (10, 13) and in vivo (14). Resnick et al (1993) identified a putative shear stress-responsive element (GAGACC) in the 5'-flanking region of the PDGF-BB gene (15). Decreased synthesis and activation of pro-MMP 2 was observed in endothelial cells after application of shear stress (12dyn/cm², 15 hours) (12) (Figure 2A). Although there was no diminution of MMP 2 mRNA, MT1-MMP levels were reduced explaining the loss of active MMP 2 (MT1-MMP is a potent activator of pro-MMP 2). A significant reduction in PDGF-BB receptor (PDGF-RB) mRNA and protein levels were observed, diminishing the migratory responsiveness of endothelial cells to PDGF-BB in the presence of shear stress. Under normal conditions, exposure of endothelial cells to shear stress inhibits migration due to a reduction in PDGF-RB mediated activation of pro-MMP 2, preventing intimal thickening and atherosclerotic plaque formation. In the presence of PDGF-BB (5ng/ml), application of cyclic strain (1 or 4%) abolished the growth factor mediated up-regulation of MMP 1 (9). This was an MMP-1 specific response as expression and activation of MMP 2 and 9 did not alter with PDGF-BB or cyclic strain. Strain-induced abrogation of MMP 1 was not exclusive to PDGF-BB as Yang et al demonstrated a similar effect with TNF-alpha stimulation. The MMP 1 promoter contains several cis-acting elements including an AP-1 site that was subsequently shown to be mecano-responsive; within 30 minutes of cyclic strain Ets-1 expression by PDGF-BB was suppressed. Ets-1 forms a complex with the AP-1 site to initiate MMP 1 transcription (16) (Figure 2B).

Smooth muscle cells located in the vessel walls also experience cyclic circumferential strains. Asanuma et al (2003) demonstrated that exposure of saphenous vein explants to a stationary strain (105% of resting length) induced a 50-fold rise in MMP 2 mRNA, significant increases in cell-associated pro-MMP 2 and 9, and secreted active MMP 2 (17). Application of cyclic strain (110% of resting length, 1Hz, 72 hours) inhibited MMP 2 production. In a normal hemodynamic environment where cyclic strain prevails, secretion of MMP 2 is inhibited, suggesting a mechanism that restricts the amount of active MMP released by smooth muscle cells. Stationary strain, as observed in diseased vessels where advanced calcification has developed, prevents expansion and recoil of the arteries creating a rise in circumferential strain and stimulation of MMP activity, promoting atherosclerotic plaque formation. TGF-beta has been hypothesised as a candidate involved in strain-induced MMP expression in smooth muscle cells. Application of a high, cyclic mechanical strain (10-16% of resting length, 1Hz, 96 hours) increased MMP 2 activity in smooth muscle cells (11). Cyclic strain rapidly increased expression of TGF-beta mRNA in a strain-dependent manner, which subsequently regulates the extent of MMP activity in the vessel wall, corroborating evidence in other cell types, i.e. aortic cells (18) and glomerular mesangial cells (19) that strain-induced TGF-beta activity regulates MMP 2 synthesis (Figure 2C).

Abnormal mechanical stretch induces reactive oxygen species (ROS) formation in vascular smooth muscle cells via NAD(P)H oxidase (20), which can contribute to vascular inflammation and remodelling via redox-sensitive signalling mechanisms. Increased mRNA expression of NOX-1 (NAD(P)H oxidase subunit) and the rapid formation of ROS was observed in vascular smooth muscle cells within 5 minutes of exposure to cyclic stretch (maximal 15% radial stretch, 0.5Hz) (21). Downstream effects included elevated MMP 2 mRNA levels, as well as increased release and activation of pro-MMP 2. Utilising a transgenic mouse deficient for one of the NAD(P)H oxidase subunits p47phox, load induced MMP 2 expression was shown to be dependent on NAD(P)H oxidase and ROS formation in smooth muscle cells (Figure 2D). Activation of MMP 2 contributes to the vascular remodelling and plaque formation observed in atherosclerosis (21).

Vascular tissue remodelling underlies the development, evolution and complication of vascular lesions in atherosclerosis, hypertension, graft failure, and after surgical interventions such as balloon angioplasty or stenting. Naturally occurring vascular pathologies i.e. atherosclerosis are limited to the arterial side of circulation where exposure to pulsatile blood flow alters the hemodynamic environment. In diseased vessels, atherosclerotic plaques preferentially form in specific areas of vasculature i.e. infrarenal aorta, coronary arteries and at bifurcation of carotid arteries (branching of the arteries). Integral to atherosclerotic plaque formation is the breaching of the internal elastic lamina by smooth muscle cells, their subsequent migration into the developing neointima and the deposition of matrix. Human and animal studies have revealed that a flow rate of 15dyn/cm² is necessary to effectively remodel arteries, and if remodelling does not occur, hyperplasia advances.

Intimal hyperplasia associates with areas of low or oscillatory shear stresses (22, 23). Altered arterial vessel hemodynamic forces, generated by a balloon injury model, demonstrated that reduced blood flow increased the expression and activation of MMPs 2 and 9 proteins, and MMP 2 mRNA within 7 days (22). Increased intimal thickening was also recorded. Arterial mural injury induced increases in MMPs 2 and 9 were significantly enhanced under low flow and inhibited under high flow, in agreement with other animal models (24). Bassiouney et al speculated that shear stress induced MMP expression may be due to a potential involvement of PDGF-BB, as has previously been reported in smooth muscle and endothelial cells (9, 10, 11, 13, 14) (Table 2). A correlation exists between MMP activity and the magnitude of hyperplasia development (25, 26), and it is widely acknowledged that biomechanical factors are integral to the MMP-driven regulation of arterial architecture.

Bidirectional shear i.e. oscillatory or disturbed flow has been shown to increase tissue factor expression conferring a pro-coagulant phenotype for endothelial cells where atherosclerotic lesions are established (27). MMP 9 synthesis was significantly increased in endothelial cells exposed to bidirectional shear (15dyn/cm², 1Hz) (23). A putative c-myc binding site in the MMP 9 promoter was verified to be responsive to hemodynamic shear in
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Table 2. Common signalling pathways involved in the transduction of mechanical forces, applied to the tissue or cell, into intracellular transcriptional and translational responses

