Mechanical stimulation of human derived cells in bioreactors for articular cartilage tissue engineering – a systematic review of the literature with a focus on stimulation protocols

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ABSTRACT

Background: Mechanical stimulation has shown to be a significant aspect of articular cartilage generation and maintenance. Many bioreactor systems have been designed and built in order to delivery specific types of mechanical stimulation. The focus thus far has been twofold, applying a type of preconditioning in order to stimulate cell growth and differentiation, and to simulate in vivo conditions in order to gain further insight into how cells would react to different stimulatory patterns in vivo. Due to the complexities of the forces at work within joints, it is extremely difficult to simulate mechanical conditions perfectly using a bioreactor

Objectives: The aim of this review is to gain a deeper understanding of the complexities of mechanical stimulation protocols by comparing those employed in bioreactors in the context of tissue engineering for articular cartilage, and to consider their affects on cultured cells.

Methods: AMED (Allied and Complementary Medicine) 1985 to January 2016, Ovid MEDLINE(R) 1946 to January Week 3 2016, and Embase 1974 to 2016 January 29 were searched using key terms. Primary research articles were subject to inclusion and exclusion criteria, and results were extracted and exported into a table and subsequently discussed.

Conclusion: Based on this review it is overwhelmingly clear that mechanical stimulation leads to increased chondrogenic properties in the context of bioreactor based articular cartilage tissue engineering using human cells. However, given the variability and lack of controlled factors between research articles, results are difficult to compare, and a standardised method of evaluating stimulation protocols proved challenging. With improved standardisation in mechanical stimulation protocol reporting, bioreactor design and building processes, along with a better understanding of joint behaviours, we hope to perform a meta-analysis around stimulation protocols, and further explore the clinical applications of articular cartilage tissue engineering.

Keywords: Bioreactor, tissue engineering, cartilage, chondrocyte, stimulation

INTRODUCTION

Articular cartilage is a specialised type of connective tissue. Though lacking blood vessels, lymphatics, and nerves, it still fulfils an important function by providing a smooth surface with a low friction index at which movement can occur in diarthrodial joints (1). Due to the nature of its role as a type of shock absorbent, it is also exposed to extreme biomechanical stresses. These mechanical stresses coupled with the catabolic cytokines and matrix-degrading proteinases they induce, and furthermore the involvement of interleukin-1 (IL-1) and tumour necrosis factor a (TNFa), play a key role in the gradual degradation of articular cartilage (2). Research thus far, has demonstrated how this process is indeed complex and multi-factorial, owing to a combination mechanical, biochemical, and genetic factors (3,4).

Extreme mechanical loading due to trauma, obesity (5), joint instability (6) are some of the known risk factors for cartilage damage and development of osteoarthritis. However, it is also important to note that within an optimal and so called physiological range, mechanical loading is central, nay essential to the development and maintenance of healthy chondrocytes (7). There are a huge array of treatment methods currently recommended for osteoarthritis. A recent systematic review discovered fifty one modalities in total when looking at existing treatment guidelines for hip and knee arthritis (8). These range from altering lifestyle through education, to rehabilitation methods, physiotherapy exercises, pharmacological interventions, and surgical methods such as joint replacements (9). However, due to the distinct lack of ability for cartilage tissue to regenerate and repair itself (10, 11, 18), the search continues for more permanent and effective methods of treatment.

Recent advances have demonstrated the clinical application of surgical procedures such as autologous chondrocyte implantation (ACI) (15), osteochondral autografts (16), allografts (17), osteochondral drilling, cartilage abrasionplasty, and microfracture, however these methods continue to give variable results, and in many cases lead to the formation of fibrous repair tissue, which ultimately is not able to perform under load bearing conditions in the same way as healthy articular cartilage (12,13). Many look to the future of tissue engineering to provide more successful and consistent results.

The science of tissue engineering is concerned primarily with understanding how tissues grow, and using this knowledge to create functional tissues that will go on to replace those in the body that have become ineffective in performing their role (14). Progress in the field of tissue engineering could pave the way for functional tissue regeneration and replacement without needing to use grafting techniques.

In short, tissue engineering for articular cartilage application generally follows a set format. Cells are isolated in the first instance, they are then seeded into a 3D structure called a scaffold (usually created from biodegradable materials), the seeded scaffold is then placed into a controlled environment for the culture time period, a bioreactor is usually used to carefully control the environmental conditions, and deliver the necessary biochemical and/or mechanical stimulation to initiate cell growth and differentiation (19, 20, 21).

Mechanical stimulation has shown to be a significant aspect of articular cartilage generation and maintenance. Many bioreactor systems have been designed and built in order to deliver specific types of mechanical stimulation. The focus thus far has been twofold, applying a type of preconditioning in order to stimulate cell growth and differentiation, and to simulate in vivo conditions in order to gain further insight into how cells would react to different stimulatory

patterns in vivo. Due to the complexities of the forces at work within joints, it is extremely difficult to simulate mechanical conditions perfectly using a bioreactor (22).

