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ENDOTHELIALIZED PERIOSTEUM-DERIVED ORGANOIDS: TOWARD A LIVING ATMP FOR CRITICAL-SIZE BONE DEFECTS

Background/Aim. Critical size bone defects leading to non-union pose a serious challenge due to the limited efficacy of current treatment strategies. To address this, there is a need to engineer large tissue implants capable of mimicking native bone defect regeneration, which relies on the formation of transient soft bone callus tissue which through endochondral ossification results in a new bone formation. **Methods.** Human periosteum-derived cells (hPDCs) and endothelial cells (hECs – HUVECs or microvascular ECs, MVECs) were self-assembled in endothelialized callus organoids (hECOs) in chondrogenic medium for 14 days. Characterization included the immunofluorescence, snRNA-Seq, cell-cell communication analysis, proteomics and secretome profiling. In vivo performance was assessed by ectopic and orthotopic tibial critical-size defect models in mice (4 and 8 weeks). **Results.** hECs self-organized within hPDC aggregates, stimulating progenitor proliferation and accelerated ECM maturation. snRNA-Seq revealed advanced differentiation trajectories toward hypertrophy, with transcriptional profiles closely resembling native fracture callus and growth plate. The key signaling pathways NOTCH, BMP, ANGPT, PDGF, SPP1, THBS were enriched in hECOs. The secretome profiling showed upregulation of CCN2, SPP1, THBS1/2 and basement membrane proteins (COL4A1/2, laminins, LOX). Ectopically, 14-day hECOs formed mineralized ossicles with bone marrow within 4 weeks. Orthotopically, MVEC-containing assembloids bridged critical-size defects within 4 weeks, with cortical remodeling and marrow formation by week 8; non-endothelialized controls failed to regenerate. **Conclusions.** Co-differentiation of hPDCs with hECs generates physiologically relevant callus organoids recapitulating vascular-skeletal coupling of fracture healing. hECOs show superior chondrogenic maturation and angiogenic priming in vitro, translating into accelerated bone regeneration in vivo. This strategy integrates with clinical reconstructive timelines (e.g. Masquelet technique), offering a ATMP living implant for challenging bone defects. **Grants/Funding.** EU MSCA4Ukraine (AvH ID 101101923); EU Horizon 2020 (No. 874837); KU Leuven Internal Funds (C24M/22/058); FWO (G0D0623N); RegMedXB.

Keywords: bone regeneration, callus organoid, periosteum-derived cells, endothelial cells, endochondral ossification, tissue engineering.

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