BONE-FIBROCARTILAGE CROSSTALK AND OSTEOCYTE LACUNO-CANALICULAR NETWORK AT THE TENDON-BONE INSERTION

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Introduction

Mechanobiological interactions between tissues are a crucial aspect of the musculoskeletal system. At joints, crosstalk between bone and cartilage cells may reach unhealthy levels in osteoarthritis [1]. The tendon-bone insertion often features fibrocartilage, a fibrous form of cartilage reinforced with minerals before anchoring to bone [2]. Tendons and bones contain cells (tenocytes and osteocytes) having communication abilities thanks to underlying networks of sub-micrometer channels. In bone, the latter is called the osteocyte lacuno-canalicular network (OLCN) whereas in tendon it is referred to as nanotube network [3]. They serve multiple functions: in addition to communication, nanotubes in tendon are believed to provide biomechanical stability [3] while the OLCN is directly involved in bone mechano-responsiveness [4] and mineralization [5]. Mineralized fibrocartilage (mFC) exhibits fibrochondrocytes (FCCs) occupying lacunae, often very close to each other. Here, we explore the possible crosstalk between osteocytes and FCCs as well as the behavior of the OLCN at the interface between the two tissues. We consider the Achilles tendon insertion into calcaneus bone, and we compare enthesis with periosteal fibrocartilage, two contiguous tissues sustaining different loading conditions: tension at enthesis and compression/shear at periosteal. We have previously shown microstructural [6] and material heterogeneity [7] of mFC. Here, we address additional mechanobiological aspects.

Methods

Rat samples (ULg IACUC-21-2340) were stained with rhodamine and then micro-computed tomography (micro-CT), quantitative backscattered electron imaging (qBEI), second harmonic generation (SHG) imaging and confocal laser scanning microscopy (CLSM) were combined on the same locations to highlight the functional porosity of bone and mFC at multiple length scales, as well as its link with mineral content and matrix organization.

Results

At the enthesis, some rows of FCC lacunae located entirely inside mFC, and therefore not directly exposed to rhodamine, got stained. Correlating micro-CT with CLSM revealed that the fluorescent molecule could reach the FCC rows through perforating channels, originating from the trabecular bone marrow space and reaching mFC. The OLCN seemed to stop or bend at the cement line separating bone from mFC, but exhibited a high connectivity with the perforating channels, providing an indirect path between osteocytes and FCCs (Fig. 1). Such connections were absent at the periosteal region. The previously highlighted enthesis anisotropy [6] was not reflected in osteocyte lacunae morphology.
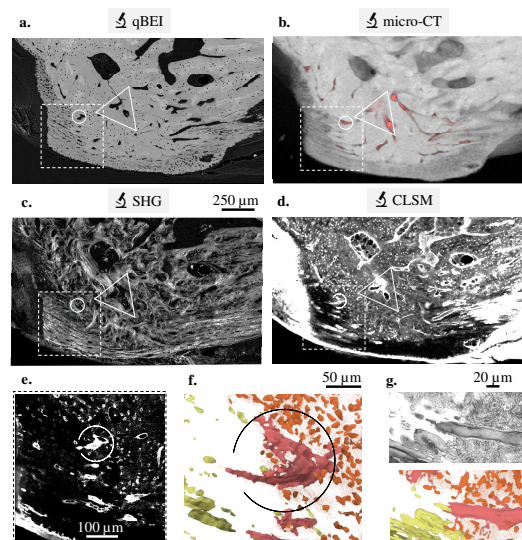

Fig. 1: Correlation of (a) qBEI, (b) micro-CT, (c) SHG imaging and (d) low-resolution CLSM overview at the enthesis. (e) CLSM representative cross-section of the frame shown in a-d. (f) 3D inset of the segmented microporosity, emphasizing a large channel (encircled in images a-f). (g) Magnified view and segmentation of CLSM data illustrating the interaction between a channel (pink), the OLCN (orange) and FCCs (yellow).

Discussion

The illustrated communication paths between bone and mFC at the insertion, together with the striking absence of such connections at the periosteal region, suggests that different level of crosstalk between bone and fibrocartilage may be required to maintain healthy enthesis, also depending on fibrocartilage morphology (thickness) and biomechanical task. Crosstalk could be further explored by quantifying the permeability of the interface using fluid flow simulations [4].

References

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