THREE-DIMENSIONAL OSTEOCYTE LACUNO-CANALICULAR NETWORK AT THE BONE IMPLANT INTERFACE

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Introduction

Osteocytes are the most abundant cell type found in bones and play a key role in the regulation of bone metabolism. In woven bone, formed after implantation, osteocytes communicate with the implant surface via lacuno-canalicular network (LCN) and detect changes in mechanical loading by releasing numerous biochemical messengers that modulate bone metabolism [1,2]. It is not fully understood how the osteocyte extensive and intricate LCN adapts to the presence of an implant, to provide the mechanical stability, exchange of nutrients, and vascularization necessary for successful osseointegration [3]. The aim of this study is to investigate the LCN formed in peri-implant newly-formed bone tissue, to understand its structural complexity and differences compared to a mature bone tissue.

Methods

Sample. Cortical bone from a rabbit tibia was retrieved after the osseointegration of a standardized coin-shaped implant for 7 weeks (TiAl6V4, Ø5-H3mm). Samples were stored immediately following surgical removal, embedded in PMMA, sectioned to a baton of 2×5×2mm^3 and polished to expose the mineralized tissue at the BII.
Image acquisition. A FIB-SEM (FEI Helios NanoLab 660) was used in BSE mode to image a 20×30×30 µm^3 region close to the implant. The 3D output images (stack of 1027 2D grayscale images) were obtained using a "Auto slice and view" software (Fig 1A), with an anisotropic pixel size of 16.78x16.78x20nm.
Image processing was carried out using Fiji. Local excessive brightness was removed using a bandpass filter. A 3D Gaussian filter was then applied to reduce noise (std dev of 3pixels). Moreover, artifacts present in the image (eg saturation close to a cavity) were reduced by applying Kuwahava filter (sampling window width of 7 pixels). Finally, the lacunae canaliculi network was segmented from the mineralized bone (visual threshold).
Data analysis. Two preferential subvolumes with limited artefacts (9x9x16µm) were isolated around the osteocyte cavity, with visual differences in porosity. Canaliculi thickness maps were extracted using BoneJ plugin, and analyzed using a matlab routine.

Results and discussion

An ellipsoidal osteocyte lacuna was observed in the center of the imaged volume, connected to a dense meshwork of canaliculi (Fig 1B) and visually aligned parallel with the direction of the implant surface. The two isolated subvolumes presented different local porosity of 0.53% (fig 1.C1) and 2.34% (fig 1.D1), associated with relative maximum canaliculi thickness of 200±60 nm (fig 1.C2) and 240±80 nm (fig 1.D2). This difference of density may be due to the heterogeneity of the newly formed bone. Further analysis is ongoing (orientation, connectivity, etc) as well as comparison with data from a mature bone region, to highlight and understand differences in the bone tissue local micro and sub-nanostructure, close to an implant[4,5].

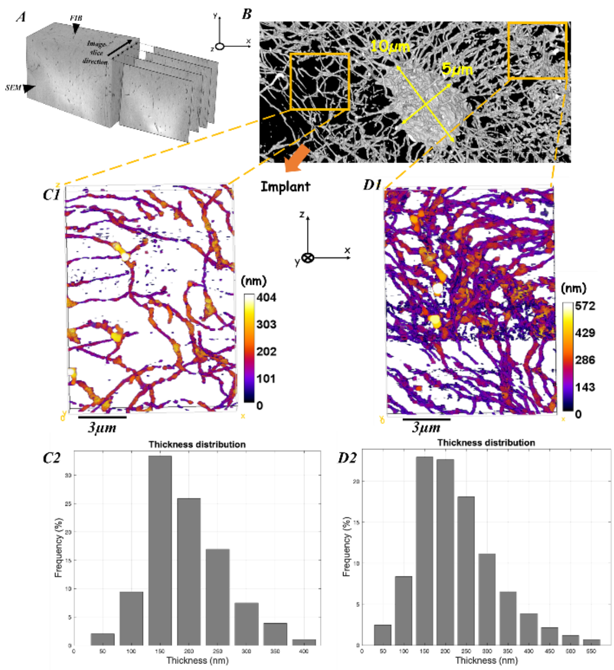

Figure 1: FIB-SEM "slice and view"(A); LCN network in the immature bone tissue (B), canaliculi connected to the same osteocyte (C1) and (D1), thickness distribution (C2) and (D2).

References

1. Haridy, Y., et al. Science advances, 14, eabb9113. 2021.
2. Shah, F.A. et al. Jour. of Dental Research,9, 977-986,2018.
3. Shah, F.A. et al., Acta Biomat., 84 :1-15, 2019
4. Buenzli, P. R., et al. Bone, 75, 144-150. 2015.
5. Turner, C. H., et al. Bone, 16, 283-285. 1995.

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