Translating growth factor beta1 enhances and modifies the bone-morphogenetic-protein-2-induced chondrogenic differentiation of bovine synovial-tissue explants

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Purpose: We have previously shown that bone morphogenetic protein-2 (BMP-2) alone cannot effect the complete differentiation of synovial tissue into a typical hyaline type of articular cartilage throughout the entire explant volume. In the present study, we investigated the combined effects of BMP-2 and either basic fibroblast growth factor (bFGF) or transforming growth factor beta 1 (TGF-b1) on the chondrogenic differentiation of synovial-tissue explants.

Methods and Materials: Synovial tissue was collected from the metacarpal joints of freshly slaughtered cows. The explants were sliced between two layers of agarose gel within 24-well plates and cultured in the absence or presence of the tested growth factors under serum-free conditions. The explants were cultured for 4 weeks in the absence or presence of BMP-2 (200 ng/mL), bFGF (50 ng/mL) or TGF-b1 (50 ng/mL) in 5% CO2. Cell morphology, proliferation and viability was measured by wound healing assay and by QPCR for aggrecan and the cartilage oligomeric matrix protein (COMP) and the process of hypertrophic differentiation was investigated. When BMP-2 was coupled with TGF-b1, the effects of its coupling with BMP-2 on the mRNA levels of cartilage-related genes were investigated. In comparison with BMP-2 alone, the mRNAs levels of cartilage-related genes were investigated. In comparison with BMP-2 alone, the mRNAs levels of aggrecan and the cartilage oligomeric matrix protein (COMP) and lowered those for collagen types I and X. The mRNA level of type II collagen was unchanged. The immunohistochemical analysis for collagen types II and X yielded results which mirrored the gene-expression findings.

Results: BMP-2 induced the chondrogenic differentiation of bovine synovial-tissue explants in a time-dependent manner. However, by the sixth week of culturing, the explants had undergone chondrogenic differentiation throughout their entire volume. And, undesirably, the cells had entered the hypertrophic phase of differentiation. When BMP-2 was coupled with TGF-b1, the chondrogenic differentiation of synovial-tissue explants was enhanced, the hyaline-like qualities of the cartilaginous tissue formed were improved (as gauged by the expression levels of cartilage-related genes), and the process of hypertrophic differentiation was suppressed. Acknowledgements: This study was supported by the NIH (NIAMS), Bethesda, MD, USA.

Synthetic growth medium and cartilage explants – a safe and improved method of culturing chondrocytes for Autologous Chondrocyte Implantation (ACI)

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Purpose: The purpose of this study was to test a Synthetic Medium (SM) which could replace serum in growth medium for culturing cells for Autologous Chondrocyte Implantation (ACI). By using an explant culture system in combination with SM, we further wanted to replace conventional chondrocyte isolation method. In this protocols cells are released from ECM by use of chondrocyte-damaging enzymes such as collagenase, trypsin and hyaluronidase (Jacob M et al., Connect Tissue Res. 2003;44(3-4):173-80).

Materials and Methods: Human cartilage pieces were obtained from the notch area of femur condyle from donors undergoing ACL reconstruction. Explants were added to: 1) Basal Medium (BM) containing antibiotic- and growth-factors in BM. BM contained hormones, attachment factors, vitamins, growth- and nutrition-factors in BM. BM were cultured for 4 weeks at 37°C, 5% CO2. Cell morphology, proliferation and viability was measured by LM. Histology on explants was also performed.

Results: BM and SM were compared for growth factors present in BM which could replace serum in growth medium for culturing cells for ACI. Repair tissue was developed on the cut surfaces. Final cell count of monolayer-cells showed that BM gave rise to an average 200 % higher cell count compared to BM+20%BS. LM showed that BM-cultured chondrocytes were more hypochromic in appearance and enhanced the accumulation of glycosaminoglycans (GAGs) and at the gene-expression level (quantitative determination of mRNA for cartilage-related genes using the real-time polymerase chain reaction).

Conclusions: BM maintain a higher chondrocyte proliferation, viability and a stable cell morphology when compared to BM+20%BS. We conclude that our SM with explants is a superior method for culturing chondrocytes for ACI.

Cellular responses in acute trauma of human ankle cartilage: Cell survival, catabolic cytokines and neuromediators

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Purpose: Investigate the effect of acute trauma to normal human adult ankle cartilage on cell death, catabolic cytokines, neuromediators, and receptors.

Methods and Materials: Loads were applied to the cartilage surfaces. Full thickness cartilage plugs were removed; divided into directly impacted core, surrounding ring, and undamaged control; and collected immediately after injury or cultured for 2 weeks. Media was analyzed for released cytokines. Tissue explants were analyzed immunohistochemically for interleukin-6 (IL-6), IL-1b, tumor necrosis factor-a (TNF- a), neuromediators (substance P, NKS, bradykinin, bradykinin receptors b1 and b2), and by TUNEL assay.

Results: The majority of apoptotic chondrocytes were localized within the impact zone (50±6%). The ring contained 3±6.5% TUNEL-positive cells; controls were almost normal (6±0.5; p<0.01). All neuromediators and cytokines were detected at baseline in controls throughout the culture. They were upregulated in response to trauma in the core and ring regions (except constitutively expressed BRb2). BRb1 was induced immediately following trauma in the upper layer of the core and ring. TNF-a levels were highest on day 2. IL-6 staining was more pronounced in the ring on days 2 and 4. Other mediators remained elevated with culture in the core and ring. Expression was elevated with culture in the ring, remaining lower in the core than in the ring.

Conclusions: Surface damage was accompanied by cell death/ apoptosis within and around the cracks and fibrillated areas. Although the tested proteins were elevated in traumatized areas, only BRb1, TNF-a and IL-6 showed time-dependent release, indicating that their activation together with cell death are the first events that should be considered for targeted intervention.