Hierarchical HRP-crosslinked silk fibroin/ZnSr-doped TCP nancocomposites towards osteochondral tissue regeneration: Biomechanical performance and in vivo assessment

Viviana P. RIBEIRO[1], Sandra PINA[1], Raphael CANADAS[1], Sabina GHEDUZZI[2], Ana ARAúJO[1], Alain DA SILVA MORAIS[1], Carlos VILELA[3], Sílvia VIEIRA[1], Ibrahim F. CENGIZ[1], Rui L. REIS[4], J. Miguel OLIVEIRA[4]
[1]3B´s Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark, 4805-017 Barco, Guimarães, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal.
[2]Centre for Orthopaedic Biomechanics, Department of Mechanical Engineering, University of Bath, Bath, UK
[3]3B´s Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark, 4805-017 Barco, Guimarães, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal.; Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Portugal; Orthopedic Department, Centro Hospitalar do Alto Ave, Guimarães, Portugal; Dom Henrique Research Center, Porto, Portugal.
[4]3B´s Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Avepark, 4805-017 Barco, Guimarães, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; The Discoveries Centre for Regenerative and Precision Medicine, Headquarters at University of Minho, Avepark, 4805-017 Barco, Guimarães, Portugal.

INTRODUCTION:Recent investigations highlight promising regenerative strategies for osteochondral (OC) tissue treatment, such as hierarchical nanocomposite scaffolds containing ionic dopants.1,2 They allow cell infiltration and ECM formation throughout the engineered cartilage and subchondral tissues. The biomechanical behavior, antibacterial properties, and in vivo performance of hierarchical nanostructures combining enzymatically crosslinked silk fibroin (SF) and ZnSr-doped β-tricalcium phosphate (ZnSrTCP) for OC tissue regeneration is herein assessed.

METHODS:Hierarchical scaffolds were fabricated using horseradish peroxidase (HRP) crosslinked SF (HRP-SF) as the articular cartilage-like layer, and HRP-SF/ZnSrTCP as subchondral bone-like layer, through salt-leaching/freeze-drying techniques. The failure behaviour of the scaffolds was evaluated under combined compression and shear loading. Antibacterial properties of the scaffolds were assessed by adhesion and biofilm formation of Escherichia coli (E. Coli) and Staphylococcus aureus (S. aureus) on its surface. In vivo OC regeneration potential of the scaffolds was evaluated in rabbit knee critical size OC defects implanted for 8 weeks. Then, explants were fixed in 10% formalin for 7 days at 4 °C and decalcified, and stained with H&E for histological and immunofluorescent analysis using transmitted and reflected light microscope.

RESULTS:The scaffolds showed capability to support tension and shear stress upon loading until 60% deformation, with a tendency of improved mechanical properties for the ZnSr-doped scaffolds. Limited E. coli and S. aureus adhesion and biofilm formation was observed on the scaffolds surface. After scaffolds implantation in knee OC defects, no evidence of adverse foreign body reactions and good integration into the host tissue was observed. Histological and immunofluorescence analysis showed positive collagen type-II and glycosaminoglycans' formation in the articular cartilage layer, and new bone ingrowth and blood vessels infiltration in the subchondral bone layer.

DISCUSSION & CONCLUSIONS:The ionic-doped scaffolds presented good mechanical properties to allow cellularity for ECM mineralization and ingrowth. The scaffolds have shown capability of preventing bacterial adhesion to its surfaces and biofilm formation. SF layer supported cartilage regeneration in knee OC defects, while the ionic incorporation into the subchondral bone-like layer favoured calcified tissue formation. Thus, the biomechanical performance together with the in vivo results shows the efficacy of these scaffolds of OC tissue regeneration.

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References:1. V.P. Ribeiro, et al. ACS Appl. Mater. Interfaces 2019;11:3781. 2. S Pina, et al. Cells Tissues Organs 2017;204:150.

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