THE NEW POSSIBILITIES OF NANOMATERIAL USAGE IN MEDICINE

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Abstract

Recently, the nanotechnology experiences a great development. Nanomaterials are applied in broad spectrum of technologies as automotive industry, construction materials, energy industry or electrotechnics. National Tissue Centre concentrates on applications of nanomaterials in medicine. Thanks to unique parameters of nanomaterials there is a wide range of applications in the field of medicine. Our group works on the selection of optimal polymer, its form and the most appropriate way of application. During the development of suitable carrier was chosen and the connection between the carrier and the nanoamaterial was optimized. The main goal is to choose the most convenient type of nanofibers and to combine them with eligible carrier. The combination is intended to allow broad usage for easy, quick and very specific application. The structure and suitability of the material was assessed by stereomicroscope and inverted microscope. If a sample passed set basic criteria, the combination of nanofibers and a carrier was further tested for pH changes and the cytotoxicity by use of cell cultures. Within the development, the unique combination of the nanofibered material and the carrier was chosen and optimized. The combination can be applied not only in the field of medicine, but also in the field of biomedicinal research. Nanofiber material offers also a new opportunity for tissue engineering and advanced therapy. The combination represents the new form for use of cellular applications to allow the use of modern, efficient and easy ways for treatment. The new quality treatment could be offered to the patients.

Keywords: nanofibers, carrier, medicine

1. INTRODUCTION

National Tissue Centre works in the field of applied research. The research concentrates on the possibilities of advanced therapy treatment. There has been several cellular products available on the market in the previous years but due to the change in the legislation there has been a step back in the use of autologous cellular therapy for the patients. We would like to overcome all the legislative difficulties to be able to revive the possibility treatment by cells, as we know, from the previous experience, the treatment using patients own cells is very effective. To restart the application of for example keratinocytes in the treatment of burns, we need a new carrier, that would pass several criteria. We were looking in several places for an ideal scaffold to carry the cells and allow an ideal application. We were looking for a biocompatible material, very fine but firm, to be sterile or sterilisable. We found a solution in the use of biocompatible nanofiber scaffold. The research in the field of nanofiber carrier was realized in cooperation with ELMARCO Company.

2. MATERIAL A METHODS

Within the development, there was 12 polymers at the beginning, among others we cans list gelatine A, Polylactid (PLA) or Poly caprolactone (PCL). All polymers were produced by the method of electrospinning [1]. Electrospinning is a method, where nanofibers are obtained from solutions of chemicals in solvents. By applying high voltage (3-30kV) between the edge of the syringe with the solution and the collector (usually in the form of a plate) the Tailor’s cone is formed from the solution droplets due to electrostatic forces [2]. At the
edge of the syringe, we can see instead of the drops observe exponentially tapering cone which diameter tends to submicrometer dimensions. The direction of the end of the cone is not constant, but spins in the surrounding of its base.

12 materials prepared by elektrospinning was assessed on the basis of their biocompatibility, biodegradability, cytotoxicity, mechanical properties in dry condition and after exposure to liquid media containing serum and the optical properties of the material were also evaluated. The materials were assessed with great emphasis on the final application as a scaffold for autologous or allogeneic cells. We have set a condition of the transparency of the material to allow the observation of the cell sheet seeded on the scaffold. Another very important parameter was determined by good handling of the material, to avoid the damage of the layer of nanofibres, but also to avoid the loss or a damage of the active layer of the cells during the final application. Once the material complied with the set of criteria, the influence of various sterilization methods on the nanofiber material was tested and afterwards wide range of primokultivated cells was seeded onto the scaffold. The growth and interactions of each culture with the material were monitored.

3. RESULTS

Within the choice of appropriate polymer, there were selected three suitable materials. As the most suitable were evaluated polycaprolacton (PCL), Polylactid type 1 (PLA 1) and Polylactid type 2 (PLA 2) - (type 1 and type 2 differed in the way of preparation).

All of the chosen materials reached the best results within the evaluated macroscopic parameters as the generation of particles, strength or the deformation of the material. The change in pH and subsequently short-degradability was evaluated at the first step of testing of the effect of commonly used tissue culture medium on the nanofiber material. For all the chosen materials there was no increase in pH on the contact with the media, on the contrary, if the pH was changed at all, the change was always in the direction towards more acidic pH, but the final change was not more than 0.5.

Fig. 1 PLA 1 - inverted microscope; magnif. 100x

Fig. 2 PLA 1 - inverted microscope; magnif. 100x

Fig. 3 PCL - inverted microscope; magnif. 100x
From selected materials, PCL nanofiber material was chosen for further technological development and work. This material was subjected to final sterilization by the vapour of ethylene oxide, broad-spectrum antibiotic solution and radiation sterilization. Subsequently, primokultivated human fibroblasts were applied. By the good growth of fibroblasts on the scaffold without contamination, the effectiveness and safety of used sterilization method was confirmed.

Furthermore, the pattern and rate of growth and the morphology of cells on the scaffold was observed. Figure 4 is taken from an inverted microscope at 120x magnification, on the picture, the PLA nanofiber scaffold, radiation sterilized with growth of primokultivated fibroblasts.

When comparing the material sterilized by various methods, the difference was not observed in any of the above parameters. Figure 4, 5 and 6 shows the growth of primokultivated cell cultures.

On the figure 4, the growth of fibroblasts on PLA is pictured, the growth of primocultivated keratinocytes is shown on figure 5. Figure 6 shows keratinocytes dyed by trypan blue solution for better visualization and detection of viability on the nanofibers made from PCL. The cells shown on the fig. 5 and 6 were seed at concentration of 5x10e5, figure 5 represents the growth following 24 hours, figure 4 and 6 reveal the confluence 48 hours following the seed of cells. Expect listed cultures, the interaction of osteocytes and mesenchymal stem cells with nanofiber materials was tested. For all tested cultures the achieved confluence was 90-100% following 48hous of culture.

The optimal surface mass of nanofibers on the carrier was tested in a wide range, but its optimum value has not yet been found. This parameter will continue to undergo development with regard to the intended application and the possible availability of optical technology.

4. CONCLUSION

Based on our previous testing and development in the field of nanofiber scaffolds for tissue engineering, polycaprolacton in the form of nanofibers seems very promising material to form very good quality scaffold
for the application of autologous or allogeneic cells in medical practice, and also is deemed as a good cell carrier for the testing of cellular responses in vitro.

During the development and testing process was the production of nanofiber scaffold was transferred from the testing to production scale by the use of Nanospider [3]. Nanospider is a variant of the electrospinning method, when Tailors cone does not arise from the edge of the syringe, but is pulled out of the level of the solution. On the level of the solution there may occur many Tailors cones, therefore much higher rate of production of nanofibres is reached (about 100x) compared to the basic method of electrospinning [4]. This technology originated at the beginning of the 21st century at the Technical University, Liberec, Czech republic and is further developed commercially by Elmarco Company. Compared to the electrospinning method, Nanospider represents especially acceleration of production, higher process stability, long term production (up to 10hours per batch) and the layer of nanofibers is in uniform, transverse direction. Nanofibers are not applied onto the static collector, but they are produced onto the moving background. It can be called a band production.

There is a Nanospider line in the National Tissue Centre to allow the production of nanofiber scaffolds for the various applications to be used to help to recover suffering patients and to bring them back to common life sooner.

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LITERATURE

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