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The Role of Mechanical Stimulation in Engineering of Extracellular Matrix (ECM)

Immanuel M. Sebastine and David J. Williams

Abstract- **The engineering of ECM *in vitro* is a critical area of research in tissue engineering. Cells respond to mechanical stimuli and regulate the metabolic functions *via* mechanotransduction and synthesise ECM. This paper reviews key pathways. *In vitro* studies of mechanotransduction on macroscopic tissues in specialised automated bioreactors that are capable of mimicking the physiological environment by applying different loads will help us to examine how mechanical loads influence intracellular signalling, subsequent behaviour of cells and the synthesis of ECM components.**

I. INTRODUCTION

The goal of tissue engineering is to repair or replace tissues and organs by delivering cells, scaffolds, DNA, proteins and/or protein fragments [1]. One tissue engineering strategy that has been widely proposed for the repair of tissue defects involves seeding isolated and expanded cells within biodegradable three dimensional (3D) scaffolds [2]. The engineering of mechanically functional three dimensional (3D) tissue *in vitro* is approached by manipulating four main variables: cell types, scaffolds, biochemical factors (peptides, growth factors), and mechanical forces [3, 4]. It is necessary to create and mimic the *in vivo* tissue microenvironment so that cells in scaffolds develop, organise, and behave as if they are in their native tissue. Cells in our body are constantly exposed to various mechanical forces both compressive and tensile, and hydrostatic pressure and these forces regulate the expression of diverse ECM components [4-7]. ECM is the substrate for cells adhesion, growth, and differentiation, and it provides mechanical support [4] and shape to tissues. ECM is composed of a great variety of molecules including the collagen family, elastic fibres, glycosaminoglycans (GAG), proteoglycans, and adhesive glycoproteins. These ECM components are synthesised by cells which reside within the ECM and some of their metabolic functions are triggered by mechanical stimulation. Hence, cells must receive external signals from the environment to proliferate, differentiate and synthesise the ECM components to form the tissue/organ [8]. However, the precise mechanism of how the load is transferred and translated into chemical and biological signals that trigger the pathway reactions and gene expression are not clearly understood [5]. This process is referred to as mechanotransduction [9].

I.M. Sebastine* and D.J. Williams are with the Healthcare Engineering Group, Wolfson School of Mechanical and Manufacturing Engineering, Loughborough University, Loughborough, LE11 3TU, UK. (e-mail:i.m.sebastine@lboro.ac.uk). *Asterisk indicates corresponding author.*

Several studies have been carried out to investigate the effect of mechanical loads on ECM synthesis via mechanotransduction mechanisms [10, 11]. These studies are carried out in microscopic tissues in order to understand the role of mechanotransduction in 3D tissue formation. However, information on mechanotransduction in macroscopic tissue experiments does not exist and it is necessary to conduct experiments in macroscopic tissues in order to gain knowledge and better understanding of synthesis of specific ECM components under specific mechanical loads. These experiments will help to understand the processes by which mechanical loads regulate the development of tissues. The objectives of this paper are to review the mechanical signals that trigger the pathways responsible for specific gene expression of ECM components, analyse the role of these signalling pathways to adjust the level of sensitivity of the cell to external mechanical stimuli and to identify requirements for characterisation and measurement of these important phenomena. This knowledge would provide extremely useful information for 3D tissue engineering and to identify the key factors that involved in synthesis of ECM components, and to design the commercial manufacturing processes for 3D tissue engineered constructs.

II. SYNTHESIS OF ECM UNDER MECHANICAL LOADS

Mechanical loading of cells has been shown to influence ECM gene and protein expression, which are dependent on the loading conditions [3]. Each organism is constantly subjected to external mechanical stress (e.g., gravity and movement) as well as to internal forces (e.g., contractile and hemodynamic) generated by both muscle and non-muscle cells [4]. Cells themselves exert forces that are generated by the cytoskeleton [11]. These forces influence cell morphology, cytoskeletal organisation, cell survival, cell differentiation and gene expression [5, 6]. Different molecular processes are responsible for the production of cellular forces. Proteins are differentially expressed according to the mechanical state of cells. Tissues such as cartilage, intervertebral discs and menisci are constantly exposed to the effect of physical forces. Several investigators have attempted to improve the functionality of engineered tissues by mimicking the *in vivo* environment through application of external mechanical loads to tissues *in vitro* [4, 5, 10, 12]. The effects of mechanical stimulation on synthesis of ECM *in vitro* are discussed in Table 1.

