

Whole organ culture of the distal femur in the development of an in vitro model of post-traumatic osteoarthritis Jessica Juergens, Aaron M. Stoker, Mary Cockrell, Keiichi Kuroki, and James L. Cook **University of Missouri College of Veterinary Medicine**, **Comparative Orthopaedic Laboratory**



Abstract

Post-traumatic osteoarthritis (PTOA) is a common sequela to joint injury and results in pain, disability, and associated financial costs of billions of dollars each year. PTOA is difficult to study in patients for many reasons, which has stimulated our efforts to develop a valid *in vitro* model for this pervasive problem. This study provides an initial step in developing this in vitro PTOA model by examining whole organ culture of the distal femur of rabbits in a rotating bioreactor. The bioreactor utilizes a rotating chamber onto which cartilage samples are mounted to more realistically mimic a functional knee joint than does static culture. For this study, the distal femurs from adult New Zealand White rabbit cadavers (n=7) were harvested and dissected free of all soft tissues such that fourteen whole organ explants were available for use. Three groups were created for comparison: time 0 control (n=4), static culture (n=5) and rotating bioreactor culture (n=5). Time 0 control explants were evaluated on the day of harvest. Explants in the static and rotating bioreactor culture groups were cultured in defined media in the respective environment (culture flask vs bioreactor) for 15 days and then harvested and assessed. All explants were assessed for cell viability using confocal microscopy, cell and tissue architecture and matrix characteristics by subjective histologic examination, and glycosaminoglycan and collagen (hydroxyproline) content via spectrophotometric assays. The data were collected and statistically analyzed for significant (p<0.05) differences among groups.

day 0 control group was performed on the medial condyle, lateral condyle and trochlear groove of each distal femur. Immediate analyses included confocal microscopic assessment of cell viability, subjective histologic assessment of cell and matrix characteristics, measurement of glycosaminoglycan (GAG) and and collagen/hydroxyproline (HP) content using spectrophotometric assays. Histological sections were cut and stained with hematoxylin and eosin (morphology), toluidine blue (GAG) and massons trichrome (collagen). All sections were subjectively evaluated by one investigator (KK) who was blinded to treatment, time frame, and results of other outcome measures performed.



Objective

To develop a valid in vitro model for study of post-traumatic osteoarthritis of the stifle/knee joint.

Hypothesis

Whole organ culture of the distal femur of rabbits in a slow-rotating bioreactor will allow for preservation of cell viability, histologic characteristics of normal articular cartilage, and GAG and collagen levels that are within 10% of Time 0 Controls.

The static group was cultured in a culture flask, and the bioreactor group was cultured in the slow-rotating bioreactor. Both groups received media changes every 3 days. At twelve days post-culture, samples from the medial condyle, lateral condyle and trochlear groove were collected and evaluated from each distal femur. Samples were analyzed via confocal microscopy, histologic assessment, and GAG and HP assays.

Data were combined for each group and means (SD) were determined. Statistical analyses (one-way ANOVA or one-way ANOVA on ranks with *post hoc* analyses) were performed using a computer software program with significance set at p<0.05.

Results

<u>Cell Viability:</u> Subjective evaluation of cell viability in cartilage sections from each group suggested that culture in the bioreactor preserves deep zone chondrocytes at a higher level than in static culture.



Matrix Composition: Glycosaminoglycan concentrations (ug/mg dry weight) were significantly higher (p≤.036) in cartilage from the bioreactor culture group than in either static culture or control groups (Fig 6). Hydroxyproline concentrations (ug/mg dry weight) were significantly higher (p<.05) in the control group than in either bioreactor or static culture groups.

Histologic Assessment of Cartilage: Subjective histology evaluation of samples for cell and matrix morphology, GAG and collagen staining, and overall articular surface architecture showed that the control group was superior (p<0.001) in histologic appearance compared to both static and bioreactor culture groups (Fig 7).



Materials and Methods

Seven adult New Zealand White rabbits were sacrificed on day 0. The distal femurs were dissected out and osteotomized immediately proximal to the trochlear ridges, and all soft tissues removed. This resulted in fourteen distal femurs which were then randomized and placed into one of three groups: day 0 control (n=4), static (n=5) and bioreactor (n=5). Immediate analysis of the



Figure 1. Method of mounting distal femurs to discs for the slow rotating bioreactor

Figure 8. Day 12 bioreactor histological section shows greater GAG levels than static culture (figure 9). Figure 9. Day 12 static culture.

Conclusions

Whole organ culture of the distal femur of rabbits in a slow-rotating bioreactor preserved cell viability and



Figure 2. rotating bioreactor mounted femurs in



Figure 5. Day 12 Static culture cartilage sample showing live (green) and dead (red cells).

articular surface structure and composition, while accentuating GAG content during a 12 day culture period. However, collagen content was not maintained at normal levels.

These data suggest that dynamic culture of joint tissues is preferable to static culture for developing a valid model of PTOA.

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