Abstract

In traumatic tissue injury with complex critical-size defects, an appropriate structural template is essential to support healing and guide tissue regeneration. A tissue analogue material or scaffold should have an adequate host response, thereby promoting tissue integration toward functional bone formation. This study hypothesizes nano- and micro-architecture, multiscale 3D-printed hybrid scaffolds along with bioinspired design strategy to mimic the structural and compositional hierarchy of native bone.

Herein, customized synthetic tissue analogues based on the bioactive glass with a biopolymer-based hybrid are designed. A 3D-printed bioactive glass scaffold with controlled lattice architectures and more than 65% interconnected porosity with a compressive strength of 7.87 MPa, similar to trabecular bone, is achieved. The scaffolds exhibited apatite layer formation on the surface after 7 days of SBF treatment, besides improved wettability and substantial cell attachment in vitro. The subcutaneous implantation *in vivo* revealed gradual mineralization and soft-tissue integration over 30 days, confirming ectopic bone formation as evident by histological study and SIMS-TOF analyses.

A CT-image-guided patient-specific design of skeletal defect model was created using bioresorbable PCL-bioactive glass composites. Additionally, decellularized extracellular matrix was infiltrated to impart bioactivity to the scaffold. These personalized scaffolds showed a regular pore size distribution with open porous network, thereby facilitating tissue ingrowth as evident by micro-CT or histology data. Quantitative gene expression analysis demonstrated a time-dependent increase in osteogenic markers, including RUNX2, ALP, COL I, and OPN, from day 1 to 21.

Thereafter, the function of native tissue was emulated by a mechano-stimuli-responsive smart bio-template using nanohybrid systems mimicking collagen-based anisotropy and piezoelectric property. The engineered bilayer scaffolds demonstrated greater piezoelectric output, from ~0.75 V to nearly 15 V, with respect to native demineralized bone matrix. These electromechanical cues promote cell alignment, matrix deposition, and functional tissue regeneration. In addition, a 3D functional organoid model with pathologic ECM components was developed to mimic the tumor niche in vitro. The decellularized skin ECM containing ~116 µg/mg of protein along with growth factors facilitates improved cell viability under native environment.

Overall, comprehensive *in vitro*, *in vivo*, and preclinical evaluation revealed scaffold resorption over time, besides enhanced tissue regeneration through AI-assisted predictive modeling. Thereby, this work presents a unified framework for designing customized 3D-printed hybrid synthetic tissue analogues towards bone regeneration for translational and cost-effective clinical uses.

Keywords: 3D-printed tissue analogue, CAD Design, Bioactive glass, Decellularization, Piezo-responsive scaffold, *in vitro* and *in vivo* bioassays, Micro-CT, Finite Element Analysis