the mESCs cultured in the hydrophilic scaffolds presented the highest viability during the period analyzed when compared with the hydrophobic (p=0.006) group. The phalloidin and DAPI dye showed that even when the mESCs were cultured for 7 days in the scaffolds they were not capable of growing in the inner core of the 3-D scaffolds. According to the data presented, it is possible to culture mESCs in scaffolds hydrophilic rather than hydrophobic. However, the cells are not able to reach the core of the scaffold. It is important that this problem be overcome in order to enable the use of the whole 3-D scaffold structure and to make it an efficient device for the growth of stem cells and their ensuing use for tissue engineering.

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EVALUATION OF ELECTROSPUN MATRICES FOR THE CO-CULTIVATION OF MESENCHYMAL STEM CELLS AND SKIN KERATINOCYTES

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The regeneration of skin is an important field for tissue engineering. Currently available treatments are insufficient to prevent scar formation and promote healing of the patient, especially in large burns and chronic wounds. Due to the great need for skin substitutes with the ability of regenerating large amounts of skin, as well as the lack of an ideal replacement, the current study has aimed to produce a cutaneous substitute with a PDLLA polymer as a biomaterial. These, in turn, must be able to serve as a suitable support for cellular growth for the period of time required for tissue regeneration. For this purpose, scaffolds were constructed by the electrospinning technique and divided into 3 groups: 1) PDLLA matrices, 2) PDLLA/NaOH, which were PDLLA scaffolds hydrolyzed with a solution of NaOH 0.75M and 3) PDLLA/Lam, also hydrolyzed with NaOH and in which the protein laminin was linked by covalent binding. They were all constructed with 2 different fiber diameters, with the smallest at the top of the scaffold. These scaffolds were characterized by morphology and fiber diameter and their hydrophilicity or hydrophobicity features. Mesenchymal stem cells were then seeded onto the bottom of the scaffold and, after 24 hours, skin keratinocytes were seeded on the other side. This procedure was performed in all the groups. The groups were evaluated for cell adhesion on the day of the seeding and on days 7, 14 and 21 for viability with WST-8 assay. From day 7, the scaffolds were submitted to an air/liquid system of culture. As a result, the scaffolds presented well formed fibers which were randomly distributed. The treatment of the matrices with NaOH for 15 minutes did not substantially affect the structure of the fibers, but it was enough to hydrophilize the surface of the biomaterials, which is necessary for laminin linkage. The fiber diameter for all the groups was 4.58 µm for the largest fibers and 574 nm for the smallest. The pore size of the scaffolds obtained were approximately 27.5 µm and 3.44 µm, respectively, for the largest and smallest fibers. The linkage of the laminin was confirmed by immunofluorescence assay. For the biological analysis, cell adhesion was greater in the PDLLA/Lam

scaffolds with absorbance of 2.268± 0.494, in comparison with 1.264±0.473 for the control (PDLLA scaffold) and 1.159±0.120 for the PDLLA/NaOH scaffold. On day 7 of the viability analysis, the absorbance for the PDLLA scaffold was 1.148±0.411, the PDLLA/NaOH group was 1.380±0.501 and the PDLLA/Lam was 1.990±0.255. On day 14, the absorbance for groups 1, 2 and 3 were 1.032±0.169, 0.755±0.016, and 1.636±0.313, respectively. On day 21, the results were 2.204±0.317, 1.437±0.024, 2.811±0.477 respectively for groups 1, 2, and 3. In general, in terms of the biological analysis, the PDLLA/Lam group showed the best results for cell adhesion and viability tests. Histological analysis is being processed for greater understanding of the behavior of the cells interacted within the scaffolds. In conclusion, the PDLLA scaffolds, mainly the PDLLA/Lam groups, showed good results for the co-cultivation of the cells, with good cell adhesion and the presence of viable cells. These biomaterials were capable of providing support for the growth of the cells, which was observed by the increase in the absorbance over time. Therefore, although histological analysis is still in progress, these scaffolds promise to be suitable biomaterials for use in tissue engineering.

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DISEASE MODELING OF LATE ONSET POMPE DISEASE SPECIFIC IPS CELLS

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Pompe disease is an inherited neuromuscular disorder caused by a deficiency of acid-glucosidase-alpha (GAA). The clinical symptoms of Pompe disease include progressive weakness, respiratory failure and ventricular hypertrophy. Based on clinical phenotype, Pompe disease is classified to infantile form and late-onset form. Hypertrophic cardiomyopathy is usually remarkable in infantile Pompe disease. Even in late-onset Pompe disease, cardiovascular complications including cardiac hypertrophy, and arrhythmia were clinically important, however cardiac involvements are less often and milder than infantile form. Enzyme replacement therapy has been shown to ameliorate these symptoms. Gene therapy using lentivirus- or adenovirus-associated vectors is another possible treatment strategy. Patient/disease-specific iPS cells have been used for disease modeling, drug screening and cell therapy. Huang et al have already reported infantile Pompe disease iPSC and successfully differentiated into cardiomyocyte like cells. (Huang et al Hum Mol Genet. 2011) We tried to generate late-onset Pompe disease iPSCs and differentiated into cardiomyocyte for disease modeling. In addition, we attempted to generate gene-corrected Pompe disease iPS cell by lentiviral gene transfer.
GAA was cloned into cDNA expressing third-generation lentiviral vectors (CS2-EF1α-GAA). To assess the transfection efficacy, Venus, an YFP variant protein, was also cloned into the vector (CS2-EF1α-Venus). Then, we transfected lentiviral vectors containing GAA and Venus into control iPSC (TkDA3-4, healthy donor) at MOI 10, 50 and 100 to determine the optimized titer for gene correction. Expressions of GAA and Venus in iPSC were observed in dose dependent manner.
Pompe disease iPSCs (HPS0175, 0176, 0177) were generated from late-onset Pompe disease patient fibroblast reprogrammed by Sendai Virus. Control iPSC (HPS0223) was also generated from healthy donor by Sendai Virus. Both healthy control (HPS0223) and Pompe disease iPSCs (HPS0175, 0176, 0177) had similar pluripotency characters shown by immunohistochemistry and RT-PCR.
Robust cardiomyocyte differentiation was conducted according to directed differentiation protocol using GSK-3 inhibitor and Wnt inhibitor. Beating cardiomyocyte was observed 10 days after the differentiation both healthy control (HPS0223) and Pompe disease iPSC (HPS0175). Cardiomyocyte derived from