0246 Investigating the interactions between breast cancer cells and bone microenvironment by a polyurethane foam 3D *in vitro* model

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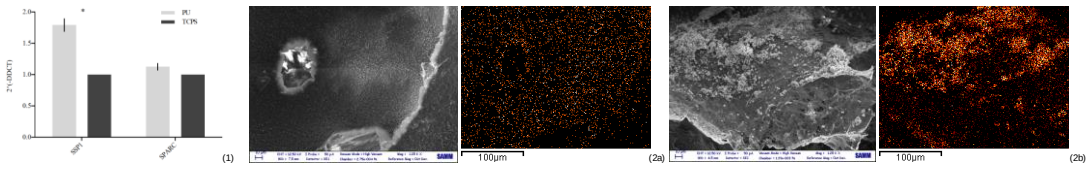
Breast cancer represents the most incident cancer case in women (29%), with high mortality rate. Bone metastases occurs in 20-50% cases and, despite advances in breast cancer research, the interactions between tumor cells and the bone metastatic microenvironment are still poorly understood. *In vitro* 3D models gained great interest in cancer research thanks to the reproducibility, the 3D spatial cues and associated low costs, compared to *in vivo* and 2D *in vitro* models.

In this study, we investigated the interactions between breast cancer stem cells and the bone microenvironment by using a poly-ether-urethane foam (PU) as 3D *in vitro* model.

The PU foam used as an *in vitro* model was synthesised by reacting an optimised poly-ether-polyol mixture with isocyanate MDI prepolymer, using Fe-AcetylAcetonate as catalyst and water as foaming agent[1]. The measured PU foam open porosity (> 70%) proved to be suitable to mimic the trabecular bone structure. PU foam showed good and stable mechanical properties under cyclic compression, even if lower than human trabecular bone. The *in vitro* bone metastasis model was produced by seeding on the PU foam patient-derived human adipose derived stem cells (ADSCs) and by differentiating them into osteoblasts for four weeks; subsequently, breast cancer derived stem cells (MCFS) were co-cultured on the PU foam with differentiated ADSCs for three weeks. Successful ADSCs attachment and elongation was shown by hematoxylin-eosin staining. Moreover, efficient osteoblastic differentiation was proved both by inorganic matrix deposition evidenced by alizarin red staining and RT-PCR (Fig1); a significant increase of osteopontin levels was shown in cells differentiated on PU foam compared to those differentiated on control tissue culture plastics (TCPS), suggesting that the PU foam can recreate a more physiological-like and biomimetic microenvironment for osteoblastic differentiation than TCPS surface.



(1) (2a) (2b)

Tumor cells agglomerates were identified on PU foam co-cultured with ADSC/MCFS by e-cadherin staining. SEM/EDX images showed a homogeneous and well-distributed deposition of Ca and P sub-micrometric particles on PU foam seeded only with ADSCs (Fig2a, Ca in red), confirming osteoblastic differentiation. On the contrary, when breast cancer derived stem cells were co-cultured with differentiated ADSCs, tumor cells agglomerates were observed (Fig2b) and a qualitative desorption of Ca and P particles in some areas of the PU foam pore walls was detected. The PU foam demonstrated to be a suitable model for reproducing a bone biomimetic microenvironment *in vitro*, allowing for the co-culturing of differentiated ADSCs and MCFS, and for the investigation of their interaction.

[1] Bertoldi S *et al.* J Mater Sci Mater Med. 2010;21(3):1005-11.