

# MINERAL DENSITY AND MICROSTRUCTURAL MORPHOLOGY OF WOVEN BONE DURING DISTRACTION OSTEOGENESIS

Juan Mora-Macías (1,2), Bert Van Rietbergen (3,4), Pablo Blázquez-Carmona (2,5), Paloma García-Florencio (6), Jaime Domínguez (2,6), Esther Reina-Romo (2,6)

1. University of Huelva, Spain; 2. Institute of Biomedicine of Seville (IBiS); Spain; 3. Eindhoven University of Technology, The Netherlands; 4. Maastricht University Medical Centre, The Netherlands; 5. University of Cadiz, Spain; 6. University of Seville, Spain

## Introduction

Characterization of bone regeneration is being studied currently for several processes, such as fracture healing or distraction osteogenesis. In many of these situations, a disorganized type of bone, woven bone, is formed first, that will reorganize and densify over time [1]. Not much is known, however, about how this process evolves, and what the relationships are between changes in tissue properties and structural remodeling. Therefore, the purpose of this study was to investigate the evolution of woven bone tissue mineralization, tissue stiffness and microstructure in *ex vivo* samples from distraction osteogenesis experiments.

## Methods

Samples of woven bone generated in a previous animal study were used [1]. In these experiments, 6 sheep underwent osteotomy followed by 15 mm distraction osteogenesis at their metatarsus to generate new bone. Samples of the callus region were harvested at days 35, 50, 79, 98, 161 and 525 after surgery (1 sample per timepoint) and scanned using microcomputed tomography (Scanco microCT100). This analysis provided the evolution of the mineral density at 4 specific interzones per sample that were used in the earlier nanoindentation studies. One of these analyzed areas is located in the original surrounding cortical bone (A in Fig 1) and the others in the newly formed woven bone (B3, B4 and B5 in Fig 1). In addition, the microarchitecture of the newly formed bone in the gap region was quantified by structural parameters such as connectivity density, trabecular number, thickness and separation.

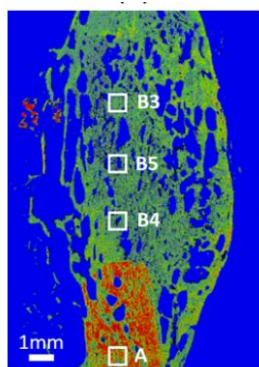


Figure 1. Mineral density distribution in one of the samples and locations of indentations in a previous study [1] in cortical bone (A) and new bone generated (B3, B4 and B5).

## Results and discussion

Results revealed that the mineral density of the woven bone tissue increased with time (from 600 to 1100 mgHa/cm<sup>3</sup>, mean values) during the regeneration process. These changes in mineralization correlated well with changes in tissue stiffness over time as measured at the same locations in a previous study [1] using nano-indentation (Fig. 2).

Over time, the bone microstructure also changed. The trabecular number decreased (3.1 to 2.2 per mm), while trabecular thickness and separation increased (0.10 to 0.38 mm and 0.35 to 0.49 mm respectively). A drastic reduction of connectivity density was found (125 to 7). All these results reflect the transition from a fine-grained disorganized structure to a coarser grained organized trabecular structure.

These results provide more insight in the transformation of woven bone into mature lamellar bone during load adaptive bone remodeling.

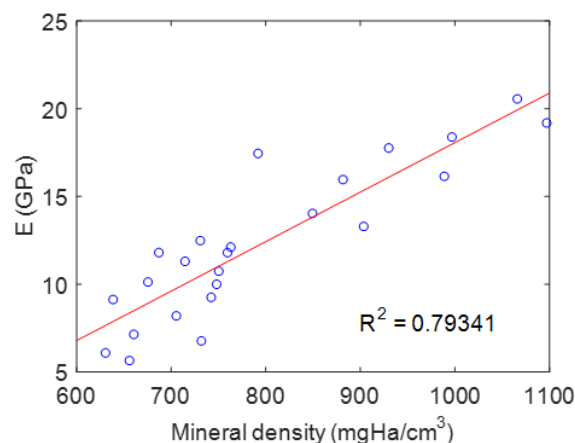


Figure 2. Elastic modulus measured versus mineral density in the same locations for all the samples

## References

1. Mora-Macías et al., J Mech Behav Biomed Mater, 74:236-244, 2017.

## Acknowledgements

This work was supported by the Spanish State Research Agency (AEI) [Grant No. PID2020-113790RB-I00 / AEI / 10.13039/501100011033]; and the European Regional Development Fund (FEDER) with the Consejería de Economía y Conocimiento, from the Junta de Andalucía [Grant No. UHU-202058]

