Utilizing hMSC/macrophage spheroids as a predictive 3D model for bone-implant interactions

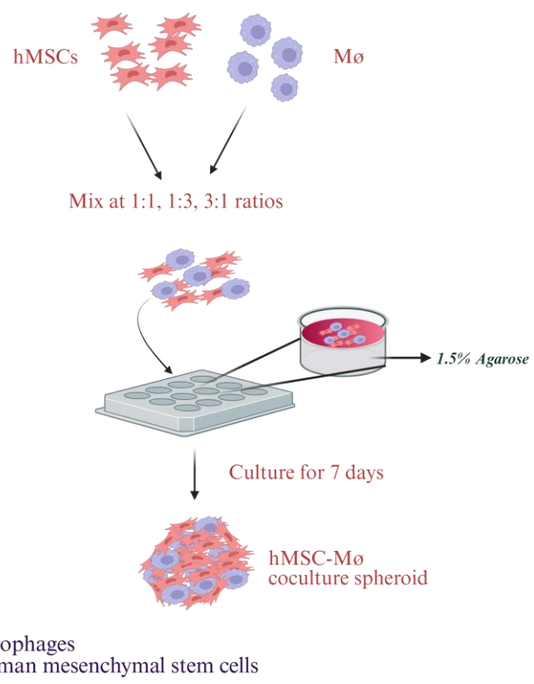
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1. BACKGROUND

2D cell culture models poorly replicate the native bone microenvironment and have limited predictive value for biomaterial evaluation.

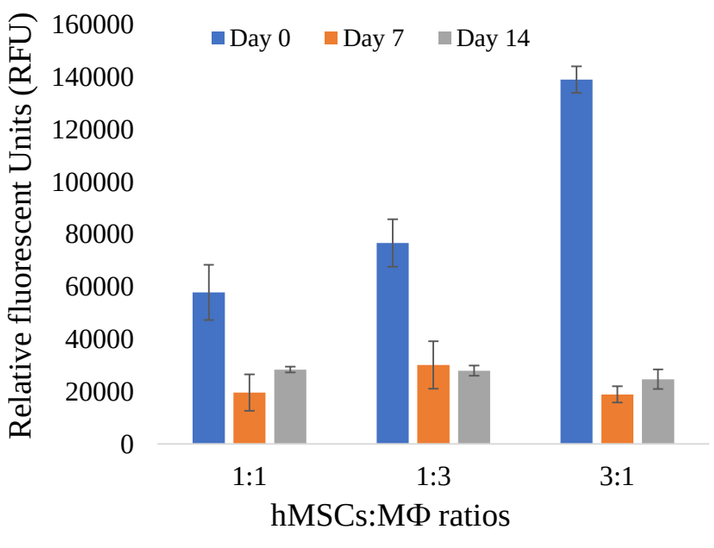
Hence, we hypothesized to use 3D hMSC-macrophage coculture spheroids for biomaterial evaluation mimicking the inflammatory phase of early bone regeneration.

2. FORMATION OF hMSC-MØ COCULTURE SPHEROIDS



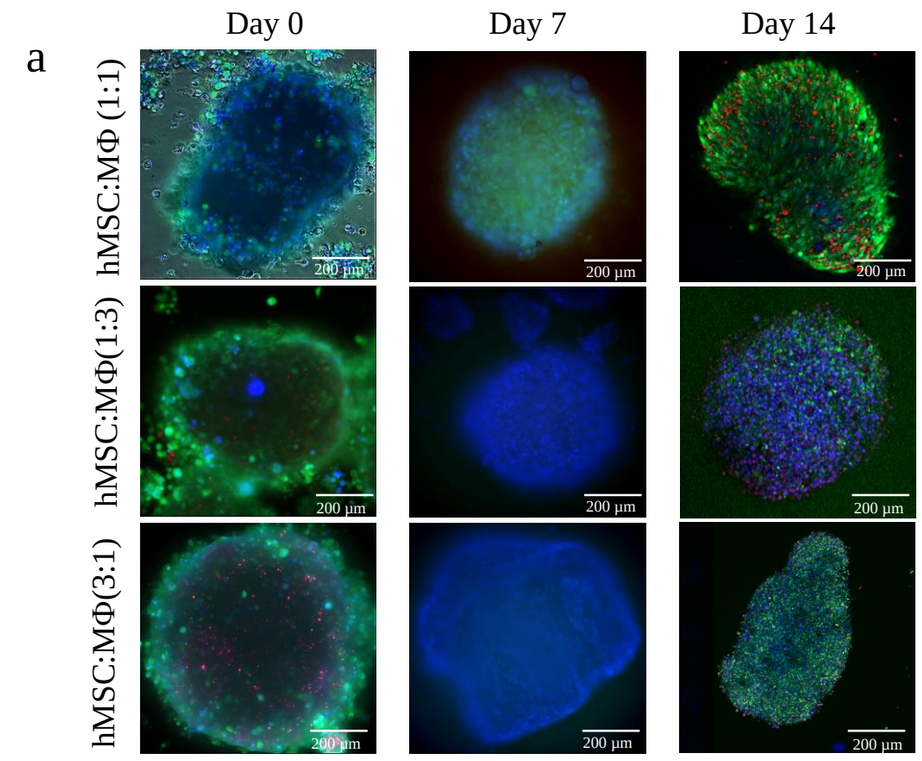
Human bone marrow-derived mesenchymal stem cells (hMSCs) were cocultured with PMMA-activated macrophages (100 ng/mL) at three hMSC:Mø ratios (1:1, 1:3, and 3:1) on 1.5% agarose-coated well plates for 7 days to facilitate scaffold-free spheroid self-assembly.

