ISOLATION AND CHARACTERIZATION OF CHONDROCYTES DERIVED FROM THE CHONDROCRANIUM OF THE DOGFISH (*SQUALUS ACANTHIAS*)

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Articular cartilage, which is present in all diarthrodial joints in mammals, is critical to proper joint function and lubrication. In the adult, articular cartilage loses the ability to regenerate itself resulting in the development of osteoarthritis, particularly in weight bearing joints such as the human knee. Recently a great deal of interest has developed in the possible use of transplantation to repair cartilage defects, employing mesenchymal stem cells or chondrocytes grown in an ex-vivo matrix. The shark chondrocranium is unique in that growth of cartilage occurs during the entire lifetime of the animal. Therefore, characterization of shark chondrocytes and their surrounding matrix might provide information concerning biologic factors that allow continued articular cartilage growth and regeneration during adulthood. The purpose of this pilot study was to determine the feasibility of isolation and culture of chondrocytes derived from *Squalus acanthias* chondrocranium and to isolate mRNA permitting comparison of matrix molecules produced by mammalian and shark chondrocytes.

The chondrocranium was harvested from dogfish following sacrifice for other studies using aseptic technique. A portion of each specimen was placed in 10% formalin for later histologic analysis and the remainder of the cranium was minced into 3 x 3 mm pieces and placed in Shark Ringer[1] (SR) containing penicillin/streptomycin (10,000/10mg per liter) for thirty minutes. The specimens were then washed with SR, finely minced and placed in 0.25 % trypsin/SR for 30 minutes on a shaker table to remove adherent fibrous tissue. Specimens were subjected to a series of digestion protocols using 0.25% collagenase and monitored hourly to determine the number of cells released. At the completion of the digestion, the cells were washed 2X with SR, cell number was determined using a hemacytometer, the cells were plated in complete shark media (Valentich et al., Am. J. Physiol. 26:C813-823, 1991)) and incubated at 17°C in an atmosphere of 5% CO_2. Cell viability was determined using a trypan blue exclusion test.

In order to determine the amount of cells available stereological techniques on histologic sections obtained from perfusion-fixed spiny dogfish were used (Hentschel et al., *Adv. Anat. Embryol. Cell. Biol* 108:1-151, 1987). After rehydration, sections stained with ethidium bromide revealed a mean value of 12,020 cells (± 1998 SEM) per mm^3 (n=26) in the hyaline cartilage of the anterior chondrocranium. In the adjacent loose connective tissue a mean value of 47,800 cells/mm^3 (n=5) was found. Cells were present in lacunae throughout the cartilage with chondroblast clusters noted at the periphery of the specimens. The dense surrounding matrix made isolation of the chondrocytes extremely difficult. Optimal yield was obtained following an 8 hour digestion at 20-25°C with 0.25% collagenase. Addition of 2 units/100 ml chondroitinase ABC did not significantly improve cell yield or viability. On average 2-4 x 10^6 cells were obtained from 20-25 mg of intact cartilage. This was approximately 1% of the cell yield anticipated from digestion of a similar volume of bovine articular cartilage. The cells were subsequently pooled for RNA extraction using the TRI Reagent protocol. These experiments form the basis for future biochemical studies, for Northern blot analysis of matrix molecules and for mechanical testing to allow comparison of shark and mammalian cartilage.