Translation through collaboration: practice applied in BAMOS project in *in vivo* testing of innovative osteochondral scaffolds

Running title: *In vivo* tests of osteochondral scaffolds in BAMOS

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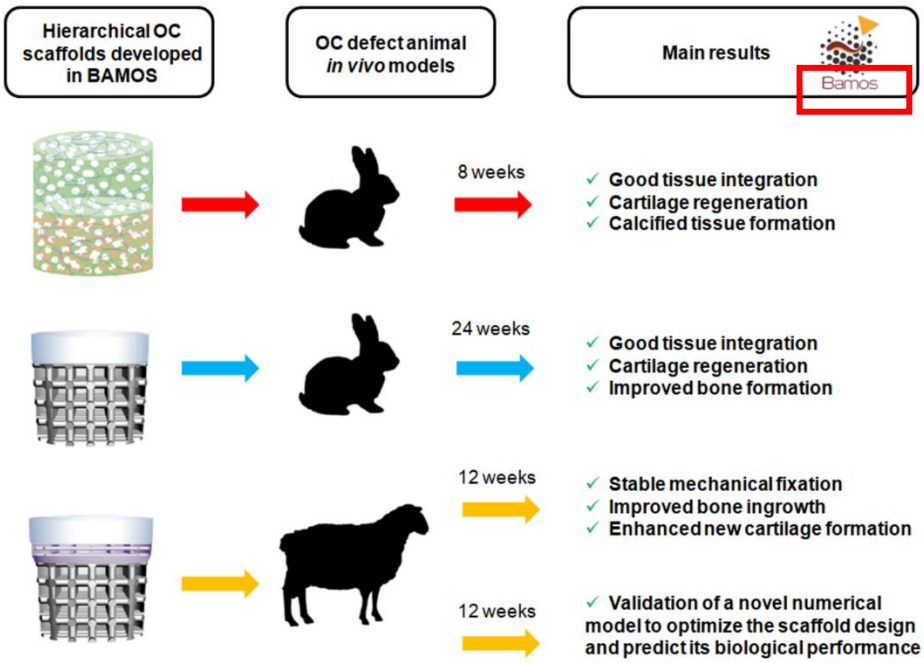
Abstract

Osteoarthritis is the most common chronic degenerative joint disease, recognized by the World Health Organization as a public health problem that affects millions of people worldwide. BAMOS project, funded under the frame of the Horizon 2020 RISE program, aims to delay or avoid the use of joint replacements by developing novel cost-effective osteochondral scaffold technology for early intervention of osteoarthritis. This project brings together five internationally leading research organisations (Universidad de Las Palmas de Gran Canaria, Spain; University of Minho, Portugal; University College London, UK; Xi'an Jiaotong University and Zhejiang University, China) and two healthcare providers (Royal National Orthopaedic Hospital, UK; and Saúde Atlântica - Gestão Hospitalar, S.A., Portugal) to work on the development, manufacturing and marketing of osteochondral scaffolds for the repair of large cartilage damages in osteoarthritis patients. The multidisciplinary consortium of BAMOS collaborates through research and innovation staff

exchanges. The project covers all the stages of the development before the clinical trials: design of scaffolds, biomaterials development, processability under additive manufacturing, *in vitro* test, and *in vivo* test. This paper reports the translational practice adopted in the project in *in vivo* assessment of the osteochondral scaffolds developed.

Graphical Abstract

The main results of the *in vivo* evaluations carried out in BAMOS project, funded under Horizon 2020 RISE program, are summarized. Animal models of osteochondral defect have been used to assess the biological performance of the different multimaterial and multilayered scaffolds developed.



Osteoarthritis is mainly characterized by articular cartilage progressive loss, osteophyte formation, synovial membrane inflammation and thickening of the subchondral bone, which leads to the generation of osteochondral (OC) defects with limited self-healing capacity. OC scaffold is a tissue engineering approach aiming to repair a joint defect and restore its function to delay or eliminate the need for a joint replacement. Although they have been established and are promising for the

treatment of small OC defects, no products to date have demonstrated the appropriate biomechanical properties required to promote successful long-lasting regeneration of large OC defects. BAMOS project addresses this challenge in osteoarthritis treatment [1] with the following main objectives: a) Define clinical specifications for OC scaffolds, b) develop new OC scaffolds biomaterials, c) develop innovative additive manufacturing techniques to produce 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.