<table>
<thead>
<tr>
<th>Cell</th>
<th>Load Stimulus</th>
<th>MMP response</th>
<th>Mediated by</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F-actin Reorganisation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermal fibroblast</td>
<td>200dyn/cm²</td>
<td>↑ pro-MMP 1, 2 &amp; 3</td>
<td>?</td>
<td>41, 42, 44</td>
</tr>
<tr>
<td>Achilles tenocytes</td>
<td>0.16-2.6MPa, 24 hours</td>
<td>↑ MMP 1 with 1 load</td>
<td>?</td>
<td>62</td>
</tr>
<tr>
<td>Synoviocytes</td>
<td>1.5dyn/cm², 6 hours</td>
<td>↑ MMP 13</td>
<td>?</td>
<td>73</td>
</tr>
<tr>
<td>Articular chondrocytes</td>
<td>17KPa (0.5Hz), 12 hours</td>
<td>↑ MMP 1, 3 &amp; 9</td>
<td>PKC</td>
<td>108</td>
</tr>
<tr>
<td>Articular cartilage</td>
<td>0.5MPa (1Hz), 3 hours</td>
<td>↑ pro/active MMP 2 &amp; 9</td>
<td>Thymosin beta4</td>
<td>90-92</td>
</tr>
<tr>
<td><strong>MAP kinase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic endothelium</td>
<td>5% (1Hz), 24 hours</td>
<td>↑ pro- &amp; active MMP 9</td>
<td>Grb2-SOS-Shc, p38, ERK1/2</td>
<td>34</td>
</tr>
<tr>
<td>Articular chondrocytes</td>
<td>1.6Pa flow, 24 hours</td>
<td>↑ MMP 9 mRNA</td>
<td>Ras-Rac-ecd42, JNK</td>
<td>101</td>
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<tr>
<td><strong>Cytokine</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Achilles tenocytes</td>
<td>1dyn/cm², 9 hours</td>
<td>↑ MMP 3</td>
<td>IL-1β &amp; COX-2</td>
<td>58, 63</td>
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<tr>
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<td>6% (0.05Hz), 4 hours</td>
<td>↑ MMP 1</td>
<td>IL-1β</td>
<td>110</td>
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<td>Articular chondrocytes</td>
<td>15KPa (0.5Hz), 48 hours</td>
<td>↑ MMP 9</td>
<td>IL-1</td>
<td>111</td>
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<td>Articular chondrocytes</td>
<td>16KPa (0.33Hz), 20 mins</td>
<td>↑ MMP 3 mRNA</td>
<td>IL-4/5 &amp; b1 integrin</td>
<td>109, 118, 119</td>
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<tr>
<td>Articular chondrocytes</td>
<td>10MPa (1Hz), 24 hours</td>
<td>↑ pro/active MMP 2</td>
<td>IL-6</td>
<td>105</td>
</tr>
<tr>
<td><strong>Growth Factor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelium</td>
<td>12dyn/cm², 15 hours</td>
<td>↑ pro- &amp; active-MMP 2</td>
<td>PDGF-BB</td>
<td>12</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>105% strain</td>
<td>↑ MMP 2 &amp; 9 mRNA/protein</td>
<td>TGF-B</td>
<td>17</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>10-16% (1Hz), 96 hours</td>
<td>↑ MMP 2 activation</td>
<td>TGF-B</td>
<td>11</td>
</tr>
<tr>
<td>Articular chondrocytes</td>
<td>20dyn/cm², 1 hour</td>
<td>↑ MMP 1 &amp; 13</td>
<td>TGF-B</td>
<td>104</td>
</tr>
<tr>
<td>Articular chondrocytes</td>
<td>5dyn/cm², 1 hour</td>
<td>↑ MMP 1 and 13</td>
<td>TGF-B</td>
<td>103</td>
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<tr>
<td>Articular chondrocytes</td>
<td>30% strain (1mm/s)</td>
<td>↑ MMP 1, 3 and 13</td>
<td>VEGFR-2</td>
<td>97</td>
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<tr>
<td><strong>Transcription Factor</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Endothelium</td>
<td>4% (1Hz), 0.5 hours</td>
<td>↑ MMP 1 mRNA</td>
<td>Ets-1</td>
<td>16</td>
</tr>
<tr>
<td>Synoviocytes</td>
<td>6dyn/cm², 6 hours</td>
<td>↑ MMP 1, 3 &amp; 13 mRNA/protein</td>
<td>Ets-1</td>
<td>72</td>
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<tr>
<td>Articular chondrocytes</td>
<td>20dyn/cm², 1 hour</td>
<td>↑ MMP 1 &amp; 13</td>
<td>Ets-1, NFkB</td>
<td>104</td>
</tr>
<tr>
<td>Articular chondrocytes</td>
<td>5dyn/cm², 2 hour</td>
<td>↑ MMP 1</td>
<td>CITED2, Ets-1, p300</td>
<td>103</td>
</tr>
<tr>
<td>Endothelium</td>
<td>24% (1Hz), 4 hours</td>
<td>↑ MT1-MMP mRNA/protein</td>
<td>Egr-1</td>
<td>32, 40</td>
</tr>
<tr>
<td>Endothelium</td>
<td>14dyn/cm², 4 hours</td>
<td>↑ MT1-MMP mRNA/protein</td>
<td>Sp1</td>
<td>32, 40</td>
</tr>
<tr>
<td><strong>ATP/ Ca²⁺ release</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anulus fibrosus</td>
<td>0.1x gravity (6Hz), 8 hours</td>
<td>↑ MMP 3</td>
<td>-</td>
<td>56</td>
</tr>
<tr>
<td>Nucleus pulposus</td>
<td>3MPa, 2 hours</td>
<td>↑ MMP 3</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td>Achilles tenocytes</td>
<td>3.5% (1Hz), 2 hours</td>
<td>↑ MMP 3 regulation</td>
<td>-</td>
<td>58, 66, 68</td>
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<tr>
<td><strong>Other pathways</strong></td>
<td></td>
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<tr>
<td>Smooth muscle</td>
<td>15% (0.5Hz), 5 min</td>
<td>↑ MMP 2 mRNA &amp; protein</td>
<td>NAD(P)H oxidase</td>
<td>21</td>
</tr>
</tbody>
</table>

Common signalling pathways involved in the transduction of mechanical forces, applied to the tissue or cell, into intracellular transcriptional and translational responses. Emerging mechanisms are evident across the cell types including reorganisation of the actin cytoskeleton, modulation of specific cytokines and growth factors and competing transcription factor binding to the MMP promoters.

3.2. Angiogenesis

Angiogenesis – the formation of new blood vessels from pre-existing ones occurs throughout development, wound healing and tumour growth. Complex signalling events cause capillary endothelial cells to initiate a proliferative and migratory phenotype, causing endothelial cells to emerge through the existing basement membrane, form contacts with the surrounding ECM and migrate along the substratum forming new blood vessels (29). Angiogenesis is a critical mechanism in the adaptations that occur in response to chronic increases in metabolic activity within skeletal muscle. Proteolysis of capillary basement membrane proteins by MMPs is crucial for angiogenesis (30). Mechanical forces, including cyclic strain and fluid shear stresses, are hypothesised to modulate the expression of key genes in endothelial cells - including the MMPs (31-35). MMPs 2 and 9 are directly involved in vascular cell migration and invasion in vitro (34-37) although the mechanism(s) of MMP regulation during angiogenesis are less well defined (Figure 3). Exposure of aortic endothelial cells to cyclic strain (5%, 1Hz, 24 hours) induced MMP 9-mediated cell migration and tube

endothelial cells. Disturbances in the haemodynamic forces experienced by smooth muscle cells increases MMP 9 production resulting in extensive proteolysis of the basement membrane and plaque formation (27). Alterations in flow rate (shear stress) regulate a fine balance between intimal thickening (low shear stress) and expansive remodeling (high shear stress) emphasizing the importance of mechanical forces in vascular tissue homeostasis (28). Using a rabbit model of bilateral carotid interposition vein grafting with unilateral distal branch ligation (allows the creation of differential wall shears at sites of bifurcation due to the constricted flow in one artery in which the ligature is placed offset by an increased flow in the other), Berceli et al demonstrated that the constricted artery (containing vein graft) exposed to a lower flow rate (~1.5dyn/cm²) had a significantly increased intimal area that correlated with increased MMP 2 and 9 activity (28). Grafting of veins to the arterial circulation, local injury and altered hemodynamic forces induces MMP expression promoting atherosclerosis through smooth muscle cell migration/ proliferation within the developing neointima.

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Figure 3. A schematic representation of how different mechanical loads acting on blood vessel endothelial cells can modulate MMP transcription, synthesis and activation. A. Cyclic strain (5%, 1 Hz, 24 hours) induced MMP 9 synthesis occurs via recruitment of the Shc-Grb2-SOS complex coupled with phosphorylation of p38 and ERK 1/2 kinases. B. Competition for the binding sites of the MT1-MMP promoter regulate its expression and the activation status of pro-MMP 2. The MT1-MMP promoter has differential binding affinities for Egr-1 and Sp1; shear stress (14 dyn/cm²) induced binding of Sp1 to the respective consensus site in the MT1-MMP promoter represses MT1-MMP transcription and abrogates pro-MMP 2 activation, whereas cyclic strain (24%, 1 Hz, 4 hours) induced Egr-1 binding promotes MMP synthesis.

