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 agent¹. 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.

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.