# Collagen Organization at Bone - Implant Interface — A Secondary Harmonic Generation Microscopy Study

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**Introduction:**

Orthopaedic implants are extensively used to replace joints affected by skeletal problems. Faster and stronger implant-bone fixation is important to the performance of artificial joints and is often achieved through porous structures on the implant surface [1]. Biological fixation of porous implants is achieved by interlocking with the ingrown bone [1]. The fixation strength strongly depends on the detailed bone structures at the interface. The objective of this study is to understand bone's lamellar structure and fibrillar organization at the implant-bone interface through Secondary Harmonic Generation Microscopy (SHG). Due to the nature of the signal (scattering by collagen molecules), SHG could directly visualize collagen fibres [2]. Its contrast depends on local fibril organization, with higher light intensity from in-plane mineralized fibrils [2]. It also has the advantage of reduced photobleaching and phototoxicity, and achieving high resolution imaging to the depths of several hundred microns [2].

**Materials and Methods:**

Cylindrical porous Ta implants (Φ 3.15 x 5 mm), with interconnected pores were used in this study. They were placed unicortically in the right anteromedial tibia of a total of ten female New Zealand white rabbits. The rabbits were sacrificed at four and eight weeks post-operatively. The animal study protocol was approved by the Animal Care Committee of the University of British Columbia. The anteromedial half of the tibia was harvested and mechanically tested. Following the push-out tests, they were fixed, dehydrated and embedded. The embedded samples were then grounded and polished perpendicular to the bone axis. Finally, they were observed by SHG microscope to examine new bone's fibril organization at the implants' interfaces.

**Results:**

Four weeks after implantation, the newly formed bone was more irregular compared to the well-organized Harversian systems in the old bone area. More importantly, the new bone area formed in a way that most fibrils preferentially grew perpendicular to the long bone axis, while most lamellae in old bone area were confined in the "dark" layer, having fibrils parallel to the long bone axis, as shown in Figure 1 (a) and (b). The major crack, created by the push-put tests, went through the new bone area instead of the old-new bone interface in the four weeks samples (Figure 1 (a)). However, after eight weeks of implantation, the specimens fractured mainly through the interface (Figure 1 (b)), indicating an increased strength in the new bone.

**Discussion:**

With the help of SHG technique, the complex bone structure at implant-bone interface could be clearly seen down to lamellar and sub-lamellar level. The area of old and new bone were clearly defined by their distinguish fibrillar organization. Analysis of the fibril orientation may reveal structure-mechanical relation at the implant interface. SHG is a promising imaging technique to understand the role of lamellar structure and fibrillar organization at the implant interface.

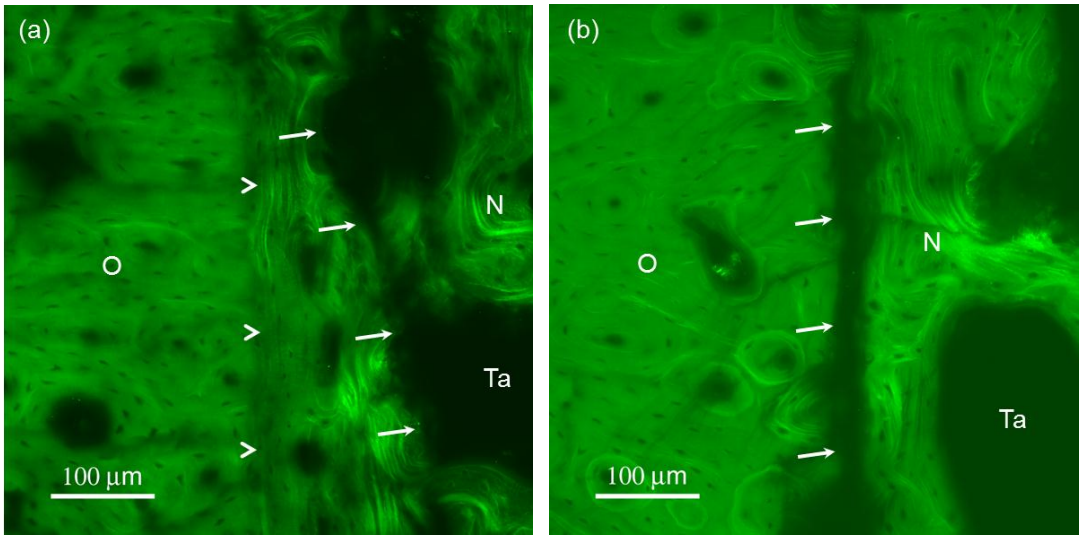


*Figure 1. Secondary Harmonic Generation (SHG) Microscopy images showing the fibrillar structure at bone-implant interface after four weeks (a) and eight weeks (b). Fracture (arrows) propagated through new bone area in the four-week sample, and mainly through the interface (arrowheads) after eight weeks. Notice the distinct lamellar structure of old (O) and new (N) bone. Bright contrast indicates in-plane organization of the mineralized collagen. Ta implants were on the right side of the pictures.*

It is well known that, after remodeling, the dominating orientation of collagen fibrils in bone corresponds to the direction in which biomechanical stress is applied. Areas with a high degree of preferred orientation are associated with the areas where the stress is highest. This is in accordance with the fact that most collagen fibrils in old bone area were parallel to the long bone axis. The newly formed collagen fibrils around the implant were mainly in-plane in the current study. This is an indication that the local biomechanical environment has been changed due to the presence of the Ta implants. The stiff Ta structure shielded the newly formed bone from the normal biomechanical loading and this changed local bone structure. Our study also emphasized the importance of time in implant-bone fixation. There was a distinct interface between the old bone and newly formed bone, which is potentially weak. However, fracture in the four-week group happened within the newly formed bone rather than the assumed weak interface. Obviously, at four weeks, the newly formed bone was still undergoing remodeling with less density and less mineralization, leading to lower shear resistance.

**References:**

[1] DS Garbuz *et al*, J Bone Joint Surg A, 90: 1090-1100, 2008
[2] PJ Campagnola *et* al, Nature Biotech, 21: 1356-1360, 2003