Engineering 3D Collagen-Nanohydroxyapatite Coculture Platforms to Uncover Drivers of Prostate Cancer Bone Metastasis

Caroline M. Curtin[1,2,3], Aamir Hameed[1], Ciara M. Murphy[1,2,3], Nezar Kamal[1, 4]
[1]Tissue Engineering Research Group (TERG), Department of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland. [2]Trinity Centre for Biomedical Engineering (TCBE), Trinity College Dublin (TCD), Dublin, Ireland. [3]Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland, [4]Department of Anatomy, King Abdulaziz University, Jeddah, Saudi Arabia.
carolinecurtin@rcsi.ie

Disclosures: Nezar Kamal (N), Aamir Hameed (N), Ciara M Murphy (N), Caroline Curtin (N)

INTRODUCTION: Prostate cancer (PCa) remains the most prevalent cancer among men in 118 countries, contributing to 15% of cancer diagnoses globally, with incidence rates steadily climbing[1]. In advanced disease, metastasis to bone is common, incurable, and represents the leading cause of PCa-related mortality[2]. Addressing this clinical challenge requires deeper mechanistic understanding of PCa's preference for bone, both to advance fundamental biology and to drive the development of targeted treatments. The bone microenvironment-characterised by a complex and diverse interplay of extracellular matrix proteins and stromal cells-plays a pivotal role in supporting and accelerating metastatic growth. Yet, existing models fail to capture the complexity of the native bone tumour microenvironment, limiting their ability to yield insights into disease progression and response to treatment. To address this gap, we developed and validated an in vitro 3D collagen-nanohydroxyapatite (coll-nHA) scaffold model to recapitulate key features of osteoblastic bone metastasis. This model employed a sequential co-culture strategy, where human mesenchymal stem cells were first induced toward osteogenesis, establishing a bone-like matrix, prior to seeding with prostate cancer cells. This design enables direct interrogation of tumour-bone interactions within a physiologically relevant microenvironment. Our model provides new opportunities to dissect the cellular and molecular interactions of PCa bone metastasis and represents a promising preclinical platform for therapeutic discovery.

METHODS: To simulate the bone metastasis microenvironment, a coll-nHA (1:2 weight ratio of coll:nHA) scaffold, developed within our lab[3], was seeded with male mesenchymal stem cells which were differentiated into osteoblasts using osteogenic media for either 7 or 21 days prior to addition of PCa cells. Cells were then cultured for an additional 14 days to determine differences between PCa cells cultured with early or late-stage osteoblasts. The co-culture model involved seeding with androgen receptor (AR)-positive and dependent (LNCaP) and AR-negative bone metastatic (PC3) cell lines. Techniques including quantitative DNA analysis, migration assays, mineral deposition assays including calcium assays and Alizarin Red staining, immunofluorescence staining, and cytokine analysis were conducted to evaluate the cellular responses of each cell line when co-cultured with osteoblasts on the bone biomimetic scaffold. Results were performed n=3, analyses of variance one-way ANOVA and two-way ANOVA were used for the statistical testing, and Tukey post-hoc test was performed.

RESULTS SECTION: The results from the co-culture model revealed that LNCaP and PC3 cells exhibit distinctly different behaviours in response to the bone microenvironment, with significant modulation of the proliferation, morphology, and migration patterns. LNCaP cells maintained a clustered morphology compared to single cell formation of PC3 cells when cultured with osteoblasts (Fig. 1A) and also significantly enhanced proliferation (Fig. 1B) and migration, particularly when cultured with early-stage osteoblasts. Calcium levels were significantly decreased in both PCa cocultured groups compared to osteoblasts alone, with an almost 75% decrease at Day 35, indicating cancer cells significantly inhibit the normal osteogenic process (Fig. 1C). Osteocalcin levels were also significantly decreased by ~ 20% in PC3 cocultured groups compared to osteoblasts alone while LNCaP cocultured groups showed a 10% increase. Vascular endothelial growth factor (VEGF) levels were significantly increased in cocultured groups suggesting bone cells may enhance the angiogenic potential of PCa cells which may contribute to increased PCa growth. Finally, vimentin levels were significantly decreased in PC3 versus LNCaP cocultured groups by ~ 10% which suggests a shift towards the mesenchymal-to-epithelial transition process (MET), suggesting adaptation to a more epithelial-like phenotype which has been shown to result in more stable colonisation at metastatic sites.

DISCUSSION: In conclusion, our findings establish 3D coll-nHA scaffolds as robust and versatile platforms for direct coculture studies, providing unprecedented insights into the cellular crosstalk that fuels metastatic progression. By recapitulating key features of the bone-tumour microenvironment, this system represents a transformative tool to deepen our mechanistic understanding of prostate cancer bone metastasis and accelerate the development of more precise, targeted therapeutic strategies.

SIGNIFICANCE: The clinical relevance of this study lies in providing a physiologically relevant in vitro model that captures the critical interactions between prostate cancer cells and the bone microenvironment, the central drivers of skeletal metastasis in advanced disease. By replicating key aspects of the bone metastatic niche, this model creates new opportunities to uncover therapeutic targets and to evaluate interventions designed to disrupt tumour-bone crosstalk, with the ultimate goal of preventing or limiting metastatic progression in patients.

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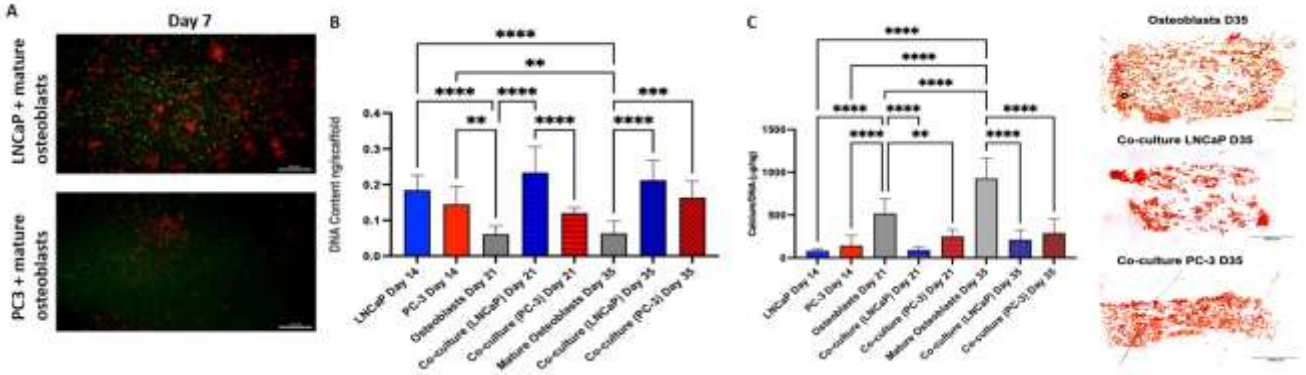

Figure 1 A) PCa cells (stained with Cell Tracker Red) and osteoblasts (stained with Cell Tracker Green) show clusters in LNCaP cells compared to singular cells in PC3 groups. B) Significantly enhanced proliferation was detected in LNCaP compared to PC3 cocultures when cultured with early-stage osteoblasts. C) Calcium levels were significantly decreased in PCa coculture groups indicating PCa cells inhibit osteogenesis.