A Qualitative and Quantitative Analysis of Bone Porosities through the Tabletop Scanning Electron Microscope

Abstract

The objective of this experiment was to access the effectiveness of using a new generation instrument, the TM-1000 tabletop scanning electron microscope to analyze cortical bone porosity in BALB mice tibiae. Scientists have been intrigued by the porosity growth of the skeleton and especially the development of diseases such as Osteoporosis and bone morphology changes during spaceflight. The tabletop SEM is proven to be very effective, as it requires minimal sample preparation, acquires resolutions of up to 30 nm and shows high contrast. Porosities were measured on the periosteal and intracortical surfaces of the bone. Intracortical porosity was analyzed on transverse sections of the tibiae that were prepared to study the way by cutting with a diamond wheel saw and by razor fracturing. Image processing techniques for background smoothing, thresholding algorithms, and noise filters were studied. A rolling ball background correction accompanied by the Shanbhag threshold and Despeckle filter proved to be most efficient on the SEM grayscale images. The techniques established here will be used to analyze periosteal and intracortical porosities on disused mouse bones.

Introduction

Pores are naturally occurring entities in bone and emerge from three main cavities that make up the porous network of the bone: Haversian/Volkmann’s canals, osteocytic lacunae, and canaliculars. Each of these cavities is intrinsic to bone modeling and remodeling processes. The Haversian/Volkmann’s canals make up most of the cortical porosity and become larger in aged and osteoporotic bone. Cortical bone perforations can tell us a great deal about bone’s material properties and processes1. Researchers and physicians have been eager to quantify pore sizes in hopes of better understanding diseases like osteoporosis and even changes in bone morphology during spaceflight.

Conventional techniques for analyzing cortical porosity include, Nuclear Magnetic Resonance (NMR), Micro-computed Tomography (µCT) and Field Emission Scanning Electron Microscopy (FESEM). However, certain limitations in each method has prevented further discovery into this field. This paper analyzes the effectiveness of studying porosity through a new generation tabletop scanning electron microscope. This instrument eliminates cumbersome sample preparation, produces higher resolutions than NMR, µCT and FESEM (~30nm) and is ideal for biological samples like the BALB mouse bones being used in this study.

New techniques for image processing of backscattering electron images from the SEM are also investigated. They provide quantitative data of the pores with the use of a public domain imaging software package, ImageJ. Automated and manual techniques are compared along with pre and post processing of the original grey scale images.

Materials and Methods

3 month old female BALB mice (right hind leg) preserved in 70% ethanol (ETOH) were obtained from the Biotechnology Center at Stony Brook University. The samples were first observed through an optical microscope and then imaged through the Hitachi TM-1000 tabletop scanning electron microscope (SEM). Sample preparation prior to SEM imaging was minimal and included the removal of the periosteum layer through fine tweezers, air drying and mounting on double sided carbon coated adhesives on aluminum SEM stubs. Periosteal surface pores were imaged on the lattitudinal direction while intracortical porosity was imaged on transversely cut bone sample, all under charge reduction mode.

Two techniques for making a transverse cut of the bone included cutting with a diamond wheel saw and with razor fracturing. Image processing techniques for background smoothing, thresholding algorithms, and noise filters were studied. A rolling ball background correction accompanied by the Shanbhag threshold and Despeckle filter proved to be most efficient on the SEM grayscale images. The techniques established here will be used to analyze periosteal and intracortical porosities on disused mouse bones.

Conclusion and Future Work

The TM-1000 was extremely efficient in capturing porosities on the periosteal and intracortical surfaces. It’s high-resolution and contrast capabilities allowed the most minute mouse cavities to be imaged and clearly differentiated between pores and soft tissues. The limited sample preparation was also one less avenue for errors. Images 8 and 9 show the intracortical surface after the diamond saw and razor fracturing. The diamond saw left behind blade marks, cracking, and covered pores on the surface. The fractured sample shows pores more distinctively however the rough and uneven surface is not ideal for scanning or image processing. A promising technique for future studies is polishing the cut sample before imaging, which might produce a smoother surface to judge intracortical porosity. With these techniques, new control and test samples from Stony Brook University will be tested. The hope is to compare the growth of pores on the periosteal and intracortical surfaces after bone disease. It is hypothesized that porosity growth increases more rapidly intracortically.

Processed images, represented by figures 3-6, used rolling ball correction that smoothed the background and allowed for better automated threshold. Many thresholding algorithms were compared including IsoData, Maximum Entropy, Otsu, Renentropy and Yen. Shanbhag produced the binary image with the least amount of noise and most realistic size of pores however slightly overestimated due to shadows around the pores. The despeckle algorithm removed noise that the threshold failed to remove and slightly eroded the pores, accounting for the overestimation of area. The pore histogram represented in figure 6 shows that the largest number of pores falls in a size range between 0 and 0.1 μm however further studies of mouse bone porosities can validate this data.

References