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EFFECTS OF DYNAMIC CELL CULTURE CONDITIONS ON 3D SPATIAL REORGANIZATION OF MSCS/HUVECS SPHEROIDS IN THE CONTEXT OF BONE TISSUE ENGINEERING

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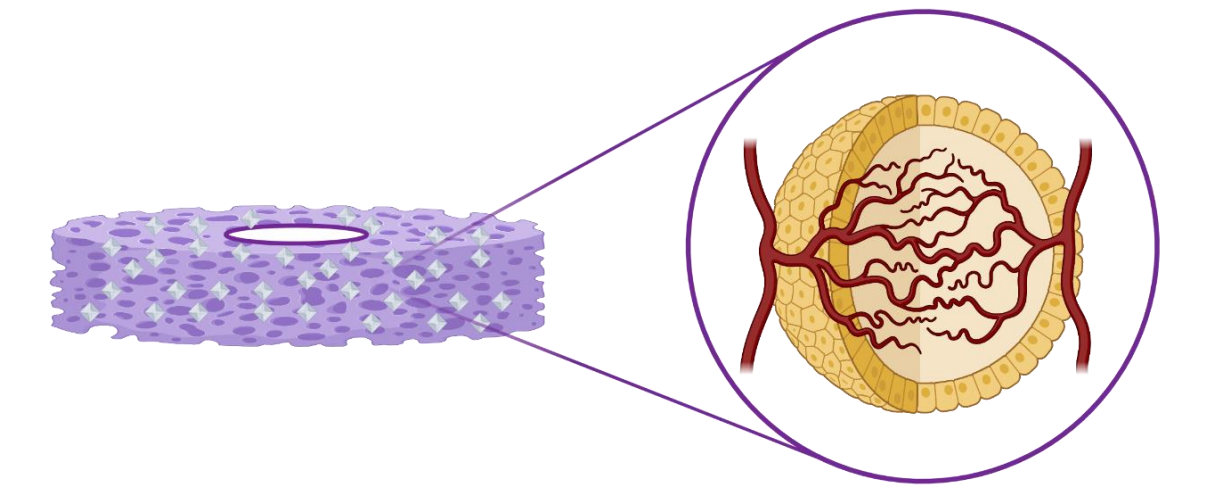
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Background

The spatial distribution of MSCs and HUVECs remains unexplored in 3D co-culture experiments that assess the symbiotic dynamic between osteogenesis and angiogenesis. Understanding such spatial cues can help us develop a prediction model for optimizing bone culture protocols.

In the context of bone tissue engineering, these properties can be exploited to produce osteogenic substitutes:

- Seeding MSCs/HUVECs on micro/macroporous scaffolds leading to the **formation of 3D aggregates**
- Supplying the scaffolds with an osteogenic factor such as **nano-hydroxyapatite crystals (nHAp)**
- Culturing under dynamic conditions in a **perfusion bioreactor** which optimizes nutrient and oxygen delivery [1]



We aim to observe the development and reorganization of MSCs and HUVECs in Pullulan/Dextran hydrogels supplied with nHAp under dynamic culture conditions in a perfusion bioreactor.

MSCs and HUVECs Rearrangement

After seeding the cells following a 1:1 ratio, the scaffolds were subjected to either dynamic or static culture conditions. Samples were taken for confocal observation from both cultures at D1, D3, D7, then every week to ensure in-depth evaluation.

As seen in **figure 1B**, following 24 hours of culture, HUVECs are spatially located at the periphery of the spheroid with MSCs occupying most of the center. As the culture progresses, we witness the migration of HUVECs to the center of the spheroid to form a distinct endothelial core starting D7 onwards.

Nearest neighbor calculations in **figure 1C** show the separation of HUVECs and MSCs into 2 distinct clusters as cells of the same type are much closer in distance to each other than to cells belonging to a different group. This finding presents a contrast to the existing literature describing the peripheral localization of HUVECs [2,3,4].

Despite the similar behavior in cellular reorganization in both culture conditions, spheroids were significantly larger in diameter under dynamic culture as seen in **figure 1D**. This can be indicative of an increased proliferative activity due to the mechanical stimulation provided by the indirect perfusion circuit.

Additionally, the early osteogenic marker ALP was measured and standardized to assess the effects of culture conditions on bone formation. ALP levels presented in **figure 1E** were found to be upregulated in scaffolds supplemented with nHAp, but no significant difference was found between dynamic and static samples.

