**Introduction:** Articular cartilage defects may occur as a result of traumatic injury, or from degenerative diseases such as osteoarthritis. Such defects have a limited capacity for repair, and may require novel regenerative approaches to restore biological and mechanical functionality of damaged or diseased tissue. There have been any different approaches to restore tissue composition, structure, and function, including the development of engineered cartilage for potential implantation. The ultimate success of cell-seeded constructs for cartilage repair depends on appropriate long-term maturation of functional repair tissue in vivo, and integration with surrounding cartilage and bone. To achieve these goals, constructs may benefit from a period of in vitro culture prior to implantation to initiate a cellular repair response (Schaefer et al., 2002; Lee et al., 2003a, b).

It was found that culture conditions may be optimized to enhance extra-cellular matrix (ECM) synthesis, or stimulate proliferation and chondrogenesis of progenitor cells. Additional studies showed that dynamic compression (Davisson et al., 2002; Lee et al., 2003a, b) and shear (Waldman et al., 2003) could affect chondrocyte biosynthesis in tissue engineering scaffolds. These studies suggest that loading may enhance the long-term deposition of ECM in cell-seeded constructs during in vitro culture. In this study, we investigated the effects of dynamic compression on cellular matrix biosynthesis and ECM retention in a chondrocyte-seeded hydrogel, a scaffold actively under investigation for applications to cartilage repair.

**Materials and Methods:** Chondrocytes were harvested from Newborn Wistar rats. After 7 days in monolayer culture, the chondrocyte suspension was embedded to yield a final cell concentration of 1 x 10^7cells/ml in 3% (w/v) agarose and cast into a custom-made mold (8 mm in diameter, 1.5 mm in thickness). The chondrocyte/agarose disks were cultured in DMEM with 10% FBS and 50 ug/ml ascorbic acid at 37°C in 5% CO2. The chondrocyte/agarose disks were measured with uniaxial un-confined dynamic compression test. The un-confined chamber was mounted in a bioreactor equipped with mechanical spectrometer. A dynamic unconfined compression test was carried out at different compressive amplitude (5%, 10%, 15%) and frequency (0.5 Hz, 1.0 Hz, 2.0 Hz, 3.0 Hz). In the second part of experiment, we choose the parameter with 10% strain, 1 Hz frequency for further unconfined mechanical compression test. In this part of study, the biochemical analysis were performed immediately, 1 day, 3 days and 7 days after 24 hrs' unconfined mechanical compression. Various biochemical analysis including cell proliferation, glycosaminoglycan (GAG) amount, total collagen amount, and nitric oxide content were performed. To clarify whether there is delayed effect of compressive stimulation on the chondrocytes; various biochemical parameters were studied in the 10% strain, 1 Hz groups for 1, 3 and 7 days after experiment.

**Results:** From the result of frozen section, we demonstrated that the distribution of chondrocytes were quite even within various depth of the agarose disc. The effect on chondrocyte proliferation by different combinations of stimulation parameters were tested in this study. In the 10% strain group, higher frequency (2 Hz, 3 Hz) compressive stimulation can significantly enhance the chondrocytes proliferation (P < 0.05 and P < 0.001, respectively). Under 24 hours' unconfined compressive stimulation, there was significant increase in GAGs secretion in the 1.0 Hz group, but significant decrease in GAGs secretion in the 0.5 Hz group. In this study, the parameter of 10 – 15 % strain with 1Hz compressive stimulation seemed to be beneficial for the GAGs secretion from the chondrocytes. There was no statistically significant change observed in total collagen synthesized. In the 10% strain group, the NO synthesis increased significantly in the 0.5 Hz group (P<0.05); while in the 15% strain group, there was significant decrease in NO synthesis in the 3.0 Hz group (P < 0.05). Although the initial proliferation of chondrocytes was not so obvious, the proliferation of chondrocytes, the secretion of GAGs was significantly increased and the effect persistent for 7 days; the NO synthesis by chondrocytes did not change in the first days’ culture, but decreased significantly at the 3rd, and 7th days culture (P < 0.01).

**Discussion:** In summary, the use of bioreactors applying dynamic mechanical deformation to engineered cartilage could be exploited not only to understand mechanisms of chondrocyte response to loading or to try to stimulate the engineering of cartilage constructs. Chondrocytes cultured in agarose synthesize, assemble and maintain an ECM containing macromolecules characteristic of the chondrocyte phenotype. We have further shown here that chondrocytes in agarose which have synthesized a cell-associated matrix exhibit a biosynthetic response to compression. Current evidence points towards the predominance of cell-matrix interactions, and informing the chondrocyte of its mechanical environment. This work demonstrates the ability of tissue engineered cartilage to respond metabolically to compressive loading. Compressive loading may therefore be used to modulate the metabolism of cartilage constructs, which in turn would be expected to modulate the growth and the resultant biomechanical properties of these constructs during culture.

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