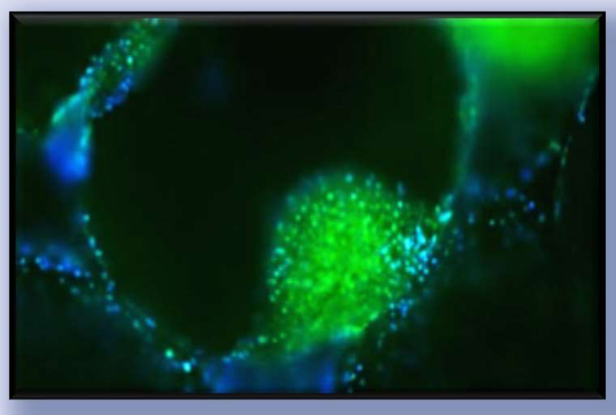
IN VITRO 3D SCAFFOLD-BASED OSTEOSARCOMA MODELS AS TUMOR ENGINEERING APPROACH AGAINST CANCER STEM CELLS NICHE



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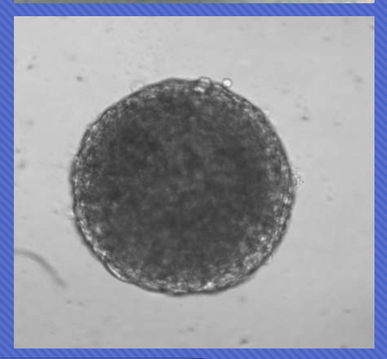
INTRODUCTION

Osteosarcoma (OS) is the most common type of bone tumour diagnosed in children and young adults[1]. The lack of specificity for Cancer Stem Cells (CSCs) subpopulation together with the poor *in vitro-in vivo* translation ability of traditional two-dimensional (2D) *in vitro* models have been recently identified as the main limitations of conventional therapies[2,3]. This work provides "Tumour Engineered" three-dimensional (3D) osteosarcoma models as new tools to improve therapy outcomes.

MATERIALS AND METHODS

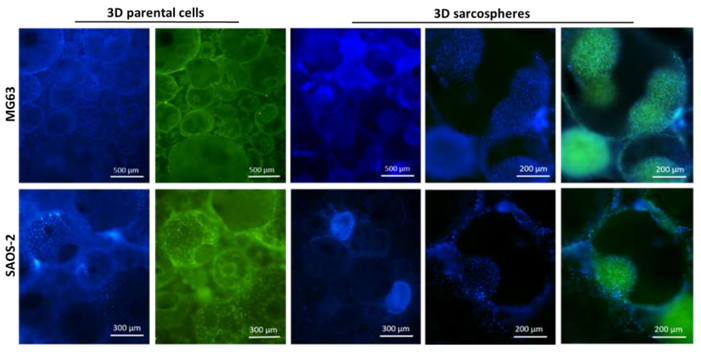
Two different hydroxyapatite-based scaffolds[3,4] to recapitulate *in vivo* 3D bone extracellular matrix (ECM) were used: a porous ceramic scaffold (HA) and a hybrid biomineralized type I collagen scaffold (MgHA/Coll). The sphere-forming culture[2] was used on MG63 and SAOS-2 osteosarcoma cell lines to enrich CSCs under spheroidal phenotype. CSC-enrichment was confirmed by gene expression of stemness genes (OCT-4, NANOG and SOX-2) of scaffold-free spheroids by qRT-PCR. The *in vitro* 3D scaffold-based spheroids were investigated by morphological evaluation, qRT-PCR and immunofluorescence of stemness and CSC niche-related genes (NOTCH-1, HIF-1α and IL-6). Actually, the variability of tumoral properties in serial spheroids passaging[5] is being investigated by analysis of proliferation, sphere-forming efficiency, migration/invasion ability, and qRT-PCR under scaffold-free conditions. Various 3D cell seeding approaches are being considered.