The localisation of the MT-MMPs to the cell surface and their ability to activate pro-MMP 2 has led to speculation that MT-MMPs are involved in controlling the proteolytic cascades characterised in angiogenesis (32, 40). MT1-MMP mRNA and protein levels were increased in rat microvascular endothelial cells exposed to cyclic strain (24%, 1 Hz, 4 hours) (22). MT1-MMP levels increased in a time dependent fashion up to 24 hours. Yamaguchi demonstrated that the mechanical regulation of MT1-MMP was through transcription factor Egr-1 induction (early growth response gene product), with Egr-1 mRNA levels peaking at 30-60 minutes. This correlates well with a study by Haas et al who demonstrated increased production and promoter binding of Egr-1 that led to the coordinate induction of MT1-MMP with MMP 2 in microvascular endothelial cells (40). MT1-MMP contains an Egr-1 consensus binding site in the promoter which is essential for cyclic strain mediated MT1-MMP expression in endothelial cells (32). Application of shear stress to microvascular endothelial cells (14 dyn/cm²) decreased MT1-MMP transcription and protein synthesis in a time-dependent manner (33). Interestingly Egr-1 mRNA levels were also rapidly and transiently elevated upon application of shear even though MT1-MMP amounts decreased. A Sp1 consensus site exists in the MT1-MMP promoter adjacent to the Egr-1 element, and in response to cyclic strain the Egr-1 displaces Sp1 promoting MT1-MMP expression (32). Shear stress induction of Egr-1 was insufficient to displace pre-bound Sp1 from the MT1-MMP promoter (33). Exposure of endothelial cells to shear stress increased Sp1 binding to the MT1-MMP promoter in a time-dependent manner; phosphorylation of serine residues on Sp1 increased the affinity of Sp1 for DNA hence abrogating MT1-MMP mechano-induction (Figure 3B).

3.3. Wound Healing (Fibroblasts)

Wound healing is a complex process requiring constant “crosstalk” between dermal fibroblasts, extracellular matrix and associated molecules. Proteolytic degradation of the matrix is an essential feature of wound repair/remodelling, and this is partially mediated by the MMPs. Mechanical loading of skin fibroblasts (tensional forces derived from contraction of the collagen lattices in which the cells were embedded) increased MMP 1 synthesis (41, 42). The importance of mechanical induction of MMPs to promote de novo tissue formation and repair after injury has been recently investigated (43-45). MMPs 2 and 9 were induced in dermal cells after application of a cyclic tensile load (480 dyns/hour, comprising 4 phases including rest); 80% of the MMP 9 observed was in its active form whereas most of the MMP 2 detected remained latent (44). MMP 3 levels decreased after mechanical stimulation. A different study demonstrated increased expression of MMPs 1, 2 and 3 in dermal fibroblasts exposed to an identical loading regime as above (43). Alterations to the collagen lattice configuration to promote highly aligned strain patterns, i.e. increased strain gradients, affected the MMP response initiated in the dermal fibroblasts (45). Under an increased pre-existing strain gradient, application of an identical loading regime further induced MMPs 2 and 9 synthesis, and decreased MMP 3 in excess of that observed under normal
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mechanical conditions (44). The authors suggest that this is indicative of a threshold to which cells are strain-sensitive.

An exact mechanism(s) of load induced MMP synthesis has not been elucidated in dermal fibroblasts, but earlier studies demonstrated the involvement of the actin cytoskeleton in tensional induction of MMP 1 (41, 42). Increased F-actin depolymerisation, due to contraction of the collagen lattices, induced MMP 1 expression, an effect substantiated by other studies involving disassembly of the actin filaments using cytochalasin D, in which MMPs 1, 2, 3, 9, 13 and MT1-MMP levels were elevated (46-48). This hypothesis is also consistent with a study conducted by Tomasek et al who suggested that the extracellular matrix and actin cytoskeleton transduce signals that modulate MMP-regulated tissue remodelling, as observed in wound repair (49). Mechanical deformation of the cytoskeleton, and hence the reorganisation of the actin networks, is a key mediator in regulating MMP production in fibroblasts and other cell types (Table 2) (refer to section 4.3).

3.4. Intervertebral Disc Degeneration

Intervertebral discs function to support compressive and bending loads whilst providing flexibility to the spinal column. The intervertebral disc, a hydrated soft tissue comprising a gelatinous nucleus pulposus surrounded by a highly organised fibre-reinforced annulus fibrosus (50), is exposed to varying mechanical stresses during daily activities (muscle forces and ligament tensions change with posture and movement). Mechanical load is essential to maintain disc health, but excessive loads contribute to intervertebral disc degeneration via MMP-mediated matrix degradation. Increased activation of MMPs 1, 2, 3, 7 and 8 have been reported in degenerated disc (51). Mechanical load has been shown to induce MMP expression and activation in the intervertebral disc in vivo (52-54) and in vitro (55, 56). Dynamic compression of rat caudal discs 8-9 in vivo (1MPa, 0.2Hz, 2 hours) increased expression of MMPs 3 and 13 in the annulus fibrosus, and immobilisation for 72 hours followed by 2 hours of loading also induced MMPs 3 and 13 in both the annulus fibrosus and nucleus pulposus (53). MMP mRNA induction by dynamic compression is elicited as early as 30 minutes after application of load (54). Application of a static load (1.3MPa, 1Hz) enhanced MMP 2 activation in murine coccygeal discs, an event sustained after 7 days of mechanical stimulation (52). Hydrostatic pressure (3MPa, 2 hours) increased MMP 3 levels in the nucleus pulposus (55) whereas vibrational stimulation (0.1x gravity, 6Hz, 8 hours) suppressed MMP 3 synthesis in the annulus fibrosus (56), demonstrating that intervertebral disc cells from the nucleus and annulus elicit distinct MMP responses to mechanical stimulation with sensitivity to magnitude, frequency and duration of load. The mechanism(s) of load-induced MMP synthesis in intervertebral disc is unknown but interestingly there is evidence that vibratory stimulation induces ATP release from annulus cells (57). As ATP has been shown to regulate mechanical stimulation of MMP synthesis in Achilles tenocytes (58) (refer to section 3.5), this mechanism may also be employed in intervertebral disc to regulate MMP activities.

3.5. Tendinosis/Tendon degeneration

Tendinosis is a common musculoskeletal disorder characterised by acute or chronic pain and degenerative changes in the matrix of the tendon tissue. The condition is often preceded by abnormal loads precipitated by an episode of extensive exercise or injury (59), and is associated with a progressive loss of biomechanical integrity followed by rupture. Tendon rupture is associated with increased activity of MMP 1 concomitant with a reduction in MMPs 2 and 3 in vivo (60). Physiological strain (1%, 0.017Hz, 24 hours) (61) and force (0.16 – 2.6MPa) (62) markedly inhibited MMP 1 expression in tendon cells – tenocytes. Increasing the magnitude and frequency of load eliminated MMP 1 expression. In the presence of a disrupted actin cytoskeleton (cytochalasin D treatment to inhibit F-actin assembly) MMP 1 mRNA expression was increased in tenocytes exposed to load (2.6MPa) (62). These studies support a role for the actin cytoskeleton in a mechanosensory capacity in tenocyte MMP regulation (Table 2). Any alteration to actin filament assembly/ disassembly by mechanical stimulation can affect the homeostatic balance of MMPs.

