Optimization of a Protocol for Visualizing Vascular and Cellular Pore Networks in Human Bone Using Multiphoton Confocal Microscopy

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1. Introduction

3 Results: Optimizing Stain Concentration Based on Depth

An optimal stain concentration should resolve the finest vascular canals by a 100–150 µm diameter for receptor-based cellar. Cellular pores include a 1 µm wide radial and 1–2 µm large diameter canalicular system. For the protocol, maximum depth refers to the deepest point in the bone where cellular ducts could be distinguished from the background. The "Image depth" used to generate 3D images was set when the distance of pore structures in the field of view could be observed. For small concentrations of 0.1% to 1%, cellular pores were observed at maximum depths ranging from 100 to 150 µm. This resolution dropped off only slightly for stain concentrations of 0.5% to 1%.

### Discussion: Recommendations

- FITC concentrations between 0.5% and 1% resulted in optimal pore structures, specifically, at depths of 100 to 150 µm. At FITC concentrations of 0.2% to 0.4%, small vascular canals, which fad outside the field of view, increased in brightness relative to background and vascular canals. Thrombosis caused cellular and structural changes to bone. Cellular pores were observed at depths of 100 to 150 µm.

2. Materials and Methods

3. Results: Optimizing Stain Concentration Based on Thresholding Capability

- Stain Concentration and Depth

4. Results: Optimizing Stain Concentration Based on Thresholding Capability

- 0.1%: 60.64 µm, 0.3%: 60.64 µm, 0.5%: 60.64 µm

5. Conclusion: Image Analysis Potential

- 0.4%: 60.64 µm, 0.6%: 60.64 µm, 0.8%: 60.64 µm

6. Conclusion: Image Analysis Potential

- 1%: 60.64 µm

- 2D Image at Top of Figure 2

- Auto Local Threshold

- Boni Optimise

- Original Image

- Boni Optimise

- Auto Local Threshold

- Original Image

- Original Image