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DEVELOPMENT AND CHARACTERISATION OF POLYPROPYLENE-PLGA ELECTROSPUN HERNIA MESHES

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Abstract: *The use of polymer hernia meshes lead to significant reduction in the rate of relapse, pain minimisation, overall improving the post-operative outcomes of abdominal hernia. Regardless of the fact that a wide range of surgical meshes have been tailored and employed in hernia healing procedures, no mesh has yet enabled a sufficiently strong structure to simultaneously promote the remodeling of the host tissue. Within this line of thought, Electrospinning is a versatile technique, being the most reliable way of manufacturing continuous fibers scaling from nano to micrometer. Electrospun fibers have high porosities and a highsurface-volume ratio perfectly mimicking the native extracellular matrix. We have used electrospinning of poly (lactic-co-glycolic acid) (PLGA) on polypropylene hernia meshes which we characterized by scanning electron microscopy. The biocompatibility of the fibers was confirmed by cell viability assay and cell morphology analysis. We show here that the meshes modified with PLGA- nanofibers harbored an excellent biocompatibility.*

Key words: *hernia mesh, electrospinning, PLGA, polypropylene meshes, biocompatibility*

1. INTRODUCTION

Electrospinning is a versatile technique, being the most reliable way of manufacturing continuous fibers scaling from nano to micrometer. Electrospun fibers have high porosities and a highsurface-volume ratio [1] and can perfectly mimic the native extracellular matrix (ECM). Many scientific papers have demonstrated the ability of micro/ nano-fibres to support cellular attachment and enhancecell proliferation. There is a wide range of materials that can be used in this technique, such as natural or synthetic polymers, ceramic, as well as composites. Typically, natural polymers used for electrospinning include gelatin, collagen, chitosan and silk fibroin, while synthetic polymers include polylactide (PLA), poly (lactic-co-glycolic acid) (PLGA) and poly (-caprolactone) (PCL) [2-3].

Within this study, we have performed electrospinning of PLGA on a polypropylene hernia mesh. Moreover, we show that surface modification has significantly improved biocompatibility of the surgical mesh.

2. MATERIALS AND METHODS

2.1. Preparation of electrospun PLGA nanofibers. PLGA was firstly dissolved in choloroform/DMF (v/v = 3:1) at a concentration of 16%. The electrospun nanofibers were prepared with an in house engineered electrospinning equipment where a stainless steel needle with an inner diameter of .19 mm mm was used. The electrospinning conditions were set at an applied voltage of 15 kV, a tip-to-collector distance of 15 cm, and a flow rate of 0.5 mL/h controlled by a syringe pump (working temperature 24°C and relative humidity of 35%)

2.2. Morphology characterisation. Morphologies of the PLGA nanofibers was analysed by a scanning electron microscopy (SEM) (type Quanta 200 (FEI, Eindhoven, Netherlands) with an operating voltage of 15 kV. Before microscopic observation, meshes were sputter coated with gold films (10 nm thickness). Fiber diameters were quantified using the Image J 1.40G software.

2.3. Biocompatibility

Biocompatibility was evaluated using the MTT and LDH tests a spreviously described [7]. MTT assay is a quantitative test used for evaluation of both cell viability and proliferation. Briefly, NCTC fibroblasts were incubated with 1 mg/ml [3-(4.5- dimethylthiazol-2yl)]-2.5-diphenyltetrazolium bromide (MTT) solution for 4 hours. Formazan crystals were solubilized with HCl-SDS, resulting in purple solution, quantified by spectrophotometry at 570 nm, using FlexStation3 (Molecular Devices, USA).

The cytotoxicity LDH test (Roche) was performed according to manufacturer's instructions. Cells that no longer have membrane integrity release lactate dehydrogenase (LDH) into the culture medium. The culture medium was collected and mixed with the kit's components in order to be evaluated 2 days of culture by spectrophotometric readings at 490 nm. Cell morphology in the presence of the electrospun meshes was evaluated by phase contrast microscopy.

3. RESULTS AND DISCUSSION

The morphology of the formed PLGA nanofibers was analysed by SEM (Figure 1). The PLGA nanofibers were shown to have a smooth and uniform morphology with a mean diameter of 112 ± 21 nm.

MTT assay results, after 48 hours of culture in standard conditions, showed an overall good viability of L929 fibroblasts cultured in contact with a simple polypropylene mesh and the polypropylene-PLGA mesh. The PLGA modified mesh exhibited a significantly increased viability (Figure 2A) compared to the simple polypropylene mesh. Since viability and proliferation were better on the PLGA-polypropelene material than on the polypropylene mesh control, this might suggest that the electrospun fibers did not affect the fibroblasts cell viability, probably due to material's high surface area to volume ratio.

