USE OF A THREE-DIMENSIONAL IN VITRO ALGINATE HYDROGEL CULTURE MODEL TO DIRECT ZONAL FORMATION OF GROWTH PLATE CARTILAGE

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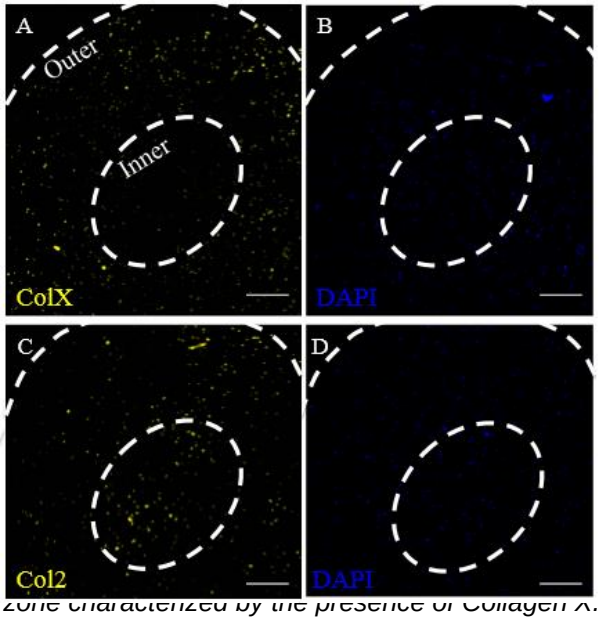
Growth plate cartilage is found at the ends of long bones, and is responsible for the growth of the bones as a person is developing. The architecture of this growth plate is very specific and contributes to proper function to allow for bone growth. Although there are many factors known to be involved in the formation of the growth plate and its proper regulation, the exact mechanisms involved in these processes are not fully understood. So far, previous attempts to recapitulate a functioning growth plate *in vitro* have been unsuccessful. In this study, a new method to study the growth plate and the mechanisms involved in its formation was developed using an *in vitro* cell culture system made of alginate hydrogel scaffolds. Chondrocytes isolated from neonatal mouse growth plates were encapsulated within hydrogel beads and cultured. The addition of exogenous factors to the culture medium allowed the study of the parathyroid-related protein-Indian hedgehog (PTHrP-IHH) pathway, a known feedback loop necessary in the regulation of bone growth. Supplemental PTHrP increased proliferation and inhibited hypertrophy of encapsulated chondrocytes, while maintaining chondrocyte differentiation. The addition of IHH or thyroxine to bead cultures stimulated the formation of a hypertrophic zone located around the surface of the beads (Figure 1). This study demonstrates the ability of this culture system to induce proper zonal architecture of the growth plate, and has lead to further studies of the mechanisms involved in this zonal formation. Currently, the addition of extra-cellular matrix binding proteins to alginate scaffold, such as heparin or chondroitin sulfate, is being studied for its ability to retain growth factors and affect architecture formation through cell migration and signaling. Also, knockout mice and other soluble factors known to be involved in the PTHrP-IHH pathway, including smoothened and Wnt5a, are being used to study the specific mechanisms and pathways involved in zonal formation of the growth plate. The use of this culture model to elucidate all the necessary factors involved in the formation of a functioning growth plate will allow the advancement of regenerative medicine in the field of developmental cartilage damage and disease.



zone characterized by the presence of Collagen X.