TS007
Mechanical liposuction vs. laser liposuction vs. surgeries – the best method of adipose tissue harvesting for ADSC isolation

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Objectives: One of the major goals of tissue engineering and regenerative medicine is search and characterize the new sources of mesenchymal stem cells, which will be better than bone marrow. Adipose tissue is easily accessible and abundant source of stem cells hence their isolation creates new opportunities for regeneration of injured tissues. The aim of this study was the comparison of the Adipose Derived Stem Cells (ADSCs) quality harvested during mechanical liposuction (ADSCsML), laser liposuction (ADSCsLL) and surgeries (ADSCsS) and to select facilely available mesenchymal stem cell source with the highest proliferative potential and ability to differentiate.
Methods: The human adipose tissue was obtained during planned surgical procedures in patients with pathological obesity ($n = 30$), mechanical liposuction ($n = 8$) and laser liposuction ($n = 10$). ADSCs after isolation were cultured in DMEM/Ham's F-12 medium supplemented with 20% (up to the first passage) or 10% (after first passage) fetal bovine serum (FBS), 1% antibiotic and basic fibroblast growth factor (bFGF). To characterize their properties the proliferation rate, differentiation potential and phenotype analysis (CD34, CD44, CD45 and CD90 expression) were performed. Results and Discussion: ADSCs harvested by three methods underwent rapid adhesion to the surface of culture dishes and began to proliferate. The proliferation rate was stable during 14 days of culture. The cells proliferated at least to the sixth passage. The success of culture establishment was 86.7%, 100% and 100% for ADSCsS, ADSCsLM and ADSCsLL, respectively. The average of cell number per 1 g/mL of tissue, at the first passage, was 2.3×10^5, 1.1×10^5 and 9.7×10^3 for ADSCsS, ADSCsLL and ADSCsLM, respectively. ADSCs cultured in medium supplemented with differentiation factors toward osteogenic, adipogenic and chondrogenic lineages showed ability to multilineage differentiation. All ADSCs types were CD44+, CD90+, CD34+ and CD45- (at third passage).
Conclusions: The results obtained in that study support the findings that adipose tissue harvested by three considered methods contains rapidly growing cells with high differentiation potential. However, concerning average of cells number at the first passage, adipose tissue collected during surgeries seems to be the most efficient. Nevertheless, liposucation is less invasive procedure so that ADSCs isolated from lipoaspirates obtained from a larger number of patients require more detailed investigation.

TS008
Microparticles loaded Gellan gum hydrogel matrices: engineering tissues for nucleus pulposus regeneration

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The intervertebral disc central core is made by a gel-like tissue structure composed of more than 80% of water, Nucleus Pulposus (NP). Proteoglycans such as versican and especially aggrecan are the main constituents of the NP matrix as well as collagen type II. The purpose of this work is creating novel Gellan gum-based (GG) hydrogel formulations. GG microparticles (MPs) dispersed in a GG matrix are the novelty for finding application as NP substitute. The ongoing experiment comprises de GG functionalization through methacrylated groups addition. In order to optimize some properties of GG, the functionalization will allow us to improve the water solubility and photopolymerization *in situ* of the biomaterial. High acyl (HA) and Low acyl (LA) Gellan Gum (GG) at different ratio [75%:25% (v/v); 50%:50% (v/v), 25%:75% (v/v)], HAGG 0.75% and LAGG 2%, were mixed in order to prepare solutions to be used as formulations of GG MPs/hydrogels matrix. The GG MP/hydrogel matrix formulations were characterized by dynamic mechanical analysis (DMA), swelling behaviour and degradation rate. The toxic effect of GG MPs/hydrogel discs leachables onto the cells was investigated *in vitro* using a mouse lung fibroblast-like cell (L929 cells) line. Live/Dead cell viability assay was performed to assess the encapsulation efficacy; meanwhile DAPI/Phalloidin staining was performed to evaluate cell morphology. The Methacrylated Gellan Gum (GG-MA) was prepared following the protocol [1]. Mechanism reactions occurred in presence of glycidyl methacrylate by addition to a solution of LAGG at 2% (w/v). The reaction was running over 24 h at room temperature controlling the pH at 8.5 with sodium hydroxide 1 M. GG MPs size was measured using a stereo microscope by staining the MPs with Toluidine Blue-O. This method also allowed evaluating the MPs dispersion and matrix cohesion. From DMA analysis it was observed that the range of 50–500 mg/mL of incorporated MPs is the optimal concentration to reinforce GG matrices. It was demonstrated the non–cytotoxic effect of MPs/hydrogels over L929 cells. In fact, L929 cells were successfully encapsulated in all GG formulations GG MPs and remaining viable over 72 h of culturing. The resulting product from methacrylation reaction was evaluated by nuclear magnetic resonance to assess the reaction efficiency and the degree of substitution. Methacrylated Gellan gum and GG MP/hydrogel matrix are promising hydrogels to be used in tissue engineering strategies for treatment of the degenerated NP.

Reference

1. Silva-Correia J, 2011 Journal of Tissue Engineering and Regenerative Medicine, 5(6): e97–e107.