EFFECT OF PLCL/PCL NANOFIBERS-BASED SCAFFOLDS WITH FIBRIN ASSEMBLY CONTAINING PLATELET LYSATE ON SKIN CELLS

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Introduction

Skin wound healing is a process that involves several cell types, such as keratinocytes, fibroblasts, endothelial cells and other immune cells. The ability of these cells to respond upon injury is critical to start the process of healing. However, skin regeneration could be reduced due to several factors including people aging and diabetes [1]. Chronic wounds are affecting millions of patients around the world and became a serious problem for health services in developed countries [2]. In this regard, the development of scaffolds and wound dressings that improve skin regeneration is necessary. Here, we show the effect of PLCL/PCL nanofibers scaffolds that have been assembled with fibrin containing platelet lysate on keratinocytes.

Materials and Methods

PLCL/PCL nanofibers were coated with fibrin assemblies with different content of platelet lysate (1%, 5%, 10%, 20%, 50%, 100% and control 0%). Human keratinocytes cell line (HaCaT cells) were cultured directly on samples to evaluate the cytocompatibility and the effect of platelet lysate on cell growth and differentiation. Cell proliferation was quantified after 1, 4, 7 and 14 days in culture using MTS assay kit (Abcam) and the results were statistically evaluated using one-way analysis of variance (ANOVA) and Bonferroni multiple comparison test (SigmaStat). Cell adhesion and F-actin cytoskeleton organization after 24h in culture were analysed by actin fibres staining with phalloidin-TRIC and visualized with confocal laser scanning microscope (CLSM) on different z-stacks. Intermediate filaments presence and assembly were evaluated by staining two different types of cytokeratin markers. Cytokeratin 14 is a marker of basal keratinocytes, whereas cytokeratin 10 is a marker of stratified differentiation. Both cytokeratins were stained using immunodetection and visualized with CLSM.

Results and Discussion

Results indicated that keratinocytes were able to adhere and grow directly on PLCL/PCL nanofibers scaffolds. After 24h in culture, randomly distributed cells were detected on samples and the cells showed a rounded shape morphology similar in all samples analysed. After 14 days in culture, differences in cell metabolic activity were observed on samples containing 50% and 100% of platelet lysate. The increase in platelet lysate content improved cell proliferation which demonstrated the safety of the polymer and the positive effect of the platelet lysate. In addition, the second piece of PLCL/PCL nanofibers-based scaffolds were added on day 7 days to compare the effect of using 1 or 2 scaffolds on cell proliferation. The results clearly indicated that the presence of the second scaffold in medium increased cell metabolic activity and proliferation in the most samples.
Images of cytokeratin 10 and 14 immunostaining showed an increased number of differentiated cells positive for cytokeratin 10 on PLCL/PCL nanofibers scaffolds with a high percentage of platelet lysate. Keratinocytes showing cytokeratin 10 were detected on the upper layers of cells (FIG. 1) in agreement with other authors that evaluated the presence of cytokeratin types according to the differentiation of stratified layers of the skin [3]. Results obtained from adhesion and proliferation are in agreement with the differentiation results, which indicate the positive effect of platelet lysate in keratinocytes regeneration. Previously, the effect of platelet lysate on HaCaT cells has been studied [4]. In the present work, we also analysed the effect of platelet lysate incorporated in fibrin assemblies on nanofiber scaffolds with interesting results on keratinocytes.

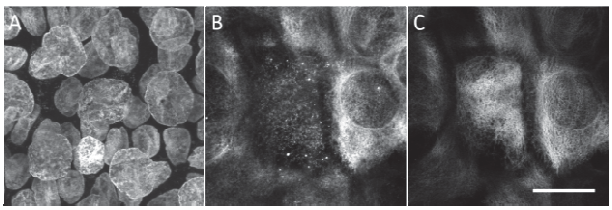

FIG. 1. Immunofluorescence staining of HaCaT cells after 7 days in culture grown on PLCL/PCL nanofibers scaffolds with fibrin assembly containing 100% of platelet lysate. Images of nuclei (A), cytokeratin 14 (B) and cytokeratin 10 (C) are shown.

Conclusions

Keratinocytes were able to spread and proliferate on PLCL/PCL nanofibers scaffolds, and the increase of platelet lysate content in fibrin assemblies on the samples improved the proliferation rate and the differentiation of keratinocytes. Both scaffold chemical properties and its biocompatibility and bioactivity in experiments on keratinocytes allow considering it to be a promising scaffold for skin wound healing. In addition, future experiments will be performed to evaluate the effect on other cell types present on skin wound.

Acknowledgments

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