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 or result from metastatic spread from other primary sites. Prostate cancer exhibits metastatic behaviour and is the second most frequent malignancy in men worldwide [1]. Research has focused on *in-vitro* models to better explain disease pathophysiology and develop more effective clinical therapies. Most of the *in vitro* studies are reported to be conducted using conventional 2D cell-culture models which lack the ability to recapitulate the properties of native tissue or cell behavior especially for cancer cells [2]. Bio-relevant 3D models are increasingly of interest [3]. 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), a bony mineral. The 3D model will be used to more effectively investigate drug treatments for metastatic prostate cancer in the bone.

Methods

RESEARCH PHASE

MICROSPHERES

ELECTROSPRAYING: Polymer microspheres were obtained by extrusion of a solution of 3.5% PLGA in DCM through a 300 µm nozzle by application of high voltage (15 kV). The distance applied during the process was 10 cm and flow rate 1ml/hr.

SCAFFOLDS

A) Plain PLGA, **B)** 2mg nHA/PLGA, **C)** 4mg nHA/PLGA.

Scaffolds were prepared using NaCl as porogen.

COMPRESSION and FOAMING: the powder mixture was compressed (1500 psi) and foamed in CO_2 (800 psi, 24 h).

LEACHING: the salt porogen was removed with deionized water to produce a porous scaffold.

CHARACTERIZATION

Microspheres and scaffolds were characterized by SEM at 5kV.

The samples were sputter coated with gold and analyzed at 70X magnification.

Porosity of the scaffolds was determined following immersion in deionized water for 30 min.

2D CELL CULTURE

Cell culture studies were carried using 20,000 cells/well for PC3; 20,000 or 40,000 cells/well for hFOB 1.19. Cell metabolic activity was determined with MTT at two temperatures (33.5°C, 37°C) at day 3 and day 7.

hFOB 1.19 BEHAVIOUR

- ALP Staining
- pNPP QUANTIFICATION

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

SEM CHARACTERIZATION

Microspheres have a collapsed, spherical morphology with a size in the range 2-6 µm. Scaffolds have a porous structure and the inner porous diameter was approximately 300 µm (**Fig. 1**).

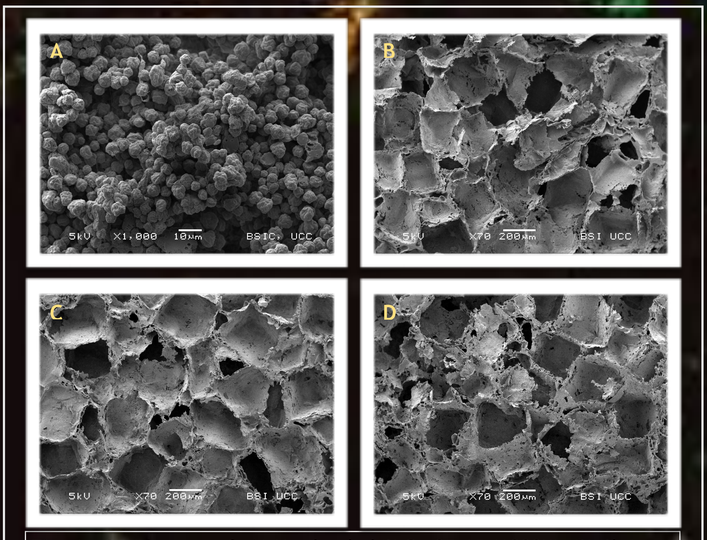

Fig. 1 - SEM Images of: **A)** Not loaded PLGA 85:15 (1000X); **B)** Plain PLGA Scaffold (70X); **C)** 2 mg nHA/PLGA scaffold (70X); **D)** 4 mg nHA/PLGA scaffold (70X).

POROSITY

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) (**Fig.2**)

SCAFFOLD	Av. POROSITY %	Std. DEVIATION
Plain PLGA	55,61%	3,20
2mg nHA/PLGA	48,20%	5,58
4mg nHA/PLGA	60,91%	12,44

Fig. 2 - Average porosity of Plain PLGA scaffolds, 2 mg nHA/PLGA scaffolds, 4mg PLGA scaffolds. Porosity was registered after the scaffolds were immersed for 30 min in deionized water.

CELL CULTURE

2D CELL CULTURE - VIABILITY

MTT assay on 2D monocultures indicated that both the cell lines were viable after 7 days. Results showed 2D co-cultures at different ratios and temperature are metabolically active after 7 days in co-culture (**Fig 3**).

