formed normally shaped round colonies and, when subjected to activin/BMP4 cardiac differentiation protocol, formed robustly beating layers of confluent cardiac myocytes.

Conclusions: MHC-I depletion does not preclude cardiac differentiation, paving the way to produce immunocompatable cardiac tissue grafts from non-autologous hESC sources. In subjects treated with hESC-cardiomyocytes to remuscularize infarcted hearts this approach will help to reduce the need for aggressive immunosuppressive treatments.

P-343: Soft Tissue Scaffold Derived from Porcine Adipose Tissue

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Despite thickness and source homogeneity limitations, naturally derived scaffolds have seen the greatest clinical use. Porcine adipose tissue (PAT) has largely homogeneous characteristics that can be sectioned into desirable thicknesses. However, intact decellularized PAT has not been explored as a tissue scaffold. Two decellularization methods aimed at maintaining the tissue's natural morphology to improve nutrient distribution by utilizing intact blood vessel channels were attempted. The first method, Methanol-chloroform submersion, destroyed the tissue and was discontinued. The second, successful method, freeze-thawing, was optimized. The number of freeze-thaw steps (1–7), tissue surface area and thickness, trypsin incubation time (1–3.5 hours), washing time (20–60 minutes), and washing solution were all examined during optimization. Initial, unsuccessful HFF cell seeding demonstrated incomplete lipid removal and led to a lipid removal strategy using short time period xylene immersion. Tensile testing was performed under hydrated conditions in PBS at 25°C and 37°C. An average of 50% swelling of re-hydrated tissue, an elastic modulus of 38.5 kPa, and an ultimate tensile strength of 50.4 kPa were observed; temperature dependent mechanical characteristics were also exhibited. Moreover, relaxation characteristics are being determined. All mechanical tests were performed following protocols congruent with SIS testing methods and the properties of both scaffolds were compared. SEM imaging was performed and demonstrated the presence of pores and capillary channels; H&E micrographs were used to confirm unaltered morphology and lack of cells in processed PAT. Finally, H&E micrographs of samples from a two-day neuroblastoma cell seeding study were examined and demonstrated the presence of cells.

P-344: Co-cultivation of Mesenchymal Stem Cells and Skin Keratinocytes on Electrospun Scaffolds

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Available treatments in skin regeneration are insufficient for promoting healing. This study has aimed to produce a cutaneous substitute combining mesenchymal stem cells (MSCs), keratinocytes and a PDLLA biomaterial, constructed by electrospinning, into 3 groups: 1) PDLLA, 2) PDLLA/NaOH, scaffolds with hydrolyzed surfaces and 3) PDLLA/Lam, hydrolyzed and with laminin binding. The MSCs were seeded at the bottom of the scaffolds. After 24 hours, skin keratinocytes were seeded at the top. The scaffolds were characterized by morphology, fiber diameter, cell adhesion on the day of the seeding and viability on days 7, 14 and 21. As a result, the scaffolds presented randomly distributed, well formed fibers. The fiber diameter for all the groups was 4.58 μm for the largest fibers and 574 nm for the smallest. The pore sizes of the scaffolds obtained were approximately 27.5 μm and 3.44 μm, for the largest and smallest fibers, respectively. In 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 PDLLA and 1.159 ± 0.120 for the PDLLA/NaOH scaffolds. There was an improvement in the number of viable cells from day 7 to day 21 of analysis. In general, the PDLLA/Lam group showed the best results for cell adhesion and viability tests. 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. Therefore, these scaffolds show promise to be suitable biomaterials for use in tissue engineering.

P-345: Measurement Assurance in Stem Cell Characterization: Quantitative Comparison of Cell Enumeration Techniques

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Cell number is a critical measurement in biomanufacturing and is required as a release criterion for cell therapy products. Methods have been developed to increase the throughput of cell counting and to improve precision. However, there are often discrepancies between counts acquired via various methods (i.e., hemocytometers, coulter counters, automated image counters), leaving the question of which method is most accurate? In this study, we developed an experimental and statistical framework for comparing the accuracy of cell-enumeration methods. Unfortunately, ''ground truth'' cell numbers cannot be easily obtained for clinically relevant concentrations of cells; therefore, we instead considered relative accuracy or self-consistency. In our analysis, we establish ranges for how far total cell count values (modeled using the most flexible response) deviate from the ideal self-consistent, proportional response. These ranges can be compared across the various cell-counting methods to determine if one has improved relative accuracy compared with another method. Our results suggest that in the enumeration of human mesenchymal stem cells (MSCs), the automated fluorescent imaging based cell counter was more precise and had higher relative accuracy when compared to traditional hemocytometer methods. These studies support the development and implementation of non-traditional cell enumeration methods for improved cell number determination for cell therapy products.

P-347: Bone Tissue Engineering for Cleft Lip and Palate Patients Using Non-Invasive Sources of Stem Cells

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Cleft lip and palate (CLP), affects the alveolar bone in the great majority of the cases, and the reconstruction of this defect still represents a challenge in the rehabilitation of these patients. The gold standard in alveolar bone reconstruction is autogenous bone grafts. However, these surgical procedures may be subjected to complications such as donor area morbidity, post-surgical reabsorption and infections. To circumvent these problems, researchers have been focusing on the development of bone tissue engineering strategies that may offer alternative methods with no donor site morbidity for bone grafts. Therefore, in order to identify a non-invasive alternative source of stem cells with osteogenic potential, we have used Orbicular Oris