Polylactide (PLA) And Poly-4-hydroxybutyrate (P4HB) Bioabsorbable Textile Scaffolds For Bone Tissue Regeneration

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INTRODUCTION: Critical-sized bone defects are defined as those that will not heal spontaneously within a patient's lifetime [1]. Tissue engineering aims to tackle such problem by combining highly porous scaffold biomaterials with cells from the body and mechanobiological cues, to generate new functional tissue. Bioabsorbable polymers are very attractive for such applications, since they can support new tissue formation, and become replaced over time [2]. Polylactide (PLA) and poly-4-hydroxybutyrate (P4HB) represent a suitable biomaterial choice for bone scaffolds, featuring well established biocompatibility profile, good mechanical properties, and predictable degradation rate [3-4]. Textile technology offers a wide range of structural design versatility and is readily scalable to large volumes of production, hence a promising manufacturing method of scaffold-based implants [5]. In this work and for the first time, MC3T3-E1 pre-osteoblast cell line and human bone marrow mesenchymal stem cells (hMSC-BM) are cultured *in vitro* on 3D bioabsorbable PLA and P4HB warp-knitted spacer fabric scaffolds, to explore their potential for bone tissue engineering.

METHODS: Warp-knitted scaffolds were manufactured using PLA (Trevira GmbH and Luxilon Industries NV) and P4HB yarns (Tepha Inc.). Each textile featured two cover areas made of multifilament yarns interconnected by a monofilament pile yarn (Figure A). After knitting, textiles were heat set in fully constrained conditions, in a convection oven at 150°C (PLA) and in water at 52°C (P4HB) for 5 minutes. Textile sheets, with a final thickness of 1-1.2 mm, porosity of 80-85% and Young's modulus of 14.5-18.5 kPa (PLA) and 24-31 kPa (P4HB), were sterilized by Ethylene Oxide. Prior to seeding, scaffolds were cut with a 6 mm diameter sterile punch and prepared following [6]. MC3T3-E1 cells (ATCC) were expanded in basal media as in [7] (using 1% penicillin/streptomycin instead), seeded at passage 22 on top of the scaffolds in 96-well plates at a cell density of 10^4 cells per scaffold, and cultured in basal media for 12 days. The same process was repeated using hMSC-BM from a healthy human donor (female, 44 years, PromoCell GmbH) which were expanded in basal culture medium (as before) and plated at passage 5 on scaffolds at a cell density of 10^4 cells per scaffold. A 2D control of MSCs plated at a density of 1500 cells/well [8] was used, due to the lower surface area available to the cells compared to the 3D scaffolds. Textiles and the control group with MSCs were cultured for 24 h in basal media, and then treated with osteogenic supplements as in [9] for 14 days, with media renewal every 2-3 days. DAPI (4',6-diamidino-2-phenylindole) fluorescent staining was performed at different time points on MC3T3-E1-loaded scaffolds and MSCs-loaded scaffolds. MSCs cells metabolic activity was measured using AlamarBlue assay (Thermo Scientific) at day 1, 4, 7 and 14. Minitab software was used to perform a one-way ANOVA followed by pair-wise comparison with Tukey's test to determine statistical comparisons between groups. Statistical significance was set as $p < 0.05$. Data from at least three replicates were averaged and are reported as mean ± standard deviation.

RESULTS SECTION: Preliminary results show that MC3T3-E1 cells attached and proliferated on both PLA and P4HB scaffolds between day 1 and 12, with a qualitative increase in the number of DAPI stained nuclei in all scaffolds at day 12 (Figure B, i-ii, MC3T3-E1s on P4HB scaffolds). MSC attachment on both PLA and P4HB textiles was also confirmed by DAPI staining, with a higher cell density observed on multifilament yarns (Figure B, iii-iv, MSCs on PLA scaffolds). AlamarBlue reduction, directly proportional to cells metabolic activity, confirms that MSCs are metabolically active during the 14 days of culture, with no difference between PLA and P4HB scaffolds at day 14 (Figure C). Cells in P4HB scaffolds had significantly higher metabolic activity than PLA at days 4 and 7. At all timepoints metabolic activity was significantly higher in the 2D control compared to the scaffolds, however it may not be appropriate to directly compare 2D to 3D results, since a 2D surface is not representative of the complex environmental cues received by cells in 3D.

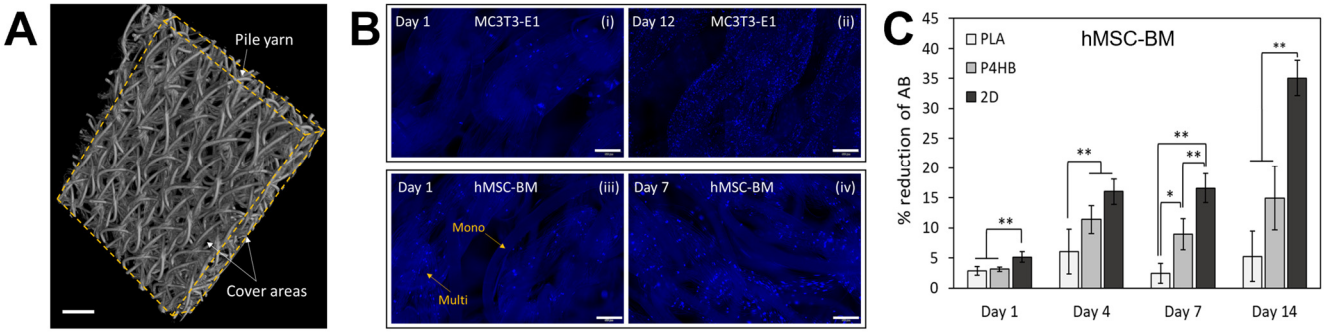

Figure: A. µCT image of warp-knitted spacer fabric. Scale bar is 1 mm long. **B.** MC3T3-E1 on P4HB textile at day 1 (i) and 12 (ii). hMSC on PLA textile at day 1 (iii) and 7 (iv). Scale bar is 500 µm long. Mono = monofilament yarn, Multi = multifilament yarn. **C.** Metabolic activity of hMSCs on scaffolds and 2D control. *, $p < 0.05$; **, $p < 0.01$.

DISCUSSION: Preliminary results show that the 3D textile scaffolds support MC3T3-E1 cells attachment and proliferation over 12 days of culture and MSC cell attachment and proliferation over 7 days. Metabolic activity results indicate that both textiles show good cytocompatibility and a suitable 3D structure for MSCs cell infiltration and growth. The limited increase of metabolic activity of MSCs on scaffold over time compared to literature [4] suggest that cell density and seeding must be improved to stimulate cell proliferation and favour osteogenesis over time. Preferential cell attachment on multifilament yarns on both scaffolds was observed and may occur since the thin filaments bundles (multifilament) feature high surface area, which is known to influence initial cell adhesion [5]. Further experimental data will be collected to investigate scaffolds osteogenic potential, by assessing cell differentiation and calcium deposition. Enzymatic degradation and its influence on the osteogenic properties of the scaffolds will also be investigated [4]. These preliminary and encouraging results show that PLA and P4HB bioabsorbable warp-knitted spacer fabric scaffolds merit further exploration for bone tissue regeneration.

SIGNIFICANCE: To the authors' knowledge, no studies have previously investigated the use of PLA and P4HB warp-knitted spacer fabric scaffolds for bone tissue engineering.

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