TABLE 1
EFFECTS OF MECHANICAL STIMULATION ON SYNTHESIS OF ECM *IN VITRO*

Cell/Tissue	Force/Load/duration	Effect	Reference
Chondrocytes/Cartilage	Dynamic compression (10 % strain, 1 Hz, 3x , 1 hour on, 1 hour off/day, 5 days/week for 4weeks)	Increased synthesis of sulphated GAG and hydroxyproline.	[13]
Rabbit intervertebral disc cells/IVD	Hydrostatic pressure (3D culture, 0–3 MPa; monolayer, 0–1.7 MPa, 1–20 Hz)	High amplitude and frequency increased protein synthesis and lowered protein degradation.	[14]
Intervertebral disc/IVD	Cyclic strain (1, 2, 4, 8% strain, 1 Hz) Hydrostatic pressure (0.25 MPa, 0.1 Hz)	Cyclic strain increased collagen II and aggrecan expression and decreased MMP-3 expression of AF cells. Intermittent hydrostatic pressure increased collagen I and aggrecan expression and decreased MMP-2 and 3 expression of NP cells.	[15]
Chick embryo fibroblasts	Equi-biaxial cyclic strain (10% strain, 0.3 Hz)	Tenacin-C mRNA and protein levels were increased twofold within 6 h compared to the resting control.	[16]
Bovine chondrocytes/Cartilage	Mechanical compression (0–10% sinusoidal strain at 0.1 Hz for 1 h, applied twice daily for 3 days)	Doubled the rate of GAG release from the constructs, but had little effect on gene expression.	[17]
Rat marrow stromal cells (MSCs)/Bone	Shear stress Fluid flow 1ml/min	Constructs demonstrated a 75-fold increase in calcium content compared with control.	[18]

These studies revealed that mechanical loads modulate cell function, including growth, differentiation, migration, gene expression, protein synthesis, and apoptosis. However, excessive/abnormal mechanical loads may alter the cellular metabolism, consequently leading to cell death and tissue degeneration. Thus, uncovering the mechanisms by which living cells sense mechanical stress lies at the core of understanding how they respond and adapt to their physical environments [6, 11].

III. MECHANOTRANSDUCTION

Mechanotransduction is a complex phenomenon requiring the selective involvement of many different signalling pathways in response to mechanical stimuli [11, 12]. The critical component of the mechanotransduction process is the ECM and the initial responses to mechanical stimuli are recorded at the proximities of cell-ECM contacts [5]. The ECM interacts with cells to provide relevant microenvironmental information, biochemically through soluble and insoluble mediators and physically through imposition of structural and mechanical constraints [19]. The major components involved in the mechanotransduction mechanism are *integrins, the cytoskeleton, soluble mediators, G proteins, receptor tyrosine kinases (RTKs), mitogen activated protein kinases (MAPKs), protein kinase C, nuclear factor kB (NFκB), guanine triphosphotases (GTPases, RhoA, Rac1, Ras, focal adhesion-associated kinases [focal adhesion kinase (FAK), rous sarcoma oncogene (Src), integrin linked kinases (ILK)], phophotidyl metabolism, reactive oxygen species and stretch-activated channels* [3, 5]. The major signalling pathways involved in gene regulation by mechanical loads are shown in Figure 1. The mechanical loads induce mechanotransduction pathways via cell and nucleus deformation due to hydrostatic pressure, compression, cyclic stretch, and electromagnetic signals [7, 12, 13-18, 20, 21].

