BAMOS project: Osteochondral scaffold innovation applied to osteoarthritis

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Abstract: Osteoarthritis (OA) is a degenerative joint disease typified by a loss of quality of cartilage and changes in bone at the interface of a joint, resulting in pain, stiffness and reduced mobility. BAMOS project, funded under the frame of the Horizon 2020 RISE program, aims to develop novel cost-effective osteochondral scaffold technology for early intervention of OA to delay or avoid the joint replacement operations. A multidisciplinary consortium of academic and non-academic partners from Europe and China collaborate in this project through research and innovation staff exchanges.

Keywords: Tissue Engineering; Regenerative Medicine; Bone; Cartilage; In vitro evaluation

Osteoarthritis (OA), also known as degenerative joint disease, is the most common form of arthritis. It involves gradual and progressive wearing of the articular cartilage, synovial membrane inflammation, and subchondral bone remodelling that generate osteochondral defects characterised by an unbalanced regeneration of articular cartilage and bone, where the intrinsic repair mechanisms are insufficient. BAMOS project particularly addresses the challenges in OA treatment by providing novel cost-effective osteochondral scaffold technology for early intervention of OA to delay or avoid joint replacement operations. The main objectives of the project have been [1]: a) Define clinical specifications for osteochondral (OC) scaffolds, b) Develop new OC scaffolds biomaterials, c) Develop innovative additive manufacturing techniques to produced patient-tailored OC scaffolds, d) Assess the OC scaffold in both in vitro and in vivo, and e) Train early-stage researchers in the context of collaborative research.

Different biomaterials have been evaluated for osteochondral (OC) scaffold manufacturing in the context of BAMOS project, including biodegradable polymers, ceramic materials, titanium alloys and naturally derived hydrogels. Multi-layered and multi-material hierarchical 3D structures with optimized designs for *in vitro* 3D models and OC tissue regeneration have been developed. One promising approach involved the use of horseradish peroxidase cross-linked silk fibroin as base material for the development of hierarchical osteochondral scaffolds [2]. While the cartilage layer of these novel biofunctional hierarchical scaffolds was solely composed of the enzymatically cross-linked silk fibroin (HRP-SF), the bone-like layer also contained a 20% (w/w) of zinc (Zn) and strontium (Sr)-doped tricalcium phosphate (TCP) (Figure 1.A). The proposed 3D structures have shown to be able to support combined compression-shear loadings for osteochondral tissue, possess a controllable porosity, memory-shape properties, and prevent bacterial biofilm formation. *In vitro* evaluation of the bilayered structures was carried out by using a co-culture system of human osteoblasts (hOBs) and human articular chondrocytes (hACs) [3]. Good adhesion, proliferation and extracellular matrix (ECM) formation was observed for both types of cells. Not only osteoblasts produced a mineralized ECM in the bone layer and chondrocytes showed glycosaminoglycan (GAG) deposition in the cartilage layer, but also the formation and adequate integration of an interface region was confirmed. The structural characteristics, mechanical properties and biological performance of these bilayered scaffolds make them potential candidates for applications in osteochondral

tissue regeneration, and as artificial extracellular matrices for *in vitro* osteochondral models. More recently [4], we explored the properties of silk fibroin, decellularized extracellular matrix and carbon nanotubes resulting in elastic and bioactive scaffolds for bone tissue engineering. Such promising carbon nanotubes-reinforced cell-derived matrix-silk fibroin hierarchical scaffolds stimulated the expression of osteogenic-related markers such as ALP, Runx-2, Col Iα and OPN. In brief, the developed 3D carbon nanotubes-reinforced cell-derived matrix-silk fibroin hierarchical scaffolds showed great potential for applications in bone tissue engineering.