MMPs 1 and 3 were elevated in achilles tenocytes after exposure to shear stress (1dyn/cm², 9 hours) in vitro, with MMP 3 protein levels increasing in a stress responsive manner (63). COX-2 and IL-1beta mRNA levels were also elevated after shear stress stimulation. IL-1beta stimulated tenocytes have previously been shown to increase endogenous IL-1beta mRNA levels indicative of a positive feedback loop in triggering tenocyte cytokine-mediated MMP matrix destruction (58). Intracellular Ca²⁺ levels were also partially modulated by mechanical shear (63). ATP modulates intracellular Ca²⁺ levels in vitro (64), and ATP is released upon exposure to mechanical stimulation (65-67). Equibiaxial stretch (3.5% elongation, 1Hz, 2 hours plus 18 hours rest period) to flexor digitorum profundus tenocytes increased MMP 3, IL-1beta and COX-2 mRNA expression (58). Addition of exogenous ATP had no effect on MMP expression alone, but in conjunction with cyclic stretch, ATP reduced load-inducible MMP gene expression. Exposure of tenocytes to mechanical stimulation by medium changing induces a transient release of ATP, which declines with time (58, 66, 68). Load-stimulated ATP secretion by tenocytes was totally blocked by gadolinium indicating the role of stretch-activated channels in this response (58). Mechanical load may stimulate ATP release from tenocytes, and the secreted ATP may act in an autocrine/ paracrine manner as a pulse dampening mechanism to modulate excess load stimuli, thereby regulating the extent of MMP synthesis in tendon tissue to confer protection against the pathology of tendinosis/ tendon rupture. Interestingly, Ca²⁺ regulates shear-induced cytoskeletal reorganisation and gene expression in osteoblasts (69) indicating the importance of the actin cytoskeleton (62) and Ca²⁺/ATP release (58, 63) in MMP regulation (Table 2).

3.6. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterised predominantly by inflammation that evokes articular cartilage and bone destruction. Destruction
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of these synovial joint components occurs through the uncontrolled proliferation of synovial cells (synoviocytes) that are believed to be responsible for the cascade of pathophysiological events, including MMP production (70). Gentle weight-bearing exercise can alleviate some of the pain and immobility associated with rheumatoid arthritis (71), whilst more strenuous loading activities propagate inflammation of the rheumatic joints. Despite this interplay of biochemical and biomechanical factors on the synovial joint it is largely unknown whether mechanical loading plays any significant role in the process of joint tissue degradation and more specifically in MMP gene expression.

Human synoviocytes, isolated from the intraarticular soft tissue of knee joints from RA patients, and subjected to gentle shear stress (1 dyn/cm²) suppressed levels of MMPs 1, 3 and 13 compared with rested controls (72). Mechanical regimes of unloading (0.2 dyn/cm²) and intermediate shear (6 dyn/cm²) had an opposing effect (72). MMP genes possess cis-acting regulatory elements including activator protein-1 (AP-1) and Ets binding sites that regulate transcription of pro-MMPs (16). c-fos and Ets-1 and -2 mRNA are differentially induced in RA synoviocytes in response to mechanical stimulation. Induction of c-fos occurred after each of the shear regimes, whilst Ets-1 levels were elevated by both unloading and intermediate shear (72). Ets-2 mRNA was present in all conditions except unloading. These findings indicate that mechanical induction of MMPs 1, 3 and 13 are regulated by Ets-1. Interestingly these responses to load were exclusive to RA synoviocytes; MMP levels in normal synoviocytes were unaffected by shear indicating that two separate mechano-regulatory mechanisms exist in normal and diseased synoviocytes, respectively, to elicit MMP synthesis.

Intriguingly, differences in cell morphology were observed upon exposure of RA synoviocytes to oscillatory shear (1.3 dyn/cm²) or unloading (0.2 dyn/cm²) for periods of up to 6 hours, compared with control synoviocytes (73). Cells were flattened with characteristic extended processes, but appeared rounded and displayed poorly developed tracks of unidentified cytoskeletal elements after stimulation of unloading (1 hour). Shear stress induced significant increases in filamenitous organisation, although unfortunately the specific involvement of F-actin in this altered morphology was not established. Oscillatory shear initially suppressed MMP 13 mRNA levels but this was temporally regulated as levels were restored after 6 hours of load suggesting desensitisation to the load stimulus. An alteration in oscillatory direction (load was applied in one direction for 3 hours followed by loading in a perpendicular direction for an equivalent time) was sufficient to suppress MMP 13 transcription (73). In contrast, unloading elevated MMP 13 expression. This is more consistent with the rounded nature of the synoviocyte suggesting a strong correlation between alterations in the actin cytoskeleton and MMP induction. We, and others have previously demonstrated that cell rounding induced by disassembly of the F-actin cytoskeleton induces MMPs 1 and 3 (46) and MMPs 3, 9, 13 and 14 mRNAs in fibroblasts (47, 48), and MMPs 2 and 9 expression and activity in chondrocytes [Blain, unpublished observations] (section 4.2.1) (Table 2).

MMPs are considered to be the most influential proteolytic enzymes in RA (74) and are induced by cytokine stimulation. Stimulation of RA synoviocytes with the pro-inflammatory cytokines IL-1beta (50 ng/ml) or TNFalpha (50 ng/ml) promoted transcription, translation and activation of MMP 1 and 13 (75). Cyclic strain inhibited expression of MMP 1 and 13 in human RA synoviocytes exposed to a 2% axial strain (1 hour) (75). However, application of cyclic strain in the presence of these pro-inflammatory cytokines suppressed MMP production and activation. Interestingly, addition of actinomycin D – a potent transcriptional inhibitor, did not affect MMP expression induced by strain or cytokine stimulation suggesting a novel mechanism for the mechano-regulation of MMPs, at least in RA synoviocytes, at the post-transcriptional level.

4. MECHANICAL STIMULATION OF MMP EXPRESSION IN ARTICULAR CARTILAGE AND ITS RELEVANCE TO THE DISEASE OSTEOARTHRITIS

Articular cartilage - a specialised tissue that is avascular, aneural and alymphatic, provides a resilient and compliant articulating surface to the bones in diarthrodial joints. The tissue functions in protecting the joint from local, high stress concentrations by dissipating the applied load. It also provides a low friction surface for smooth locomotion. The cartilage ECM comprises heterotypic collagen fibrils, proteoglycans and water, which governs the tissue’s specialised mechanical properties. Any alteration to cartilage homeostasis will affect the functional integrity of the tissue increasing the likelihood of deterioration and the development of joint diseases such as osteoarthritis. Osteoarthritis is characterised by focal areas of articular cartilage damage, osteophyte formation and often inflammation in the joint, creating a painful and debilitating disorder (76). Cartilage degradation arises due to a homeostatic imbalance favouring increased catabolism of the ECM. In recent years many catabolic agents have been identified as potentiators of cartilage destruction in osteoarthritis. Of key importance is the activity of the chondrocyte-derived MMPs that promote degradation of the cartilage collagens and proteoglycan. MMPs 1, 2, 3, 7, 8, 9 and particularly the cartilage-specific collagenase MMP 13 have all been implicated in osteoarthritis with increased activities at lesion sites (77-82), and in a murine spontaneous OA strain (STR/ort) (83). Osteoarthritis is a multi-factorial disorder but as lesions are often located in areas of weight-bearing cartilage or to sites of trauma, repetitive mechanical insult has been hypothesised as a key signalling event for osteoarthritis initiation and progression.