Therefore we have set out to review the literature with the aim of gaining a deeper understanding into the complexities of mechanical stimulation protocols in the context of articular cartilage tissue engineering.

METHODS

For the purposes of this review we used the PRISMA checklist (23) to guide our reporting methods. Our topic of interest was pertaining to mechanical stimulation delivered via bioreactor systems to human derived cells in the context of articular cartilage tissue engineering. This was formulated into a focused question: "In the context of bioreactor culture systems used for articular cartilage tissue engineering, how do the different mechanical stimulation protocols used during in vitro experimentation affect outcomes relating to differentiation and expression of chondrogenic properties in human derived cells, compared to the absence of mechanical stimulation?"

Search

AMED (Allied and Complementary Medicine) 1985 to January 2016, Ovid MEDLINE(R) 1946 to January Week 3 2016, and Embase 1974 to 2016 January 29 were searched. Search terms used include "tissue engineering" AND "bioreactor*" AND "stimulation" AND "cartilage OR chondrocyte". Duplicates were removed, and the remaining full text articles were assessed and subjected to the following inclusion and exclusion criterion.

Inclusion criteria:	Exclusion criteria:
1)Primary research articles	1) Bioreactors not utilised
2) Experimental data acquired from using bioreactor systems to analyse the effects of cell stimulation	2) Culturing cells with intent of engineering tissue types other than articular cartilage e.g. bone
3) Within the context of tissue engineering for articular cartilage	3) Experiments using animal derived cells
4) Experiment used only human derived cells	4) Non-English language
	5) Non-experimental studies with no primary research and data collection i.e. systematic reviews
	6) Conference abstracts or pending publications

Figure 1Inclusion and exclusion criterion

Full text analysis was performed on the primary research articles included for review. A standardised form was created to aid extraction of relevant information and comparison of studies. The most relevant information was extracted from these forms and exported into a table, with column headings including "cell type", "scaffold", "bioreactor", "stimulation", "comparison or control", and "outcome and outcome parameters pertaining to mechanical stimulation". We looked for outcomes and outcome parameters which indicated how cultured cells responded to the different mechanical stimulation protocols. Though each study may have characterised this response using slightly different measures, we attempted to identify those which represented chondrogenic expression or demonstrated the absence thereof.

RESULTS

Through the search strategy outlined above 196 records were identified. After removal of duplicates 136 records remained. 136 records were screened and 69 excluded due to using animal derived cells. From the remaining 67 records, full-text assessment for eligibility was performed based on the aforementioned inclusion and exclusion criterion as a result of which 55 articles were removed. 12 articles were carried forward for the purposes of the systematic review.

