BONE BIOMIMETIC 3D SCAFFOLDS AS PLATFORMS TO ELUCIDATE THE BEHAVIOR OF HUMAN FETAL OSTEOBLAST CELLS WITH PC3 A PROSTATE CANCER CELL LINE

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Background: Prostate cancer is the second leading cause of death in men worldwide[1]. It arises *in situ* in the prostate but can spread elsewhere in the body with prevalence in the bone. External and microenvironmental stimuli influence cell behavior and responses to drug treatment[2]. 2D cell-cultures are relatively cheap and practical but fail to recapitulate cell behavior *in-vivo*[4]. 3D biomimetic scaffolds more closely resemble the natural composition and cues in the cells' microenvironment. Here, we aim to study the behavioral responses of hFOB 1.19 (human fetal osteoblasts) in mono and coculture with PC-3, a prostate cancer cell line using 3D porous scaffolds composed of PLGA and nano-hydroxyapatite (nHA), a bony mineral.

Methods: Scaffolds are produced following a multi-sequenced process which include: i) tableting of powdery mixtures, ii) CO_2-foaming, iii) leaching of the porogen (NaCl). Three batches of scaffolds were produced: i) Plain PLGA, ii) 2mg nHA/PLGA, iii) 4mg nHA/PLGA. Cell viability was assessed by quantifying the amount of DNA produced (Picogreen) at Day 3 and Day 7. The test was first carried in 24 well-plates in mono ($2x10^4$ cells/well) and coculture ($2.5x10^4$ cells/well hFOB 1.19/PC-3 ratio 4:1) prior to conduct it in 3D scaffolds ($2x10^5$ cells/scaffold, hFOB 1.19/PC-3 ratio 4:1). Scaffold colonization was assessed by performing histology after staining hFOB 1.19 and PC3 with fluorescent dyes DiO and DiI. hFOB 1.19 differentiation behavior was evaluated by staining of alkaline phosphatase (Fast-Blue) prior to imaging. Collagen production in coculture was assessed by staining the different scaffolds slides at Day 7 with 0,5% Fast Green FCF. Ongoing studies are focusing on assessment of PC3 and hFOB 1.19 behavior (RT-qPCR) and quantification of live/dead cells pre- and post-treatment with Docetaxel (10nM).

Results: 3D mono- and cocultures confirmed cell viability over 7 days. 4mg nHA/PLGA scaffolds provided higher viability at Day 7 corresponding to higher amount of DNA released in comparison with the other batches examined. DiO/DiI staining confirmed the cells successfully colonized the scaffolds and migrated deeply in the structure. Fast-Blue staining confirmed the presence of differentiated hFOB 1.19 in the scaffolds. Fast Green FCF staining assessed the cells in coculture produced collagen with the higher amount produced in the scaffolds with the highest nHA loading. Further quantification (RT-qPCR) is ongoing to investigate whether it is the amount of nHA or the presence of PC3 to have impacted on the differentiation rate of osteoblasts. Preliminary results on 3D cytotoxicity (Picogreen) showed Docetaxel is cytotoxic to the cells in coculture after 72h treatment compared to relative controls (no drug applied) in all the batches produced. Ongoing studies are also quantifying the cytotoxic effect between 2D and 3D but first indicative results show that Docetaxel seems less effective in 3D.

Conclusions: nHA/PLGA-mixed scaffolds can be used as biomimetic models to elucidate cells' behavior in metastatic prostate cancer. nHA loadings can impact on the viability of the cells. Additional studies are assessing the impact of nHA loadings on the differentiation of hFOB 1.19 cells. Ongoing studies are focusing on the impact of Docetaxel on cells in pre- and post-treatment phase in both 2D and 3D.