Influence of Flow in The Adhesion and Proliferation of Cells on Hydroxyapatite Integrated in a Microscale Culture

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INTRODUCTION: Synthetic biomaterials, such as calcium phosphate cements (CPCs), are a promising alternative to autologous bone to enhance bone regeneration. Calcium-deficient hydroxyapatite (CDHA), the end-product of apatite cements, matches the inorganic phase of the bone and exhibits excellent biocompatibility *in vivo* [1]. However, *in vitro*, CDHA uptakes calcium ions (Ca^{2+}) from cell culture medium [2], causing detrimental effects on cell activity and function [3]. The aim of this work was to integrate CDHA into a microfluidic chip that provides continued culture medium supply, and to evaluate cell adhesion and proliferation as compared to standard well plates.

METHODS: CDHA was integrated in a polydimethylsiloxane (PDMS)-glass microfluidic chip (CDHA-on-chip). PDMS was cured in a 3D-printed mould at 60°C for 2h. α-tricalcium phosphate was mixed with 2.5% w/v $Na_2HPO_{4(aq)}$ (liquid-to-powder of 0.65 ml/g) and the CPC was cast within a PDMS pocket. The CPC was immersed in an aqueous solution at 37°C for 10 days to ensure full transformation to CDHA. Through plasma treatment, a glass slide was bonded to the PDMS holding the CDHA, thus forming a 0.5mm channel above the CDHA. CDHA samples were pre-incubated for 24h in minimum essential media (MEM) supplemented with 10% FBS and 1% penicillin-streptomycin (sMEM). Pre-osteoblasts (MC3T3-E1) were seeded at 50,000 cells/cm^2 and after a cell adhesion period of 2h, flow was applied for 72h through the chip at different rates: 2, 8 and 14 µl/min. A static (0 µl/min) chip condition was included, where sMEM was manually replaced every 24h. CDHA discs (⌀=6mm, h=2mm) placed in a 96-well plate were used as a standard static control (200 µl sMEM replaced every 24h). At 6h and 72h, the cells were stained with a calcein, propidium iodide and Hoechst triple-stain to assess their adhesion and proliferation, respectively. In a separate experiment, sMEM was flown through the chips for 24h at the aforementioned flow rates, and Ca^{2+} concentration was quantified via inductively coupled plasma-optical emission spectroscopy (ICP-OES). As control, sMEM in contact with CDHA discs for 24h was evaluated.

RESULTS: A larger number of cells adhered on the CDHA-on-chip under flow as opposed to both static CDHA-on-chip and CDHA disc in a well plate. Differences in cell adhesion between the flow conditions were negligible. Cell proliferation at 72h was significantly increased under flow compared to CDHA disc samples (Fig.1A). Static CDHA-on-chip showed almost no viable cells. 2 and 8 µl/min flow conditions showed the greatest cell counts, followed by the 14 µl/min flow condition. At higher flow rates, Ca^{2+} concentrations were closer to in fresh medium (Fig.1B).

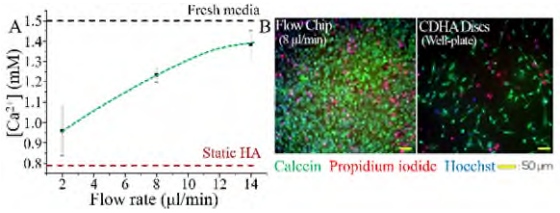

Fig. 1: A) Calcium concentration in sMEM. B) Stained cells after 72 h of culture.

DISCUSSION & CONCLUSIONS: The static CDHA-on-chip and disc samples displayed a low degree of cell adhesion and proliferation, which seemed to indicate that ionic exchange led to detrimental cell behaviour. Cells displayed the greatest degree of adhesion and proliferation at a flow rate of 2 and 8 µl/min, probably due to more optimal Ca^{2+} concentrations. At 14 µl/min, the degree of cell adhesion and proliferation decreased, which could be ascribed to adverse effects of shear stress.

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