Mechanical loading of articular cartilage is essential in regulating the metabolic activity of chondrocytes (the only cell type in cartilage), and is required for maintaining normal extracellular matrix properties. In vivo, articular cartilage is subject to many different loading parameters including dynamic cyclic and
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<table>
<thead>
<tr>
<th>MMP</th>
<th>Loading Parameter</th>
<th>Response/Mechanotransduction Pathway</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50% final strain (1mm/s) 20dyn/cm2, 1 hour</td>
<td>↑ pro-MMP 1 synthesis - via ↑ VEGFR-2 activation of ligand</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>17KPa (0.5Hz), 12 hours 6% (0.05Hz), 4 hours</td>
<td>↑ MPP 1 mRNA, protein and enzyme activity - TGF-beta mediated interaction of CITED2 with p300 and other transcription factors e.g. Ets-1 and NFXb</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>16KPa (0.33Hz), 20 mins</td>
<td>↑ MPP 1 mRNA - possible induction via cytoskeletal reorganisation and PKC activation</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>1 and 5MPa, 24 hours</td>
<td>↑ MPP 3 mRNA</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>11.5MPa (0.2Hz), 24 hours</td>
<td>↑ pro-MMP 3 synthesis</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>50% final strain (1mm/s) 17KPa (0.5Hz), 12 hours</td>
<td>↑ MPP 3 mRNA - possible induction via cytoskeletal reorganisation and PKC activation</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>16KPa (0.33Hz), 20 mins</td>
<td>↑ MPP 3 mRNA - via alpha5-beta1 integrin dependent IL4 autocrine/paracrine route</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>0.5MPa (1Hz), 3 hours</td>
<td>↑ MPP 9 mRNA - mediated through TRE element via activation of JNK by the GTPases Ras, Rac &amp; cdc42</td>
<td>90,91,92</td>
</tr>
<tr>
<td></td>
<td>1MPa, 5.0 - 24 hours</td>
<td>↑ MPP 9 mRNA - possible induction via cytoskeletal reorganisation and PKC activation</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>17KPa (0.5Hz), 12 hours</td>
<td>↑ MPP 9 mRNA, protein and enzyme activity - via load induced IL-1 activity</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>15KPa (0.5Hz), 48 hours</td>
<td>↑ pro-MMP 9 synthesis and activation - F-actin reorganisation via thymosin beta4</td>
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<tr>
<td>2</td>
<td>0.5MPa (1Hz), 3 hours</td>
<td>↑ pro-MMP 2 synthesis and activation - F-actin reorganisation via thymosin beta4</td>
<td>90,91,92</td>
</tr>
<tr>
<td></td>
<td>10MPa, (1Hz), 0 - 24 hours</td>
<td>↑ pro-MMP 2 synthesis and activation - via ↓ IL-6 levels</td>
<td>105</td>
</tr>
<tr>
<td>3</td>
<td>50% final strain (1mm/s) 4KPa (0.5Hz), 3 hours/day</td>
<td>↑ pro-MMP 3 synthesis - via ↑ VEGFR-2 activation of ligand</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>5MPa (0.5Hz), 3 hours/day</td>
<td>↑ MPP 13 mRNA, protein and enzyme activity - TGF-beta mediated interaction of CITED2 with p300 and other transcription factors e.g. Ets-1 and NFXb</td>
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<tr>
<td></td>
<td>5MPa (0.5Hz), 3 hours/day</td>
<td>↑ MPP 13 mRNA</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>5MPa (0.5Hz), 3 hours/day</td>
<td>↑ MPP 13 mRNA</td>
<td>107</td>
</tr>
</tbody>
</table>

The effect of diverse loading parameters on cartilage chondrocyte MMP expression. MMPs 1, 2, 3, 9 and 13 mRNA, protein synthesis and activation are all responsive to dynamic compression, static compression, shear stress and tensile strain and share common signalling pathways. Physiological loading regimes appear to consistently increase MMP expression in both cartilage tissue and cell. Hypothesis only (no substantiating data).

4.1 In vivo Loading

In vivo, articular cartilage is predominantly subjected to dynamic compression (which the cell also perceives as hydrostatic pressure generated by fluid flow and tensile strain (Figure 1)) as the joints are exposed to loads generated by body weight during movement. Physiological dynamic compression varies depending on activity; loads of between 0.5-5MPa have been recorded whilst walking, loads of 5-10MPa during running and loads exceeding 20MPa when falling from a height (2). In an in vivo rat model, excessive load application (intensive running) induced knee osteoarthritis which was coincident with enhanced MMP 3 activity (84). Where an irreversible insult to cartilage during mechanical injury has been incurred i.e. after an anterior cruciate ligament or meniscal injury, radiographic signs of OA are evident within 10 years (85). One of the most commonly used animal models to mimic post-traumatic OA is the combined ligament transection and meniscectomy model. In this model surgical transection of the ligament and a full thickness cut through the meniscus occurs followed by normal loading activity. Surgical intervention induces joint instability and abnormal loading of the joints, which then gives rise to OA within weeks. In vivo studies have shown a direct correlation between increased expression of MMPs 1 and 3 (86), and MMP 13 (87) and onset of cartilage degradation. Interestingly, administration of a broad spectrum MMP inhibitor prevented cartilage degradation in a rat meniscal tear model (88). These in vivo studies demonstrate a direct involvement between abnormal loading and cartilage degeneration via MMP activity.

4.2. In vitro Loading

Undoubtedly, human and animal studies have provided much of the data relating mechanical load to cartilage matrix degradation via MMP induction in vivo. However, there is an increasing use of in vitro loading models (tissue and isolated cells) to analyse the response of the tissue, allowing the minute dissection of the underlying cellular and molecular mechanisms of MMP mechano-regulation in articular cartilage to be elucidated (Table 3).
regulated is still unclear, a G-actin sequestering protein called thymosin beta4 was elevated after 10 and 30 minutes of cyclical loading (91). We have demonstrated that addition of exogenous thymosin beta4 (2-10µM) to high-density chondrocyte monolayer cultures induces expression and activation of MMP 2 within 2 hours; pro-MMP 9 levels were also significantly elevated (92). Thymosin beta4 promotes disassembly of the F-actin cytoskeleton sequestering the monomeric actin moieties until cellular demands dictate otherwise. Using cytochalasin D (a potent F-actin disrupting agent) we, and others have demonstrated that disassembly of the F-actin cytoskeleton induces MMPs 1 and 3 (46) and MMPs 3, 9, 13 and 14 mRNAs in fibroblasts (47, 48) and MMPs 2 and 9 expression and activity in chondrocytes [Blain, unpublished observations]. We hypothesise that mechanically induced MMP expression and activation is potentiated through the re-organisation of the F-actin cytoskeleton in chondrocytes (Figure 4A), as has been delineated in other cell types (Table 2).

In our studies, a physiological load of 0.5MPa initiates a classical ECM “turnover” response in articular cartilage with elevated MMP levels counterbalanced by increased expression of type II collagen and aggrecan to maintain tissue homeostasis [Blain, unpublished observations]. In other studies where greater loads corresponding to impact or injurious loads have been applied induction of MMPs is in a purely catabolic response (93-95). Mature bovine articular cartilage explants exposed to increasing severities of load culminating in mechanical injury demonstrate a differential MMP response. At a lower load magnitude of 1MPa, MMP 3 was not induced until at least 24 hours of exposure to cyclic deformation; this load induced MMP 3 expression was also observed when a load of 5MPa was applied (95). Increased MMP3 observed after application of a 5MPa load (24 hours) coincided with cell death and denatured type II collagen. MMP 3 mRNA was also observed in bovine articular cartilage explants after injurious compressive loading (11.5MPa, 0.2Hz, 24 hours) (93) (Figure 4B). An 11.5MPa (1mm/second) injurious load applied to explants (equating to 50% compression of the tissue) revealed a 10-fold increase in MMP 3 mRNA after mechanical injury (93), but no changes in MMP 13 mRNA were observed. MMP 3 elevation after mechanical injury correlates well with in vivo data where it was reported that circulating MMP 3 levels in synovial fluid were 50-100 times higher in patients who had previously had anterior cruciate ligament or meniscal tears compared with healthy patients (96). Clearly, injurious loads appear to elevate MMP 3 levels in articular cartilage (Table 3) which can contribute to cartilage degeneration and OA pathology (77-82).

