STUDY DESIGN/SETTING: An experimental study.
METHODS: PMSCs were cultured and adhered to plate and were harvested by trypsin digestion. Their membrane markers were tested by flow cytometry methods (FCM) and the differentiation ability was identified by ossification and fat formation induced tests. PMSCs were cultured with medium which contained transforming growth factor-β1 (TGF-β1) under different oxygen concentrations (5%, 21%). The proliferation of differentiated cells was detected by method of CCK-8 on day 1, day 3, day 5, and day 7. The mRNA expression of HIF-1α, Sox-9, Collagen II and Aggrecan were detected by method of real-time fluorescence PCR on day 3 and 7. The expression of collagen type II in differentiated cells was detected by method of immunofluorescence after 2 weeks. Biological effects of different oxygen concentrations on differentiation from PMSCs to nucleus pulposus cells were compared by statistical analysis.
RESULTS: PMSCs were successfully isolated and cultured in vitro with high growth rate. The positive antigens of CD73, CD29, CD44 and CD105 or negative antigen of CD34, CD45 and HLA-DR on PMSCs membrane were detected by FCM. Cells were maintained in undifferentiated state and be induced to differentiate into osteoblast and lipid cells. There were no significant differences in the growth rates of the two groups ($p>0.05$). The proliferation of the hypoxic group, however, was significantly higher than the normoxic group ($p<0.05$). Their proliferation rates reached their highest peaks on day 5. The peak of the hypoxic group was significantly higher than normal oxygen group ($p<0.05$). After day 5, both groups' proliferation rate decreased with no significant differences ($p>0.05$). The mRNA expressions of Sox-9 between the induced group of PMSCs and the control group were significantly different ($p<0.05$). The expression level in the hypoxic group was higher than the normoxic group ($p<0.05$). No significant differences were shown on the mRNA expression of Collagen II and Aggrecan ($p>0.05$). After 7 days of induction, the mRNA expression of Sox-9, Collagen II, and Aggrecan all increased ($p<0.05$) and the expression level of the hypoxia group was higher than that of the normoxia group ($p<0.05$). Under the same hypoxic conditions, the mRNA expression level of HIF-1α to hypoxia group was higher than normoxia group at different times ($p<0.05$). After 2 weeks, cells in both groups could express type II collagen and the expression level of the hypoxic group was higher than that of the normoxic group.
CONCLUSIONS: PMSCs can be successfully cultured in vitro with stable biological characteristics and could be produced in large quantities for tissue engineering research. Nucleus pulposus-like cells were cultured from the differentiated PMSCs under monolayer culture conditions. Results showed that hypoxic conditions could have an effect on early PMSCs proliferation and the stem cells could be induced to express mRNA of Sox-9, Collagen II, and Aggrecan. The expressions were enhanced hypoxic conditions via HIF-1α pathway.
FDA DEVICE/DRUG STATUS: This abstract does not discuss or include any applicable devices or drugs.

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86. A Novel Model for Intervertebral Disc Degeneration Using Whole Organ Explants in a Rotating Bioreactor

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BACKGROUND CONTEXT: Intervertebral disc (IVD) disorders resulting in pain and disability are extremely prevalent and current treatment options do not restore tissue integrity or function. Mechanisms of IVD degeneration are not fully understood. As such, in vitro culture models are being used to investigate these mechanisms. Models using cells deprived of extracellular matrix (ECM) result in loss of differentiation and viability (Haschtmann 2006) and limit the use of important outcome measures, such as biomechanics. While tissue culture of IVD explants allows for maintenance of cell morphology, preservation of ECM, and biomechanical testing, the biologic and biomechanical influences of endplate cartilage and bone are lost (Ariga 2003). For these reasons, our goal is to develop and validate effective models for translational research. Ideally, our model will provide long-term maintenance of cell and tissue integrity, architecture, composition, viability and allow for clinically relevant assessment of IVD health and disease. To our knowledge, few studies on whole organ culture for the study of IVD disorders have been published and none have established a validated model addressing the optimal criteria outlined above.
PURPOSE: The objective of this study was to investigate a novel methodology for IVD whole organ culture using biological and biomechanical assessments.
METHODS: Under ACUC approval, tails were collected from mature Sprague Dawley rats (n=12). Soft tissues were aseptically removed and bone-disc-bone explants were prepared by sawing the proximal and distal vertebral bodies in half. Explants were randomly assigned to either the Injured group (n=20) (posterolateral annulus fibrosus–AF) was penetrated with a 20 G needle to enter the nucleus pulposus (NP), which was aspirated with a 1 mL syringe to 0.5 mL), or the Uninjured group (n=22) (no insult). Explants were cultured in a rotating bioreactor (Synthecon) at 50 rpm for 14 days in supplemented DMEM+ITS media (changed on days 1 and 7). Cell viability in each IVD was determined using calcein-AM and ethidium homodimer-1 at day 0, 1, 7 and 14. IVDs were processed for histology and stained with hematoxylin eosin (HE), Safranin-O and Trichrome, and assessed by a pathologist, blinded to treatment, for cell and tissue morphology and architecture. Biomechanical testing of explants was performed at days 0 (after injury) and 14 by loading in axial compression at 0.001 mm/s. Stiffness was determined from the linear regions of the force-displacement curves. Cervical IVDs were then obtained from canine cadavers (n=7) and processed for culture, uninjured, in a similar manner. Cell viability of the canine whole organ explants was assessed on day 14 of culture.
RESULTS: Uninjured rat tail and canine cervical IVDs maintained cell viability through day 14 of whole organ culture. Injured rat tail IVDs showed viability similar to uninjured at day 1, but viability became notably lower at days 7 and 14, particularly within the NP. Histologically, uninjured IVDs maintained normal characteristics of cells and ECM through day 14. Uninjured IVDs retained more collagen in AF and proteoglycan in NP. Injured IVDs show histologic evidence of cell death and loss of proteoglycan staining in NP and loss of collagen staining in AF. Stiffness for uninjured IVDs was similar at days 0 (80.8 ± 34.8 N/mm) and 14 (89.2 ± 23.1 N/mm), while stiffness of injured IVDs was lower than the uninjured group at day 0 immediately after injury (52.9 ± 1.7 N/mm) and then increased approximately 2.5 times by day 14 (130.8 ± 37.0 N/mm) significantly (t-Test, $p<0.05$) more stiff than day 14 uninjured and day 0 injured.
CONCLUSIONS: The results of this study suggest that whole organ (bone-disc-bone) IVD explants can be cultured in a rotating bioreactor such that cell viability, tissue architecture, and material properties are maintained for at least 14 days. The injury used in this model produced pathologic changes similar to those seen in human IVD degeneration, including cell death, abnormal ECM, and increased stiffness over time. In light of previous studies that reported significant loss of cell viability using other culture models (Gawri 2011), we suggest that the use of the rotating bioreactor is critical for providing nutrient supply to IVD explants, thus preserving biologic and biomechanical properties. Ongoing research in our laboratories is aimed at further optimizing and validating the rat tail and canine cervicalmodels in order to investigate mechanisms of disease and test therapeutic interventions.
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