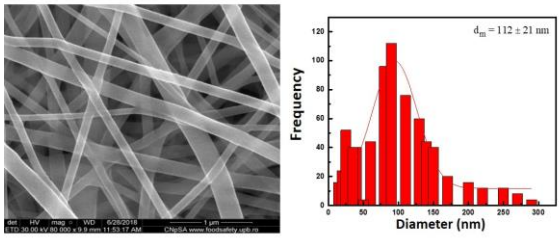

***Fig. 1**: PLGA nanofibers -SEM analysis*

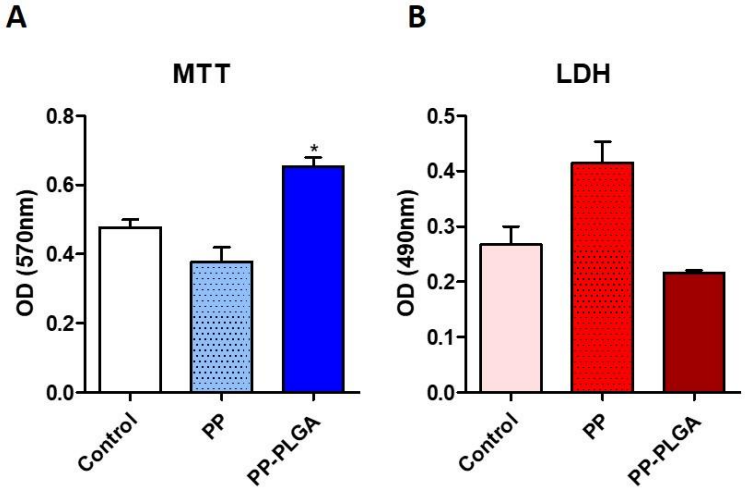

Fig. 2. *Biocompatibility analysis. A. MTT test; B. LDH cytotoxicity test*

After two days of culture in standard conditions, LDH assay indicated a similar cytotoxic effect (Figure 2B) for the PLGA-polypropylene mesh compared to the cell control, since a low number of dead cells were found after they were kept in contact with the materials. The results suggest that electrospun surgical meshes did not exert a significant cytotoxic effect on the cellular component. The results of these biochemical tests were also correlated with the microscopic analysis of the cells cultivated in the presence of the material (Figure 3). L929 cells cultured in the presence of PLGA-polypropylene meshes were shown to have a typical fibroblast shape whereas the cells cultured in the presence of simple polypropylene meshes had round morphology, typical of a stress response. Altogether, these data highlight the fact that hernia meshes surface modified by PLGA electrospinning exhibit an improved biocompatibility.

PLGA has been extensively investigated for medical uses, including suture materials, implants, prosthetic devices, bone fixation, surgical sealant films as well as drug delivery systems [4]. Recently electrospun PLGA nano- and microfibers have also been studied for tissue engineering, bone regeneration and wound dressings [5].

Our data is in agreement with other published papers. For instance, Zhang et al showed that PLGA electrospun meshes accelerated the excretion of extracellular matrix providing a promising technique to control the migration of cells [6].

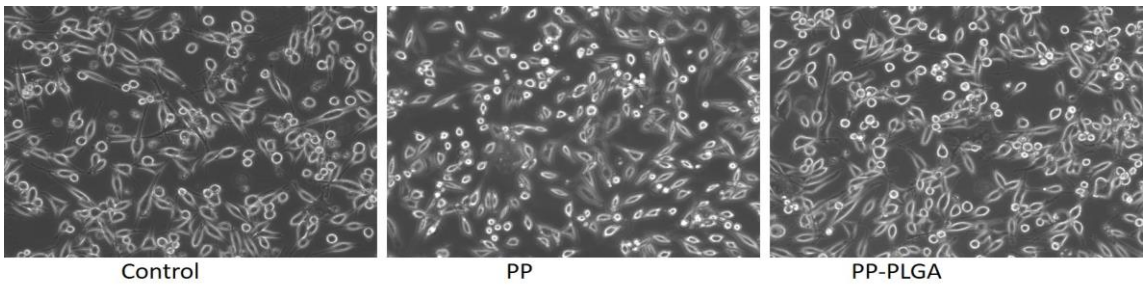

Fig. 3. *Cell morphology in the presence of PLGA electrospun meshes – phase contrast microscopy (20x magnification)*

Böhm et al showed using an *in vivo* diaphragmatic hernia model that PLGA modification of polypropylene meshes lead to increased biocompatibility compared to a standard polypropylene-based mesh [7-8].

4. CONCLUSIONS

Abdominal hernia is routinely rpaired by surgical meshes fabricated various biomaterials but , so far, no ideal hernia mesh exists. Nowadyas, surgical meshes with different fiber diameters and porosity are being developed by various manufacturing methods and implantation procedures. Surface modification can be used to retain material strength while enhancing biocompatibility of available meshes. Meshes manufactured using textile technologies reinforced with electrospun nanofibers were shown to yield scaffolds with exceptional mechanical properties and cytocompatibility.

We describe here the development of new polypropylene meshes coated with PLGA nanofibers designed for improved hernia repair. We show that these meshes harbores good cytocompatibility *in vitro* but, nevertheless future *in vivo* approaches are needed.

ACKNOWLEDGEMENTS

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