Processed Lipoaspirate (PLA) Cells Seeded on 3-Dimensional Calcium Phosphate-Collagen-Chitosan-Based Spongy Scaffolds for Potential Bone Tissue Engineering Application

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Statement of Purpose: Processed lipoaspirate (PLA) cells have attracted increasing interest as a seeding source for tissue-inducing scaffolding because of their mesenchyme-like morphology, plasticity and multilineage differentiation potential ability (Zuk PA. Tissue Eng. 2001;7:211-218).Calcium phosphates constructs, particularly combinations of hydroxyapatite (HA), dicalcium phosphate (DCP), and tricalcium phosphate (TCP) have been commonly studied as implantable biodegradable systems for tissue-inducing scaffolding using mesenchymal stem cells (Lechner S. Artific Organs. 2006;30:770-774). Collagen and chitosan have also been combined with hydroxyapatite and others calcium phosphates for potential application in cell-based tissue engineering (*Wang Y. J Biom Mat Res .2008;86:244-258*). They are used to develop interpenetrating polymeric network (INP)-based scaffolds using glutaraldehyde as a cross-linking agent. This work towards evaluating the best physicochemical and microstructural characteristics of different three-dimensional, porous biodegradable calcium phosphate-collagen-chitosan-based scaffolds for promoting the colonization, expansion and differentiation of PLA cells *in vitro* for bone tissue-inducing scaffolding.

Methods: This study was conducted and approved by the Institutional Review Board of Universidade Federal de Minas Gerais. Tissue specimens were collected with the informed consent of patients. PLA cells were obtained from raw human lipoaspirates and cultured according to technique previously reported (Zuk PA. Tissue Eng. 2001;7:211-218). After attaining a subconfluent state, the cells were harvested between passages 3 to 7 for seeding. 3D-biodegradable calcium phosphate-collagen-chitosan-based spongy scaffolds were prepared by chemical crosslinking of chitosan and collagen with glutaraldehyde in the presence of different calcium phosphate (HA, DCP and TCP) combinations. Chitosan (SIGMA-ALDRICH) with deacetylation degree about 85% was dissolved in 0.5 M aqueous acetic acid at room temperature. Collagen (VETEC, Brazil) and different calcium phosphate combinations were dissolved in PBS (phosphate-buffered saline). These solution was then mixed and crosslinked with 25% wt glutaraldehyde aqueous solution (SIGMA-ALDRICH), poured in 48 wells culture plates, incubated at 37 °C and frozen to −20 °C *overnight*. The frozen scaffolds were then lyophylized *overnight*. 50% of the sponge samples were sterilized by gamma irradiation at 15 kGy . The non-sterilized samples were then re-crosslinked with glutaraldehyde, incubated, frozen, lyophilized, neutralized and sterilized under the same conditions above. Microstructures of the scaffolds, and PLA cells were characterized using scanning electron microscopy and optical microscopy, respectively. Expanded cell detection and surface marker expression were performed by flow cytometry. DAPI (4',6-diamidino-2-phenylindole dihydrochloride) stain was used to assess attachment and proliferation of viable cells on scaffold surfaces. Alkaline phosphatase activity was evaluated by the BCIP-NBT assay, followed by mineralized matrix detection using Von Kossa's method.

Results: PLA cell culture appeared as a monolayer of healthy, large and flat cells, approaching confluence and assuming a spindle-shaped, fibroblastic morphology. The cell surface marker expression profiles of the PLA cell population indicated significative levels of CD49d, CD29 and CD44 were observed, which are consistently expressed on MSCs (mesenchymal stem cells) and adipose stem cells (ASC). Although primary cultures of stromal cells isolated from adipose tissue also include a small number of hematopoietic cells, pericytes, endothelial cells, and smooth muscle cells, the PLA cell cultures were negative for both, the endothelial and hematopoietic marker CD34 and CD106 confirming that they are not contaminated by cells of hematopoietic origin. The cell colonization process on the all scaffold samples seems to indicate the presence of less cells on their surfaces. Low cellular concentration to colonize all available surface area of the scaffold can have not provided sufficient cell spreading and proliferation. Additionally, either partial loss of the seeded cells by spontaneous cellular detachment from the scaffold surface, and bulk incorporation during scaffold biodegradation can also have favored a poorer cell colonization on the 3D-scaffold surfaces. However, colonization of viable cells on the glutaraldehyde re-crosslinked scaffolds seems to have taken place due to the cell attachment and proliferation events. This behavior suggests that potential cytotoxicity of glutaraldehyde was not evidenced. The staining for alkaline phosphatase activity on the PLA cells seeded on the scaffords was taken as AP-positive cells suggesting the assembled multiple distinct colony formation. Von Kossa staining of PLA cells seeded on the 3D-spongy scaffold samples suggested mineralized matrix can have been formed after osteoblastic induction process. Although similar phase composition (collagen and calcium phosphate) and highly porous microstructure to those found in natural trabecular bone have been exhibited for the PLA cell-seeded scaffolds after osteogenic induction, mainly those glutaraldehyde re-crosslinked , additional qualitative and quantitative analysis are yet necessary to confirm it.

Conclusions: Under the conditions used in this study, foam-like scaffolds were synthensized resembling chemically and structurally natural trabecular bone with biomimicry for promoting PLA cell attachment, proliferation and differentiation *in vitro* into osteoblast-like cells.