**ENGINEERNG OSTEOGENIC BIOHYBRID IMPLANTS COMPOSED OF CARTILAGINOUS MICROTISSUES & TAILORED MELT ELECTROWRITTEN MESHES**

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Progenitor cells from the periosteum are major contributors in fracture healing with contribution to the formation of the cartilaginous fracture callus. It was previously demonstrated that microspheroids of human periosteum derived cells, differentiated towards the chondrogenic lineage, could be assembled into scaffold-free constructs that healed murine critical-size long bone defects [1]. However, the stability of such scaffold-free implants can be compromised when scaling-up. In this work, cartilaginous microspheroids were combined with tailored melt electrowritten (MEW) meshes to create an engineered biohybrid callus-like membrane able to induce bone formation via endochondral ossification. Human periosteum-derived cells (hPDCs) were seeded in non-adherent microwells (AggreWell™400, STEMCELL Technologies) to form microspheroids with approximately 250 cells. The microspheroids were cultured in a chemically defined chondrogenic media (CM) for 4 days where after they were seeded onto MEW polycaprolactone (PCL) meshes with defined pore size. The PCL meshes were printed with Spraybase® Melt Electrospinning instrument with an average fiber diameter of 10.9 ± 2.3 µm and coated with 0.1% gelatin before seeding. Microspheroids on MEW-PCL meshes were differentiated in CM for an additional 14 days before gene expression analysis or subcutaneous implantation in immunodeficient mice was performed. Gene expression analysis demonstrated up-regulation of chondrogenic (9-fold: *SOX9,* 140-fold: *COL2*) and prehypertrophic (71-fold: *IHH*) gene markers. To assess the bone forming capacity of the “living membranes” (day 14), they were implanted subcutaneously with MEW-PCL meshes only as control. No mineralization was detected in mesh-only explants but bone, bone marrow and mineralized cartilage was detected in all “living membranes”. A total amount of 23 ± 3 % (MV/TV) mineralized tissue was observed. These data demonstrated differentiation towards prehypertrophic chondrocytes *in vitro* and bone formation *in vivo*. The high versatility of this biofabrication approach lies in the possibility to tailor the scaffolds to shape and dimensions corresponding to the clinical indication and the individual patient using robust bone forming building blocks. We believe that these strategies will be instrumental in the development of designed living tissues with predictive clinical outcomes.

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**Reference**: [1] G. Nilsson Hall, L.F. Mendes, C. Gklava, L. Geris, F.P. Luyten, I. Papantoniou, Developmentally Engineered Callus Organoid Bioassemblies Exhibit Predictive In Vivo Long Bone Healing, Adv. Sci. 7 (2020) 1–16. doi:10.1002/advs.201902295.

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