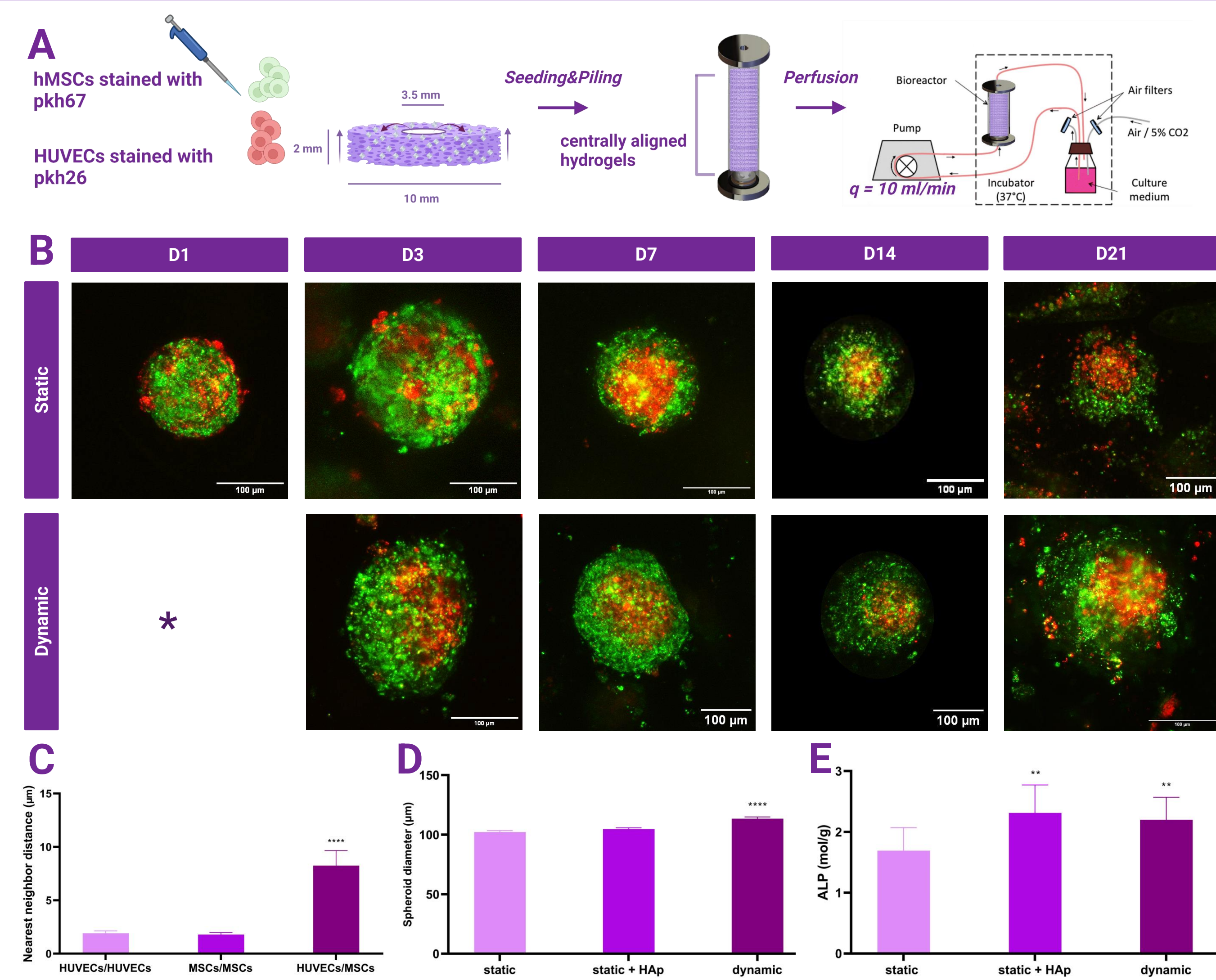


Figure 1: (A) Protocol of cell seeding and culture in a perfusion bioreactor. (B) Distribution and rearrangement of spheroids in the macroporous scaffolds throughout the 21 days of culture under dynamic and static conditions. (C) Nearest neighbor distance (µm) between cell types. (D) Evolution of spheroid diameter (µm) under different culture conditions. (E) Standardized ALP (mol/g) measurements in different conditions.

Cell Viability and Oxygen Consumption

Viability tests were conducted using a Live/Dead kit throughout the culture. In accordance with literature, viability was superior under dynamic culture conditions:

- **At D1 and D7:** viability rate of **97%**
- **At D14:** viability rates decrease to reach **83.2%** in static culture, remain at **95%** in dynamic culture
- **At D21:** viability rates stay stable at **94%** in dynamic culture with most cell death occurring on the surface, while it plummets to **54%** in static culture with most cell death occurring in the center and on the surface

The difference in viability between dynamic and static groups can be attributed to oxygen consumption; an indirect measure of metabolic activity. Using the Michaelis-Menten equation, we estimated the oxygen consumption in the bioreactor to be **13%** of the total oxygen inlet, meaning that there is an abundance of oxygen in dynamic culture.

