Mechanical Ex Vivo Stimulation of Cartilage Tissue: Effect on Nutrient Supply

Subject: Human Interface Technology / Tissue Engineering
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Articular cartilage covers the surface of the bones within synovial joints where it absorbs loads and provides a low friction interface to the articulating surfaces of the joints.Within this study the hypothesis was put forward that the positive effect of loading on cartilage is (partly) due the supporting effect of the diffusion of nutrients, which is essential for the cellular function within the tissue.

Introduction

Articular cartilage covers the surface of the bones within synovial joints where it absorbs loads and provides a low friction interface to the articulating surfaces of the joints. It measures 1 to 3 mm in thickness and as an avascular tissue it relies on diffusion from the synovial fluid for supply with nutrients. Cyclic loading of the tissue, such as during gait, which exerts a pumping effect, is essential as it supports the diffusion processes. Mechanical loading is therefore an essential factor for the formation of cartilage tissue and the maintenance of its integrity.

Within this study the hypothesis was put forward that the positive effect of loading on cartilage is (partly) due the supporting effect of the diffusion of nutrients, which is essential for the cellular function within the tissue. The primary aim of this study was to establish a method enabling to measure the diffusion process of ions into cartilage tissue and to assess the diffusion behavior of these substances with and without mechanical loading.

Approach

Cylindrical cartilage pins with underlying bone tissue (diameter 10 mm) were harvested from. bovine knee joints (6 to 36 month old) within less than 24 hours after slaughter. Four model elements from alkali metals and alkaline earth metals, namely Rb^+, Cs^+, Sr^{2+}, and Ba^{2+} were used to assess diffusion into cartilage tissue.

These elements are closely related to Na^+, K^+, Mg^{2+} and Ca^{2+}, respectively which are commonly found in the tissue. The ions were allowed to either diffuse with or without mechanical loading for up to 24 hours into the tissue. Mechanical loading (dynamic compression up to 25 N was applied in a custom-made bioreactor system during 5 min per hour. Thereafter the cartilage was separated into different sections and was subjected to different digestion protocols prior to measuring the ion content by inductively coupled plasma with mass spectrometer analyser.

Results

After extensive studies, a simple digestion protocol based on 1.2 mL 40% H_2O_2 and 1.8 mL of a 70% HNO_3 at 55 °C for 15 h was established for the preparation of the cartilage section for ICP MS analysis.

All data have to be normalized to enable comparison between different pins and experiments. It was decided that the top layer, most outer section was set to 100% of each pin since there are no other factors that allow for normalization. By doing so, quantitative comparison between stimulated and non-stimulated samples is lost.

There is a trend that the center section of the top layer of the mechanical loaded 24 h samples had the highest diffusion rate followed by the center of the next layer as compared to controls. This can be explained that the center part of the pins is more loaded due to the curvature of the loading piston. A second finding indicates that double charge ions (Sr^{2+}, Ba^{2+}) are more readily diffusing than single charged (Rb^+, Cs^+) in the tissue. More experiments are currently done to verify these findings.

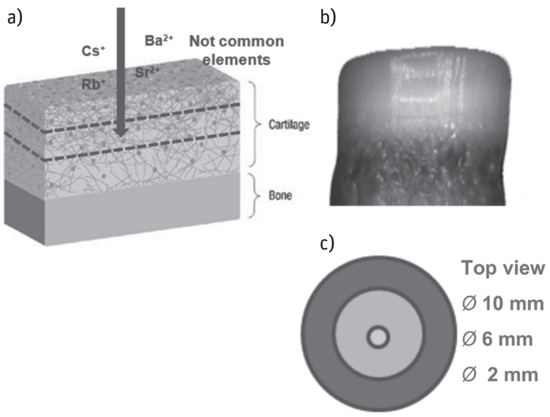

Figure 1: Experimental design
a) sketch of diffusion; b) cartilage pin and section, c) different cartilage sections

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