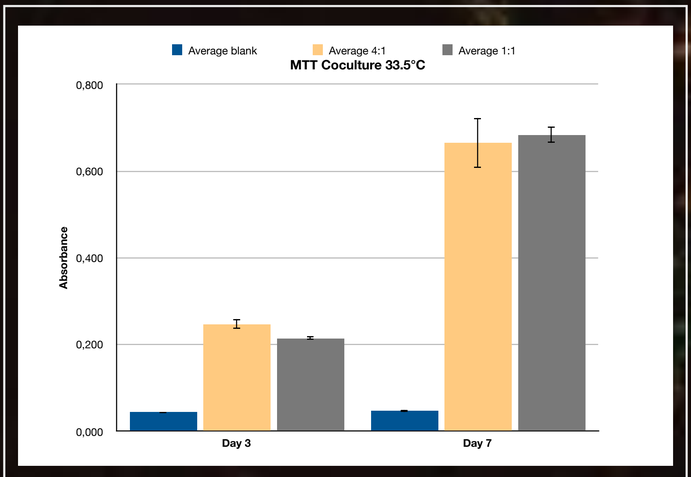

Fig. 3- Metabolic activity (MTT) expressed by cells in co-culture at different time points (Day 3 and Day 7) and different temperatures (33.5°C, 37°C).

2D CELL CULTURE - BEHAVIOUR

ALP staining and pNPP test hFOB 1.19 demonstrated higher differentiation at higher temperature (37°C) (**Fig. 4**), cell density (40,000 cells/well) and in co-culture at 4:1 ratio when compared to 1:1 (**Fig. 5**)

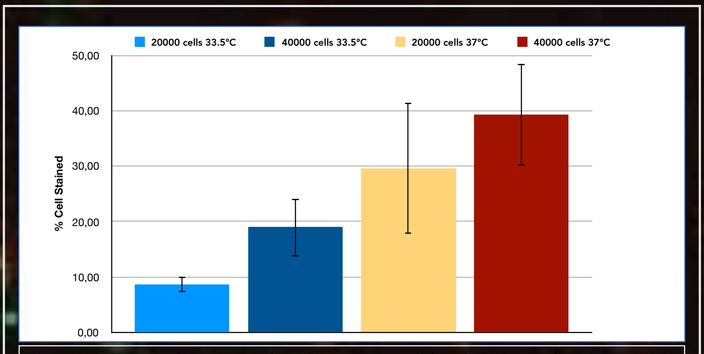

Fig. 4 - Quantification of ALP expressed by hFOB 1.19 with pNPP: **LIGHT BLUE**) 20000 cells 33.5°C, **BLUE**) 40000 cells 33.5°C, **YELLOW**) 20000 cells 37°C, **RED**) 40000 cells 37°C.

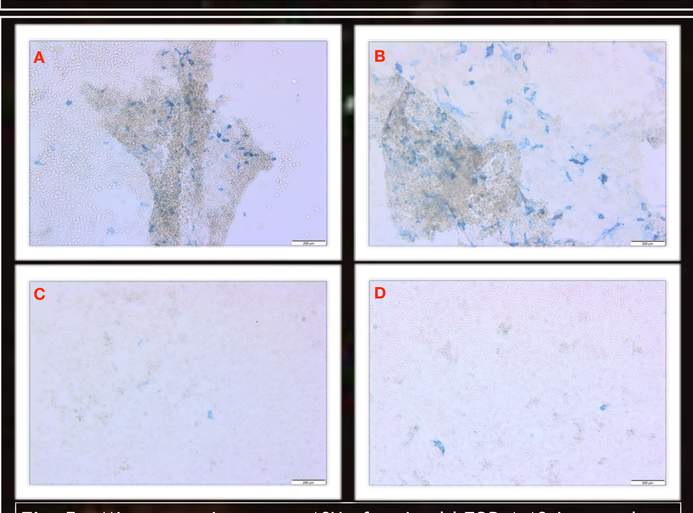

Fig. 5 - Microscope images at 10X of stained hFOB 1.19 in co-culture (25000 cells/well) with PC-3 at different hFOB/PC-3 ratios and different temperatures: **A**) 4:1 ratio 33.5°C, **B**) 4:1 ratio 37°C, **C**) 1:1 ratio 33.5°. **D**) 1:1 ratio 37°C.

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. 3D mono and co-culture studies are currently under investigation.

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