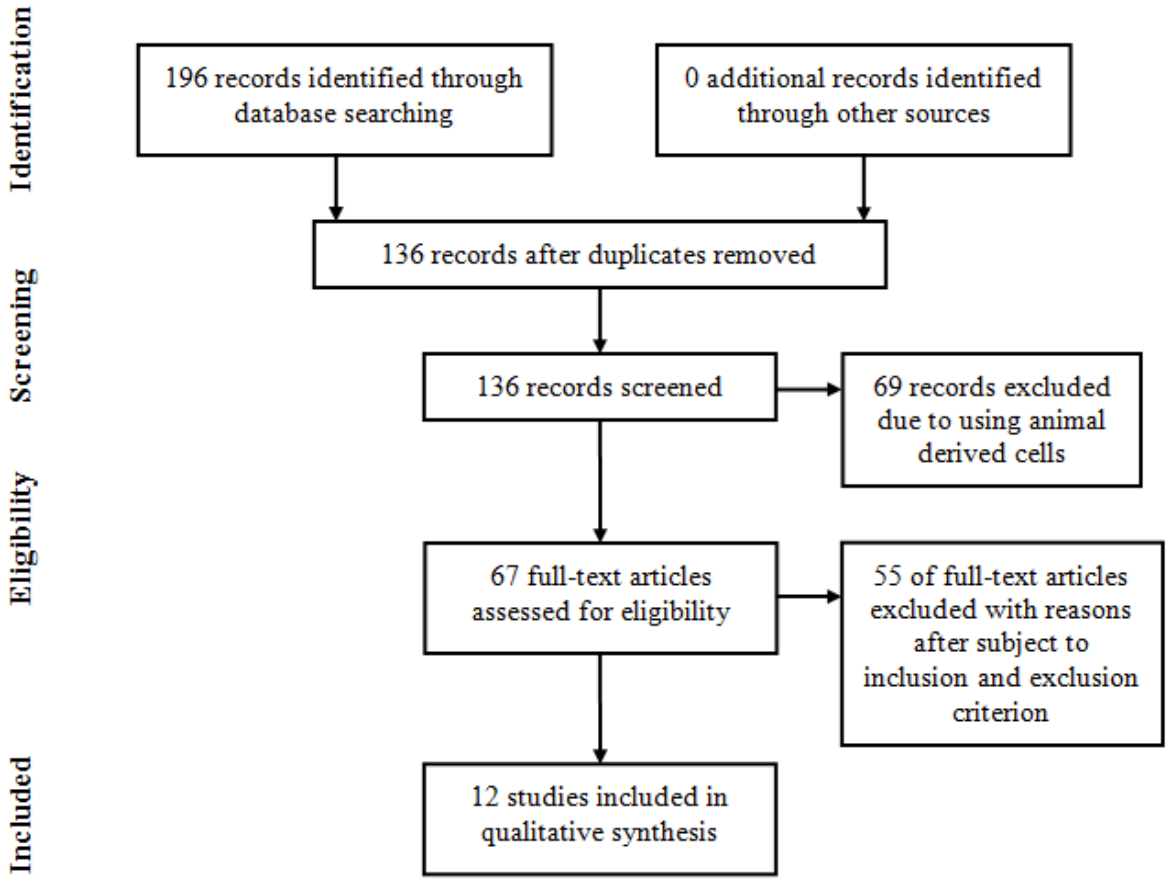

Figure 2 PRISMA flow chart detailing selection procedure

Study	Cell type	Scaffold	Bioreactor	Stimulation	Comparisons or controls	Outcomes and outcome parameters pertaining to mechanical stimulation
(25)	hACPCs	Biodegradable, cylindrical (8x4 mm), Fibrin-Polyurethane composite scaffold (pore size 90 - 300 um) (26)	Custom made bioreactor capable of generating joint-like movements (27)	Mechanical loading protocol was used (28): • Dynamic compression: 1Hz, 0.4–0.8 mm • Shear stress (rotation): 1 Hz ±25° • Superimposed on a static offset strain of 0.4mm • Equivalent to cycling at 5% - 10% strain. Duration: • Mechanical stimulation applied over 7 or 28 days • In both 7 and 28 days group: 1 h of mechanical stimulation applied per day, for 6 days per week (loaded).	Comparison groups: 1. Mechanical stimulation alone 2. Viral transduction (adenoviral-mediated over-expression of BMP-2) alone 3. 1 and 2 in combination 4. Unstimulated but transduced 5. Tansduced but unstimulated	Overall: mechanical stimulation was beneficial for in vitro chondrogenesis of hACPCs. Biochemical analysis: significant increase in total GAG and GAG/DNA ratio. Gene expressions analysis: chondrogenic marker genes up regulated.

(29)	HACs	Scaffold free	Custom built microfluidic bioreactor with integrated USWT	Mechanical stimulation was delivered by:
				• Continuous perfusion of the culture medium
				  ○ Low shear rates: 1.32ml / hour
				• Sweeping acoustic drive frequencies
				  ○ Range: 890 to 910 kHz
				  ○ Sweep rate of 50 Hz
				
				Duration of ultrasound application: 21 days
					Neocartilage was created in the described bioreactor system and mechanical stimulation was applied via ultrasound and perfusion as described.
					
					Next, partial thickness defects were created in human femoral head articular cartilage pieces.
					
					Two experimental groups were set up:
					
					Group 1: One neocartilage graft was inserted into each defect.
					
					Group 2: cartilage defects left empty (control)
					
					Both groups were cultured for 16 weeks
					.
						IT-AFM (30) was used to evaluate the mechanical properties of cartilage pieces dissected from femoral heads, and the neocartilage produced in the bioreactor system. No statistically significant differences were discovered between them.
						
						Histology of group 1 and group 2:
						
						Group 1 revealed hyaline cartilage-like repair tissue in the defect, significantly improving the overall tissue architecture.
						
						Group 2: absence of regeneration.

(31)	Cocultured human intervertebral disc cells and hMSCs	Nanofibrous strips formed the outside of the constructs in the shape of rings, seeded with hAF and hMSCs. The core was occupied by hydrogel, seeded with hNP and hMSCs.	Perfusion bioreactor (ElectroForce 5200 BioDynamic; Bose, Eden Prairie, MN, USA)	Mechanical stimulation: • Cyclic compression: 10% strain • Frequency: 1 Hz • Duration: 1 hour per day for 21 days	Different ratios of hAF/hMSCs in nanofibers and hNP/hMSC in hydrogels were compared.	Optimum ratios of cells were detailed in the study results: • Nanofibers: 2:1 ratio of hAF to hMSCs • Hydrogels: 1:2 ratio of hNP to hMSCs Study demonstrated that compressive loading caused: • tensile stimulation in nanofrous strips seeded with hAF cells and hMSCs • compressive stimulation in hydrogels seeded with hNP cells and hMSCs This demonstrates how the structural properties of a scaffold can influence the effects of compressive loading on cells. Compressive stimulation did not demonstrate increased GAG production per cell in the bioreactor tissue engineered IVD cultures.
(32)	Primary Articular Chondrocytes	Alginate hydrogel scaffold housed within microcell culture system. (33)	High throughput perfusion microcell culture system made up of 12 individual microbioreactors (33)	Dynamic compressive loading delivered using a pneumatically-driven membrane-based actuation scheme: • Strain: 20% and 40% • Frequency: 0.5, 1.0, and 2.0 Hz; • Daily regimen: 3 consecutive cycles of 1 hour loading and 1 hour relaxation • Duration: up to 5 days	Results were compared between: • a control (no stimulation) • Group 1: 20% strain • Group 2: 40% strain With each group split into three groups operating at different frequencies of stimulation i.e. 0.5, 1.0, and 2.0 Hz.	Cell viability: • Technique: fluorescent dye staining and image analysis • no statistically significant difference with mechanical stimulation. Articular chondrocyte proliferation: • Technique: DNA content measured • no statistically significant difference with mechanical stimulation. Chondrocyte metabolic activity:

						• Technique: lactic acid production measured • 18.77% statistically higher than control in under 40% strain at frequency of 2Hz. Biosynthetic activity: • Technique: GAGs synthesis measured • 20% and 40% strain groups at 1Hz and 2Hz stimulation frequencies revealed total GAGs synthesis at statistically higher levels than control. Collagen production was not affected at statistically significant amounts.
(34)	hUC-derived hMSCs	No scaffolds	Programmable voice coil actuator vibration mechanobioreactor	Vibratory stimulation: • Sinusoidal stimulus applied • Frequencies: 1 and 100Hz • Duration: 1-min intervals with a 15-min rest for 15 hours per day for 10 days.	Experimental groups at 1 and 100 Hz compared against controls.	1 Hz resulted in cartilage phenotype Demonstrated by GAG deposition and COLII/COLI mRNA ratio 100 Hz resulted in bone phenotype Demonstrated by calcium deposition and the expression of BMP2 mRNA.
(28)	Bone marrow-derived hMSCs	Biodegradable, cylindrical (8x4 mm), Fibrin-Polyurethane composite scaffold (pore size 90 - 300 um) (26)	Custom built bioreactor that is able to generate joint-like movements (27)	Mechanical loading protocol was used (28): • Dynamic compression: 1Hz, 0.4–0.8 mm • Shear stress (rotation): 1 Hz ±25° • Superimposed on a static offset strain of 0.4mm • Equivalent to cycling at 5% - 10% strain. Duration: • Mechanical stimulation applied over 7 or 28 days • In both 7 and 28 days group: 1 h of mechanical stimulation applied per day, for 6 days per week (loaded).	Comparison groups: 1. Mechanical stimulation alone 2. Viral transduction (adenoviral-mediated over-expression of BMP-2) alone 3. 1 and 2 in combination 4. Unstimulated but transduced 5. Tansduced but unstimulated	Mechanical stimulation demonstrated upregulation of chondrogenic genes, but also small increases in hypertrophic marker Col X. Expression of hypertrophic marker Col x was reduced/delayed upon viral transduction. Mechanical stimulation increased GAG/DNA ratios. Though mechanical stimulation and viral transduction worked synergistically at around day 7. Transduction also showed a trend towards decreased GAG/DNA ratios at later stages.

(35)	Bone marrow derived hMSCs	Biodegradable, cylindrical (8x4 mm), Fibrin-Polyurethane composite scaffold (pore size 90 - 300 um) (26)	Custom built bioreactor that is able to generate joint-like movements (27)	Shear stress and dynamic compression: • Shear stress (rotation): ±25° oscillation at 1 Hz. • Dynamic compression: sinusoidal pattern, 10% strain at 1 Hz on a 10% static load. • Duration: 1 h on alternating days for 14 days (adding up to seven 1-h loading periods).	Two main experimental groups were created: Group 1: with mechanical loading Group 2: free swelling Each was split into further subgroups with addition of IGF-1 and/or TGF-B1, or alone. on chondrogenesis of bone narrow derived hMSCs,, and compared with free-swelling controls.	Mechanical stimulation increased chondrogenesis. Single recombinant TGF-β1 results in stronger chondrogenesis compared to mechanical load. Single recombinant IGF-1 does not result in significant chondrogenesis. The combination of mechanical load, TGF-b1 and IGF-1 resulted in the largest overall chondrogenic differentiation.
(36)	Chondrocytes isolated from human knee articular cartilage	Chitosan (biocompatible and biodegradable polysaccharide) scaffolds (37)	Pendulum stirred-type spinner flask bioreactor.	Mechanical stimulation via intermittent flow cycles: • For the initial 72 hours: ○ Regime of 60 rpm during 5 min, followed by 15 min in stasis, repeated. • Then, continuous stirring at 80 rpm until 28 days achieved.	Static versus dynamic conditions. Control groups without cells but subject to the same experimental interventions were also run.	Results revealed greater chondrogenesis potential under mechanical stimulation. This was demonstrated by: Immunostaining assays under static conditions showing chondrocytes expressed type I, type II collagen, and aggrecans; Ki-67, and actin cytoskeleton. Under stirred flow, cells kept a rounded morphology over 28 days, produced predominantly GAG and type II collagen.
(38)	Chondrocytes isolated from human fetal epiphyseal cartilage after 16–20 weeks of gestation	PGA-alginate scaffold	Custom built mechanobioreactor, delivering shear and compressive forces simultaneously	Magnetic stirrer operated: 65rpm Intermittent shear and compressive loading: • Frequency: 0.05 Hz • Strain: 2.2% superimposed on a static axial compressive strain of 6.5%. • Duration: 10 min each day, up to 2.5 weeks.	Cultures were compared with and without mechanobioreactor loading.	Mechanical stimulation increased both the amount and quality of cartilage produced, GAG synthesis, and collagen type II.