4.2.2. Static Compression

Static compression has also been utilised as a model of blunt end trauma to mimic the catabolic phenotype observed in post-traumatic OA. Induction of MMPs 1, 3 and 13 were observed in bovine cartilage explants after application of static compression to a 50% final strain (1mm/s) (97). Expression of vascular endothelial growth factor (VEGF) was shown to be directly involved in the mechanical regulation of MMPs 1, 3 and 13 in cultured OA chondrocytes (97), although stress-induced VEGF expression was first demonstrated in the ventricular wall of the heart in response to increased diastolic pressure (98). In normal chondrocytes VEGF is not expressed, but its presence has been demonstrated in OA chondrocytes, in conjunction with its receptor VEGFR-2 (flk-1) (97, 99) (Table 4). The mechanism-responsiveness of the VEGF gene is believed to arise from the presence of a hypoxia induced transcription factor 1alpha (HIF-1alpha) element found in the VEGF promoter (100). Mechanical load (trauma) activates/stabilises HIF-1alpha which increases VEGF levels in the chondrocytes, leading to the subsequent induction of MMPs 1, 3 and 13 in chondrocytes (97) (Figure 4C). Pufe et al demonstrated that the mechanical induction of MMPs was the result of autocrine activity by VEGF, whereby MMP induction is partially dependent on VEGFR-2 activation by the ligand. Elevated VEGF expression perpetuates mechanical up-regulation of MMP expression in pathological human OA cartilage, an effect not observed in normal tissue (97).

4.2.3. Fluid Shear

Detailed studies to determine the intricate signalling mechanisms involved in mechanical shear regulation of MMPs in chondrocytes have been conducted. Utilising high-density chondrocyte monolayer cultures derived from skeletally mature rabbit articular cartilage, cells were exposed to a laminar flow equating to a shear stress of 1.6Pa over a loading duration of 30 minutes to 24 hours (101). A 300% increase in MMP 9 mRNA expression was observed compared to unloaded control cells; a further 30% increase in MMP 9 mRNA was measured after 24 hours of stimulation (Figure 4C). The MMP 9 gene contains a 12-O-tetradecanoylphorbol 13-acetate responsive element (TRE) in the promoter that has been previously reported to be mechano-responsive (102). The shear stress response was shown to be mediated through the TRE element, as deletion of the TRE site in the MMP 9 promoter abolished shear stress induced MMP 9 expression. The TRE binds predominantly to the transcription factor AP-1 (either as a heterodimer of fos/jun or as a jun homodimer) and the MAPK family are upstream regulators of such transcriptional activities. After exposure of chondrocytes to shear stress JNK, ERK and p38 activities were all initially elevated within 30 minutes. A direct correlation between MAPK activity and shear induced MMP 9 expression was verified using dominant negative MAPK mutants; the JNK mutant attenuated the effect of shear induced MMP 9, whereas ERK and p38 had no effect. Further studies assessing the regulation of JNK activity identified the involvement of the small GTPases Ras, Rac and cdc42, where dominant negative mutants of all three attenuated the signal from an MMP 9 luciferase promoter. This provides direct evidence that in chondrocytes shear stimulation activates Ras, Rac and cdc42, which subsequently activate JNK to induce TRE-mediated MMP 9 expression (101) (Figure 4C). It is hypothesised that mechanical stimuli regulate JNK and MMP 9 expression via GTPase activity. Although the GTases have different intracellular functions, all are involved in regulating actin-based cytoskeletal...
Figure 4. Schematic representation of how mechanical stimulation modulates MMP expression in articular cartilage chondrocytes. A. Cyclical, dynamic compression (0.5MPa, 1Hz, 3 hours) reorganises the architecture of the actin cytoskeleton resulting in increased synthesis and activation of MMP 2 and 9 in intact tissue. Thymosin-beta 4 causes the net depolymerisation of the actin microfilaments via sequestration of G-actin moieties. Disruption of the actin cytoskeleton using the chemical agent – cytochalasin D also induces MMP expression in cartilage chondrocytes. Larger forces amounting to injurious loads (> 5MPa, 24 hours) induce MMP 3 expression, although the exact mechanism(s) has not been determined. B. In osteoarthritic chondrocytes, static compression (final strain of 50%) regulates expression of MMP 1, 3 and 13 via VEGF binding to its receptor VEGFR-2 (flk-1). Activation of a mechano-sensitive HIF-1alpha element in the VEGF promoter induces VEGF, which upon binding to its receptor activates MMP transcription. This signalling pathway is not evident in normal chondrocytes. C. Shear stress (1.6Pa, 24 hours) induced reorganisation of the actin cytoskeleton and complexing of Ras-Rac-cdc42 at the cell surface activates JNK. A shear-responsive element 12-O-tetradecanoylphorbol 13-acetate responsive element (TRE) then binds to the AP-1 transcription factor at the MMP 9 promoter initiating mRNA synthesis. MMPs 1 and 13 are differentially regulated by shear stress (5-20dyn/cm²) through interaction of a transcriptional co-activator CITED2:p300 with Ets-1 (to inhibit MMP expression) or AP-2 (to enhance MMP expression). D. Tensile strain utilises cytokine signalling to regulate MMP production. Physiological, cyclic strain (16KPa, 0.33Hz, 20 minutes) inhibits MMP 3 mRNA expression through an alpha5-beta 1 integrin-dependent IL-4 signalling mechanism, a mechanism that is absent in osteoarthritic chondrocytes. F-actin reorganisation, coincident with PKC activation promotes transcription of MMPs 1, 3 and 9 in response to physiological strain (17KPa, 0.5Hz, 12 hours). Non-physiological strains (6%, 0.05Hz, 4 hours) have been shown to induce the expression of the pro-inflammatory cytokines TNF-alpha and IL-1beta that are then hypothesised to induce MMP 1 gene expression.
architectures. Re-organisation of the F-actin networks could potentially activate the GTPases and subsequent downstream pathways mediating MMP expression, as observed in other cell types (Table 2).

