Measuring permeability and anisotropy effects of cortical bone using FRAP

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Introduction

The specific aims of this study were threefold, (i) to measure the currently unknown diffusion constants of various size macromolecules through the lacunocanalicular network, (ii) to compare differences in permeability in the transverse and longitudinal planes in cortical bone, and (iii) to compare permeability across length scales including the matrix microporosity, cellular syncytium, and "bulk tissue" permeability.

Methods

Diffusion constants were calculated based on FRAP experiments conducted on a laser scanning confocal microscope (SP2 AOBS, Leica Microsystems, Mannheim Germany). Longitudinal and transverse sections were obtained from the mid-diaphysis of a fresh bovine femur cortex. Samples were soaked in tracer solution until sample was saturated. Acid Yellow Fluorescein free dye (Sigma) and fluorescein conjugated dextran molecules of 3, 10, 40, and 70 kDa tracer molecules were chosen to achieve our aims. Bleach regions included tissue, cell, and subcellular length scale areas. Ten measurements were made per sample (n = 3); for each sample, diffusion constants were reported as the mean (± standard error) of these ten measurements.

Results

The measured diffusion coefficient decreased exponentially with increasing molecular weight (Fig. 1). Significant differences were also observed in coronal and transverse diffusion constants (Fig. 2) for small molecular weight (MW) free dye (300 Da), but not for the larger MW dyes. The permeability of cortical bone is highly dependent on length scale measured.

Discussion

These studies show, for the first time to our knowledge, that the permeability of cortical bone is not only dependent on the size of the molecule being transported but also on the direction of transport, and the length scale of the system through which transport occurs. Of particular interest was the observation that the low pass filter function of bone's molecular sieve shows anisotropy, whereby small molecules (300 Da) are transported more rapidly in the longitudinal plane than in the transverse plane. This anisotropy may confer an additional means by which low molecular weight substances are transported preferentially, *e.g.* in the direction of growth during bone modeling.

Figure 1

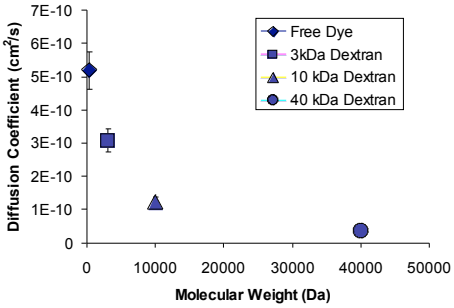

Figure 2