Exploring cellular crosstalk between neuroblastoma tumor cells and their bone marrow microenvironment

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BACKGROUND

- Neuroblastoma (NB) is the most common and deadly extracranial solid tumor in children under 5 years old.
- The considerable clinical and biological heterogeneity contributes to a survival rate of 40% in high-risk patient groups.
- Bone marrow (BM) metastasis is the most common adverse event.
- There are still no *in vitro* or *in vivo* models to best represent this disease.

AIM

The aim of this project is to mimic 3D bone marrow niche using a 3D scaffold made of β tricalcium phosphate with interconnected channels of tailored shape and size and investigate the key signaling events that sustain the growth of NB cells disseminated to the bone/BM niche.

METHODS

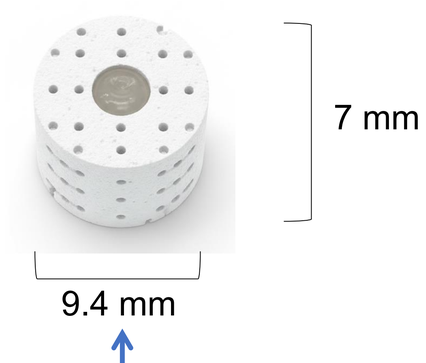
β-TCP slurry inside wax mold

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Sintering of the ceramic material at 1020°C

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β-TCP scaffold with interconnected channels



↑

Seeding of MSC, HUVEC, HSC, and NB cells inside the smaller channels

Cell viability analysis

- CCK-8 assay
- Immunofluorescence

β-TCP scaffold characterization

- Scanning Electron Microscopy (SEM)
- X-Ray Diffraction (XRD)

RESULTS

1.a MSC metabolic activity

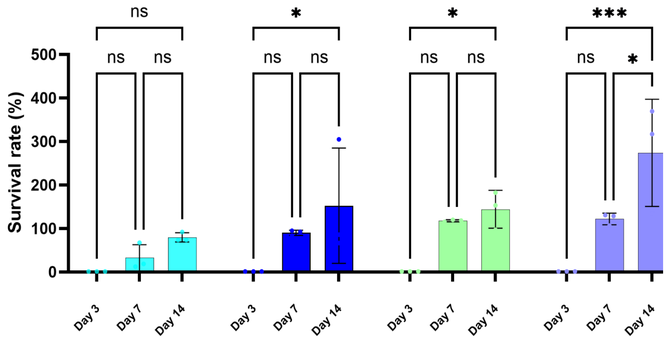


Figure **1.a**: CCK-8 viability assay of four different β-TCP scaffolds containing Mesenchymal Stem Cells (MSCs) at Day 3, Day 7 and Day 14. The metabolic activity increases with the duration of the cell colture as observed on days 7 and 14. Results are normalized to Day 3.

1.b Complete cellular metabolic activity

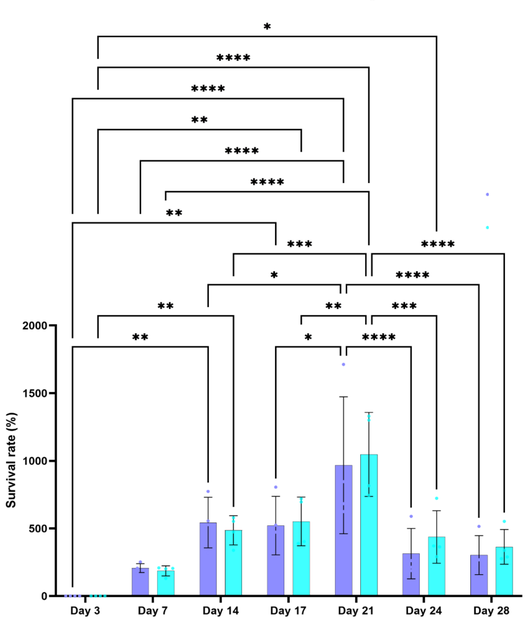
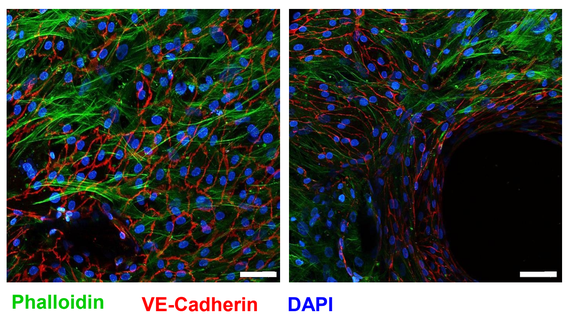


Figure **1.b**: CCK-8 viability assay of two different β-TCP scaffolds containing MSCs, HUVEC, Hematopoietic Stem Cells (HSCs) and NB cells at different days. Also in this case, from Day 3 to Day 28, the metabolic activity of the cells increases. Results are normalized to Day 3.

