DEVELOPMENT AND CHARACTERIZATION OF 3D HYDROXYAPATITE-PLGA SCAFFOLDS TO STUDY METASTATIC PROSTATE CANCER IN THE BONE

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Background: Bone-related cancers can arise directly in the bone structure such as primary cancers osteosarcoma or result from metastatic spread from other primary sites. Prostate cancer is a metastatic type of cancer and the second most frequent malignancy in men worldwide, which accounted for nearly 3,8% of all cancer-related deaths in men in 2018. Advancements of new oncology treatments are poor and research has focused on *in-vitro* models to address the need to explain disease pathophysiology and to develop more effective clinical therapies. Still, 70-80% of *in vitro* cancer studies are reported to be conducted using conventional 2D cell-culture models despite the fact that they fail to recapitulate the properties of native tissue and that cancer cells exhibit markedly different behavior in vivo compared to in 2D cell-cultures. 3D models which recreate the cues in the biological environmental are increasingly of interest.
Here we aim to develop a physiologically relevant 3D model of the bone using PLGA (polylactic-co-glycolic acid 85:15) mixed with nHA (nano-hydroxyapatite), which is chemically similar to bone mineral. The scaffolds will be used to study more effectively metastatic prostate cancer in the bone by conducting 3D culture studies of hFOB 1.19 (human fetal osteoblasts) and PC3 (prostate cancer cell line) in mono and co-culture.

Methods: Polymer microspheres were obtained by electro spraying, a process by which a solution of 3,5% PLGA in DCM is extruded through a nozzle by application of high voltage (15 kV). The microspheres produced were used to obtain different types of scaffolds: A) Plain PLGA B) 2mg nHA/PLGA C) 4mg nHA/PLGA, using NaCl as porogen. After the powder mixture was compressed (1500 psi) and foamed in CO_2 (800 psi, 24 h), the salt porogen was leached to produce a porous scaffold. For cell culture studies, scaffolds were sterilized with multiple washings of 70% ethanol, PBS and FBS and left to dry prior to use. Microspheres and scaffolds were characterized by SEM. The scaffolds porosity was calculated following immersion in deionized water (30 min). Prior to 3D cultures, common 2D mono and co-culture controls were set up: 20,000 cells/well for PC3; 20,000 or 40,000 cells/well for hFOB 1.19. Cell metabolic activity was conducted by performing MTT assay. hFOB 1.19 behaviour was tested by staining ALP and quantified with pNPP. Current 2D co-culture studies (25,000 cells) were conducted at two different ratios (hFOB 1.19: PC,3) of 4:1 and 1:1 at 33.5°C and 37°C.

Results: The SEM characterization of microspheres indicated particles has a collapsed, spherical morphology with a size in the range2-6 µm. SEM characterization of scaffolds revealed the inner porous structure diameter was approximately 300 µm. The average porosity of the scaffolds was 55%±3.2 (plain PLGA), 48,2%±5,58% (2mg nHA/PLGA) and 60,91%±12,44 (4mg nHA/PLGA). 2D monocultures indicated that both the cells lines were viable after 7 days. Based on ALP staining and pNPP test hFOB 1.19 demonstrated higher differentiation at higher temperature (37°C), cell density (40,000 cells/well) and in co-culture at 4:1 ratio when compared to 1:1. Studies on mono and co-culture in 2D and 3D are undergoing investigation, however, first indicative results suggest hFOB 1.19 display a better growth profile in hHA/PLGA scaffolds compared to the one in plain PLGA scaffolds.

Conclusions: A porous, composite scaffold was produced and sustained hFOB 1.19 and PC3 growth for 7 days. 2D mono and co-culture studies show that cell behavior is influenced by seeding density, ratio of different cells and incubation temperature. This suggests that initially, PC3 could generate an osteoblastic lesion in the bone leading hFOB 1.19 to deposit more bone mineral. 3D co-culture studies are currently under investigation.