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Purpose: Soft tissue engineering holds great promise for the restoration of breast defects by the combination of bio-compatible scaffolds and growth factors. To date, there is no optimal biomaterial by which to deliver adipose tissue within a scaffold. Polycaprolactone (PCL) scaffolds are medical grade biodegradable biomaterials that offer great potential for breast tissue engineering due to its ability to be 3D-printed according to patient specific defects. Platelet Rich Plasma (PRP) is a component of blood plasma that is rich in growth factors and shown to enhance adipocyte proliferation but their ability to support soft tissue formation in vivo is unknown. This study aimed to investigate the optimal 3D-printed PCL scaffold architecture to support adipose tissue delivery and determine the beneficial effect of PRP.

Methods: Breast scaffolds were designed using SolidWorks and then 3D-printed using Fused Deposition Modelling (FDM). Different PCL scaffolds porosities (20, 30, 40, 50, 60, 80%) and pore architectures (square, honeycomb and triangle) were evaluated to closely mimic human breast tissue compressive mechanical properties. The internal structure and surface architecture of the scaffolds were assessed using scanning electron microscopy (SEM). The scaffolds were further characterised including the wettability (contact angle assessment), surface chemistry (X-ray photoelectron spectroscopy) and mechanical properties (Youngs elastic modulus in compression). The biocompatibility of the 3D-printed scaffolds was assessed by differentiating 3T3-L1 adipocytes in vitro over 14 days. The adipocyte cell adhesion, proliferation, gene expression of adipogenic markers using RT-qPCR and immunocytochemistry was evaluated. Following 4 weeks of adipocyte differentiation in vitro, the optimised PCL scaffolds were seeded with 3T3-L1 adipocytes and then implanted in the dorsum of mice with and without PRP coating from donor mice for 6 and 12 weeks. The integration, vascularisation and adipocyte formation were assessed at 12 weeks using histology and immunohistochemistry.

Results: PCL scaffolds with 40% porosity and square pores demonstrated similar compressive properties to human breast tissue. Surface hydrophobicity and chemistry was significantly affected by porosity and pore architecture (p < 0.05). The PCL scaffolds with 40% porosity and square pores showed the greatest adipocyte cell adhesion and proliferation over 14 days. The gene and protein expression of PPARγ, C/EBPα and FABP4 was significantly upregulated on scaffolds with 40% porosity at 7 and 14 days (p < 0.05). The PCL scaffolds with PRP coating demonstrated greater adipocyte formation, tissue integration (H&E and masons trichome staining) and vessel formation (CD31 staining) in vivo at 12 weeks than without PRP treatment (p < 0.05).

Conclusion: PRP enhances adipocyte cell proliferation and differentiation on 3D-printed PCL scaffolds. The combination of PCL scaffolds with PRP holds promise for the effective delivery of adipose tissue for breast reconstruction.

131 Dynamic Self-regenerating Cartilage Construct For Cartilage Repair In The Hand

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Purpose: Cartilage is avascular with limited capacity for repair and self-regeneration. Articular cartilage lesions in large joints have been successfully treated with autologous chondrocyte implantation (ACI) with or without a matrix scaffold (e.g., MACI). However, lesions in small joints of the hand and wrist cannot be easily treated with current ACI techniques. Dynamic Self-Regenerating Cartilage (dSRC) has been shown successfully engineers contiguous articular cartilage matrix. The purpose of this study was to develop a new strategy that generating new cartilage matrix using dSRC to treat cartilage lesions in the joints of the hand and wrist.

Methods: To form the dSRC, 10⁷ freshly harvested autologous swine chondrocytes were placed into a 15-cc polypropylene tube and cultured on a rocker at 40 cycles per minute for 14 days at 37°C. During this time the chondrocytes aggregate and begin to make hyaline cartilage, forming a pellet of dSRC. To evaluate the cartilage formation of dSRC, additional culture was performed for 2, 4, 8, 10, and 12 weeks. Media changes were performed every 3-4 days. All constructs were evaluated histologically and immunohistochemically for cartilage formation, and biomechanical analysis.
Results: Photograph showed the dSRC was injectable on week 2 and became thicker and more solid after 2 month and 3 months. After 8 weeks in vitro, dSRC generated contiguous new cartilage matrix, the cell number of week 8 dSRC was significantly decreased, compared to week 2 dSRC that showed hypercellular formation. All dSRC groups demonstrated intense staining with Safranin-O and Tolutidine blue stains indicating high glycosaminoglycan (GAG) production. Immunohistochemical staining further confirmed that the matrix of dSRC was typical of normal hyaline cartilage, rich in collagen type II and no collagen type I, similar to native cartilage. Aggregate modulus of the 3 month dSRC was ~20% of native cartilage. Results of this study demonstrate that dSRC itself can successfully engineer contiguous articular cartilage matrix in in vitro environments.

Conclusion: dSRC demonstrates successful hyaline cartilage formation in all different time points. Such a strategy could be employed to inject or implant dSRC in the smaller joints of hand and wrist to treat cartilage defects or osteoarthrosis.

Reconstruction Of Craniofacial Structural Defects Through Patient-specific 3d-printed Custom Beta Tricalcium Phosphate Scaffolds: Development Of A Translational Porcine Model

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Purpose: 3D-printed bone scaffolds can be commercially printed within days using CT guidance to reconstruct complex craniofacial bony defects. Additional autologous stem cell seeding of scaffolds may enable improved regeneration of normal bony architecture. However, the ability of 3D-printed scaffolds to regenerate load-bearing bone is untested in large animal models.

Methods: A craniofacial porcine model was developed testing the ability of custom 3D-printed bone scaffolds to heal non-critical (<6 cm) and critical (>6 cm) bony defects in Yucatan pigs. Simultaneous full-thickness defects were made in the body of the right zygoma (2 cm) and the angle of the left mandible (6 cm) using custom cutting guides. In the negative control (n=4), no construct was placed. In the experimental arm (n=8), beta-tricalcium phosphate (β-TCP) defect-specific bone scaffolds were 3D-printed from preoperative CT scans and placed into bony defects based on promising in vitro cell adhesion and viability assays. Animals were followed until a six-month study endpoint obtaining CT imaging at three and six months. After surgical site explanation, bony regeneration was evaluated histologically and via μCT scanning. Additional bone strength testing was performed on implant cores.

Results: All animals reached the 6-month study endpoint. In the negative control group, CT and gross evaluation of zygomatic and mandibular defects was consistent with incomplete heterotopic ossification. Masson’s trichrome staining, picrosirius staining, and μCT confirmed the presence of dystrophic bone formation at the ostomy sites with disruption of normal bone architecture compared to control sites. Biomechanical testing was not performed due to inadequate regeneration. In the experimental arm, scaffolds maintained high fidelity to preoperative surgical plan with CT evidence of bone regeneration at three months. Evaluation at study endpoint demonstrated bone regeneration throughout the implants with bony integration at the implant/native bone interfaces. Histologic, μCT and biomechanical testing is ongoing.

Conclusion: Our model has broad applicability in preclinical evaluation of bone regeneration scaffolds. 3D-printed, defect-specific β-TCP scaffolds demonstrated biocompatibility with bone regeneration and osseointegration. Insights from this model may realize the possibility of reconstructing bony defects of any size, shape, and thickness by harnessing the power of 3D-printing and autologous stem cell seeding for congenital, post-traumatic, and oncologic reconstruction.