(39)	Bone marrow derived hMSCs	Biodegradable, cylindrical (8x4 mm), Fibrin-Polyurethane composite scaffold (pore size 90 - 300 um) (26)	Pin-on-ball bioreactor system based on previous designs (27).	Oscillation of the ball at an axis perpendicular to the scaffold exerted shear force. Superimposed compressive strain was applied along the length of the scaffold. Duration: 1 h a day for 5 consecutive days per week, over 3 weeks. Compression: • Frequency: 1 Hz • Amplitude: 0.4 mm Shear: • Frequency: 1 Hz • Amplitude: ±25° Preload: • 0.4 mm	Experimental groups consisted of a free swelling culture, compression alone, shear alone, and combination of compression and shear.	Shear or compression alone was not enough for chondrogenic induction. However, shear superimposed upon dynamic compression resulted in significant increases in chondrogenic gene expression, evidenced by increased GAG, and chondrogenic markers Col2, AGG, COMP, and Sox9. Importantly, there were no significant increases in hypertrophic (Col 10) and osteogenic (Col 1 and ALP) gene markers with the addition of surface shear.
(40)	Human chondrocytes	A type I collagen hydrogel scaffold system, Amedrix (Esslingen, Germany).	Mechanobioreactor, which uses an engine driven eccentric to drive a vertical piston which continuously compresses the specimens, as described in (41)	Mechanical stimulation protocol: • Frequency 0.3 Hz; • dynamic compressive strain 10% (0.3 mm). • Duration: 14 days	Groups were compared with or without compressive loading, for a period of 14 days.	Mechanical stimulation was found to produce overall, more chondrogenic phenotypes when considering cell morphology and function. Histological and immunohistochemical analyses: • No significant differences in: col-I, aggrecan and MMP-13 gene expression. • Significant increase under stimulation in: col-II gene expression and the col-II/col-I mRNA ratio were significantly increased. However, biomechanical properties of chondrocytes were decreased in both groups.

(42)	chondrocytes derived from human articular cartilage	Hyaluronic acid (HA) derivative used to create a pad.	Custom made perfused-column bioreactor, exposing cells to convective solute transports and flow induced shear stress.	Perfusion flow rate: 0.5 ml/min, with cyclical flow inversion every 1 min (inlet fluid velocity of 44.2 lm/s). A computational fluid-dynamic (CFD) model was used to predict the shear stress on cells. Median shear stress imposed on the cells in the bioreactor culture, as predicted by the CFD model, is 3 x 10^{-3} Pa (0.03 dyn/cm2).	Static cultures were compared to dynamic cultures in bioreactor environment.	Cultures in dynamic flow: higher structural integrity in comparison to static controls. However, higher levels of HA scaffold deformation and biodegradation were found. It was thought that perhaps stresses of forces were being transmitted to the cells and therefore further investigation is needed to establish the stress distribution of acting forces on cells.

Figure 3 Summarised table of results.

Abbreviations: hACPs: Human Articular cartilage progenitor cells, GAG: glycosaminoglycans, HACs: Human articular chondrocytes, USWT: ultrasound standing wave traps, IT-AFM: Indentation-type atomic force microscopy, hMSCs: Human mesenchymal stem cells, hAF: human annulus fibrosus, hNP: human nucleus pulposus, hUC: Human umbilical cord, PGA: polyglycolic acid,

DISCUSSION

Based on this review it is overwhelmingly clear that mechanical stimulation leads to increased chondrogenic properties in the context of bioreactor based articular cartilage tissue engineering using human cells. As the focus of this review was to consider the different mechanical stimulation protocols employed, other components such as cell type used, scaffold, and bioreactor design varied significantly across reviewed articles. In addition to this, the techniques used to measure outcomes in order to establish chondrogenic expression of cells was also approached in a variety of ways by the studies included for review. Despite this, all studies concluded that mechanical stimulation inferred some kind of beneficial influence on chondrogenic expression of cultured cells.

In this review we have extracted from research articles the mechanical stimulation methods and protocols that have attempted to simulate in vivo conditions of chondrocytes, and examined to what degree, when comparing studies, this simulation has helped to upregulate chondrogenic properties of cultured cells. Furthermore, we will explore how these findings can be carried forward into a clinical setting, and contribute to future therapies aimed at treating articular cartilage defects.

