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Chapter 16. IMPACT OF THE MULTI-WALL MWCNT CARBON NANOTUBES ON THE BIOCOMPATIBILITY OF MICRO AND NANOFIBERS OF COMPOSITE POLYCAPROLACTONE MATRIX

16.1. Introduction

Biomanufacturing is an indispensable part of regenerative medicine. It is a field of research that can use knowledge of cells and tissue together with biomaterial production techniques to manufacture cell and tissue scaffolds (Fig. 16.1) [1]. Biomaterial is a material intended for integration with biological systems in order to treat, augment or replace any tissue, organ or their function [2]. Biomaterials need to meet numerous of requirements to be qualified as a material for scaffolds production. First of all, it has to be biocompatible which means it needs to be acceptable by human body without any immunity response or allergic reaction. Biocompatibility as the basic requirement that each biomaterial must meet is much more difficult to achieve then it seems, because it is complicated process depending on various factors, such as surface or structural biocompatibility, function, period of the implant application and most important its interaction with the surroundings [3]. Furthermore, biodegradable biomaterials must be able to degrade to non-toxic products within required time. It should also support cell growth and proliferation, which is necessary to heal a tissue defect. Moreover, good biomaterial should be easy to form into specific shapes with required porosity and meet appropriate mechanical requirements. The low cost of production is an addition advantage [4].

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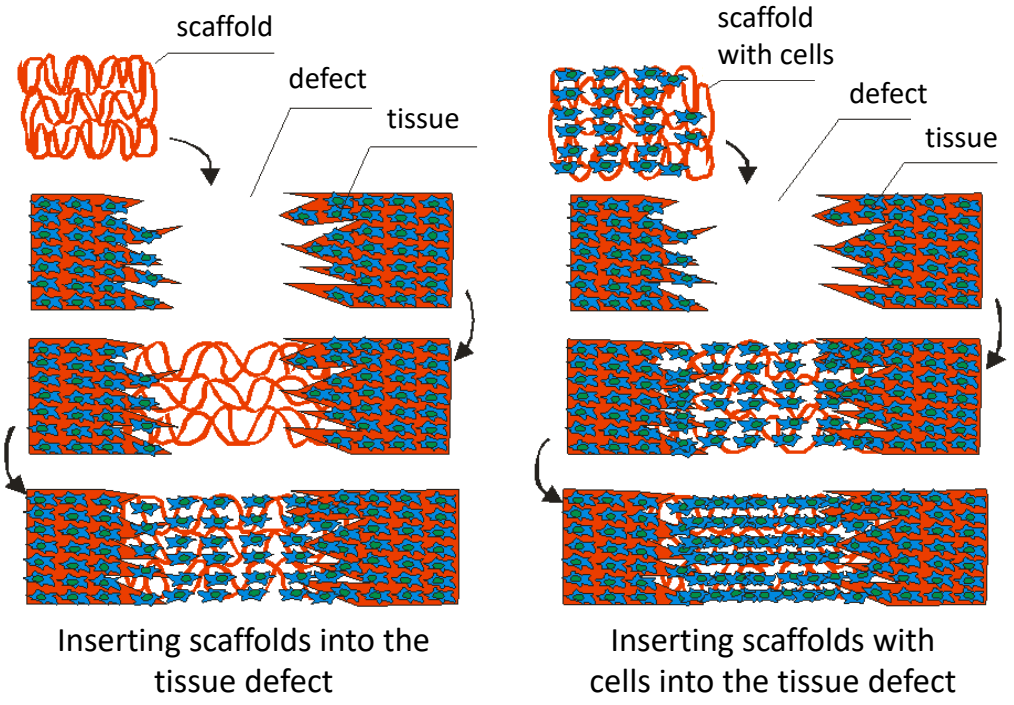

Fig. 16.1. Cell scaffolds in regenerative medicine
Rys. 16.1. Rusztowania komórkowe w medycynie regeneracyjnej

One group of materials that meet these requirements are synthetic polymers. Apart from meeting the basic biomaterial requirements, such as biocompatibility and good mechanical properties, their advantages include being cost efficacy and uniformly in large quantity production. In addition, their tensile strength, elasticity and degradation rate are similar to the bones, so they are readily used as scaffolds for bone regeneration. The group of these polymers among others includes polycaprolactone (PCL), which stands out from other polymers in this group by slower rate of degradation- up to two years, which made it attract the attention of many researchers in recent years [5].