Shear flow modulated MMPs 1 and 13 mRNA, protein and enzyme activities in a stress-dependent manner (1-20 dyn/cm², 1 hour) in an immortalised human chondrocyte cell line (C-28-I2). Interestingly, exposure of chondrocytes to 5 dyn/cm² decreased MMP 1 and 13 mRNA, protein and enzyme activities, which were partially restored at 10 dyn/cm², and elevated above unloaded control levels at 20 dyn/cm² shear flow. MMP 1 levels appeared to be altered to a greater extent than MMP 13 at 5 and 20 dyn/cm². Previous studies purported that a transcriptional co-activator - CITED2 (CBP/CREB binding protein/p300-interacting transactivator with ED-rich tail 2) is involved in regulation of stress-responsive genes (103). In conjunction with down-regulation of MMP 1 (5 dyn/cm²) CITED2 mRNA and protein levels were elevated directly correlating CITED2 induction with reduced MMP 1 expression. Over-expression of CITED2 in chondrocytes resulted in inhibition of MMPs 1 and 13 mRNA and reduced enzyme activities; abrogation of CITED2 expression using antisense technology in the presence of 5 dyn/cm² shear increased MMP 1 mRNA expression (103). Chondrocytes were stimulated with 5 dyn/cm² shear stress in the presence of IL-1beta or TGF-beta – growth factors reported to elicit opposing effects on matrix synthesis and MMP expression. IL-1beta stimulated MMP 1 mRNA expression in chondrocytes was significantly inhibited when treated in combination with shear stress; however IL-1beta had no effect on CITED2 mRNA. In contrast TGF-beta stimulated CITED2 mRNA in a dose-response manner. CITED2 and Ets-1 interact with p300; complexing of p300 with Ets-1 was observed in chondrocytes stimulated with 25 ng/ml IL-1beta, a complex that was also observed in cells exposed to 20 dyn/cm² shear stress alone. Chondrocytes stimulated with 25 ng/ml TGF-beta promoted complexing of p300 with CITED2, and again this complex was observed in cells subjected to 5 dyn/cm² shear flow only. CITED2-mediated mechanical down-regulation of MMP 1 and 13 appear to be regulated by TGF-beta (Figure 4C). CITED2 orchestrates MMP expression by regulating interactions between p300 and other transcription factors that either down-regulate (AP2) or up-regulate (Ets-1 and NFKB) MMP gene expression (Figure 4C). These findings are corroborated by the known role of TGF-beta in regulating MMP 1 expression in chondrocytes (104), and other cell types (Table 2).

4.2.4. Hydrostatic Pressure

Studies have been conducted on the responsiveness of OA chondrocytes to hydrostatic pressure using MMPs as a marker of altered chondrocyte expression. High density monolayer cultures of human OA chondrocytes, derived from total knee replacement but devoid of visible signs of cartilage softening/fibrillation, subjected to 10 MPa intermittent hydrostatic pressure (1 Hz, 6, 12 or 24 hours) decreased secretion of MMP 2 (105). This inhibition of MMP 2 release was time-dependent. Active MMP 2 levels were also decreased at all durations of loading when compared with non-loaded control cultures. As IL-6 levels were also decreased after hydrostatic pressure it is believed that this influences MMP activity, as it has previously been reported that shear stress can induce IL-6 (106) which can modulate MMP expression (70). They further suggest that the ability of the OA chondrocytes to decrease IL-6, and subsequently MMP expression, in response to hydrostatic pressure may be a mechanism to preserve the integrity of the remaining articular cartilage tissue surrounding the lesion site.

4.2.5. Tensile strain

Several studies have utilised the Flexercell loading device in both normal (107-110) and OA chondrocytes (111, 112) to determine how cyclic tensile strain affects MMP expression. Tensile strain (17 KPa, 0.5 Hz, 12 hours), applied to immature rabbit chondrocytes, induced expression of MMPs 1, 3 and 9 (108) (Figure 4D). Elevated transcription of the cytokines IL-1beta and TNF-alpha, known MMP mediators, were also observed in response to strain which corroborates other studies demonstrating the induction of MMPs 1, 2, 3 and 9 after stimulation with IL-1beta and TNF-alpha in cultured chondrocytes (113-115). Mechano-stimulation of cytokine expression may be a common intermediate in regulating MMP production in chondrocytes as has been previously described in other cell types (73, 110) (Table 2). Interestingly, tensile strain altered the cellular morphology of these chondrocytes from polygonal to a spindle-like shape that aligned perpendicular to the load direction, further demonstrating the importance of the actin cytoskeleton in mechanotransduction (Table 2). Malek and Isuma demonstrated that cytoskeletal reorganisation after shear application is dependent on a mechanism involving tyrosine kinase activity, intracellular calcium and an intact microtubular network in endothelial cells (116). MMPs are also reported to be regulated by protein kinase C (PKC) activation in chondrocytes (114) and that PKC, itself, is activated by cyclic strain (117) suggesting that this kinase may act as a signalling intermediate in mechanos-regulation of MMPs. Honda et al hypothesised that high tensile loads regulate expression of MMPs 1, 3 and 9 via cytoskeletal reorganisation and PKC activation following changes in chondrocyte cell shape (Figure 4D). However we must consider that in the in vivo situation extensive alteration to chondrocyte morphology by mechanical stimulation would not result due to an extensive ECM framework. There are likely to be adaptations to the cytoskeletal elements in response to mechanical strain to elicit an effect, via deformation of the tissue and thus the cell, as was observed in our system (90-92).

The magnitude of cyclic tensile force (CTF) can also affect induction of MMP expression. Application of high CTF (30 cycles/minute) reduced collagen and proteoglycan synthesis (111). The tensile stretch-mediated inhibition of proteoglycan could be partially restored upon addition of an MMP inhibitor, which led to speculation that mechanical stimulation of MMPs contributed to the degradative effects observed. IL-1 and MMP 9 mRNA levels were initially elevated. IL-1 mRNA levels returned
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Table 4. Diseased osteoarthritic cartilage responds differently to normal cartilage when subjected to an identical mechanical stimulus

<table>
<thead>
<tr>
<th>Load Stimulus</th>
<th>Normal articular chondrocytes</th>
<th>Osteoarthritic articular chondrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% final strain (1mm/s)</td>
<td>Absence of VEGF expression</td>
<td>Induction of MMP 1, 3 + 13 via VEGF binding to its receptor VEGFR-2</td>
</tr>
<tr>
<td>0.16KPa (0.33Hz), 20 mins</td>
<td>Decreased MMP 3 mRNA expression via alpha5-beta1 integrin-dependent IL-4 autocrine/paracrine pathways</td>
<td>Unaltered MMP 3 mRNA expression</td>
</tr>
</tbody>
</table>

Diseased osteoarthritic cartilage responds differently to normal cartilage when subjected to an identical mechanical stimulus. VEGF signalling has been shown to modulate MMP synthesis in OA cartilage in response to static compression (50%, 1mm/second). However, VEGF is not normally expressed in healthy tissue indicating a unique mechanotransduction mechanism. The well characterised alpha5-beta1 integrin/IL-4 signalling pathway involved in MMP 3 mRNA suppression identified in normal chondrocytes is aberrant in OA chondrocytes. Under an identical loading regime (0.16KPa, 0.33Hz, 20 minutes) MMP 3 expression is unaffected in the diseased cell.

Cyclic tensile strain can modulate MMP expression and confer protection to the cartilage in the presence of cytokines (110). IL-1beta is a potent stimulator of MMP synthesis and has been demonstrated to rapidly increase MMP 1 mRNA levels in chondrocytes. Lapine articular chondrocytes exposed to cyclic strain (6%, 0.05Hz, 4 hours) in the presence of lng/ml IL-1beta abolished MMP 1 mRNA and protein expression (110). Pre-incubation of chondrocytes with IL-1beta for more than 1 hour, prior to mechanical stimulation, did not reverse the strain-induced abolition of IL-1beta mediated MMP expression, therefore the effect was not thought to be mediated via an IL-1 receptor. The presence of a pro-inflammatory signal is a prerequisite for cyclic tensile strain induced suppression of MMP 1. As demonstrated with IL-1beta hence cytokine strain may be involved in the disruption/dysregulation of a critical step(s) in IL-1beta signal transduction cascades. The magnitude of strain is crucial to the adaptation of articular cartilage; at low strains the tissue can combat inflammatory responses, but under higher strains this effect is negated and a degradative phenotype is witnessed (110, 111) (Figure 4D).

