A Humanized 3D Bioprinted Model of Breast-to-Bone Metastasis

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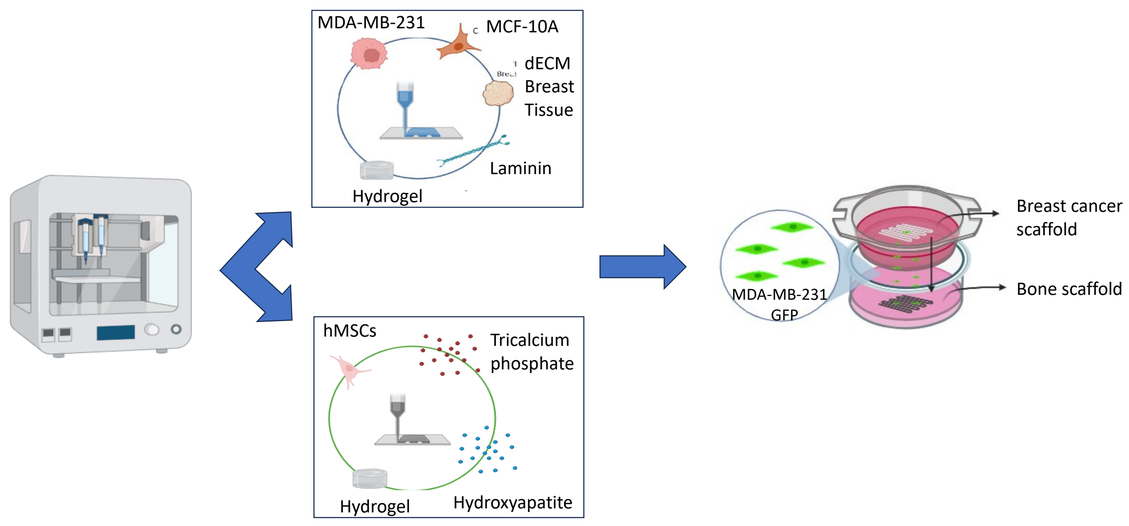
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INTRODUCTION & AIM

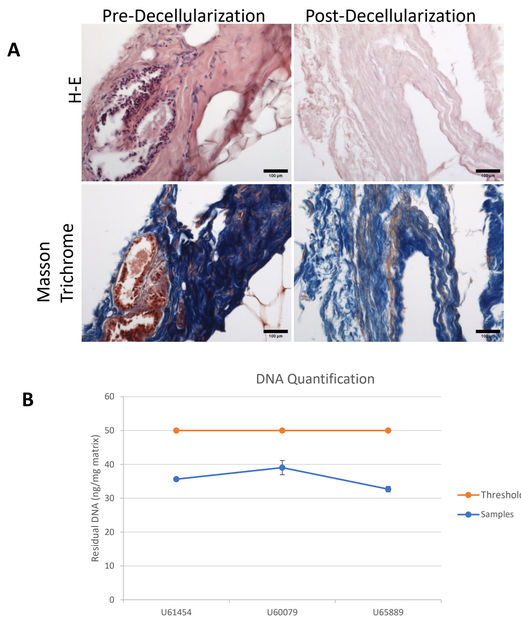
Bone metastases are debilitating complications of breast cancer. One of the main limitations of the *in vitro* models currently used is the inability to perfectly mimic physiological conditions. In this scenario, 3D bioprinting technology has revolutionized the field of tissue engineering due to its excellent potential in reproducing the geometry and complexity of constructs. This project aims to develop a cell culture system, using a sophisticated tissue engineering and 3D bioprinting approach, to recreate a human bone tissue and human breast cancer model in vitro, which will be connected in order to mimic a metastatic environment.