PCL was firstly synthesized in 1930s by F. J. Van Natta and co-researchers [6]. It is hydrophobic, linear, aliphatic, semi-crystalline material with melting point at 59°C-64°C which is relatively low. PCL surface properties can be easily modified by addition of appropriate functional groups, such as hydroxyapatite or calcium carbonate. Sufficient additions can render its hydrophilic, adhesive or biocompatible. Thereat researchers found a variety of application for this biomaterial [7]. One of the first uses was in drug delivery systems to extend the time of drug delivery in the controlled manner [8]. Another example can be a scaffold manufacturing for various of tissue type, such as skin, bone or periodontal tissues [9-11]. Taking into account the emerging new

applications for the biomaterial, after discovering its properties in combination with new additives, research in that field carry a potential for discoveries important in field of regenerative medicine.

16.2. Material and methods

16.2.1. Biomaterial production

To obtain polycaprolactone in a form of micro and nanofibers firstly polymer material (Sigma Aldrich) in form of granules with or without addition of carbon nanotubes (Ssnano) has been converted into a solution with a solvent. Used solvent was a mixture of dimethyl sulfoxide, dimethylformamide and tetrahydrofuran (Chemland). Next, obtained solution was transformed into micro or nanofibers with an electrostatic field. The size of obtained fibers was depended on the viscosity of the polymer solution.

16.2.2. Cell culture

Biocompatibility tests of PCL and PCL/C 3% were carried out using human dermal fibroblasts adult (HDFa). Cells were cultured in Dulbecco's Modified Eagle Medium (Gibco, 2007787) with addition of 10% fetal bovine serum (Corning, 35079002), 2mM L-Glutamine (Corning, 34717007) and 1% Anti-Anti (Gibco, 1989506) and incubated at 37°C, 5% CO_2.

16.2.3. Biomaterial sterilization

Appropriate amount of biomaterial was placed in multiwell plate. Next, wells with biomaterials were filled to the brim with 70% ethanol and incubated for 1h. After incubation time, biomaterials were washed twice with Phosphate Buffered Saline (Lonza, 0000708833) and left to dry.

16.2.4. Biomaterial extract preparation

To obtain 100% biomaterial extract, 6 cm^2 of sterilized biomaterial was placed in 1 ml of DMEM with addition of 10% FBS, 2 mM L-Glutamine and 1% Anti-Anti and has been incubated for 24 h at 37°C, 5% CO_2 in 6-well plate. After incubation time obtained extract was portioned and diluted with cell medium to get following concentration: 75%, 50% and 25%.

16.2.5. MTT Assay

HDFa were seeded into 96-well plate at density 10 000 cells/well. After 24 h cell medium from 96-well plate was replaced with 100 µl of fresh medium, biomaterial extract at appropriate concentration or 5% DMSO (Corning, 25-950-CQC) as control. Cells were incubated for another 24 h at 37°C, 5% CO_2. After incubation time, MTT solution has been prepared by dilution of 3 mg Thiazozyl Blue Tetrazolium Bromide (Sigma, MKCK7253) in 3 ml DMEM without phenol red (Gibco, 2036286). Thereafter cell medium and biomaterial extracts were replaced with 50 µl MTT solution. After 2 h of incubation, MTT solution was replaced with 100 µl isopropanol which was used to dissolve formazan crystals. The absorbance was determined spectrophotometrically at 570 nm.

16.3. Results

16.3.1. PCL micro and nanofibers

The study of structure of the micro and nano fibers obtained in electrostatic field that was performed with use of SEM Scanning Electron Microscope showed differences between obtained fibers (Fig. 16.2-16.3). The following fibers and their characteristics are obtained in PCL samples: (i) fiber's diameter of less than 1000 nm (30% of all studied fibers), (ii) fiber's diameter between 1001 and 1500 nm (15% of all studied fibers), (iii) fiber's diameter above 1501 nm (55% of all studied fibers).

Adding 3% share of carbon nanotubes to the solution changes the diameter of the obtained fibers. In a PCL sample containing 3% share of carbon nanotubes, the

following fibers were found: (i) of diameter below 1000 nm (25% of the sample), (ii) of diameter between 1001 and 1500 (40% of the sample), (iii) of diameter above 1501 nm (35% of the fibers).

The total number of fibers below 1500 nm in diameter stands for 45% of the total share while 1500 nm in diameter stands for 55% share in the tested PCL sample. In the sample containing 3% addition of carbon nanotubes fibers with a diameter below 1500 nm constitute 65% and those above 1500 nm only 35% of all fibers.