The ECM-integrin-cytoskeleton pathway is one of the signalling pathways most studied. Integrins connect cells to their surrounding ECM, link the actin cytoskeleton, transmit signals across the membrane, transduce into a chemical response through changes in the cytoskeletal structure at the site of receptor binding or at other locations inside the cell and regulate various cellular functions, including cell attachment, proliferation, migration and differentiation [6, 20]. Also, mechanical forces on a cell bring conformational changes to G proteins that initiate signalling cascade of down stream signalling events which causes a generation of second messengers [3]. The routes of activation of

mechanotransduction in various cell types are mediated by integrins, and the accompanying formation of cytoskeletal complexes, can regulate the RTK/Ras/MAPK and IKK/ NFκB pathways [4, 22, 23]. MAPK is crucial for the conversion of mechanical load to tissue adaptation inducing signalling from the cytosol to the nucleus [7], and leading to gene expression and protein synthesis [3]. Besides protein kinase signalling molecules, the activation of mechanosensitive ion channels has also been proposed as a transduction mechanism [12]. Stretch-activated ion channels allow the movement of ions like Na^+, K^+ and Ca^{2+} in and out of cells [3]. As a result, an ECM component could be regulated at the gene transcription level by at least three mechanisms [10]:

(i) *Primary response:* An already available transcription factor, such as NFκB is activated and binds to a mechanoresponsive element in the ECM gene,

(ii) *Secondary response:* A mechanical signal first induces the transcription and synthesis of a nuclear factor and this transcription factor transactivates a specific ECM gene,

(iii) *Autocrine or paracrine loop:* The synthesis and/or release of growth factors are triggered upon mechanical stimulation and in turn indirectly regulate ECM gene expression.

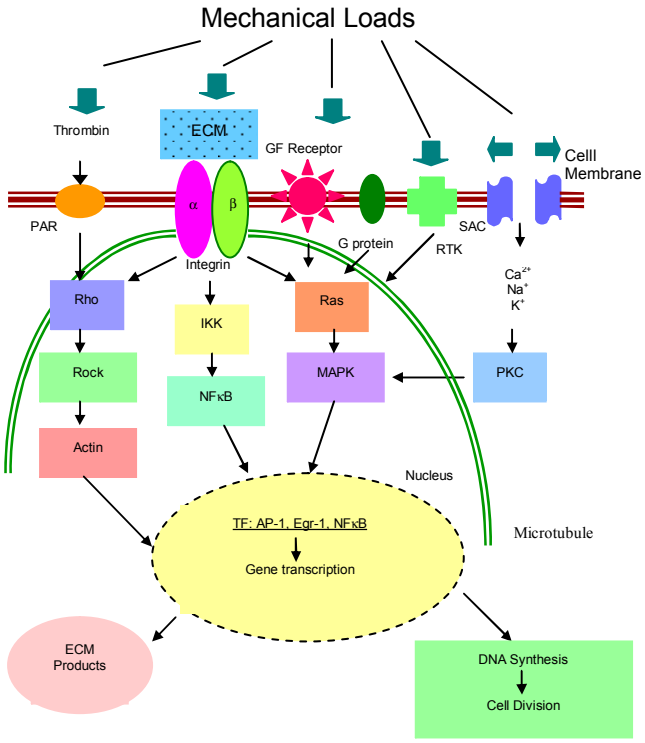

Figure 1. Diagram showing major signalling pathways involved in gene regulation by mechanical loads

PAR- proteinase activated receptor, ROCK- Rho dependent Kinase, NFκB – Nuclear factor kappa B, IKK – Inhibitor of NFκB kinase, MAPK – mitogen activated protein kinases, TF – transcription factors, GF – Growth factors, PKC – protein kinase C, SAC-Stretch activated channel, Rho and Ras- Proteins (molecular switches), EGR1 (early growth response 1), AP1-transcription factor

It is evident that mechanical loads activate various pathways and regulate the expression of ECM components. Studies of mechanotransduction are required in order to examine how mechanical loads influence intracellular signalling and subsequent behaviour of cells [5, 6].

