SEEDING DENSITY INFLUENCES THE DEVELOPMENT OF MECHANICAL PROPERTIES IN AGAROSE CONSTRUCTS

INTRODUCTION: The generation of a successful cartilage substitute will require both the mechanical and biochemical properties of the engineered tissue to approach that of native cartilage. Recent studies have shown that hydrogels and other 3D matrices can support the development of a functional extracellular matrix (1, 2, 3). These studies have been carried out with a range of cell seeding densities, from 1 to 125 million cells/ml (6). Agarose is a well-characterized hydrogel that is used to maintain 3D chondrocyte cultures for cartilage biology, bioengineering and tissue engineering investigations (3). Motivated by our interest in tissue engineering of cartilage using an agarose scaffold, we sought to characterize the effect of initial cell seeding density on the development of biochemical and mechanical properties in free-swelling chondrocyte-seeded agarose constructs. In this novel study of agarose constructs, we investigated three different chondrocyte seeding densities: 10, 20, and 60 x 10^6 cells/ml over a nine week period.

METHODS: Cell Culture: Cell-seeded agarose hydrogels were prepared as described previously (1). Isolated bovine chondrocytes were resuspended in fully supplemented DMEM and mixed 1:1 with 4% agarose to produce agarose slabs with cell densities of 10 x 10^6, 20 x 10^6 and 60 x 10^6 cells/ml. The agarose slabs were gelled and disks (4.76 mm x 1.6 mm) were cored with a trephine. Disks were cultured in 60 mm tissue culture dishes (20-25 disks per plate) at 37°C and 5% CO2 in 10 ml of fully supplemented DMEM and 50 µg/ml ascorbate (Sigma). Media (with fresh ascorbate) were changed daily. Disks (3-4) were removed for analysis at weekly intervals over the first three weeks, and then at bi-weekly intervals for the remaining six weeks.

Mechanical Testing: Mechanical testing of tissue constructs was performed in confined and unconfined compression (not reported). To minimize variability, each sample was first equilibrated in creep to a load of 2 grams. From this tare offset, stress relaxation tests were performed with a ramp speed of 1 µm/s until reaching 10% strain. The equilibrium confined compression aggregate modulus (H_A) was calculated from the equilibrium stress. All properties of chondrocyte-seeded agarose constructs. In our previous study dramatically influence the development of biochemical and mechanical properties in free-swelling chondrocyte-seeded agarose constructs. In this novel study of agarose constructs, we investigated three different chondrocyte seeding densities: 10, 20, and 60 x 10^6 cells/ml over a nine week period.

RESULTS: For the agarose constructs inoculated with 10 x 10^6 cells/ml, we found an increase in H_A by ~10-fold by day 21 (p=0.015, Fig. 1) which was attributed to diffusion limitations resulting from matrix elaboration and the increased number of cells associated with these higher density cultures. Our results highlight the necessity for optimal culture conditions, wherein waste is removed and nutrients are supplied to meet the stringent demands of the growing construct. Under the conditions of this study, increases in seeding density elevate the metabolic demands of the growing construct. Incorporation of continuous feed bioreactors (7), coupled with the application of in vivo physiologic conditions, may further enhance the growth of articular cartilage in these chondrocyte-seeded agarose constructs.


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