3D-Printed Composite Scaffolds for Customised Bone Defect Reconstruction in Low Osteogenic Environment: In Vitro Analysis

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INTRODUCTION: Three-dimensional (3D) printing emerges as a novel approach for fabricating customised (patient-specific) bone scaffolds with controlled geometry and porosity. Such design flexibility is particularly valuable for addressing large or irregular bone defects, which remain an unmet clinical challenge due to their complexity and limited regenerative capacity. These scaffolds are generally composed of biodegradable polymer-ceramic blends such as polycaprolactone-hydroxyapatite (PCL-HA) or polycaprolactone-tricalcium phosphate (PCL-TCP). Previous studies have shown that combining HA and TCP can improve both the mechanical strength and bioactivity of such constructs. Therefore, this work investigates different PCL-HA-TCP (PHT) formulations to optimise the performance of 3D-printed bone scaffolds in low osteogenic environment.

METHODS: This in vitro study compared three Polycaprolactone-Hydroxyapatite-Tricalcium phosphate (PHT) composite formulations (PHT90-6-4, PHT70-18-12, and PHT50-30-20), using pure PCL as the control. The 3D-printed scaffolds were modified by O_2 plasma surface treatment and subsequently characterised. Their biocompatibility (PicoGreen dsDNA, PrestoBlue assay) and osteogenic potential (ALP activity (colorimetric), Alizarin red staining and quantification) were evaluated over 28 days using human adipose-derived mesenchymal stem cells (ADSCs) (40,000 cells/well, n=4 per group) without the addition of osteogenic supplements/growth factors. The best performing composition was further tested for angiogenic potential (chorioallantoic membrane/CAM assay) and scaffold stability/integration under surgical fixation procedure (screw pull out test).

RESULTS SECTION: Increased ceramic content raised the printing temperature (from 120°C for PCL to 180°C for PHT50-30-20) and required higher extrusion pressure due to increased viscosity, as confirmed by rheology**.** The addition of ceramic fillers also enhanced wettability (though not statistically significant), while the compressive modulus increased with ceramic content (from 104.57±9.72 MPa in PCL to 148.41±28.51 MPa in PHT70-18-12) before slightly decreasing at higher loading (134.42±32.23 MPa in PHT50-30-20). dsDNA concentration increased similarly across all groups, while metabolic activity peaked between days 14-21 before declining (Fig. 1a). The early osteogenic marker ALP gradually increased, peaking at day 21 in all groups before decreasing (Fig. 1b). In contrast, mineral deposition (Alizarin Red, background-corrected) remained consistently highest in PHT50-30-20 throughout 28 days (Fig. 1c). Based on these findings, PHT50-30-20 was identified as the most promising composition and underwent further analyses. ADSCs were well distributed and firmly attached to the PHT50-30-20 scaffolds, as observed under fluorescence microscopy (Fig. 1d). The CAM assay revealed a strong angiogenic response surrounding the scaffold (Fig. 1e), and the pull-out strength test demonstrated adequate mechanical performance (734.35±207.59 N) (Fig. 1f).

DISCUSSION: Our dsDNA and metabolic activity assays demonstrated that all PHT compositions of 3D-printed scaffolds support ADSCs proliferation to a similar extent. The early osteogenic marker ALP exhibited a delayed peak at day 21 (typically seen around days 7-14) in all groups, which is likely attributed to the absence of osteogenic supplements/growth factors and possibly to the intrinsic characteristics of ADSCs compared with bone marrow-derived MSCs. However, the higher ceramic content (PHT50-30-20) induced osteogenic differentiation (characterised by mineral deposition) even without osteogenic supplements/growth factors. Thus, these finding indicates promising osteoinductive potential under low osteogenic milieu. In addition, PHT50-30-20 scaffolds promoted not only cell adhesion and osteogenic differentiation but also angiogenesis, an essential feature for tissue regeneration. With pull-out strength within the range of cancellous bone, these scaffolds can be fixated using standard cancellous bone screws. Our main limitations include the absence of additional osteogenic marker analyses (e.g., gene expression via PCR) and in vivo validation. Nevertheless, this novel PCL-HA-TCP composition demonstrates favourable cell-material interactions and adequate mechanical performance, highlighting its strong potential for clinical translation in large bone defect reconstruction.

SIGNIFICANCE/CLINICAL RELEVANCE: This study addresses the unmet clinical need for bioactive, biodegradable, and patient-specific bone graft substitutes that promote both osteogenesis and angiogenesis in large or irregular defects, particularly in low osteogenic environments where current grafts often fail. By optimising 3D-printed PCL-HA-TCP scaffolds, this work advances translational biomaterials that enhance bone regeneration, reduce reliance on auto-/allografts, and may prevent limb amputation in severe cases.

IMAGES AND TABLES:

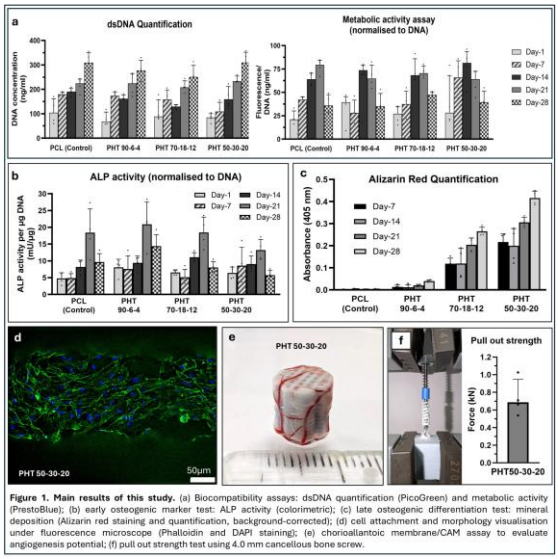

Figure 1. Main results of this study. (a) Biocompatibility assays: dsDNA quantification (PicoGreen) and metabolic activity (PrestoBlue); (b) early osteogenic marker test: ALP activity (colorimetric); (c) late osteogenic differentiation test: mineral deposition (Alizarin red staining and quantification, background-corrected); (d) cell attachment and morphology visualisation under fluorescence microscope (Phalloidin and DAPI staining); (e) chorioallantoic membrane/CAM assay to evaluate angiogenesis potential; (f) pull out strength test using 4.0 mm cancellous bone screw.