IV. CONCLUSIONS

It is well known that mechanical stimuli are important modulators of cell physiology, and regulate the expression of diverse ECM components *in vitro*. Although the experiments conducted in microscopic tissues have demonstrated a strong correlation between mechanical forces and changes in cell behaviours, the mechanotransduction mechanism by which cells convert mechanical signals into a specific response in ECM gene expression is poorly understood. Transmission of mechanical loads may alter the shape of the cells and deform the nucleus, and there by regulate the import of transcription factors. Studies on mechanotransduction on macroscopic tissues are required to understand how these changes in cell shape influence the behaviour of cells, cytoskeleton, cell membrane, release of chemical and biological molecules/signals and the pathways and subsequent synthesis of ECM. Better understanding of mechanotransduction will help us to apply appropriate mechanical stimulation on cells in scaffolds *in vitro* for the expression of a specific gene of interest or creation of particular constructs.

In order to improve or accelerate ECM synthesis, identification and quantification of the key steps and molecules involved in the mechanotransduction mechanism which are responsible for the initiation of gene expression of specific protein is required. This will require novel experimental and theoretical methodologies to determine how mechanical loading exerts effects at the cellular, molecular and transcriptional level. *In vitro* studies in specialised automated bioreactors that are capable of mimicking the physiological environment by applying different loads and flow patterns simultaneously to improve or accelerate ECM synthesis will be useful to study 3D tissue construction prior to implantation *in vivo*, and to conduct macroscopic tissue experiments to study the effects of mechanical loading (i.e., dynamic compression, hydrostatic pressure and their combinations) on cells and their gene expression and ECM synthesis and also to assess the edge effects of the 3D tissue constructs. Development of new tools or sensors to observe the changes in cells during mechanotransduction and to analyse the genes, mRNA, and proteins expressed within the tissue construct will open new avenues of research.

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REFERENCES

[1] D.L. Butler, S.A. Goldstein and F. Guilak, "Functional tissue engineering: The role of biomechanics," *Journal of Biomechanical Engineering,* vol. 122, pp. 570-575, 2000.

[2] R. Langer and J.P. Vacanti, "Tissue Engineering," *Science,* vol. 260, pp. 920-926, MAY 14. 1993.

[3] J.H.-. Wang and B.P. Thampatty, "An introductory review of cell mechanobiology," *Biomechanics and Modeling in Mechanobiology,* vol. 5, pp. 1-16, 2006.

[4] M. Chiquet, "Regulation of extracellular matrix gene expression by mechanical stress," *Matrix Biology,* vol. 18, pp. 417-426, 1999.

[5] A. Sarasa-Renedo and M. Chiquet, "Mechanical signals regulating extracellular matrix gene expression in fibroblasts," *Scandinavian Journal of Medicine and Science in Sports,* vol. 15, pp. 223-230, 2005.

[6] C.S. Chen, J. Tan and J. Tien, "Mechanotransduction at cell-matrix and cell-cell contacts," *Annual Review of Biomedical Engineering,* vol. 6, pp. 275-302, 2004.

[7] M. Kjaer, "Role of Extracellular Matrix in Adaptation of Tendon and Skeletal Muscle to Mechanical Loading," *Physiol REv,* vol. 84, pp. 649-698, 2004.

[8] F. Rosso, A. Giordano, M. Barbarisi and A. Barbarisi, "From Cell-ECM Interactions to Tissue Engineering," *Journal of Cellular Physiology,* vol. 199, pp. 174-180, 2004.

[9] H. Huang, R.D. Kamm and R.T. Lee, "Cell mechanics and mechanotransduction: Pathways, probes, and physiology," *American Journal of Physiology - Cell Physiology,* vol. 287, 2004.

[10] D. MacKenna, S.R. Summerour and F.J. Villarreal, "Role of mechanical factors in modulating cardiac fibroblast function and extracellular matrix synthesis," *Cardiovascular Research,* vol. 46, pp. 257-263, 2000.