$$\frac{CO_2 \text{ consumed by cells}}{\text{total } CO_2 \text{ in}} = \frac{n_{cell} V_{max} C_{O_2}}{q C_{O_2} (in) + K_M} = 0.13 \quad \text{with} \quad \begin{aligned} n_{cell} &= 16,100,000 \\ K_M &= 4.05 \times 10^{-3} \text{ (mol.m}^{-3}\text{)} \\ V_{max} &= 2.778 \times 10^{-17} \text{ (mol.cell}^{-1}\text{.s}^{-1}\text{)} \end{aligned} \quad \begin{aligned} CO_2 \text{ concentration inlet} &= 0.20 \text{ (mol.m}^{-3}\text{)} \\ \text{Flow rate } q &= 10 \text{ (ml.min}^{-1}\text{)} \end{aligned}$$

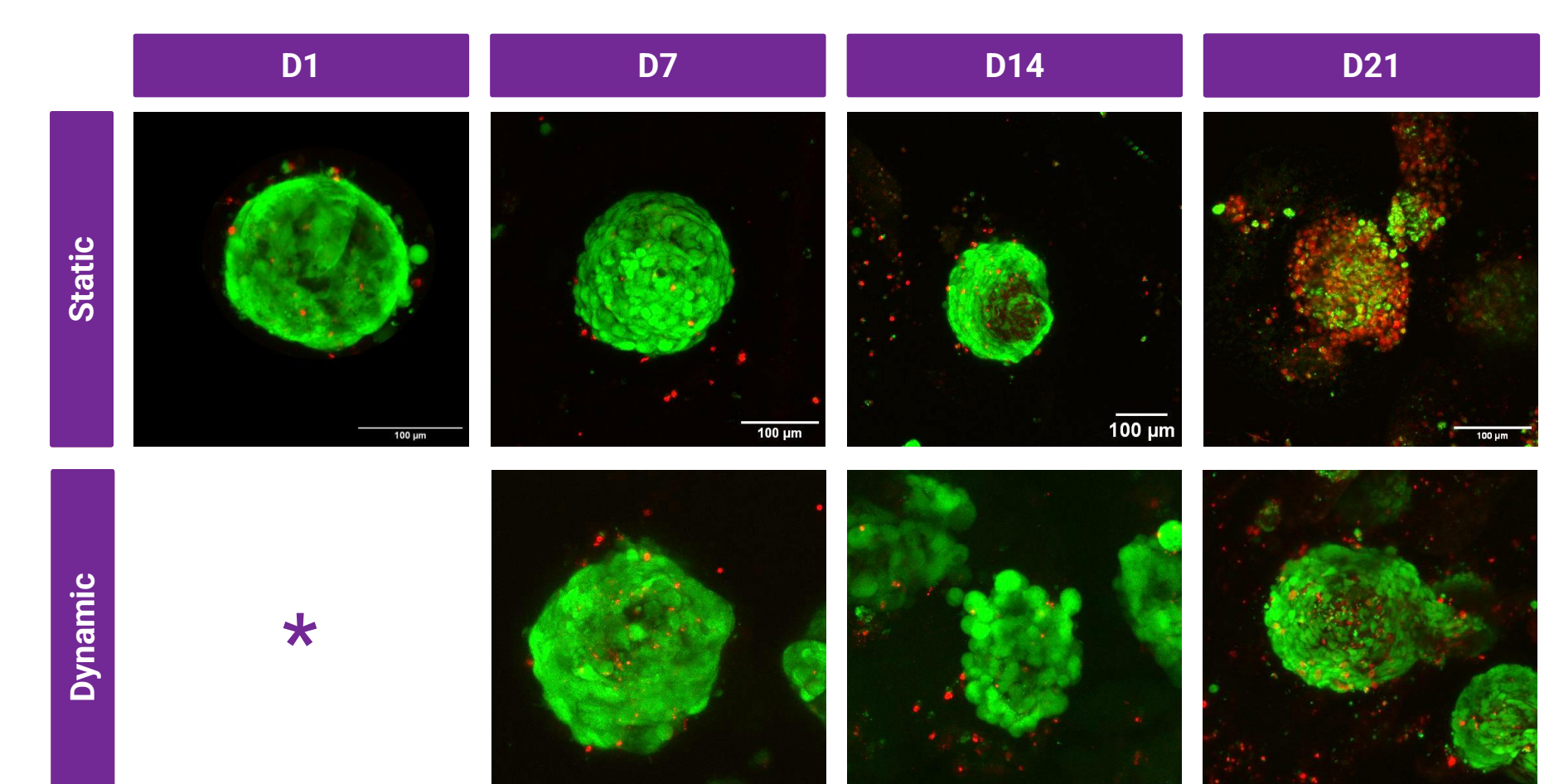


Figure 2: Imaging of Live (in green) and Dead (in red) staining during the culture period under dynamic and static conditions. *D1 is a common sample for both dynamic and static culture conditions

Conclusion

- Our results show that co-culturing HUVECs and MSCs in a porous hydrogel under dynamic culture conditions leads to viable and well-developed spheroids.
- We plan on monitoring the regulation of osteogenic and angiogenic markers according to the spatial distribution of cells.
- Oxygen and glucose gradients need to be assessed locally to investigate their effect on spatial distribution.

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