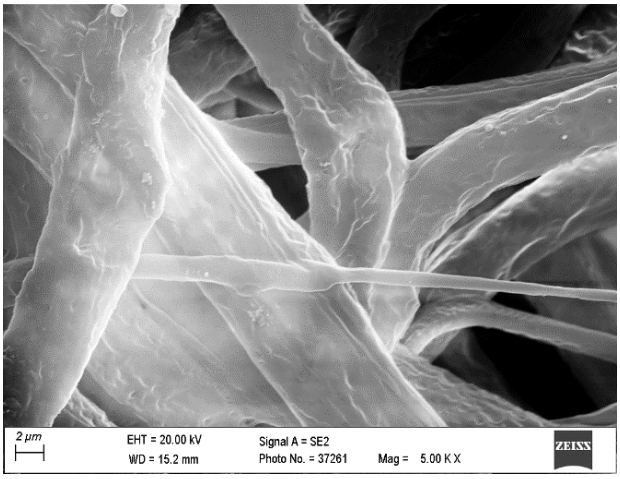

Fig. 16.2. The structure of the polycaprolactone fibers. Images taken using a scanning electron microscope (SEM)

Rys. 16.2. Struktura włókien polikaprolaktonu. Zdjęcia wykonane z zastosowaniem skaningowego mikroskopu elektronowego (SEM)

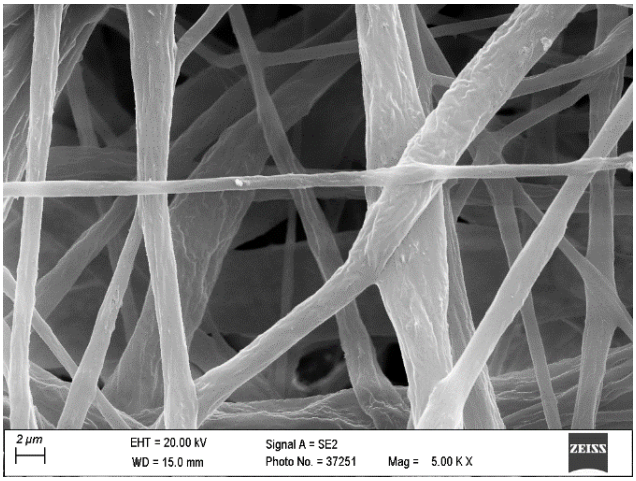

Fig. 16.3. The structure of the polycaprolactone fibers with carbon nanotubes. Images taken using a scanning electron microscope (SEM)

Rys. 16.3. Struktura włókien polikaprolaktonu z nanorurkami węglowymi. Zdjęcia wykonane z zastosowaniem skaningowego mikroskopu elektronowego (SEM)

16.3.2. Biocompatibility of PCL extract

The experiment was carried out once with three replications for each extract and six replications for controls. No toxic effect of the PCL extract on HDFa was found (Fig. 16.4). The lowest cell viability was observed in 75% PCL extract, where it was decreased by 23.1% and the highest in 50% extract, where it was decreased by 9%.

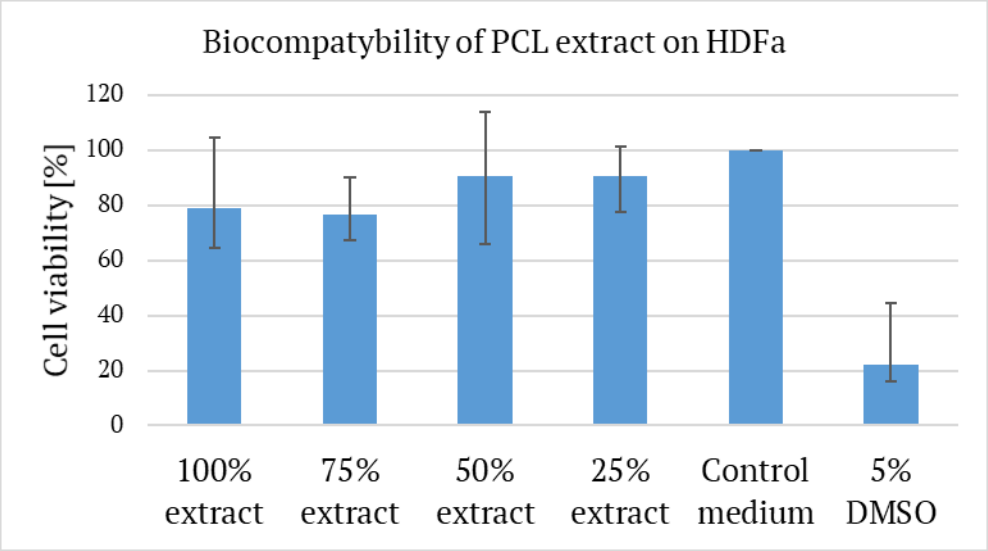

Fig. 16.4. Biocompatibility of PCL extract on HDFa
Rys. 16.4. Biokompatybilność ekstraktu z PCL testowana na komórkach HDFa

16.3.3. Biocompatibility of PCL/C 3% extract

The experiment was carried out once with three replications for each extract and six replications for controls. No toxic effect of the PCL/C 3% extract on HDFa was found (Fig. 16.5). The lowest cell viability was observed in 50% extract and it was decreased to 77.3%. Furthermore in 75% extract HDFa viability was increased to the value of 108.3%.