MATERIAL & METHOD



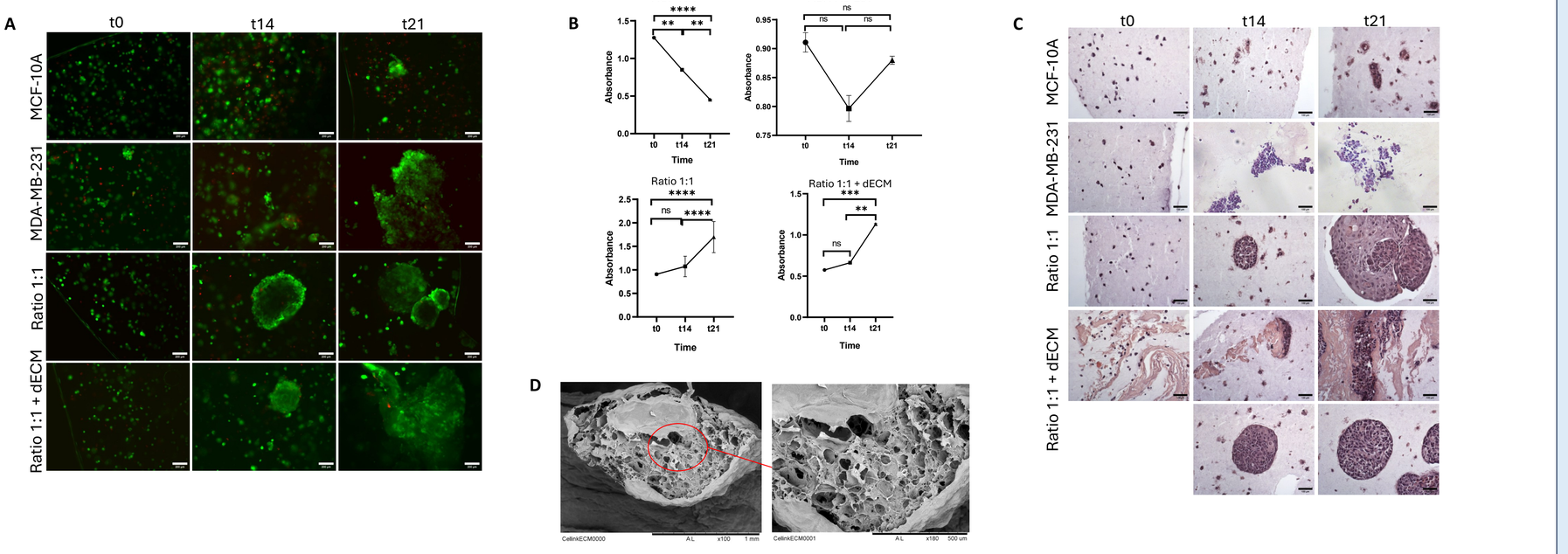
IN VITRO HUMAN BREAST CANCER TISSUE

dECM healthy Breast Tissue characterization



Decellularization Heatlhty Human Breast Tissue.
A. Characterization Healthy Breast Tissue before and after decellularization with Hematoxylin and Eosin staining to evaluate cellular content. Characterization Healthy Breast Tissue before and after decellularization with Masson Trichrome to evaluate collagen and fibrous tissue in blue or green, muscle and cytoplasm in red, and nuclei in dark purple to black.
B. Quantification of DNA on three different samples (U61454, U60079, U65889) to evaluate DNA content after decellularization

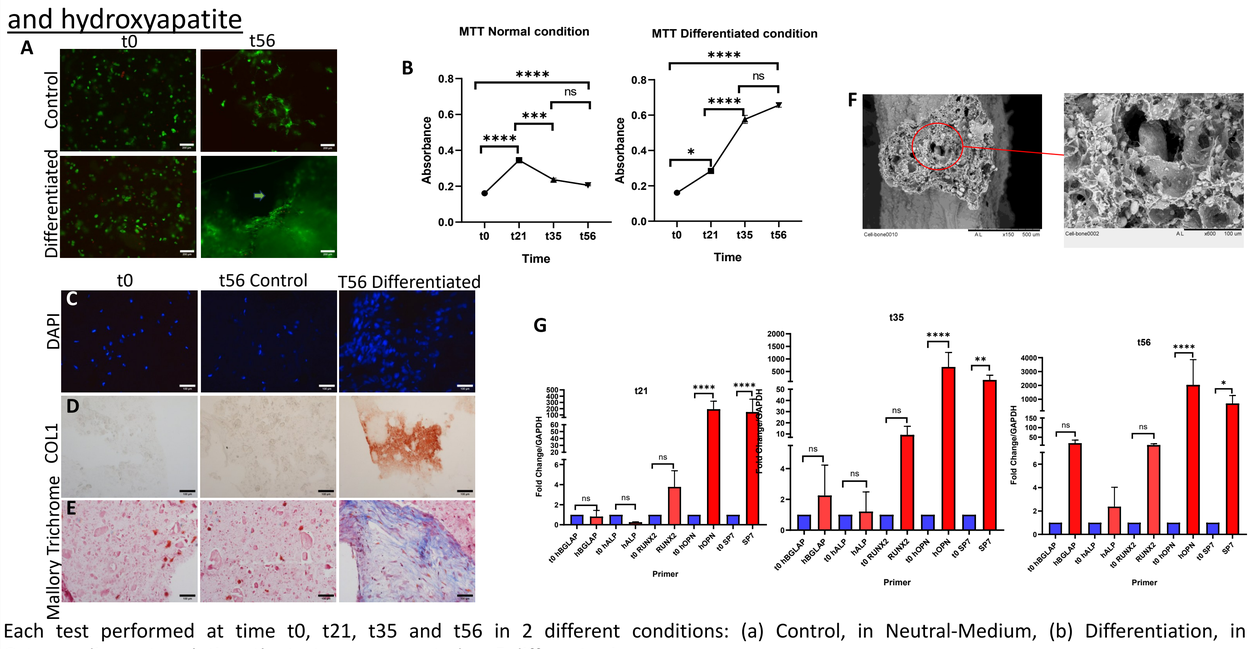
Bioprinting MDA-MB-231 mixed with Laminink 111 (Cellink), alginate ink enrich with laminin



