

Decellularization of rat and human omentum to develop novel scaffolds to be recellularized with adipose derived stem cells

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Background. The omentum may be a source of decellularized scaffold maintaining a complex 3D structure, completed with vessels, to be recellularized by autologous cells such as components of the stromal vascular fraction (SVF), isolated from lipoaspirate samples. After colonization of scaffolds with cells, the matrix could provide a suitable microenvironment with numerous application in the field of reconstructive surgery.

Methods. Adult rat and human omenta were treated with an adapted decellularization protocol involving freeze-thawing cycles, enzymatic digestions with trypsin, deoxyribonuclease, lipase and ribonuclease, and lipids extraction to yield a collagenous natural matrix, i.e., decellularized adipose tissue (DAT). The scaffolds obtained were studied with histological (haematoxylin-eosin, azan-Mallory, Van Gieson, Oil Red and Sudan) and immunohistochemical (anti-CD31) stainings to highlight the absence of cells and lipids, and the persistence of frames of the vascular network. On the other hand, cells of the SVF, such as endothelial and inflammatory components and also mesenchymal stem cells, were isolated from human lipoaspirate through incubation with collagenase and then cells were maintained in DMEM for 48 hours in incubator. After 4 culture passages, cells were subcultured with the scaffold, in DMEM in incubator for 1 week, changing medium every 72 hours. Scaffold recellularized was fixed in formalin and analyzed with histological (H&E, Van Gieson, Azan Mallory) and immunohistochemical (Ki67, CD31, CD34) reactions.

Results. Histological stainings confirmed the effectiveness of the decellularization protocol, resulting in a cell-free scaffold with no residual cells and lipids. Azan Mallory and Van Gieson stainings identified a large amount of collagen and elastic fibers, organized in a quite complex three-dimensional network that still preserved vascular structures. The volume of samples was quite preserved during the decellularizing passages. Preliminary trials with cells seeding in the decellularized matrix showed the possibility for cells derived from lipoaspirates to attach to the collagenous matrix and start to proliferate.

Conclusions. The fat-rich and well vascularized omental adipose tissue may be decellularized to realize complex tridimensional scaffolds preserving the architecture of tissue and suitable for recellularization without any rejection. Preliminary analysis suggests the possibility of recolonization in vitro of the scaffold by Adipose Derived Stem Cells (ADSC), that are members of the SVF.

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