Biodegradable nanofibers scaffolds containing platelet lysate for skin wound healing

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The number of patients suffering from non-healing wounds is reaching epidemic proportions and it is estimated that 1-2% of the population will experience chronic wounds during their lifetime. The prevalence of chronic wounds increases along with vascular diseases and diabetes mellitus, and along with systemic factors such as advanced age [1]. Therefore, it is necessary to develop new drugs and medical devices to improve wound healing. In this regard, nanofibrous scaffolds are promising materials to regenerate the damaged skin, because they can act as a protective barrier against penetration of microorganisms, allow gas exchange, absorb the exudate and mimic the fibrous component of the extracellular matrix. On the other hand, platelet lysate (PL) has been considered to heal skin wounds due the accelerating effect of growth factors and bioactive molecules present in platelets [2]. In the study, polymeric scaffolds containing PL were developed as skin dressings with a controlled release of growth factors and bioactive molecules. Here, we show the *in vitro* effect of two types of biodegradable scaffolds on cell types involved in skin wound healing.

Both polymeric biomaterials, poly(vinyl alcohol) (PVA) and poly(L-lactide-co-ε-caprolactone) blend with poly-ε-caprolactone (PLCL/PCL), were fabricated by electrospinning in order to obtain a mesh of nanofibers. Two different strategies were used to assemble the PL according to the biodegradability of synthetic polymers. In the case of PVA, PL were introduced into the dissolvent to obtain PL-loaded nanofibers. In case of PLCL/PCL nanofibers, the nanmofibers were coated with fibrin assemblies together with PL.
Human keratinocytes (HaCaT), mouse fibroblasts (3T3) were cultured with nanofibrious scaffolds in Dulbecco's Modified Eagle Medium supplemented with 2% of fetal calf serum. Primary human saphenous vein endothelial cells were cultured in EGM-2 medium (Promocel) without growth factors. Cell metabolic activity was quantified on days 1, 3 (4), 7, and 14 days after seeding using MTS assay kit (Abcam). Cell migration was evaluated only for EC and measured by transmigration assay using Corning FluoroBlock cell culture inserts with the pore size of 8 μm. Cell differentiation were detected by immuno-fluorescence staining of specific markers. Basal cytokeratin 14 and differentiated cytokeratin 10 were analyzed for keratinocytes, whereas von Willebrand factor and platelet endothelial cell adhesion molecule (CD31) were analyzed for endothelial cells. For fibroblasts, type I collagen was analyzed.

For PVA samples, cell metabolic activity of keratinocytes, fibroblasts and endothelial cells was increased when PVA contained PL compared with pure PVA. In addition, images of cytokeratin 10 and 14 immunostaining on keratinocytes showed an increased number of differentiated cells positive for cytokeratin 10 on PVA containing PL. Cytokeratin 10 was detected on cells which grew on the upper layers in agreement with other authors that evaluated the presence of different types of cytokeratin according to the stratified layer of skin [3]. Endothelial cells were positively stained for von Willebrand factor and CD31. 3T3 fibroblasts started to produced type I collagen.
Similar results were obtained for PLCL/PCL scaffolds. The higher values of metabolic activity of keratinocytes and endothelial cells were observed on samples containing PL. The number of cells positively stained for cytokeratin 10 was higher on scaffolds with PL. Well-developed cytoskeleton of cytokeratin 10 and 14 can be observed in cells growing on samples (Fig. 1A). Endothelial cells staining for von Willebrand factor and CD31 indicated the presence of both differentiated markers (Fig. 1B). Moreover, endothelial cells migration towards the scaffold was significantly increased when samples contained PL.

The *in vitro* results indicate that both polymers could be considered as candidates for biomedical applications. However, the potential therapeutic application of each scaffold is different due to their physical and chemical properties and biodegradation rate. PVA nanofibers do not allow cells to migrate and grown on samples, so they can be used as dressing for wound healing. On the other hand, PLCL/PCL nanofibres could be used as scaffolds for skin regeneration with cells colonization.

References

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Figures

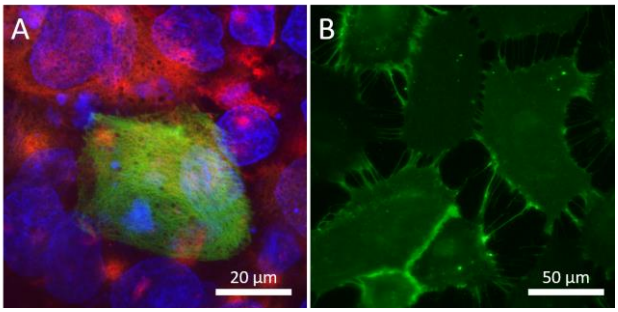

Figure 1. Immunofluorescence staining of cytokeratin 10 (green) and cytokeratin 14 (red) in HaCaT cells 14 days after seeding (A); and immunofluorescence staining of CD31 (green) in endothelial cells 7 days after seeding in medium with PLCL/PCL nanofibers scaffolds containing PL.