Each test performed at time t0, t14 and t21 in 4 different conditions: (a) MCF-10A bioprinted, (b) MDA-MB-231 bioprinted, (c) MCF-10A and MDA-MB-231 bioprinted together, (d) MCF-10A and MDA-MB-231 and dECM bioprinted together; at each time the tests were repeated 4 time.
A. Live and Dead Assay performed at time t0, t14 and t21. It displays an elevated vitality, while mortality is almost zero.
B. MTT assay performed at time t0, t7, t14 and t21; at each time the MTT was repeated on 3 different scaffolds. MTT assay. Statistical test: two-way ANOVA analysis.
C. Hematoxylin and Eosin histology. Time t0, t14 and t21 were evaluated. At each time 3 scaffolds with cells and 2 scaffold without cells (empty) were included.
D. SEM analysis ink with dECM

IN VITRO HUMAN BONE TISSUE

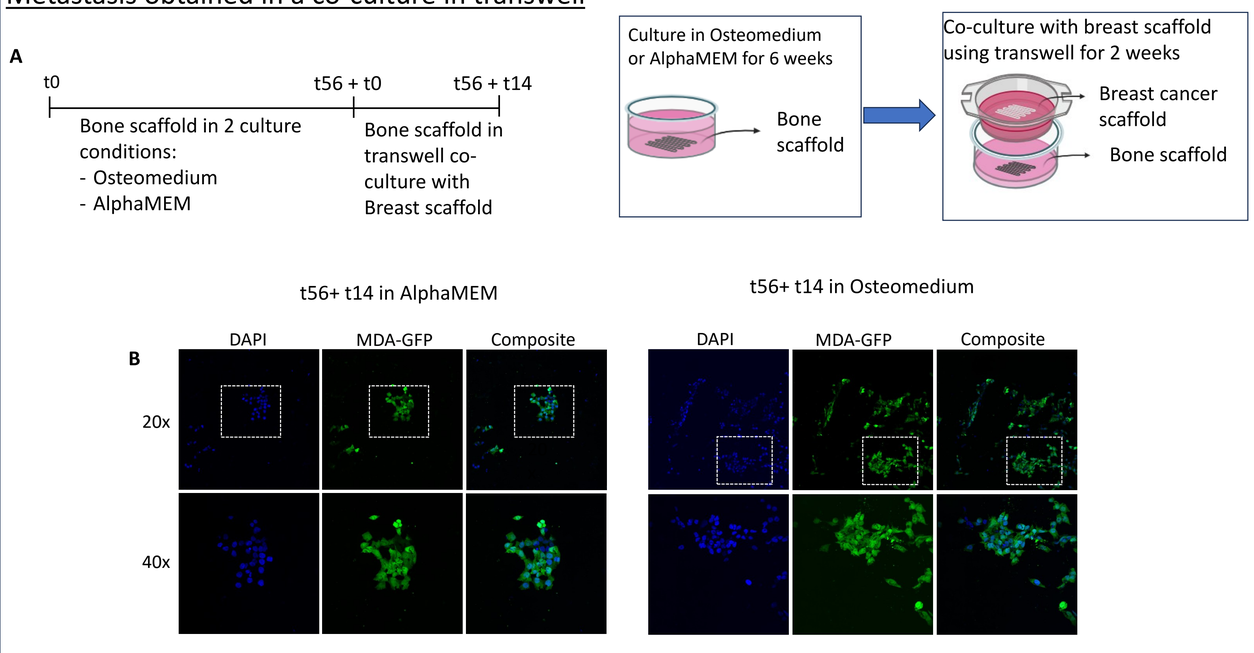
Bioprinting hMSCs mixed with Cellink Bone (Cellink), alginate ink enrich with tricalcium phosphate and hydroxyapatite



