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HEALTH AND MEDICINE

Spheroid-laden hydrogels with dual biomimetic intra- and perispheroidal niches for enhanced bone healing

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Stem cell spheroid–hydrogel platforms offer promise for bone defect repair, but implantation-associated oxidative stress, poor vascular ingrowth, and insufficient osteogenic cues limit their clinical translation. Here, we developed dual-NICHE (dual niche-instructive construct for bone healing and engraftment), a gelatin methacryloyl hydrogel containing human adipose–derived stem cell spheroids with spatially distinct intra-/perispheroidal niches. Within a single construct, these two niches provide complementary functions to simultaneously address the key bottlenecks in bone regeneration. The intraspheroidal niche incorporated bone morphogenetic protein-2–immobilized fibers to promote osteogenic differentiation, whereas the perispheroidal matrix contained Sr^{2+}–tannic acid nanoparticles (NPs). Upon implantation, the NPs mitigated oxidative stress and supported vascular infiltration. Concurrently, the osteogenic cue promoted differentiation, while the NPs facilitated mineral nucleation and growth, as further supported by molecular dynamics simulations. Together, this spatial integration enhanced vascularized bone formation with a bone volume fraction of ~13.5% using only 5208 cells per defect in a mouse calvarial model, providing a potentially translatable framework for bone regeneration.

INTRODUCTION

Most bone defects heal spontaneously, but those associated with systemic diseases remain difficult to repair, posing a substantial challenge. Autografts and allografts are still the clinical mainstay, but they are limited by donor site morbidity and restricted availability, highlighting the need for alternative therapies (*1*, *2*). Stem cell delivery has been pursued to promote repair by providing osteogenic progenitors and modulating the local microenvironment (*2*). However, the outcomes of those treatments remain inconsistent because the transplanted cells often exhibit poor in vivo persistence and fail to achieve sustained osteogenesis (*2*–*4*). To overcome these limitations, stem cell spheroids combined with extracellular matrix–mimicking hydrogels have been explored; the spheroids provide enhanced paracrine factor secretion, and the carriers support spheroid sprouting, retention, and host integration (*5*, *6*). Nevertheless, bone regeneration involves multiple biological processes, including inflammatory regulation, vascularization, mineralized matrix formation, and remodeling, so spheroid-laden hydrogels must be programmed to support the complex in vivo milieu after implantation. Therefore, integrated systems capable of addressing these complementary regenerative processes are needed (*7*).

In the early stage of bone injury, vascular rupture–induced hypoxia and inflammation generate excessive reactive oxygen species (ROS) that compromise the viability and osteogenic potential of transplanted cells (*8*–*10*). To mitigate that issue, codelivery of ROS scavengers and stem cells has been explored (*8*, *11*). Polyphenols are particularly attractive in this context because their abundant hydroxyl groups confer strong redox activity; they have thus been incorporated into coatings, conjugates, and hydrogel chemistries for cytoprotection of transplanted cells (*12*–*14*). However, when polyphenols are incorporated as coatings or cross-linking components, some phenolic moieties may become less accessible to reactive species or become depleted at the implantation site, potentially limiting local cytoprotective activity (*12*–*14*). These considerations highlight the need for antioxidant materials that remain localized within the implant microenvironment and mitigate excessive oxidative stress while supporting transplanted cell function.

Successful bone regeneration requires rapid vascular penetration into the transplanted cell-laden hydrogel to promote oxygen delivery and couple the implant with the host repair program. This coupling proceeds through two opposing remodeling axes within the hydrogel matrix: spheroid-driven inside-out sprouting and matrix reorganization, and host-driven outside-in infiltration of endogenous cells. Although strategies using matrix metalloproteinase (MMP)–cleavable motifs or adhesive ligands can enhance inside-out remodeling, they often fail to ensure complete matrix reorganization or achieve robust vascularized bone regeneration (*15*–*17*). Conversely, outside-in approaches using chemokine delivery to recruit host vasculature have primarily been restricted to acellular scaffolds, impairing rapid inosculation (*18*, *19*). Together, these observations indicate that enhancing either remodeling axis alone is insufficient to achieve a rapidly perfused architecture. This underscores the need for integrated systems that complement spheroid-driven inside-out sprouting with on-demand outside-in endogenous infiltration to drive complete vascularized bone regeneration.

Diffusion-limited delivery of signaling cues within spheroid-laden hydrogels often restricts the osteogenic uniformity of stem cells (*7*). Although localizing bone morphogenetic protein-2 (BMP-2) via intraspheroidal carriers such as fibers or micro/nanoparticles enhanced osteogenic differentiation compared with soluble delivery, it failed to establish the perispheroidal mineralizing environment required for robust bone formation (*5*, *20*–*22*). During native

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