# Engineering Vascularized Bone Grafts by Integrating a Biomimetic Periosteum and β-TCP Scaffold

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## Supporting Information

**Mineral bone volume measurement using MicroView based on MicroCT images**

Micro-computed tomography (MicroCT; Imtek MicroCAT II; Knoxville, TN) at a resolution of 80 μm was used to scan the change in bone volume with implantation time. 4 Live mice were scanned at 2, 4, and 8 weeks. Before scanning, a calibration phantom was tapped on the MicroCT bed. Images were further analyzed by GE MicroView 2.2 (General Electric Co.). The algorithm in MicroView used to calculate the mineral volume in the implants depends on the calibration of the scanner using the phantom provided with the system (see the instruction manual of MicroView). Mean gray values within air, water and "bone" (phantom) from an acquired MicroCT image were obtained using a region of interest (ROI). The air and water values acquired with arbitrary units were converted to Hounsfield units (HU) by scaling the air to -1000 and the water to 0. From these air and water HU values, the system then determines a "bone" HU value based on an extrapolation. Using this thresholding procedure, the HU of a sample in a mouse at a designated time point was calculated. A standard ROI that contained a portion of the implant was defined to determine a grayscale values (Figure S1). The histogram of voxel grayscale values was then plotted (Figure S2). To prevent air from artificially reducing the calculation of a mean, a grayscale value for lower exclusion ADU was selected. Similarly, a grayscale value for an upper exclusion ADU was used to prevent metal from artificially increasing the mineral volume measurement. These two entries were used to limit the range of grayscale

values. Only voxels with grayscale values between the value for lower exclusion ADU and upper exclusion ADU were used to calculate the mean (Figure S2). The voxels of the sample at week 2 were used as the starting time point. The increase percentage of the HU at 4 and 8 weeks is designated as the increase volume ratio of the newly formed bone based on that of 2 weeks (n=4).


**Figure S1.** A 3D MicroCT image demonstrated the location of implants: Upper-left: OM/UM/β-TCP; Upper-right: β-TCP; Lower-left: HUVEC-UM/OM/β-TCP; Lower-right: OM/HUVEC-UM/β-TCP. One sample as a representative was selected using a standard ROI (yellow box) to calculate the volume of bone mineral tissue. The size of ROI box was carefully drawn to include the whole implant but not mouse host bone.

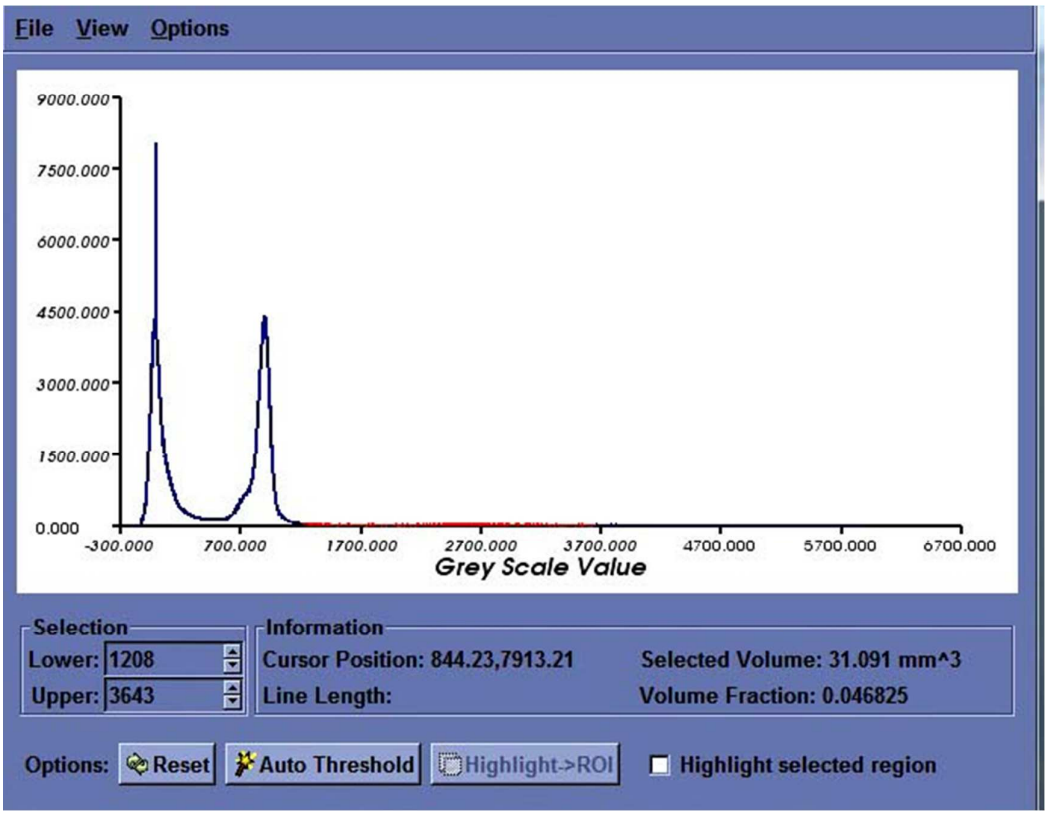


**Figure S2.** A histogram of voxel grayscale values was performed and a selected range was plotted (red line) to obtain the volume of mineral tissue.