Human embryonic stem cells (hESC) can propagate indefinitely *in vitro* under appropriate conditions. The use of proteins, such as vitronectin or laminin, promote adhesion and maintain the pluripotency of ES in monolayer cultures. The growth of ES in tridimensional scaffolds could facilitate cell engraftment and potentially be used as a first step prior to the application of specific differentiation protocols for regenerative medicine. The aim of this study has been to evaluate the capacity of different poly(lactic-co-glycolic acid) (PLGA) scaffolds to support the growth of human ES *in vitro*. Scaffolds made of 30% PLGA in THF/DMF (7:3) were produced by electrospinning and treated with 0.25M NaOH. hESC (H9) were cultivated in vitronectin-coated plates. 5×10^4 cells were plated on NaOH-treated, NaOH-treated + vitronectin or NaOH-treated + matrigel scaffolds. After 1, 7 and 14 days, the viability was analyzed by the Wst-8 method. Viability was accessed using fluorescein diacetate and propidium iodide. Electrospun fibers with an average diameter of $2.9\pm0.31\mu m$ were obtained. The viability increased in all the groups from day 1 to 14 except on the NaOH-treated scaffolds. The maximum increase was observed on the tissue culture plate. Microscopy analysis revealed that the cells formed round colonies in the NaOH-treated group and bigger and more spread colonies in the protein-coated scaffolds. No apparent differences in the number of dead cells were observed. In conclusion, H9 cells were able to grow in vitronectin and matrigel coated-PLGA scaffolds.

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Preparation and Physicochemical Characterization of Small Intestinal Submucosa-Chitosan Hydrogels as a New Material for Tissue Engineering Applications

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Hydrogels have been used in different medical applications due to their high hydrophilicity and soft structure. Nevertheless, body tissues have a wide range of physicochemical properties. We propose a Small Intestinal Submucosa and Chitosan (SIS/Ch) hydrogel as a new copolymer for tissue engineering (TE) applications. This hydrogel could promote hemostasis, angiogenesis and tissue regeneration. The objective of this study was to design a SIS/Ch material, using glutaraldehyde (GA) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as crosslinking agents (CLA), with tunable physicochemical properties. SIS-Ch hydrogels ($n=3$) were fabricated using high and low Ch and CLA concentrations. Functional groups were studied by Fourier Transformed Infrared Spectroscopy (FTIR). Characteristic decomposition patterns were assessed by thermogravimetry. Morphology was determined by Scanning Electron Microscopy (SEM). Swelling and degradation tests were performed. Rheology analysis included: flow, amplitude sweep and oscillation tests. Collagen structural stability and chitosan addition was confirmed by their FTIR and decomposition patterns, suggesting the production of a functional SIS/Ch hydrogel. SEM results showed that increasing CLA led to higher network density. Obtained porous and interconnected microstructure is optimal for cell infiltration. Although GA caused lower water uptake, hydrogels absorbed large quantities of water (30–120%), suggesting that all formulations could be used for bioactive molecules encapsulation maintaining a moisturizing environment that enhances regeneration. Degradation time was doubled by Ch and GA addition. Rheology evidenced hydrogels injectability and solid-like behavior; adding Ch and increasing CLA concentration improved network stability. In conclusion, we designed a new SIS-Ch copolymer hydrogel with tunable physicochemical properties for TE applications.

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Highly Controlled Polymer Synthesis and Additive Manufacturing Allow for Complex Scaffolds with Tunable Degradation

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The use of poly(propylene fumarate) (PPF) in tissue engineering dates back 20 years. However, only recently have we developed adequate process control of PPF synthesis and additive manufacturing (AM). In this work, a new synthesis method for PPF is combined with an additive manufacturing process to achieve fabrication of highly complex scaffolds possessing controlled chemical properties, porous architecture, and external geometry derived from patient data to optimize scaffold efficacy. A ring-opening polymerization of maleic anhydride and propylene oxide (coupled with a post-polymerization isomerization reaction) was used to produce large, highly-controlled batches of PPF. Low molecular mass oligomers, ranging from 700 to 3000 Da, were produced with narrow mass distributions (<1.6). A resin suitable for 3D printing via photocrosslinking was formed by dissolving PPF into diethyl fumarate (DEF) in a 1:1 ratio. Photoinitiators, Irgacure 784 and Irgacure 819, and light absorber, oxybenzone, were are added to enable and control the crosslinking reaction. Biocompatibility of 3D printed PPF was confirmed by cytotoxicity testing with L929 mouse fibroblasts and human mesenchymal stem cells according to ISO Standard 10993-5. Scaffolds were manufactured with two polymer molecular weights (1500 Da, 2450 Da) and two architecture styles (200 μm, 400 μm struts). Degradation was assessed in an accelerated *in vitro* environment with a 0.1M NaOH solution at 37 deg C. Over 30 days, molecular weight ($p<0.001$) and strut size ($p<0.05$) were both shown to have significant effects on scaffold degradation rate.

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No-Crosslinking Scaffold of Collagen Support the Three-Dimensional Culture of Human Coronary Artery Endothelial Cell

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Introduction: Most studies assessing endothelial activation have been made in culture dishes. However, experimental evidence supports that chemical composition and structure of the surface in which endothelial cells are seeded influence their response. This work studied the influence of scaffolds -made with collagen I using different processing methods- on the behavior of primary human coronary artery endothelial cells (HCAEC) cultured on their surface.

Methods: A collagen suspension was poured on membranes of transwell inserts, frozen (−20°C or −80°C) and lyophilized to obtain 3D-20 and 3D-80 scaffolds. The same suspension supplemented with Geltrex™ Basement Membrane Matrix also was processed to obtain 3DG-20 and 3DG-80 scaffolds. The 3D-20 and 3D-80 scaffolds were cross-linked (3DC-20; 3DC-80) or not with glutaraldehyde, the 3DGs were not cross-linked. HCAEC were seeded on the scaffolds to obtain 3DCE-20, 3DCE-80, 3DE-20, 3DE-80, 3DCG-20 and 3DCG-80 cultures. Monolayer formation, cell viability and inflammatory cytokine (IL-6, IL-8, TNF-α, IL-12 p70, IL-1β, IL-10) secretion were then evaluated.

Results and Discussion: HCAEC cultured on all the scaffolds formed monolayers independently of the scaffold manufacturing methodology used. All cultures secreted Il-6 and IL-8 but not the other cytokines and IL-6 and IL-8 secretion was significantly lower in the 3DE-80 and 3DCG-80 than in the other cultures ($p<0.05$).

Conclusion: Data indicate that 3DE-80 and 3DCG-80 have better biocompatibility with cultured HCAEC and suggest that 3D scaffolds might have differential effect on cells seeded on their surface.

Reference

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