A number of factors caused limitations in our review. The vast terminology utilised in describing mechanical stimulation methods may have resulted in studies not being detected in the initial search. We also found that due to a lack of standardisation and format in approaching mechanical stimulation protocols, as well as the methods used to measure outcomes, it was hugely challenging to compare and contrast studies, their loading parameters, and results. Related to this, is the number studies that utilised custom made bioreactors or built their own, and as such were devoid of any form of standardisation in their calibration and application. Therefore, any mechanical loading parameters reported may not have been true values and a true reflection of the forces applied.

In its day to day functioning, articular cartilage is subject to complex mechanical forces. It is generally accepted that these forces are an important factor in maintaining and developing articular cartilage tissue. In order to simulate in vivo conditions, novel bioreactor systems have been designed and built, each with the remit of producing certain environmental conditions. One endeavour to better understand the complexities of in vivo articular forces was the application of tribological principles (43) (which in brief is the science of interacting surfaces in relative motion) to natural joints, and to produce from this a new bioreactor concept for articular cartilage tissue engineering. Out of this was born a "pin-on-ball" bioreactor system capable of producing joint-like movements (27). This bioreactor design went on to be employed in several studies as the bioreactor of choice for articular cartilage tissue engineering experiments (25, 28, 35, 39) as it was able simulate joint like movements, and its claim to do so backed up by a scientific mechanical engineering model. With the advent of the digital age, much research has been focused into developing computational mechanics, and its application to orthopaedic biomechanics will shed much light on the mechanical behaviour of joints within computational joint models (44). We hope that as this field advances, we will learn more about the intricacies of joint behaviours and the forces involved, with a view to build bioreactor systems that mimic joint conditions more accurately.

It is also noteworthy to mention that mechanical stimulation can and should not be considered in isolation. Although its application is largely dependent on the bioreactor system employed to deliver the stimulus, other factors such as the geometry of the scaffold being used can impact the way stimulation is received by cells. The primary research article by Tsai et al (31) demonstrates this point well. For the purpose of tissue engineering intervertebral discs, a complex scaffold was used which consisted of nanofibrous strips forming the outside of the constructs in the shape of concentric rings, seeded with human annulus fibrosis cells and human mesenchymal stem cells, whilst the core of the structure was occupied by a hydrogel, seeded with human nucleus pulposus cells and human mesenchymal stem cells. With the application of compressive loading the nanofibrous strips experienced tensile stimulation, whilst the compressive hydrogels underwent compressive stimulation. This demonstrates how the structural properties of a scaffold can influence how compressive loading is transmitted on to cells. These apparent subtleties of mechanical stimulation must however not be overlooked, as such differences as tensile and compressive stimulation can lead cells to differentiate into separate cell types. For example previous studies have reported that tensile loading is able to enhance osteogenic (45) and tenogenic (46) differentiation but inhibits chondrogenic differentiation of

mesenchymal stem cells (47). In contrast, compressive loading can enhance chondrogenic differentiation of mesenchymal stem cells (48-50).

Given the numerous challenges we face in simulating in vivo conditions, some attention has been given to considering in vivo tissue engineering. In order words using the living joint itself as the bioreactor. Some advances have been made with this approach in the area of bone tissue engineering (51). However, at present further research is necessary to explore the plausibility of this approach including animal studies and experimentation. If successful, it may lead to the field of tissue engineering somewhat diverting its focus away from in vitro bioreactor based processes, and considering the human body as the perfect bioreactor.

References

1. Sophia Fox A, Bedi A, Rodeo S. The Basic Science of Articular Cartilage: Structure, Composition, and Function. Sports Health: A Multidisciplinary Approach. 2009;1(6):461-468.

2. Goldring M. The role of the chondrocyte in osteoarthritis. Arthritis & Rheumatism. 2000;43(9):1916-1926.

3. Koopman W, Moreland L. Arthritis and allied conditions. Philadelphia: Lippincott Williams & Wilkins; 2005.

4. Radin E, Burr D, Caterson B, Fyhrie D, Brown T, Boyd R. Mechanical determinants of osteoarthrosis. Seminars in Arthritis and Rheumatism. 1991;21(3):12-21.

5. Felson D. Osteoarthritis: New Insights. Part 1: The Disease and Its Risk Factors. Annals of Internal Medicine. 2000;133(8):635.

6. Brandt K, Myers S, Burr D, Albrecht M. Osteoarthritic changes in canine articular cartilage, subchondral bone, and synovium fifty-four months after transection of the anterior cruciate ligament. Arthritis & Rheumatism. 1991;34(12):1560-1570.

7. Kurz B, Lemke A, Fay J, Pufe T, Grodzinsky A, Schünke M. Pathomechanisms of cartilage destruction by mechanical injury. Annals of Anatomy - Anatomischer Anzeiger. 2005;187(5-6):473-485.

8. Zhang W, Moskowitz R, Nuki G, Abramson S, Altman R, Arden N et al. OARSI recommendations for the management of hip and knee osteoarthritis, Part I: Critical appraisal of existing treatment guidelines and systematic review of current research evidence. Osteoarthritis and Cartilage. 2007;15(9):981-1000.