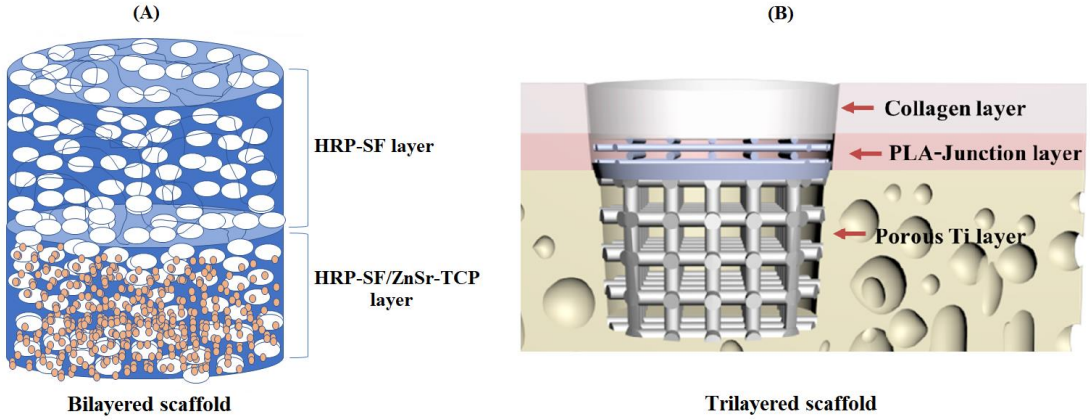

Figure 1. Main hierarchical osteochondral scaffolds developed in BAMOS.

Following a different design strategy, tri-layered osteochondral scaffolds were also developed by integrating various Additive Manufacturing (AM) technologies and biomaterials (Figure 1.B) [5]. Thus, a porous titanium matrix obtained by powder bed fusion of metal (DMLS) was used as bone layer. On the other hand, a collagen/PLGA composite layer, produced by casting and freeze-drying methods, was intended for cartilage regeneration. These two components were joined by a two-part junction polylactic acid (PLA) layer manufactured by material extrusion, commonly referred as fused deposition modelling (FDM). This junction layer served as calcified cartilage of the engineered OC scaffold, providing a graded structure in terms of morphological, mechanical and compositional features. The biocompatibility of the multilayer scaffold, as well as the cell viability and proliferation on the scaffold layers, was evaluated using sheep bone marrow mesenchymal stem cells (sBMMSCs). The *in vitro* assays performed for up to 14 days on the titanium, PLA and collagen-PLGA layers confirmed their biocompatibility. Results of the Live/Dead assay confirmed the viability of cells throughout 14 days of culture, with sBMMSCs evenly distributed in the samples. Finally, the proliferation analysis using the AlamarBlue^{TM} (Thermo Fisher, UK) reagent revealed a continuous increase of viable cells on the samples on days 1, 7 and 14.

Polymeric-based scaffolds obtained by 3D printing were also developed in BAMOS project to serve as support structures for the regeneration of subchondral bone in OC defects. Specifically, polycaprolactone (PCL) and PLA were used as matrices, to which natural or ceramic additives were incorporated to enhance their biofunctionality. Calcium carbonate and β-TCP particles were used as additives in the case of PLA-based scaffolds [6]. Apart from an increase of the porosity, hydrophilicity and surface roughness, composite scaffolds showed a very highly statistically significant ($p<0.001$) improvement of the metabolic activity of human osteoblastic osteosarcoma cells (SaOS-2) after 7 days of culture. Improved mechanical and biological properties were also obtained by introducing microcrystalline cellulose (MCC) as an additive to the PCL matrix when manufacturing 3D printed bone scaffolds [7]. The results of the proliferation assay of sBMMSCs cells after 1, 3 and 8 days of culture showed a significantly higher ($p<0.05$) value for the composite PCL-based scaffolds containing 2% MCC.

Another strategy explored in the context of BAMOS to improve the biological performance of PLA and PCL bone scaffolds involved the application of surface treatments to the 3D structures. In this sense, a novel surface treatment method has been developed, comprising the

use of oxygen plasma and the subsequent application of an *Aloe vera* bioactive coating. In the first published work regarding this method [8], Aloe vera-coated PLA scaffolds showed an improvement in terms of cell metabolic activity of human fetal osteoblastic (hFOB) cells cultured *in vitro* for 10 days. Surface-modified PLA- and PCL-based scaffolds have shown improved biofunctionality and thus, great potential to be applied for bone tissue regeneration or as a bone component of an OC scaffold.

Interestingly, different bioreactors for the *in vitro* and *ex vivo* culture of tissue engineered constructs, human osteochondral tissue, and other tissue interfaces (gradients) are being developed. These are of great interest for applications involving 3D dynamic cell culture, tissue engineering strategies, tissue modelling and personalised medicine.

Statements and Declarations:

Funding: The authors would like to thank H2020-MSCA-RISE program, as this work is part of developments carried out in BAMOS project, funded from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 734156.

Competing interests: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data availability: The data presented in this study are available on request from the authors.

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