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Mini Review

Pre-Osteoblast Differentiation and Proliferation Following Combined Mechanical Stimulation

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Abstract

A bioreactor system that can simultaneously stimulate cells with cyclic strain and ultrasound, both of which are known to effectively stimulate the regeneration of bone tissue, was developed for this study. Due to their osteoblast-like characteristics, MC3T3-E1 pre-osteoblasts were chosen for use in bone tissue engineering. Poly-L-lactic acid and polycaprolactone were used in the salt leaching process to create three-dimensional scaffolds. The bioreactor used ultrasound and cyclic strain to stimulate the cells. For ultrasound, the bioreactor was set to 1.0 MHz and 30 mW/cm2 at 1.0 Hz and 10% strain for cyclic strain. A control group and three experimental groups—ultrasound, cyclic strain, and combined stimulation—were examined. For 20 minutes each day, each group was stimulated. When measured using the cell counting kit-8, mechanical stimuli had no significant effect on the proliferation of MC3T3-E1 cells for up to ten days. However, MC3T3-E1 cells' matrix maturation was accelerated by the combination of mechanical stimulation, as shown by gene expression analysis of osteocalcin, RUNX2, and osterix.

Keywords: Bioreactors, Cell differentiation, Physical stimulation, Tissue engineering, Ultrasound

INTRODUCTION

In order to create functional engineered tissues in a three-dimensional (3-D) culture environment, cultured cells must grow on a biodegradable scaffold in order to engineer living tissues in vitro. In addition to solid free-form fabrication using computer-aided design, various technologies for fabricating optimal scaffolds have been developed (Davisson T, 2002). The creation of in vitro environments for growing cells or tissues that are comparable to native tissue is another growing problem in the field of tissue engineering. It is believed that the multiple internal and external environments that living tissues are exposed to have an impact on the regeneration of tissues or organs (Griffith LG, 2002). These environments contain both internal and external biochemical, biophysical, and mechanical stimuli. Mechanical stimuli, such as those produced by ultrasound or cyclic strain, have not been the subject of extensive research, despite the fact that these stimuli have been found to aid in bone wound healing (Kokubu T, 1999). Both cyclic compressive strain and repeatable tensile strain are examples of cyclic strain. Because these tissues are constantly subjected to cyclic strain when bodies are moving, it was prompted by a bio-mimicking concept for the regeneration of bone and cartilage (Lan PX, 2009). When strain is applied, pre-osteoblast MC3T3-E1 cells express specific bone-related genes like bone morph genic protein-2 (BMP-2), runt-related transcription factor-2 (RUNX2), and MAD homolog 5 (SMAD5) 2008). Additionally, osteoblasts with a higher concentration of osteopontin and alkaline phosphatase can be produced by cyclic strain. The term "ultrasound" refers to cyclic sound pressure with a frequency greater than 20 kHz, which is the maximum that a person can hear. Since it was first shown that continuous-wave ultrasound could make rabbits' bone calluses grow, ultrasound has been used for treatment and diagnosis in many different areas (Mauney JR, 2004). The healing of neural tissue, cartilage, tendon, and bone is accelerated by especially low intensity ultrasound stimulation, typically administered at the diagnostic intensity level of less than

50 mW/cm2. In addition, it has been demonstrated in mice that low-intensity ultrasound increases prostaglandin-E2 production by inducing cyclo-oxygenase-2 mRNA. Low-intensity ultrasound can also boost ECM synthesis (Owen TA, 2006). Despite the fact that native cells or tissues are inherently exposed to multiple stimuli simultaneously, the majority of researchers continue to investigate the effects of single mechanical stimuli like compression, shear stress, and ultrasound. There were no studies on the effects that can occur on cells when two or more stimuli are applied simultaneously (Rath B, 2008). As a result, we set out to create a novel bioreactor system that combines simultaneous ultrasound treatment of cells on a scaffold with controlled mechanical cyclic strain. The developed bioreactor system was used to further investigate the effects of combined stimulation, specifically ultrasound and cyclic strain, on pre-osteoblast MC3T3-E1 cells in 3-D environments in vitro.

Cells are exposed to a variety of mechanical stimuli

Ultrasound, combined stimulation, and cyclic strain were the three groups that received stimulation. After seeding the cells onto the scaffolds, differentiation and cell proliferation were tested seven and fourteen days later (Saito M, 2004). Stimulation: At the bottom of the water tank, three ultrasound transducers could produce acoustic waves with intensities ranging from 30 to 100 mW/cm2. Cell-seeded scaffolds were positioned on a six-well plate in the water tank's plate holder after the ultrasound equipment was warmed up. The height between the transducer and the well plate was equal to the travel distance of the ultrasound because the well plate was floating directly on the water's surface (Wang J, 2006). When calculating the expected ultrasonic intensity for the cells, this was crucial. Because they can obstruct ultrasonic waves, air bubbles between the plate and the water's surface were removed. On the scaffold, cells were stimulated with ultrasound at a frequency of 1.0 MHz and an intensity of 30 mW/cm2 in a continuous sine wave for twenty minutes each day. At a frequency of 1.0 Hz, cyclic strain was 10% of the scaffold's thickness. Each scaffold's surface was just covered by the sterile Teflon® stimulators. The same frequency and amplitude as for ultrasound and cyclic strain were used for combined stimulation (Yang RS, 2005). Each stimulus lasted for 20 minutes per day.