Bilayered OC scaffolds: After a complete physicochemical and *in vitro* characterization, the different hierarchical OC scaffolds developed in the context of BAMOS project were evaluated using *in vivo* models. Thus, for example, a rabbit knee critical size OC defect model was used for assessing *in vivo* OC regeneration when implanting a horseradish peroxidase cross-linked silk fibroin-based (HRP-SF) scaffold [2]. These three-dimensional structures were prepared by combining two distinct layers: an HRP-SF cartilage-like layer, and a subchondral bone-like layer composed of HRP-SF and beta-tricalcium phosphate (β-TCP) particles doped with zinc and strontium (HRP-SF/ZnSr-β-TCP) [3]. After 8 weeks of implantation, the hierarchical scaffolds showed good tissue integration (with no signs of inflammatory reactions), cartilage tissue regeneration and calcified tissue formation. Formation of collagen type II and glycosaminoglycans in the HRP-SF articular cartilage-like layer was confirmed by histological analyses, while *de novo* bone ingrowth and blood vessels infiltration were observed in the HRP-SF/ZnSr-β-TCP bone-like layer [2]. These OC bilayered scaffolds have also shown to possess adequate structural integrity, memory-shape properties, and excellent mechanical and *in vitro* biological properties, even preventing bacterial biofilm formation [3], which in sum confirms the potential of these structures to be used in OC tissue engineering applications. Also using a rabbit model, bilayered scaffolds were implanted into OC defects created at the distal femoral trochlea and tested for 24 weeks [4]. These scaffolds were composed of a titanium (Ti) matrix that served as bone layer, and a collagen/poly(lactic-co-glycolic acid) layer intended for cartilage regeneration. We concluded that the mechanical support provided by the Ti layer promoted subchondral bone formation and new tissue integration, which led to better cartilage regeneration.

Trilayered OC scaffolds: Following a different approach, multi-material trilayered OC scaffolds were also developed in BAMOS by combining additive manufacturing and other conventional technologies [5]:

- Casting and freeze-drying methods were used to obtain a collagen/poly(lactic-co-glycolic acid) composite layer to act as cartilage-like layer.

- Material extrusion of polymers from a heated nozzle (MEX-TRB/P), commonly referred as fused deposition modelling, was used to manufacture a polylactic acid-based two-part junction layer that served as calcified cartilage of the hierarchical scaffold.
- Powder bed fusion of metal (PBF-LB/M) was used to produce a porous Ti matrix intended for bone regeneration.

The 12-week *in vivo* evaluation of the proposed OC trilayered scaffold, carried out in a sheep stifle condyle model, showed a stable mechanical fixation of the three-dimensional structure on the implantation site with no adverse effects observed on the surrounding tissues [6]. Improved bone ingrowth into the Ti matrix, as well as enhanced formation of hyaline-like cartilage tissue, were reported after histological examinations. The up-regulation of the cartilage-related markers aggrecan and collagen type-II confirmed the capacity of the proposed three-dimensional structures to regenerate cartilage tissue. In summary, the results obtained showed the potential of these cell-free scaffolds to be applied in the treatment of large OC defects.

Other *in vivo* tests carried out in BAMOS: Interestingly, the incorporation of bone marrow concentrate into the developed trilayered scaffolds has led to a non-significant improvement in bone regeneration when treating OC defects [7]. In this case, an ovine stifle condyle model was used during a 6-month test. Despite obtaining no significantly higher quantity of newly formed bone when using the Ti-polylactic acidPLA-poly(lactic-co-glycolic acid)/collagen scaffold, the results suggested that enhanced bone homogeneity and biomechanical durability were obtained when implanting the trilayered scaffolds (seeded with bone marrow concentrate), thus producing a higher quality of new subchondral bone tissue. Similarly, no statistically significant differences in terms of OC regeneration were obtained between collagen/hydroxyapatite scaffolds with or without bone marrow concentrate when tested *in vivo* using an ovine femoral condyle model for 6 months [8].

An ovine condyle model was also used to validate a novel numerical model developed in the context of BAMOS project, which is intended to optimize the scaffold design and material properties but also to predict its final biological performance [9]. The simulated cell distribution in the scaffold matched well with the *in vivo* regenerated bone tissue distribution. Therefore, this model could help reduce the number of preliminary time- and cost-consuming *in vivo* and *in vitro* tests needed to optimize the scaffold design.

Author contributions

MM: Resources, Writing—Original Draft, Project administration, Funding acquisition. Ricardo Donate: Writing—Original Draft, Visualization. Maryam Tamaddon: Writing—Review and Editing. Viviana Ribeiro: Writing—Review and Editing. Chaozong Liu: Resources, Writing—Review and Editing. J. Miguel Oliveira: Resources, Writing—Review and Editing. All authors approved the final version of this manuscript.

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Not applicable.

Conflicts of interests statement

The authors have no competing interests to declare.

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