Human chondrocyte monolayer cultures subjected to pressure induced strain (16KPa, 0.33Hz, 20mins) significantly inhibited MMP 3 mRNA expression after 1 hour; basal expression returned by 24 hours (109). Having previously identified a role for the alpha5-beta1 integrin in mechanotransduction (118), and the involvement of IL-4 activity in potentiating the mechanical signal (119) alpha5-beta1 integrin and IL-4 were shown to be involved in MMP 3 mechanoregulation (109) (Figure 4D). Addition of the ion channel blockers – gadolinium (stretch-activated calcium channels) and apamin (small conductance calcium dependent potassium channels) demonstrated that stretch-activated calcium channels are also involved. Thus strain-induced inhibition of MMP 3 is transduced via an integrin-dependent IL-4 autocrine/paracrine route, although the downstream effectors have yet to be fully identified.

Comparisons were made between the mechanical signalling pathway(s) in OA versus normal chondrocytes in response to pressure-induced cyclic strain. The pathway delineated in normal chondrocytes (109) was aberrant in OA chondrocytes (Table 4). Pressure induced cyclic strain did not affect MMP 3 mRNA levels, and transduction of mechanical signals were observed to occur independently of the IL-4 pathway utilised in normal chondrocytes (112). It is unclear why chondrocytes from osteoarthritic cartilage respond differently to mechanical load compared with normal cells, although the range of integrin and other adhesion molecules expressed by chondrocytes is modified in OA, hence increasing the potential for altered cell-matrix interactions. Also, it has recently been demonstrated that there are structural differences in cytoskeletal organisation between normal and OA human articular cartilage chondrocytes; in both human OA cartilage (121) and a rat model of osteoarthritis (122) actin localisation was less well defined and appeared diffusely throughout the cytoplasm, or was limited to the periphery of the cell. As the actin cytoskeleton is involved in mechanoregulation of MMPs (41, 42, 46-49, 91, 101) (Table 2) it might be that such an alteration to the dynamics of actin filament assembly may compromise the interaction of the extracellular matrix with the chondrocyte focal contacts to respond appropriately to a mechanical stimulus.

5. MECHANICAL REGULATION OF TIMP EXPRESSION

This review has focused on the mechanoregulation of MMP expression at both the mRNA and protein levels, the story would not be complete without a mention of the effects of mechanical stimulation on TIMP expression. Clearly different mechanical regimes influence the extent of MMP production, and usually increased MMP production is counterbalanced by the presence of TIMPs to prevent deleterious consequences. It is interesting to note that in most of the studies reporting an effect on MMP modulation there are no concomitant correlations with increased TIMP expression (23, 90, 105, 110, 111). In studies where there is an mechanical-responsiveness by TIMPs, TIMP 1 appears to be more sensitive to mechanical stimulation with the greatest induction in response to fluid shear (106, 120), hydrostatic pressure (55, 107), and cyclic
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tensile strain (9, 108, 110), which may reflect the inducible nature of TIMP 1. Elevated levels of TIMP 2 were observed after exposure of chondrocytes to cyclic strain (107) and in rheumatoid synoviocytes subject to shear, where it was demonstrated to be under the control of c-fos activity (72). It appears that MMPs are much more responsive to mechanical forces than the TIMPs. Whether the mechanical induction of TIMP expression is sufficient to neutralise the elevation in MMP activity remains to be answered. This concept fits in with our knowledge to date indicating that mechanical induction of MMPs may reflect a mechanism by which pathology is instigated whereby an imbalance in tissue homeostasis favours catabolism and hence the onset of pathology.

6. CONCLUSIONS AND PERSPECTIVES

As highlighted in this review, mechano-regulation of MMPs can be both necessary - in maintaining tissue homeostasis, and deleterious - in inducing pathological conditions. It is evident from these studies that physiological loads induce MMP expression purely for matrix turnover, whereas abnormal or non-physiological loading induces MMP-mediated damage of the tissues. In their entirety, these studies demonstrate the exquisite sensitivity of cells to mechanical stimuli (Tables 1 and 3), and the powerful influence mechanical forces have on MMP-mediated tissue homeostasis and disease. Even small mechanical deformations can exert a powerful influence on MMP-mediated ECM degradation.

Clearly, there is commonality in the signal transduction pathways utilised by the diverse cell types to regulate MMP synthesis in response to mechanical stimulation (Table 2). Interestingly, MMP mechano-regulation is not only initiated at the cell surface i.e. binding of cytokines or growth factors, but is propagated through intracellular events including cytoskeletal reorganisation, phosphorylation cascades and ultimately altered binding of transcription factors to the MMP promoters. Elevated expression of pro-inflammatory cytokines is a classical sign in several synovial joint pathologies i.e. in osteoarthritis (77-83) therefore it is not surprising that mechanical load induces IL-1beta cytokine mRNA expression and that this in turn regulates MMP activity (58, 63, 110, 111). Conversely mechanical induction of the anti-inflammatory cytokine IL-4 suppresses MMP 3 expression in articular chondrocytes conferring a chondro-protective effect (109, 118, 119). Apparent in several cell types is the involvement of growth factors in MMP mechano-regulation. TGF-B plays a pivotal role in MMP synthesis both in smooth muscle cells and chondrocytes. Smooth muscle cell production of MMPs via TGF-B – receptor binding appears to be independent of strain magnitude/ frequency (11, 17), but in chondrocytes different loading parameters elicit opposite effects. Low, physiological shear stresses suppress TGF-B induced collagenase production (103), whilst higher, non-physiological stresses promote TGF-B mediated MMP synthesis (104) demonstrating how MMPs can degrade the cartilage in response to abnormal loading. When cells perceive a mechanical stimulus, reorganisation of the actin cytoskeleton occurs (41, 42, 44, 62, 73, 90-92, 108) which instigates downstream effects such as propagation of the MAP kinase signalling pathways (34, 101), and ATP/ Ca²⁺ release (55-58, 66, 68) to evoke MMP production. In reality these cells would be protected by an extensive extracellular matrix, but we have demonstrated that even in intact tissue cell deformation does occur coupled by an alteration in the dynamics of actin turnover (90-92), and therefore believe that the actin cytoskeleton plays a pivotal role in orchestrating the other signalling pathways identified (Table 2). The other cooperative pathway involved in transducing a mechanical stimulus into an MMP response occurs at the transcriptional level. The binding of several transcription factors appear to be crucial in determining whether the mRNA synthesis of a particular MMP is repressed or activated by mechanical stimulation. The collagenases, MMP 1 and 13, are regulated by the binding of the Ets-1 transcription factor to the promoter; if there is a reduction in Ets-1 or it is displaced from the MMP cis-element, then MMP synthesis is abrogated (16, 72, 103, 104). Competition for binding to the cis-element has been demonstrated in MT1-MMP regulation where Sp1 represses and Egr-1 activates MT1-MMP transcription under different loading regimes (32, 40).

But where are we going with MMP mechanotransduction? A great deal has yet to be discovered! The interplay between biology and biomechanics is relatively new and we are only now beginning to unravel the complexities that exist in mechanotransduction pathways and their direct involvement in MMP regulation and tissue homeostasis. Clearly, a major challenge is defining precisely how mechanical forces become biochemical signals that initiate MMP expression or activation; experimental findings to date suggest that cells use several pathways (Table 2), although a specific pathway may be dominant in a given circumstance. As the mechanisms by which mechanical forces lead to the eventual biochemical and molecular induction of MMPs remains largely undefined, unravelling this mystery will undoubtedly provide new insights, and in particular may offer scope for targeting drugs to specifically inhibit cartilage degradation in osteo- and rheumatoid arthritis, prevent atherosclerotic plaque formation, and promote wound healing.

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