03-151 Production of PLGA/fibrin scaffolds and in vitro test

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Tissue engineering involves the search for biological substitute materials favorable for the development and regeneration of new tissues. Poly(lactic-co-glycolic acid) (PLGA) is a synthetic polymer that presents good mechanical properties and processibility, and has been employed to develop scaffolds for tissue regeneration. In contrast, fibrin is a natural polymer, widely used in medicine, with excellent biological properties. The aim of this study was to test cell viability of keratinocytes (HaCaT cells) on PLGA scaffolds with fibrin incorporation. To produce the scaffolds, PLGA (MW=80,000; Mw 50-75 kg/mol; 75:25 lactide/glycolide; PURAC Biochem BV) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol and formic acid (50:50) and 2% polyethylene glycol (PEG; Mn 20,000) to create a 40% (w/v) solution. The set-up to conduct the electrospinning included a high voltage of 19 kV, a collector to needle distance of 20 cm, and a constant flow rate of 0.03 mL/h. Scaffolds of PLGA and PLGA/fibrin were produced. The fibrin concentration was 0.7% per scaffold. A total of 25,000 cells/well were seeded in tissue culture plates (TCP) used as a control group, or on the scaffolds, using a 24-well plate. The cell viability analysis was performed after 6 days by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test and live/dead assay. Statistical analyses were performed by ANOVA (P<0.05). The characterization by scanning electron microscopy showed uniform nanofibers. The average ± standard deviation values of absorbance and statistical difference obtained related to cell viability were: 0.60 ± 0.10 for the TCP, 0.17 ± 0.08 for the PLGA (p < 0.01) and 0.28 ± 0.03 for the PLGA/fibrin (p < 0.01). A significant statistical difference was obtained between the PLGA scaffolds and PLGA/fibrin scaffolds (p < 0.05), with evidence of the presence of a greater number of cells on the PLGA/fibrin scaffolds. Importantly, it was not possible to solubilize completely the formazan crystals of the PLGA/fibrin group, which resulted in an apparent lower viability. Both the live/dead viability test and the DAPI adhesion test showed a higher number of live cells in fibrin scaffolds compared to fibrin free scaffolds. A similar quantity of cells was possible to observe in PLGA/fibrin scaffolds compared with TCP group. It can be concluded that the incorporation of fibrin into scaffolds improved the viability of keratinocytes, being a biomaterial with potential for use in skin regeneration.