9. Hinton R, Moody R, Davis A, Thomas S. Osteoarthritis: diagnosis and therapeutic considerations. Am Fam Physician. 2002;65(5):841-849.

10. Tuan R. A second-generation autologous chondrocyte implantation approach to the treatment of focal articular cartilage defects. Arthritis Res Ther. 2007;9(5):109.

11. Vinatier C, Bouffi C, Merceron C, Gordeladze J, Brondello J, Jorgensen C et al. Cartilage Tissue Engineering: Towards a Biomaterial-Assisted Mesenchymal Stem Cell Therapy. CSCR. 2009;4(4):318-329.

12. Wood J. Autologous Cultured Chondrocytes: Adverse Events Reported to the United States Food and Drug Administration. The Journal of Bone and Joint Surgery (American). 2006;88(3):503.

13. Revell C, Athanasiou K. Success Rates and Immunologic Responses of Autogenic, Allogenic, and Xenogenic Treatments to Repair Articular Cartilage Defects. Tissue Engineering Part B: Reviews. 2009;15(1):1-15.

14. MacArthur B, Oreffo R. Bridging the gap. Nature. 2005;433(7021):19-19.

15. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of Deep Cartilage Defects in the Knee with Autologous Chondrocyte Transplantation. New England Journal of Medicine. 1994;331(14):889-895.

16. Boulas H, Herren A, Büchler U. Osteochondral metatarsophalangeal autografts for traumatic articular metacarpophalangeal defects: A preliminary report. The Journal of Hand Surgery. 1993;18(6):1086-1092.

17. MEYERS M. Resurfacing of the Femoral Head with Fresh Osteochondral Allografts Long-term Results. Clinical Orthopaedics and Related Research. 1985;(197):111-114.

18. Hunter W. Of the Structure and Diseases of Articulating Cartilages, by William Hunter, Surgeon. Philosophical Transactions of the Royal Society of London. 1742;42(462-471):514-521.

19. Bhardwaj N, Devi D, Mandal B. Tissue-Engineered Cartilage: The Crossroads of Biomaterials, Cells and Stimulating Factors. Macromol Biosci. 2014;15(2):153-182.

20. Mahmoudifar N, Doran P. Chondrogenesis and cartilage tissue engineering: the longer road to technology development. Trends in Biotechnology. 2012;30(3):166-176.

21. Roberts S, Howard D, Buttery L, Shakesheff K. Clinical applications of musculoskeletal tissue engineering. British Medical Bulletin. 2008;86(1):7-22.

22. Grad S, Eglin D, Alini M, Stoddart M. Physical Stimulation of Chondrogenic Cells In Vitro: A Review. Clinical Orthopaedics and Related Research®. 2011;469(10):2764-2772.

23. Moher D, Liberati A, Tetzlaff J, Altman D. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med. 2009;6(7):e1000097.

24. Sackett D. Evidence-based medicine. New York: Churchill Livingstone; 1997.

25. Neumann A, Gardner O, Williams R, Alini M, Archer C, Stoddart M. Human Articular Cartilage Progenitor Cells Are Responsive to Mechanical Stimulation and Adenoviral-Mediated Overexpression of Bone-Morphogenetic Protein 2. PLOS ONE. 2015;10(8):e0136229.

26. Gorna K, Gogolewski S. Biodegradable polyurethanes for implants. II.In vitro degradation and calcification of materials from poly(epsilon-caprolactone)-poly(ethylene oxide) diols and various chain extenders. Journal of Biomedical Materials Research. 2002;60(4):592-606.

27. Wimmer M, Grad S, Kaup T, Hänni M, Schneider E, Gogolewski S et al. Tribology Approach to the Engineering and Study of Articular Cartilage. Tissue Engineering. 2004;10(9-10):1436-1445.

28. Neumann A, Alini M, Archer C, Stoddart M. Chondrogenesis of Human Bone Marrow-Derived Mesenchymal Stem Cells Is Modulated by Complex Mechanical Stimulation and Adenoviral-Mediated Overexpression of Bone Morphogenetic Protein 2. Tissue Engineering Part A. 2013;19(11-12):1285-1294.

29. Li S, Glynne-Jones P, Andriotis O, Ching K, Jonnalagadda U, Oreffo R et al. Application of an acoustofluidic perfusion bioreactor for cartilage tissue engineering. Lab Chip. 2014;14(23):4475-4485.

30. Stolz M, Gottardi R, Raiteri R, Miot S, Martin I, Imer R et al. Early detection of aging cartilage and osteoarthritis in mice and patient samples using atomic force microscopy. Nature Nanotech. 2009;4(3):186-192.

31. Tsai T, Nelson B, Anderson P, Zdeblick T, Li W. Intervertebral disc and stem cells cocultured in biomimetic extracellular matrix stimulated by cyclic compression in perfusion bioreactor. The Spine Journal. 2014;14(9):2127-2140.

32. Lin W, Chang Y, Wang H, Yang T, Chiu T, Huang S et al. The Study of the Frequency Effect of Dynamic Compressive Loading on Primary Articular Chondrocyte Functions Using a Microcell Culture System. BioMed Research International. 2014;2014:1-11.

