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Two-layer Membrane of Calcium Phosphate/Collagen/PLGA Nanofibres with a Composition Similar to Bone: in vitro Biomineralisation and Osteogenic Differentiation of Human Mesenchymal Stem Cells

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INTRODUCTION: Guided bone regeneration is one of the present challenges in orthopaedic and dental surgery. Implants need to be osteoinductive and it would be advantageous to have an implant material presenting a chemical composition and a structure as close as possible to those in bone tissue. Dry bone tissue consists of hydroxyapatite (75 wt%) and collagen Type I fibrils (25 wt%). Collagen has a large range of biomedical appliance due to its antigenicity and fibre forming ability. Furthermore, collagen can be used as carrier of bioactive components such as calcium phosphates. The latter are widely used in biomaterials as they are biocompatible, and osteoconductive. In the present study the elaboration of an anisotropic bilayer is described.

METHODS: Amorphous calcium phosphate (a-CaP) nanoparticles, produced by flame spray synthesis, were combined via electrospinning with collagen (Col) Type I and poly(lactide-co-glycolide) (PLGA). The fibres' transformation during crosslinking and biomineralisation was investigated. To obtain a bifunctional membrane dyed a-CaP/Col/PLGA fibres were electrospun on top of pure PLGA. A cell culture study with human mesenchymal stem cells was conducted to analyse differentiation of the cells and exclude any cytotoxic effects of the scaffolds by alamarBlue, alkaline phosphatase activity and confocal laser scanning microscopy. Ca and collagen contents were followed by Alizarin red S and Sirius red staining.

RESULTS: The fibres' morphology depended on the chemical composition. Fibres that contained PLGA were stable enough to undergo crosslinking and biomineralisation experiments. The surface appearance of fibres that contained a-CaP dramatically changed after biomineralisation. The double membrane presented PLGA fibres on its white side and a-CaP/Col/PLGA fibres on the blue side (Fig. 1a-c). *In vitro* proliferation of the cells seeded on the membrane was successful and neither side showed cytotoxicity. Differentiation into the osteogenic lineage was better than in 2D control. Further an augmented content of Ca and collagen was confirmed.

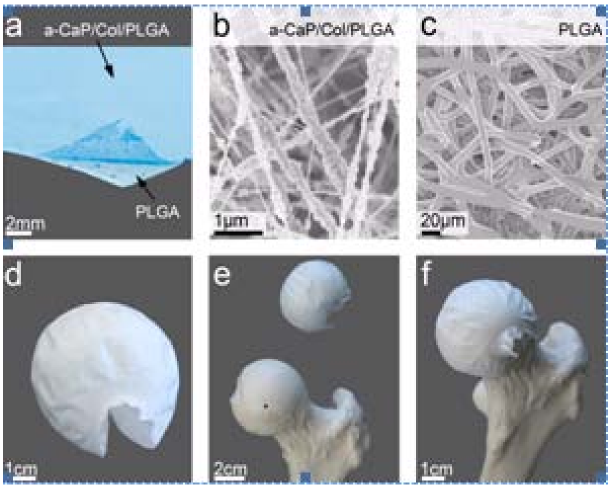

Fig.1 (a) Bilayer with dyed a-CaP/Col/PLGA (SEM in (b)) on one side and PLGA (SEM in (c)) on the other. The bilayer can be electrospun into a spherical shape (d) that can be positioned on a femur head model (e and f).

DISCUSSION & CONCLUSIONS: a-CaP nanoparticles were combined with collagen in a weight ratio similar to the chemical composition of bone tissue and the fibres were strengthened with PLGA. The hydroxyapatite formation indicates the osteoconductivity of the a-CaP-scaffolds. The anisotropic double membrane is easy to handle and can be shaped to be used as a bone wound dressing material. (Fig. 1d-f)

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