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# High-resolution imaging of the Osteocyte Lacuno-Canalicular Network of peri-implant newly formed bone

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## 1. Introduction

Bone implants are widely used in orthopedic and dental surgery to restore bone function and structure. However, the interface between the implant and the bone can be a site of complications such as implant loosening, bone resorption, and inflammation.

The bone tissue is highly hierarchical. At the submicron scale, the osteocyte lacuno-canalicular network (OLCN) is a complex system of channels that interconnects the osteocytes and allows for the exchange of nutrients, waste products, and signaling molecules. During bone modelling and remodelling, this network plays a crucial role in maintaining bone health and responding to mechanical loading. Understanding the formation and maintenance of the OLCN at the implant interface is essential for developing new strategies to enhance osseointegration and prevent implant failure (Haridy et al., 2021; Shah et al., 2018).

To access the level of resolution needed to investigate the OLCN, high-resolution imaging techniques can be used, such as scanning electron microscopy (SEM) (Schneider et al., 2010) which can be combined with Focused Ion Beam (FIB-SEM) to extend from 2D to 3D information, or nano-computed tomography (nano-CT) (Peyrin et al., 2014). Such high-resolution techniques are only usable with adapted image processing and analysis methods, to quantify the structural properties of the OLCN, such as the degree of porosity, the dimensions of osteocyte lacunae and canaliculi, and the connectivity of the network. Applied to the bone tissue, such approaches have provided valuable information on the morphology and organization of the OLCN (Hemmatian et al., 2021), revealing for instance the spatial heterogeneity of the network (Meijering et al., 2016) or that the geometrical properties of osteocyte lacunae are almost independent on age (Wittig et al., 2016). Work investigating the adaptation of the bone tissue to the presence of an implant is still scarce, data suggests for instance that collagen fibrils are oriented parallel with the implant surface.

Using a bone chamber implant model integrated into rabbit bone, we propose to investigate the newly-formed bone tissue close to a metallic implant with FIB-SEM and image processing.

## 2. Methods

**Sample.** A standardized coin-shaped TiAl6V4 implant (Ø5mm, H3mm) with a bone chamber (Fig 1A) was osseointegrated for 7 weeks in rabbit cortical bone (tibia). After harvesting, the sample (bone and implant) was embedded in PMMA and carefully sectioned to expose the newly-formed bone at the implant surface (Fig 1B). To minimize damage to the newly-formed bone and reduce the risk of bone detachment, a low-speed rotating diamond wire saw was used, followed by gentle manual polishing (SiC #1200).

**Image acquisition.** A  $20 \times 30 \times 30 \mu\text{m}^3$  region close to the implant (roughly at a distance of  $20 \mu\text{m}$ ) was imaged using a FIB-SEM (FEI Helios NanoLab 660) in back-scattered electron (BSE) mode, and “Slice and View” software (Fig 1C). The resulting 3D images stack consists of 1027 2D grayscale cross-sections (pixel size  $16.8\text{nm}$ ) with a distance of  $20 \text{nm}$  between each slice.

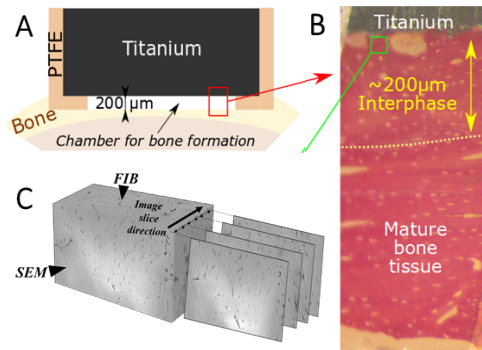


Figure 1. A. Bone-chamber implant, B. Sample with bone tissue stained in red (picrofushin), C. FIB-SEM “slice and view”

**Image pre-processing.** The acquired images were processed using Fiji to remove brightness and reduce noise and artifacts. First, a 3D Gaussian filter with a standard deviation of 3 was used to reduce noise. Then, a bandpass filter with a high spatial frequency of 150 pixels and a low spatial frequency of 3 pixels was applied to remove localized brightness, mostly due to charging effects around cavities (e.g. osteocytes cavities). Finally, to eliminate the curtaining artifacts coming from the etching with the ion beam, Fast Fourier Transform (FFT) was applied to obtain the frequency spectrum. Periodic horizontal bands were observed and identified as the artifacts and subsequently removed.

