BIO Web of Conferences **129**, 19008 (2024) https://doi.org/10.1051/bioconf/202412919008
*EMC 2024*

# Investigating Bone Microstructure with ATUM-SEM: Implications for Pathological Conditions

**Ph.d Valeria Vistoso**[1], Dr. Tom Fertè[1], Dr. Federica Buccino[2], Professor Laura Maria Vergani[2], Professor Ovidiu Ersen[1], Professor Adele Carradò[1]
[1]Institut de physique et chimie des Matériaux de Strasbourg, University of Strasbourg, Strasbourg, France, [2]Department of Mechanical Engineering, Politecnico di Milano, Milan, Italy

Background incl. Aims
Bone tissue's hierarchical structure is pivotal for various mechanical, biological, and chemical processes essential to health. However, the mechanical properties of bones are susceptible to deterioration, leading to an increased risk of fragility fractures associated with ageing, vitamin D deficiency, and bone density pathologies, such as osteoporosis [1]. The prevalence of these chronic illnesses, coupled with rising life expectancy, has become a significant public health concern. Additionally, the COVID-19 pandemic potentially exacerbates bone health issues, leading to decreased bone mass and strength in long-term hospitalised patients [2]. Bone fractures result in significant psycho-social and economic burdens, yet current understanding is limited to macro- and mesoscale levels. The challenge lies in comprehending the mechanobiological perspective at the multiscale level (between the mm and nm ranges). This knowledge gap, coupled with the influence of other intricate structural parameters, underscores the need for advancements in research tools and technical approaches.

Methods
Automated Tape Collecting Ultramicrotome Scanning Electron Microscopy (ATUM-SEM) has been introduced as an effective method for capturing high-quality images of hard tissues [3]. In this case study, the choice of using ATUM-SEM was driven by the need for a non-destructive approach that could enable the examination of the largest possible volume. The method was chosen for its flexibility, which allows cutting slices sections with a chosen thickness while preserving all of them for subsequent observations. Despite a thorough literature search, no suitable protocol was found to prepare human trabecular bone compatible with the ATUM-SEM-based approach. Consequently, it was determined that the best course of action was to utilise the same workflow previously used in the analysis of biological samples, with some adapted modifications made on a case-by-case basis. Trabecular bone samples from female and male patients diagnosed with different clinical conditions, namely osteoporotic, COVID-19, and a healthy control group, were evaluated. The specimens were processed by cutting them into macroscopic shapes appropriate for applying the ATUM-SEM approach, followed by careful cleaning and dehydration to improve their embedding in the resin. The resin embedding process was optimised for efficient sectioning, and a good trimming and polishing of the face of the specimen block was crucial for a stable section collection protocol. Slices, with thickness ranging from 300 nm to 400 nm, were collected onto Kapton tape using an Ultramicrotome. The imaging workflow that was utilised to reconstruct the acquired volume was managed using Atlas 5 Array tomography software and Fiji. Creating a 3D representation requires accurate positioning of images in a virtual volume through registration. The alignment of the image stacks was accomplished using the TrakEM2 plug-in. Finally, Data visualisation is achieved using the 3D volume rendering present in Fiji.

Results
The ATUM-SEM data sets have yielded both qualitative and quantitative analyses, revealing distinctions in lacuna size and shape between osteoporotic and non-osteoporotic cases. Specifically, the osteoporotic and COVID-19 lacunae were larger, less stretched, and more rounded, with the average dimensions ranging from 6.12 µm x 12.27 µm x 10.8 µm and 7.17 µm x 20.96 µm x 6.9 µm, respectively. In the femoral head, lacuna volume exhibited internal

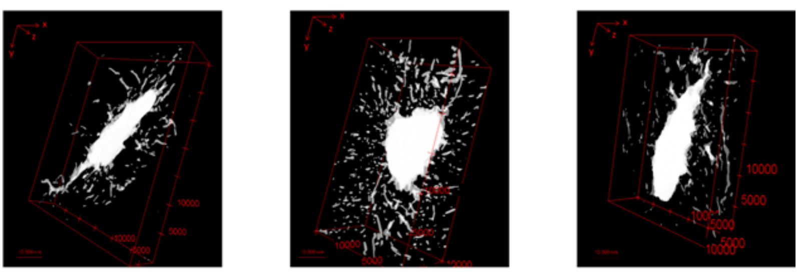
variability, with osteoporotic and COVID-19 cases showing pronounced differences compared to healthy specimens (Figure 1). In the femoral head, the volume of the lacunae presents internal variability, ranging from 26% in healthy specimens to 14% in osteoporotic ones and 18% in COVID-19 patients. The volume difference is more pronounced when comparing the physiological state with the two pathological conditions. The volume of the osteoporotic lacuna is approximately 39% larger than the healthy sample, while the volume of the COVID-19 lacuna is around 53%. The complexity of the COVID-19 pathology requires further cellular-level studies to isolate its direct effects on bone microstructure and understand lacunar alterations among different variants, even if the possibility of bone micro-structural deterioration due to COVID-19 is proven [4]. The results of increased lacunar sphericity in osteoporotic subjects are consistent with other research works demonstrating that lacunar stretch is reduced in patients affected by this pathology [4]. Nevertheless, image segmentation and processing still need to be improved for quantitative analysis, demanding improvements in automated segmentation routines. While there are ongoing debates regarding the impact of age on bone microstructure, the study has identified age-related variations in the characteristics of canaliculi and lacunae.

Conclusion

This study highlights the importance of examining bone microstructure at finer levels to understand bone health and disease. ATUM-SEM provides high-resolution imaging and detailed analysis of bone microstructures, leading to better diagnosis and treatment of bone-related disorders. Exploring ATUM-SEM's clinical applications for early bone pathology detection shows promise and may lead to targeted therapies. These future directions in ATUM-SEM analysis of bone pathologies pave the way for enhanced research and clinical applications in the field, leading to a future where targeted interventions can mitigate the burden of bone-related disorders

**Graphic:**

**Figure 1:** The following illustrations depict an example of the structure of a single lacuna with a partial reconstruction of the canaliculi system for each specimen analysed. (a) healthy specimen, (b) osteoporotic specimen, (c) COVID-19 specimen.



a) b) c)

**Keywords:**

Bone-microstructure, Osteoporosis, Lacunae-canaliculi-system, ATUM-SEM, 3D-imaging.

**Reference:**

[1] Natacha Rosa et al. From mechanical stimulus to bone formation: a review. Medical engineering & physics, 2015.
[2] Sara Cromer and Elaine Yu. Challenges and opportunities for osteoporosis care during the covid-19 pandemic. The Journal of Clinical Endocrinology & Metabolism, 2021.
[3] Patricia Goggin et al. Development of protocols for the first serial block-face scanning electron microscopy (SBF-SEM studies of bone tissue. Bone, 2020.

[4] Federica Buccino et al. Osteoporosis and COVID-19: Detected similarities in bone lacunar-level alterations via combined AI and advanced synchrotron testing. Materials & Design, 2023