3. METABOLIC ACTIVITY



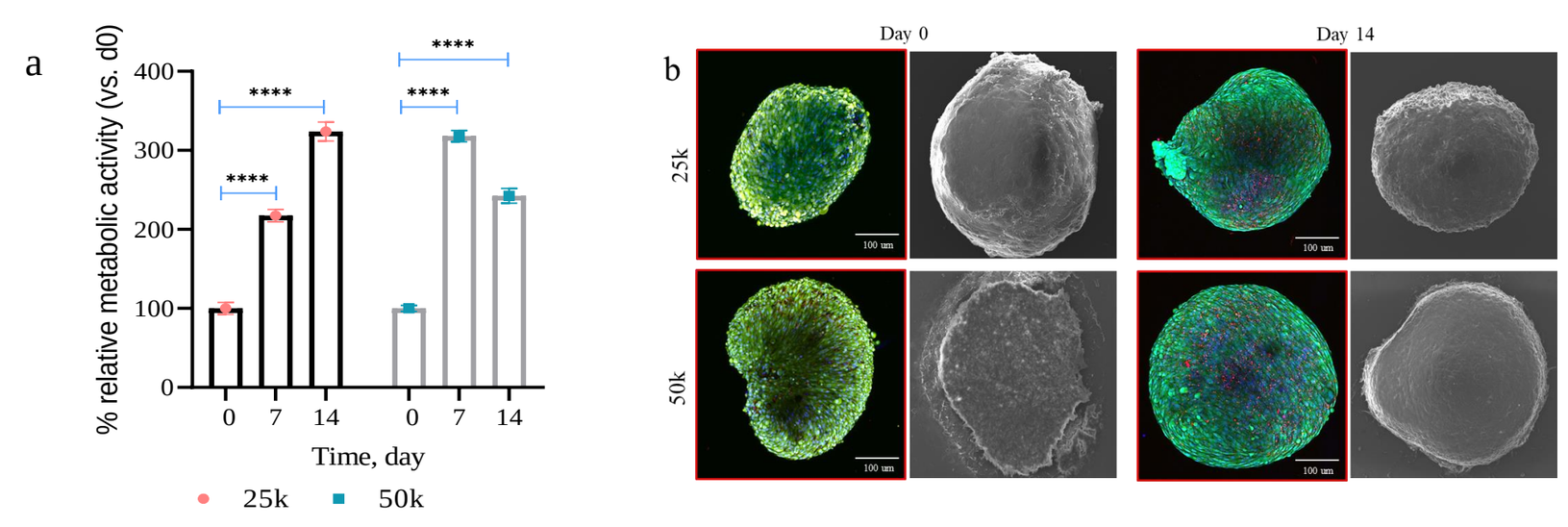
Cell metabolic activity of hMSC-MΦ spheroids was evaluated at days 0, 7, and 14 using the AlamarBlue™ assay (n=3). Fluorescence measurements indicated that the 3:1 coculture ratio sustained the highest cellular metabolic activity at the point of spheroid maturation.

4. VIABILITY AND MORPHOLOGICAL CHARACTERIZATION OF hMSC-MØ COCULTURE SPHEROIDS



Cell viability within hMSC-MΦ coculture spheroids was assessed by live/dead fluorescence assay. Confocal micrographs (a) revealed predominant viability throughout the full spheroid depth across all time points, with minimal cell death detected; Calcein AM (green) to label viable cells, Ethidium Homodimer (red) to label non-viable cells, and NucBlue (blue) to visualize nuclei. Complementarily, SEM micrographs (250x and 1000x) confirmed the presence and distinct morphology of both cell types within a compact and structurally stable spheroid architecture; (green arrow: hMSCs, red arrow: MΦ).

5. EFFECT OF INCREASED CELL DENSITY



Metabolic activity of 3:1 hMSC:MΦ spheroids at seeding densities of 25k and 50k cells was quantified using the AlamarBlue™ assay (a) over 14 days (n=3), revealing a significant increase in metabolic activity over time. (b) Confocal live/dead fluorescence imaging confirmed predominant cell viability throughout the spheroid structure, while scanning electron microscopy (250x) revealed compact and stable spheroid architecture at the respective seeding densities.

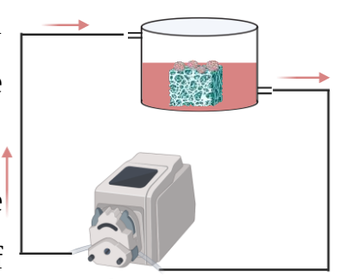
6. CONCLUSION

- 3:1 hMSC:Mø ratio shows favorable spheroid viability and stability.
- Increased seeding density enhances compactness and markedly improves viability (323.8% for 25k; 242.6% for 50k by day 14 vs. day 0; $p < 0.0001$).
- SEM analysis confirms formation of compact and structurally stable 3D spheroids.

7. FUTURE STUDIES

Future work will focus on validating the 3:1 hMSC:MΦ spheroid model to characterize Mø phenotype and osteogenic potential.

Subsequently, will be employed to evaluate the immunomodulatory capacity of biomaterials and their ability to drive macrophage polarization toward the pro-regenerative M2 phenotype.



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REFERENCE

Sun, J., Chen, C., Zhang, B. *et al.* Advances in 3D-printed scaffold technologies for bone defect repair: materials, biomechanics, and clinical prospects. *BioMed Eng Online* **24**, 51 (2025). https://doi.org/10.1186/s12938-025-01381-w

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