[11] F.J. Alenghat and D.E. Ingber, "Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins." *Science"s STKE [Electronic Resource]: Signal Transduction Knowledge Environment,* vol. 2002, 2002.

[12] S. Hughes, A.J. El Haj, J. Dobson and B. Martinac, "The influence of static magnetic fields on mechanosensitive ion channel activity in artificial liposomes," *European Biophysics Journal,* vol. 34, pp. 461-468, 2005.

[13] R.L. Mauck, M.A. Soltz, C.C.B. Wang, D.D. Wong, P.-.G. Chao, W.B. Valhmu, C.T. Hung and G.A. Ateshian, "Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels," *Journal of Biomechanical Engineering,* vol. 122, pp. 252-260, 2000.

[14] M. Kasra, V. Goel, J. Martin, S.-. Wang, W. Choi and J. Buckwalter, "Effect of dynamic hydrostatic pressure on rabbit intervertebral disc cells," *Journal of Orthopaedic Research,* vol. 21, pp. 597-603, 2003.

[15] C. Neidlinger-Wilke, K. Wurtz, A. Liedert, C. Schmidt, W. Borm, A. Ignatius, H.J. Wilke and L. Claes, "A three-dimensional collagen matrix as a suitable culture system for the comparison of cyclic strain and hydrostatic pressure effects on intervertebral disc cells," *Journal of Neurosurgery-Spine,* vol. 2, pp. 457-465, APR. 2005.

[16] M. Chiquet, A. Sarasa-Renedo and V. Tunç-Civelek, "Induction of tenascin-C by cyclic tensile strain versus growth factors: Distinct contributions by Rho/ROCK and MAPK signaling pathways," *Biochimica Et Biophysica Acta - Molecular Cell Research,* vol. 1693, pp. 193-204, 2004.

[17] C.R. Lee, S. Grad, K. Gorna, S. Gogolewski, A. Goessl and M. Alini, "Fibrin-polyurethane composites for articular cartilage tissue engineering: A preliminary analysis," *Tissue Engineering,* vol. 11, pp. 1562-1573, 2005.

[18] N. Datta, Q.P. Pham, U. Sharma, V.I. Sikavitsas, J.A. Jansen and A.G. Mikos, "In vitro generated extracellular matrix and fluid shear stress synergistically enhance 3D osteoblastic differentiation," *Proceedings of the National Academy of Sciences of the United States of America,* vol. 103, pp. 2488-2493, 2006.

[19] A.M. Pizzo, K. Kokini, L.C. Vaughn, B.Z. Waisner and S.L. Voytik-Harbin, "Extracellular matrix (ECM) microstructural composition regulates local cell-ECM biomechanics and fundamental fibroblast behavior: A multidimensional perspective," *Journal of Applied Physiology,* vol. 98, pp. 1909-1921, 2005.

[20] J.P.G. Urban, "The chondrocyte: A cell under pressure," *British Journal of Rheumatology,* vol. 33, pp. 901-908, 1994.

[21] D.E. Ingber, L. Dike, L. Hansen, S. Karp, H. Liley, A. Maniotis, H. McNamee, D. Mooney, G. Plopper, J. Sims and N. Wang, "Cellular tensegrity: Exploring how mechanical changes in the cytoskeleton regulate cell growth, migration, and tissue pattern during morphogenesis," *International Review of Cytology,* vol. 150, pp. 173-224, 1994.

[22] R. Aikawa, T. Nagai, S. Kudoh, Y. Zou, M. Tanaka, M. Tamura, H. Akazawa, H. Takano, R. Nagai and I. Komuro, "Integrins play a critical role in mechanical stress-induced p38 MAPK activation," *Hypertension,* vol. 39, pp. 233-238, 2002.

[23] F. Mercurio and A.M. Manning, "Multiple signals converging on NF-κB," *Current Opinion in Cell Biology,* vol. 11, pp. 226-232, 1999.