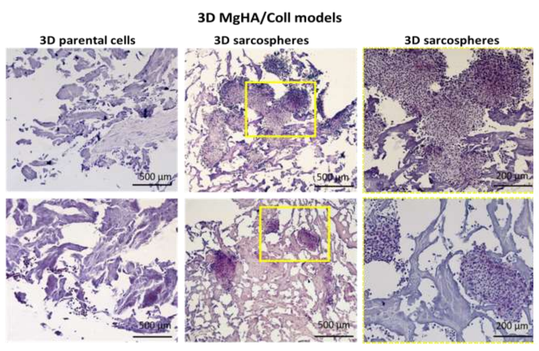


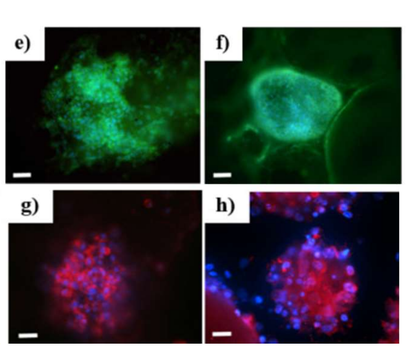
RESULTS AND DISCUSSION

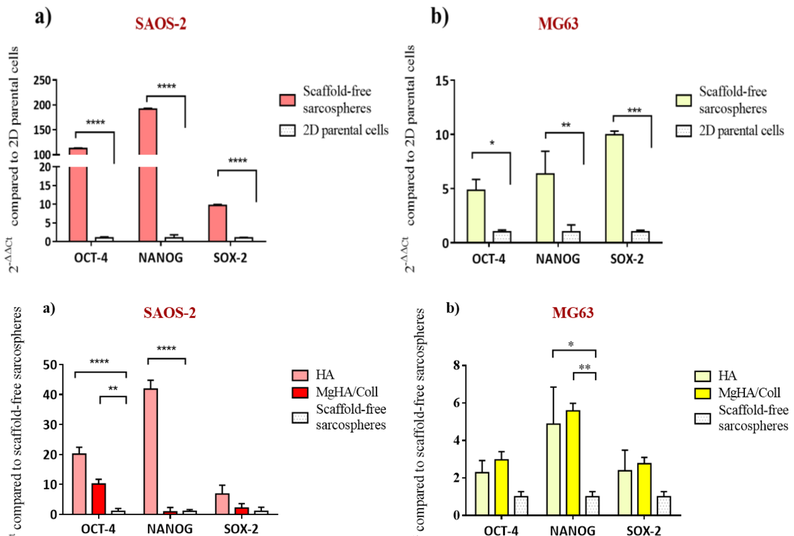
The enrichment CSCs was confirmed by expression of stemness markers on scaffold-free spheroids.

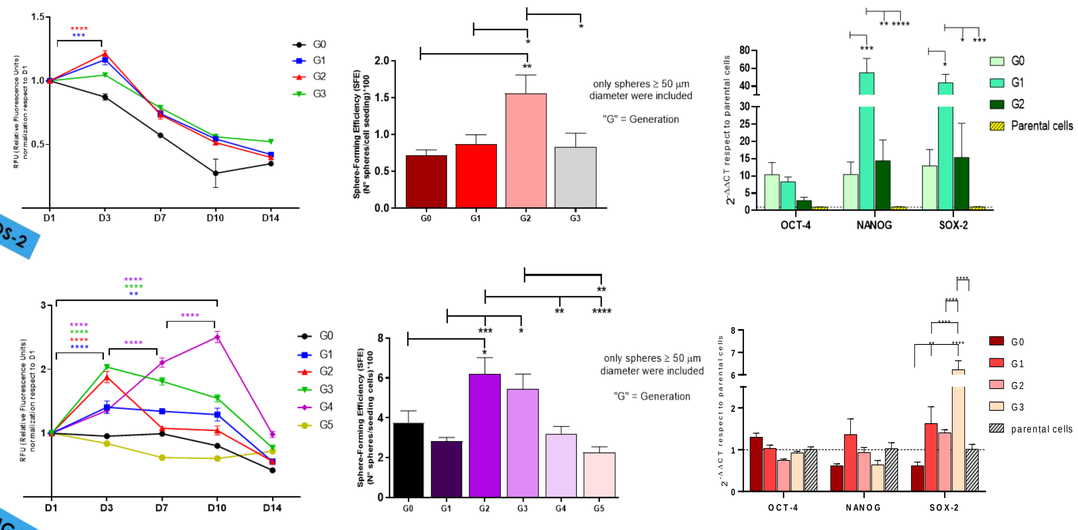
The morphological evaluation of *in vitro* 3D scaffold-based models highlights the ability of spheroids to maintain their phenotype and they showed a significantly higher expression of stemness markers compared to 2D conditions.



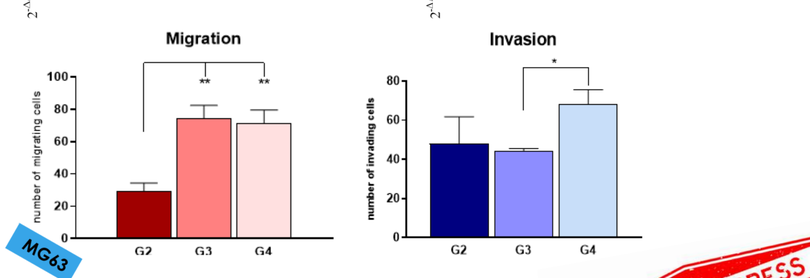








The serial passaging spheroids reported variability in gene expression and sphere forming efficiency of both osteosarcoma cell lines, highlighting different tumoral properties.



WORK IN PROGRESS

CONCLUSIONS

The scaffold manufacturing and 3D cell seeding approaches are still being optimized. Secretome and hypoxic conditions will be considered. Primary CSCs from human biopsy will be included. These 3D *in vitro* tumour models could improve the predictivity of preclinical studies and enhance the clinical translation outcomes.

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