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Multimodal and multiscale characterization of bone and bone interfaces in health and disease

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer att offentligen försvaras i hörsal Arvid Carlsson, Medicinaregatan 3, måndagen den 12:e juni 2023, klockan 13:00.

av Chiara Micheletti

Fakultetsopponent:

Prof. Håvard J. Haugen, Avdeling for biomaterialer, Institutt for klinisk odontologi, Universitetet i Oslo, Norge

Avhandlingen baseras på följande delarbeten

I. Micheletti C, Jolic M, Grandfield K, Shah FA, Palmquist A. Bone structure and composition in a hyperglycemic, obese, and leptin receptor-deficient rat: Microscale characterization of femur and calvarium. Bone. 2023;172:116747.

II. Micheletti C, DiCecco L-A, Larsson Wexell C, Binkley DM, Palmquist A, Grandfield K, Shah FA. Multimodal and multiscale characterization of the bone-bacteria interface in a case of medication-related osteonecrosis of the jaw. JBMR Plus. 2022;6:e10693.

III. Micheletti C, DiCecco L-A[#], Deering J[#], Chen W, Ervolino da Silva AC, Shah FA, Palmquist A, Okamoto R, Grandfield K. Micro-to-nanoscale characterization of the osseointegration and lacuno-canalicular network at the interface with an additively manufactured implant for local genistein delivery. *Submitted for publication.*
[#]Equal contribution.

IV. Gomes-Ferreira PHS, Micheletti C, Buzo Frigério P, de Souza Batista FR, Monteiro NG, Bim-júnior O, Lisboa-Filho PN, Grandfield K, Okamoto R. PTH 1-34-functionalized bioactive glass improves peri-implant bone repair in orchiectomized rats: Microscale and ultrastructural evaluation. Biomater Adv. 2022;134:112688.

V. Micheletti C, Gomes-Ferreira PHS, Casagrande T, Lisboa-Filho PN, Okamoto R, Grandfield K. From tissue retrieval to electron tomography: Nanoscale characterization of the interface between bone and bioactive glass. J R Soc Interface. 2021;18:20210181.

VI. Micheletti C, Shah FA, Palmquist A, Grandfield K. Shedding light (… electrons) on human bone ultrastructure with correlative on-axis electron tomography and energy-dispersive X-ray spectroscopy tomography. *Submitted for publication.*
Preprint available in *bioRxiv*, DOI: 10.1101/2023.04.20.537681.

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR KLINISKA VETENSKAPER

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Multimodal and multiscale characterization of bone and bone interfaces in health and disease

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Abstract

Seeing is believing. Our understanding of phenomena often involves their direct observation. However, bone architecture is challenging to visualize given its multi-level hierarchical organization. In this thesis, bone and bone interfaces are characterized via multimodal and multiscale platforms, combining different techniques across several length scales. Imaging techniques across the micro-nano continuum are complemented by spectroscopy methods to explore, respectively, the structure and composition of bone and bone interfaces, using both light and electron probes. By applying a characterization methodology more typical of materials science, this thesis aims to unveil structural and compositional abnormalities of bone induced by disease [Papers I-II], and bone response to functionalized biomaterials in compromised conditions [Papers III-IV]. Additionally, it expands three-dimensional (3D) characterization opportunities at the nanoscale in both native and peri-implant bone [Papers V-VI].

This characterization approach uncovered changes in bone quality (structure and/or composition) in the compromised conditions under investigation in this thesis, i.e., leptin receptor (LepR) deficiency and medication-related osteonecrosis of the jaw (MRONJ) [Papers I-II]. In a preclinical model of LepR deficiency for type 2 diabetes/obesity, multimodal characterization of bone at the microscale showed structural abnormalities indicative of delayed skeletal development, despite unaffected bone matrix composition [Paper I]. A combination of multiscale imaging and spectroscopy techniques spanning the micro-to-nanoscale enabled a detailed study of the interface between necrotic bone and bacteria in a case of MRONJ, shedding light on possible mechanisms of bone degradation. When applied to bone-biomaterial interfaces, the application of a multimodal and multiscale characterization workflow informed perspectives on bone response to novel biomaterial solutions aimed to promote osseointegration in osteoporotic conditions via local drug delivery of phytoestrogens [Paper III] or anabolic agents [Paper IV]. This highlighted the importance of studying peri-implant bone at the mesoscale [Paper III] and of confirming biomaterial behaviour *in vivo* in the presence of surface functionalization [Paper IV]. Lastly, this thesis emphasized the importance of 3D imaging at the nanoscale with electron tomography to resolve bone ultrastructure at biomaterial interfaces [Paper V] and in native conditions [Paper VI]. Specifically, in Paper VI, artifact-free on-axis electron tomography resolved some long-debated aspects regarding the organization of mineralized collagen fibrils, the fundamental building block units of bone.

Keywords: bone; osseointegration; characterization; mineralization; ultrastructure; diabetes; osteoporosis; MRONJ; surface functionalization; local drug delivery; titanium; bioactive glass; imaging; spectroscopy; microscale; nanoscale; micro-computed X-ray tomography; micro-Raman spectroscopy; scanning electron microscopy; transmission electron microscopy; PFIB-SEM tomography; electron tomography.

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