Each test performed at time t0, t21, t35 and t56 in 2 different conditions: (a) Control, in Neutral-Medium, (b) Differentiation, in Osteomedium; at each time the tests were repeated on 5 different primary.
A. Live and Dead Assay. It displays elevated vitality, while mortality is almost zero
B. MTT assay; at each time the MTT was repeated on 3 different scaffolds. MTT assay. Statistical test: two-way ANOVA analysis.
C. DAPI Histology. At each time 3 scaffolds with cells and 2 scaffold without cells (empty) were included
D. Col1. At each time 3 scaffolds with cells and 2 scaffold without cells (empty) were included
E. Mallory Trichrome Histology. At each time 3 scaffolds with cells and 2 scaffold without cells (empty) were included
F. SEM analysis ink without cells
G. Realtime qPCR of osteo-differentiation

METASTASIS

Metastasis obtained in a co-culture in transwell



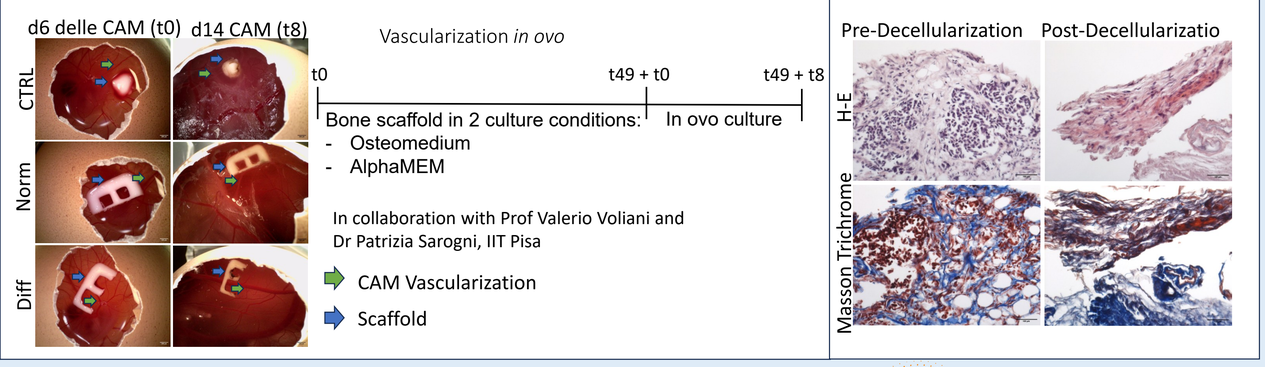
Each test performed at time t14. Test repeated 3 time
A. Experimental plan: bone scaffold bioprinted and cultured in 2 conditions: (a) Control and (b) Differentiation. After that co-cultured with breast scaffold with dECM, MCF-10° and MDA-MB-231-GFP+ for 14 weeks in transwell.
B. On fixed scaffold, evaluation of presence of MDA-MB-231 GFP+ in bone scaffold after 14 days of co-culture. Test done on conphocal microscope to evaluate metastasis and osteotropism of the tumor

DISCUSSION & CONCLUSION

The integrated system successfully reproduced breast cancer osteotropism, as evidenced by the migration of GFP-labeled MDA-MB-231 cells toward bone constructs and the activation of tumor–bone crosstalk.
ONGOING EXPERIMENT:
- In ovo chorioallantoic membrane (CAM) assays were initiated to promote pre-vascularization of bone scaffolds, supporting vessel ingrowth and improved nutrient diffusion
- Decellularization of neoplastic mammary tissue was performed to generate tumor-specific ECM-based bioinks

This humanized and modular 3D bioprinted platform provides a reproducible tool for modeling breast cancer bone metastasis. The integration of vascularization strategies and tumor-derived extracellular matrices further enhances physiological relevance, supporting applications in metastasis research, drug screening, and translational oncology while reducing reliance on animal models.

ONGOING EXPERIMENT



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