2.a



2.b

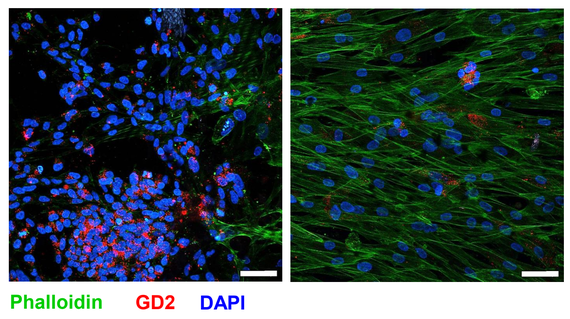
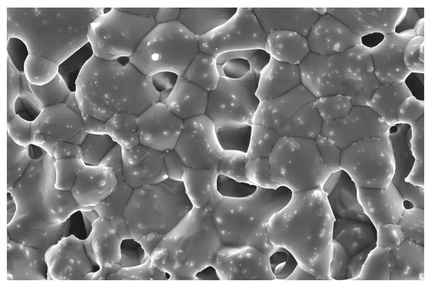


Figure **2.a**: Acquisition of Phalloidin and VE-Cadherin stainings by two photon microscopy. The presence of HUVEC and MSC cells is clearly shown inside the scaffolds and especially along the microchannels. Scale bar = 60 µm.
Figure **2.b**: Acquisition of Phalloidin and GD2 stainings by two photon microscopy. The presence of HUVEC and NB cells is shown inside the scaffolds. Scale bar = 60 µm.

3.a



3.b

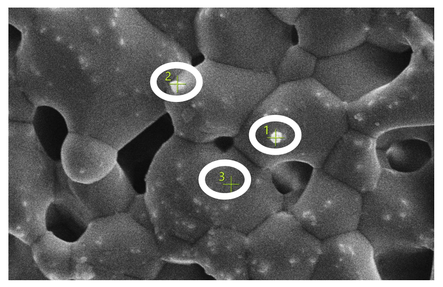


Figure **3.a**: Scanning Electron Microscopy (SEM) analysis of the surface of the β-TCP scaffolds in 5000X magnification.
Figure **3.b**: in 8000X the points surrounded by white circles indicate sites made of specific atomic composition (O, P and Ca elements).

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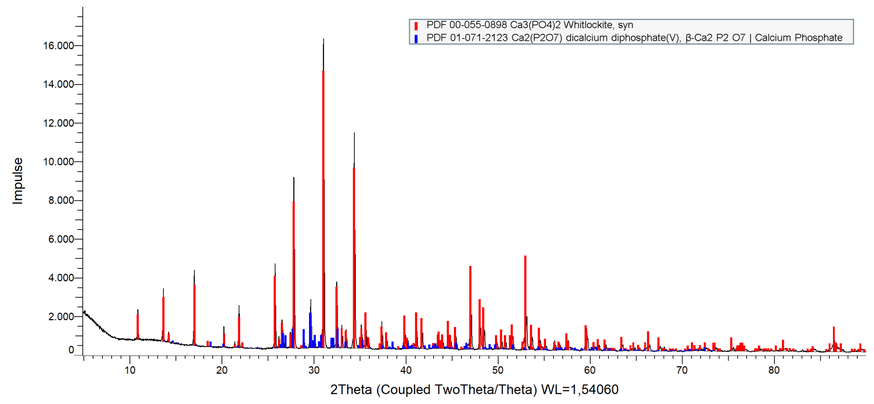


Figure **4**: X-Ray Diffraction (XRD) of known β-TCP (black) and produced β-TCP scaffolds (red). X-axis shows the count of the reflected electrons, while Y-axis shows the angle of the detector.

CONCLUSIONS

The preliminary results confirm the high potential of our 3D model for simulating cellular crosstalk in the *in vitro* bone marrow (BM) niche. This opens new possibilities for NB *in vitro* studies, improving our understanding of NB cells' interactions with cells of 3D bone and BM-like systems.

TERMIS EU-Chapter 2026 PALMA DE MALLORCA 21-24 APRIL / SPAIN

Fondazione città della speranza

DFG Deutsche Forschungsgemeinschaft Project number: 352001

Disclosure Statement of Financial Interest: I, Sara, Andriotto, and all co-authors, DO NOT have a financial interest or affiliation with one or more organizations that could be perceived as a real or apparent conflict of interest in the context of the subject of this presentation.

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