DISCUSSION

A novel bioreactor system for generating cyclic strain and ultrasonic waves to stimulate cells on 3-D PCL/PLLA scaffolds was developed to demonstrate this. A hydrophone was used to measure the ultrasonic transducers' properties, allowing for their easy location and prediction of the transferred intensity. For ease of use, the designed bioreactor had a well plate that could be purchased in stores. In addition, the bioreactor was small enough to fit in a standard incubator or on a clean bench. During the experiments, PCL/PLLA scaffolds were made using a salt leaching technique to create a three-dimensional environment for cells. Since a number of studies had shown that each stimulus has a positive effect on cell proliferation in a specific range of intensity or strain, we hypothesized that a combination of stimuli might do so. In contrast, when cells on 3-D PCL/PLLA scaffolds were subjected to cyclic strain, ultrasound, or both simultaneously in our system, the CCK-8 results indicated that there were no significant differences between the groups. However, we discovered that all groups' cells multiplied over time. As a result, we believe that pre-osteoblast proliferation was unaffected by mechanical stimuli. Bone formation is linked to numerous genes. We selected OSX, COL-I, OC, RUNX2, and other available genes. A vitamin K- and vitamin D-dependent protein produced by osteoblasts, OC, also known as bone gamma-carboxyglutamate (gla) protein, is a marker of bone formation and the most abundant and extensively studied of the non-collagenous proteins in bone. The other gene we looked at was COL-I, which is the most common collagen in the bone's extracellular matrix (ECM). These genes' expression levels can indicate three stages of osteoblast differentiation when taken together. After the proliferation stage, pre-osteoblasts enter the calcification stage and secrete ECM components. During this time, high levels of ALP expression may cause cells to transition from the proliferation stage to the ECM secretion stage. After that, when the cell status reaches the calcification step, OC and osteopontin are expressed; during the processes of mineralization, both are involved in the binding of hydroxyapatite and calcium ions. Osteocalcin is one of the osteoblast-specific genes that depend on the oestrogenic regulators RUNX2 and OSX. When we compare the levels on days 0, 7, and 14, mechanical stimuli may increase gene expression. Mechanically stimulated groups had higher levels than CN, despite the fact that the oestrogenic medium was treated to raise CN levels. According to gene expression levels related to bone formation, simple stimuli (U and C) or combined stimulation (U+C) could improve pre-osteoblast differentiation into osteoblasts. At 7 and 14 days after cell seeding, the higher levels of COL-I and OC (U+C) compared to single stimuli or no stimulation suggest that the cells were in the early stages of calcification.

REFERENCES

1. Davisson T, Kunig S, Chen A, Sah R, Ratcliffe A (2002). Static and dynamic compression modulate matrix metabolism in tissue engineered cartilage. J Orthop Res. 20: 842–848.

2. Griffith LG, Naughton G (2002). Tissue engineering-Current challenges and expanding opportunities. Science. 295: 1009–1014.

3. Kokubu T, Matsui N, Fujioka H, Tsunoda M, Mizuni K (1999). Low intensity pulsed ultrasound exposure increases prostaglandin E2 production via the induction of cyclooxygenase-2 mRNA in mouse osteoblast. Biochem Biophys Res Commun. 256: 284–287.

4. Lan PX, Lee JW, Seol YJ, Cho DW (2009). Development of 3D PPF/DEF scaffolds using micro-stereolithography and surface modification. J Mater Sci-Mater Med. 20: 271–279.

5. Mauney JR, Sjostorm S, Blumberg J, Horan R, O'Leary JP, et al (2004). Mechanical stimulation promotes Osteogenic differentiation of human bone marrow stromal cells on 3-D partially demineralized bone scaffolds in vitro. Calcif Tissue Int. 74: 458–468.

6. Owen TA, Aronow M, Shalhoub V, Barone LM, Wilming L, et al (2006). Progressive development of the rat osteoblast phenotype in vitro: Reciprocal relationships in expression of genes associated with osteoblast proliferation and differentiation during formation of bone extracellular matrix. J Cell Physiol. 143: 420–430.

7. Rath B, Nam J, Knobloch TJ, Lannutti JJ, Agarwal S (2008). Compressive forces induce Osteogenic gene expression in calvarial osteoblasts. J Biomech. 41: 1095–1103.

8. Saito M, Fujii K, Tanaka T, Sochi S (2004). Effect of low- and high-intensity pulsed ultrasound on collagen post-translational modifications in MC3T3-E1 osteoblasts. Calcif Tissue Int. 75: 384–395.

9. Wang J, Asou Y, Sekiya I, Sotome S, Orii H, et al (2006). Enhancement of tissue engineered bone formation by a low pressure system improving cell seeding and medium perfusion into a porous scaffold. Biomaterials. 27: 2738–2746.

10. Yang RS, Lin WL, Chen YZ, Tang CH, Huang TH, et al (2005). Regulation by ultrasound treatment on the integrin expression and differentiation of osteoblasts. Bone. 36: 276–283.