Bioreactor platform combining perfusion and PEMF stimulation for in vitro bone research

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Background In bone tissue engineering, bioreactors represent powerful tools for developing functional substitutes to be used as *in vitro* models for bone research [1]. *In vitro*, flow-induced shear stress promotes bone cell proliferation, differentiation, and matrix mineralization [2]. In clinical practice, pulsatile electromagnetic field (PEMF) stimulation is empirically adopted to foster bone healing [3].
Aim Development of a bioreactor platform, combining tunable perfusion and PEMF stimulation, for studying the bone response to combined biophysical stimuli.
Material and Methods The 3D-printed bioreactor allows housing cylindrical scaffolds of different size. Uni-/bi-directional flow (0.006-24 ml/min) can be applied for cell seeding or perfusion culture. A commercial device provides PEMF stimulation (1.5 mT, 75 Hz). Fluid flow and magnetic field within the bioreactor were modelled (COMSOL). Human mesenchymal stem cells were seeded into commercial scaffolds and cultured for 15 days under unidirectional perfusion (0.3 ml/min).
Results and Discussion In-house tests and simulations confirmed that the bioreactor prevents air bubble entrapment and recirculation regions, with shear stress values (0.8-7.8 mPa) in the range known to promote calcium deposition [4]. Simulations showed that the construct is exposed to a homogeneous magnetic field of 1.5 mT. In biological tests, direct perfusion significantly increased alkaline phosphatase release compared to control. Tests under combined stimulations are ongoing.

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Magneto-responsive core-shell microbeads for engineering peristalsis and alveolar breathing in-vitro

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Background Physiologically relevant in-vitro models need to reproduce both structural and mechanical features of the in-vivo environment. However, despite the complexity of current systems, to date, the replication of three-dimensional stretching is a challenge [1, 2].
Aim For this reason, we developed a new bioprinting strategy to obtain core-shell structures, able to replicate the structure and motility of the intestinal and alveolar barrier thanks to magneto-responsive materials.
Material and Methods. A core-shell microbead (COSMIC) generator was designed and fabricated using commercial coaxial needles. The core-shell structures were characterised using 1 and 2% w/v alginate in the shell and i) air, ii) 0.1% liquid pluroinic or iii) 1 - 2 % w/v FITC-alginate (Creative PEGWorks, USA) in the core. A 0.1 M calcium chloride (CaCl2) solution was placed under the needle to allow alginate crosslinking. Spheres dimension was quantified in function of different extrusion velocities (10, 20, 40 μL/s) using brightfield and fluorescence images (Olympus, Japan) and the Image-j software. The deformation of 0.5 and 0.75% w/ agarose gels loaded with 5, 10 or 15% w/v magnetite nanoparticles was measured using image analysis [3]. Cell viability tests were performed using Caco-2 cells with an encapsulation density of 1 million/mL.
Results and Discussion Results show that our strategy is suitable to obtain cell-laden core-shell structures, which can be embedded in magneto-responsive gels to mimic physiological strain and deformation mechanisms. Thus, this study represents a step further towards the definition of physiologically relevant in-vitro models, which improve the translation between research and clinical applications and also have the potential to reduce animal *tests as required by EU directives.*

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