doi:10.1017/S1431927618014873
Microsc. Microanal. 24 (Suppl 2), 2018

Characterization of Zn-Substituted Carbonated Apatite Implanted in Rat Tibia by Synchrotron Radiation X-Ray Microtomography and 3D Image Analysis.

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Anionic and cationic substitutions in calcium phosphate have been intensively investigated in order to improve osteogenic properties of synthetic implants for bone regeneration [1]. Substitution of phosphates with carbonates in hydroxyapatite increases the structure dissolution rate and improves the implant bioresorption and bone formation. Additionally, zinc is an essential metal that plays a crucial role in bone metabolism [2].

In the present study, a zinc substituted carbonated apatite was synthesized and the influence of the zinc in bone regeneration was evaluated using Thermo Scientific™ Avizo™ software after image acquisition with synchrotron radiation x-ray microtomography. The measurements were conducted at IMX beamline at the Brazilian Synchrotron Light Laboratory (LNLS) in Campinas, Brazil. A CCD camera (PCO.2000) was used to acquire 1001 projections over a 180° rotation. Pink beam, with an energy peak around 11 keV, was used to illuminate the 1.6 mm cylindrical samples using count mode (250k) to guarantee the same flux on all projections. The reconstructed image was created using RAFT, an in-house reconstruction software, resulting in 3D data with a voxel size of 0.82 μm.

Experimental methods included the generation of zinc substituted carbonated hydroxyapatite (ZnCHA) microspheres from powdered ZnCHA nanoparticles using the biopolymer sodium alginate to achieve the spherical shape. Those ZnCHA microspheres were then implanted in the tibia of a rat and were then analyzed after 7 and 21 days. In addition to the synchrotron radiation x-ray microtomography of the implanted samples, a histomorphometric analysis of hematoxylin and eosin stained slides was conducted using light microscopy. The microtomography 3D data was analyzed with Avizo™ to obtain 3D statistics of the ZnCHA microspheres to achieve a descriptive analysis of the tissue response to the biomaterials. An assessment of the presence of connective tissue, new bone formation and microsphere resorption was the goal.

One week after implantation, small islands of newly formed bone had been formed around the ZnCHA microspheres. After three weeks, the microspheres had fragmented and an important region of new bone was observed connected to those fragments. The results indicate new bone growth at the surface of the old bone. It could also be shown that the microsphere's internal porosity had increased over implantation time to facilitate the integration into the bone structure.

An Avizo™ workflow consisting of filtering and a watershed-based segmentation method was generated that led to the desired results. The overall difficulty of analyzing the x-ray microtomography lied in the nature of similar contrasts of biodegraded ZnCHA microspheres and both the old and new bone structures, especially after three weeks. A labeled dataset showing this separation at three weeks was obtained using masking and watershed segmentation (Figure 1).

Additionally, after three weeks of implantation time, most of the microspheres had fragmented into small porous particles and a comparison of 7 days with 21 days can be seen in Figures 2a and b. The new bone grows from the cortical bone in direction to the microsphere fragments and the fragments itself interconnect with the new bone which could be visualized using volume renderings (Figure 2c).

ZnCHA was biocompatible and promoted osteoconduction, meaning bone growth from previous bone on a surface, but the presence of zinc did not induce completely new bone formation from the sphere itself. Using synchrotron radiation x-ray microtomography revealed the changes of the ZnCHA microspheres after implantation and led to relevant information on the dynamics of new bone formation. In a next step, the same study is being performed for a strontium-based carbonate hydroxyapatite (SrCHA) to understand similarities or differences in the bone behavior [3].

References:

[1] W Habraken *et al*, Materials Today **19** (2016), p. 69.
[2] S Dorozhkin, Materials Science and Engineering C **55** (2015), p. 272.
[3] The authors acknowledge funding from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). The Brazilian Synchrotron Light Laboratory and its researchers are thanked for many useful discussions and contributions to this work.

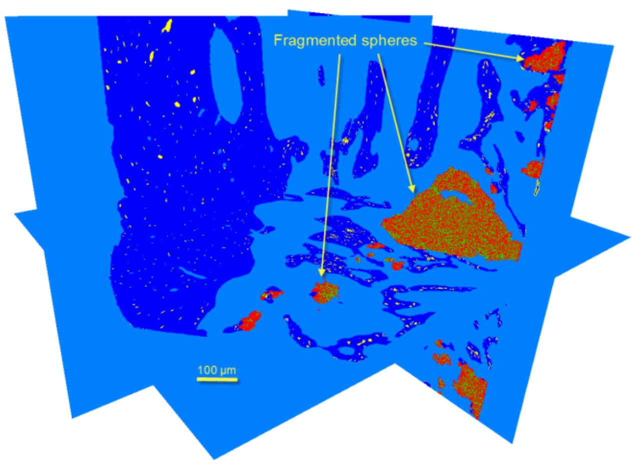

Figure. 1. Label field data of implanted ZnCHA microspheres into rat's tibia after three weeks showing the fragmented spheres (orange) with porosity using three orthogonal slices. The scale bar denotes 100 µm.

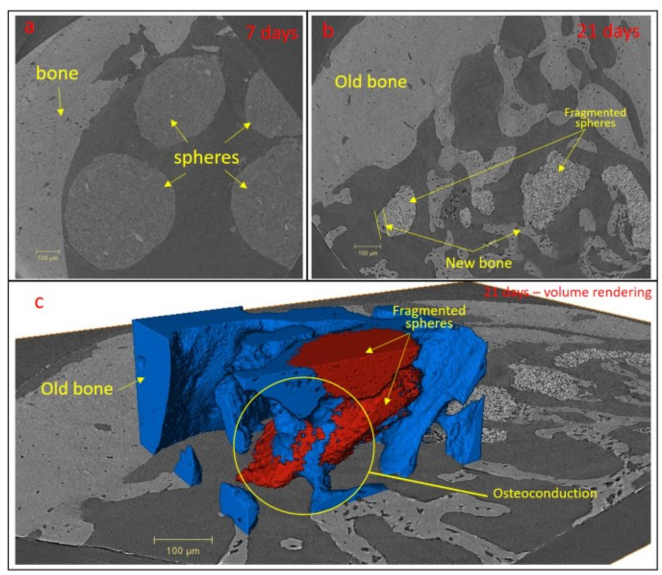

Figure. 2. Synchrotron radiation x-ray microtomography images and segmentation of implanted ZnCHA microspheres and its fragments over time. Greyscale images after a. one week and b. three weeks after implantation. c. Volume rendering of fragmented spheres showing osteoconduction at three weeks of implantation.

https://doi.org/10.1017/S1431927618014873 Published online by Cambridge University Press