**Image analysis.** Once cleaned, the images were threshold-segmented to isolate the OLCN network (low gray values) from the mineralized bone (high

gray values). Two preferential subvolumes (9x9x16µm) were identified around the osteocyte cavity, which displayed visual differences in porosity. Canalliculi thickness maps were extracted (BoneJ plugin and MATLAB routine).

### 3. Results and discussion

After a healing period of 7 weeks, the analyzed peri-implant bone region presented a well-defined lacuno-canalicular network, with an osteocyte lacuna connected to a dense meshwork of canaliculi (Fig 2A). The ellipsoidal lacuna was of approximate dimensions of 10µm in the large axis and 5µm in the small axis, and appeared visually aligned parallel with the direction of the implant surface. The two isolated subvolumes of the newly formed bone region presented different local porosity of 1.1% (Fig 2B) and 5.8% (Fig 2C) which is consistent with literature data on bone (Buenzli et al., 2015). The relative maximum canaliculi thickness was observed to vary between the subvolumes, with the higher porosity subvolume exhibiting a denser and thicker network of canaliculi (average of 240±80 nm) compared to the lower porosity one (average of 200±60 nm). The subvolume presenting lower porosity and thinner canaliculi being closer to the implant, this heterogeneity may be due to the adaptation of the tissue to the implant, which could influence the local bone formation process (Robling et al., 2020).

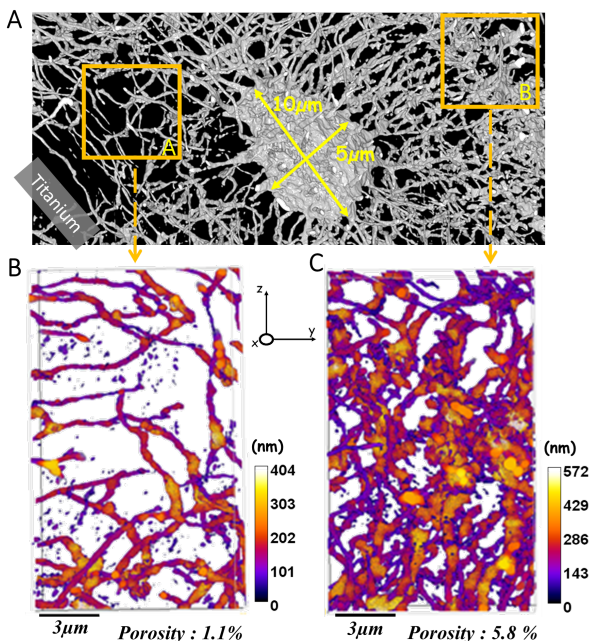


Figure 2. A. OLCN network in the immature bone tissue and the two subvolumes of interest. B-C. Thickness distribution and average porosity of canaliculi connected to the same osteocyte.

### 4. Conclusions

The bone regeneration process exhibits inherent heterogeneity, as evident from the observed variations in local porosity and canaliculi thickness within the peri-implant newly formed bone tissue. .

These findings highlight the critical importance of considering the local sub-microstructure, such as variations in porosity and canaliculi thickness, in the design and evaluation of bone implants. Further exploration of the OLCN orientation and connectivity is essential to gain insights into the three-dimensional adaptation of bone near the implant site. Future work will focus on comparing these observations with mature bone regions, to enhance our understanding of the local micro and sub-nanostructure. By incorporating these insights into bone implant designs, researchers can develop more precise and targeted approaches, optimizing long-term success. Ultimately, understanding these local variations enables the development of personalized implant solutions, leading to improved outcomes and a better understanding of the bone tissue's local micro and sub-nanostructure.

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