33. Wu M, Wang H, Tai C, Chang Y, Chen Y, Huang S et al. Development of perfusion-based microbioreactor platform capable of providing tunable dynamic compressive loading to 3-D cell culture construct: Demonstration study of the effect of compressive stimulations on articular chondrocyte functions. Sensors and Actuators B: Chemical. 2013;176:86-96.

34. Cashion A, Caballero M, Halevi A, Pappa A, Dennis R, van Aalst J. Programmable Mechanobioreactor for Exploration of the Effects of Periodic Vibratory Stimulus on Mesenchymal Stem Cell Differentiation. BioResearch Open Access. 2014;3(1):19-28.

35. Petrou M, Niemeyer P, Stoddart M, Grad S, Bernstein A, Mayr H et al. Mesenchymal stem cell chondrogenesis: composite growth factor–bioreactor synergism for human stem cell chondrogenesis. Regenerative Medicine. 2013;8(2):157-170.

36. García Cruz D, Salmerón-Sánchez M, Gómez-Ribelles J. Stirred flow bioreactor modulates chondrocyte growth and extracellular matrix biosynthesis in chitosan scaffolds. Journal of Biomedical Materials Research Part A. 2012;:n/a-n/a.

37. García Cruz D, Gomes M, Reis R, Moratal D, Salmerón-Sánchez M, Gómez Ribelles J et al. Differentiation of mesenchymal stem cells in chitosan scaffolds with double micro and macroporosity. Journal of Biomedical Materials Research Part A. 2010;95A(4):1182-1193.

38. Shahin K, Doran P. Tissue engineering of cartilage using a mechanobioreactor exerting simultaneous mechanical shear and compression to simulate the rolling action of articular joints. Biotechnol Bioeng. 2011;109(4):1060-1073.

39. Schätti O, Grad S, Goldhahn J, Salzmann G, Li Z, Alini M et al. A combination of shear and dynamic compression leads to mechanically induced chondrogenesis of human mesenchymal stem cells. European cells & materials. 2011;22:214-225.

40. Nebelung S, Gavenis K, Rath B, Tingart M, Ladenburger A, Stoffel M et al. Continuous cyclic compressive loading modulates biological and mechanical properties of collagen hydrogels seeded with human chondrocytes. Biorheology. 2011;48(5):247-261.

41. Stoffel M, Yi J, Weichert D, Zhou B, Nebelung S, Müller-Rath R et al. Bioreactor cultivation and remodelling simulation for cartilage replacement material. Medical Engineering & Physics. 2012;34(1):56-63.

42. Raimondi M, Boschetti F, Falcone L, Fiore G, Remuzzi A, Marinoni E et al. Mechanobiology of engineered cartilage cultured under a quantified fluid-dynamic environment. Biomechanics and Modeling in Mechanobiology. 2002;1(1):69-82.

43. Czichos H, Dowson D. Tribology: A systems approach to the Science and Technology of friction, lubrication and wear. Tribology International. 1978;11(4):259-260.

44. Kazemi M, Dabiri Y, Li L. Recent Advances in Computational Mechanics of the Human Knee Joint. Computational and Mathematical Methods in Medicine. 2013;2013:1-27.

45. Sumanasinghe R, Bernacki S, Loboa E. Osteogenic Differentiation of Human Mesenchymal Stem Cells in Collagen Matrices: Effect of Uniaxial Cyclic Tensile Strain on Bone Morphogenetic Protein (BMP-2) mRNA Expression. Tissue Engineering. 2006;12(12):3459-3465.

46. Barber J, Handorf A, Allee T, Li W. Braided Nanofibrous Scaffold for Tendon and Ligament Tissue Engineering. Tissue Engineering Part A. 2013;19(11-12):1265-1274.

47. Haudenschild A, Hsieh A, Kapila S, Lotz J. Pressure and Distortion Regulate Human Mesenchymal Stem Cell Gene Expression. Annals of Biomedical Engineering. 2009;37(3):492-502.

48. Huang C. Effects of Cyclic Compressive Loading on Chondrogenesis of Rabbit Bone-Marrow Derived Mesenchymal Stem Cells. Stem Cells. 2004;22(3):313-323.

49. Kisiday J, Frisbie D, McIlwraith C, Grodzinsky A. Dynamic Compression Stimulates Proteoglycan Synthesis by Mesenchymal Stem Cells in the Absence of Chondrogenic Cytokines. Tissue Engineering Part A. 2009;15(10):2817-2824.

50. Li Z, Kupcsik L, Yao S, Alini M, Stoddart M. Mechanical load modulates chondrogenesis of human mesenchymal stem cells through the TGF-β pathway. Journal of Cellular and Molecular Medicine. 2009;14(6a):1338-1346.

51. Stevens M, Marini R, Schaefer D, Aronson J, Langer R, Shastri V. In vivo engineering of organs: The bone bioreactor. PNAS. 2005;102(32):11450-11455.