Final Report

Project acronym: *BiogenInk*

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Publishable project summary

BiogenInk project aimed the development of bioinspired and bioresorbable inks for additive manufacturing, composed of marine collagen and ionic-doped calcium phosphates, as building blocks to produce advanced scaffolds towards bone regeneration, promoting innovation in health sector, mainly on orthopaedic therapies. During the execution of the project this was divided into four main tasks (i) extraction, purification, and characterization of the collagen and crosslinker factor, (ii) preparation of ionic doped calcium phosphates nanopowders (iii) optimizing materials and 3D printing and (iv) evaluation of biological performances.

BiogenInk consortium comprised three academic partners (UMinho, UPB, and WU) and one SME (REGEMAT 3D) from different European locations. UMinho had expertise in the processing and biological characterization of natural biomaterials for biomedical applications, and in particular for bone tissue engineering applications. UPB was focused in the assessment of mechanical and thermophysical properties of the developed materials, as well as of degradation in simulated fluids. WU was involved in the discovery and exploitation of marine bioactive molecules, namely in sponges and corals, here focusing on mariculture of sponges as sustainable production platform for biopolymers. REGEMAT 3D is a biotech company focused on using 3D printing technologies for regenerative therapies, pioneering the development of 3D printing, bioprinting and regenerative medicine solutions, at both technical and equipment development levels.

Collagen was extracted from the *C. reniformis* mesohyl, based on the protocol developed by Matsumura (1974) for sea cucumbers, and was characterized by SIRCOL assay, SDS-PAGE, and FTIR. Regarding the *C. reniformis* stiffening factor, the attempts to characterize the stiffening factor after extensive purification of a *C. reniformis* raw protein extract were not conclusive, as there was not enough quantity to obtain a good identification of the protein. Due to the lack of access to the marine sponges, it was not possible to obtain the *C. reniformis* stiffening factor, making it impossible to use it as a collagen crosslinker, as initially intended.

Regarding the calcium phosphate powders, they were obtained from calcination of codfish bones (Gadus morhua) captured in North Atlantic fishing-grounds and kindly offered by a local industry, Soguima (Guimarães, Portugal). After characterization, and considering the important biological role of strontium, zinc, silver, copper and cobalt, the CaPs extracted from fish bones were doped with these ions aiming to improve their biomedical performance.

Collagen extracted from the *C. reniformis* mesohyl was used the to prepare and optimize the collagen(s)/ CaP formulations for the 3D printing. Given the lack of the marine sponge-derived crosslinker factor, it was decided to use alginate mixed with collagen to study the printing process and to determine the printing parameters. Alginate is a non-toxic, biodegradable and biocompatible natural polysaccharide and it is reasonably easy to print, with the capacity to crosslink with divalent cations. After inks formulation has been optimized (collagen, alginate, CaPs, crosslinker concentration), the printing parameters were tuned and established to produce the 3D printed scaffolds. These scaffolds were then physical-chemically characterized, followed by the biological assessment with *in vitro* culture of Saos-2 cells, showing the better performance of the scaffolds produced by bioinks composed by *C. reniformis* collagen, alginate and codfish bones-derived calcium phosphates dopes with Sr, regarding printing fidelity, mechanical properties and cell adhesion and proliferation.