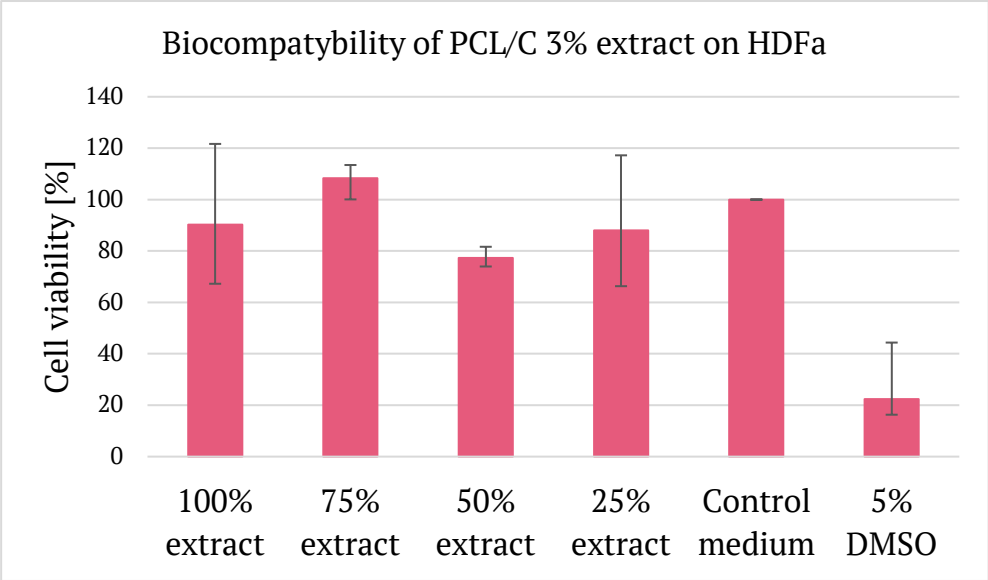

Fig. 16.5. Biocompatibility of PCL/C 3% extract on HDFa
Rys. 16.5. Biokompatybilność ekstraktu z PCL/C 3% testowana na komórkach HDFa

16.4. Discussion and Conclusion

PCL is well known biomaterial with well-known properties. Thanks to the possibility of combining, it with various additives and a relatively low production cost, it has found wide range of application in regenerative medicine. Its biocompatibility was repetitively confirmed in its basic form. However, taking in consideration a complexity of cell viability and proliferation processes, any modification in structure of PCL has to be tested for its biocompatibility.

The conducted research has shown that electrically conductive additives such as carbon nanotubes are conducive to the production of fibers with a smaller diameter. Furthermore, based on the performed analysis, it can be concluded that produced PCL extracts and PCL with 3% carbon nanotubes have no toxic effect on cells. According to UNE-EN ISO guidelines cell viability must be reduced to <70% in comparison to cells in control medium to state a cytotoxic potential of tested material [12]. In the performed analysis, cell viability was not reduced below 70% in any of the tested extracts. The lowest cell viability for PCL were observed in 100% extract and 75% extract, where it was 79.02% and 76.91% respectively. As the concentration decreased, the viability was less reduced and amounted to 99.9% in 50% extract and to 90.72% in 25% extract. The result suggests that PCL extract can exert an effect on cell viability, but it is not intense enough to be determined as a toxic biomaterial. Similarly, induced modification of

biomaterial in the form of carbon nanotubes also did not show toxic effect. Cell viability was decreased the most in 50% extract to 77.25% and in 25% extract to 87.98%. In 100% extract cell viability was less reduced to the level of 90.29%. Furthermore, in 75% extract cell viability was increased by 8,25% compare to cells in control medium. In contrast to PCL extracts, in the case of PCL/C 3% extracts, a lower cell viability is observed in extracts with lower concentrations. Moreover, observed cell viability increase in 75% extract suggest potential viability stimulating effect of tested biomaterial on HDFa. However, it should be taken into account that the results may have been affected by differences in cell density, so the number of test repetitions should be increased. Additionally, considering that the biomaterials are produced to be used as cells scaffolds, direct contact tests are planned, which will provide more information on the impact of the addition of carbon nanotubes to PCL on the viability of HDFa.

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IMPACT OF THE MULTI-WALL MWCNT CARBON NANOTUBES ON THE BIOCOMPATIBILITY OF MICRO AND NANOFIBERS OF COMPOSITE POLYCAPROLACTONE MATRIX

Abstract

Regenerative medicine uses tissue scaffolds made of various polymeric materials to rebuild tissue. One of the biomaterials that scientists pay a lot of attention to is polycaprolactone (PCL), which is widely studied for its use as a base for an electrically conductive biomaterial. This study focused on the preparation of fibrous tissue scaffolds obtained in an electrostatic field with the addition of MWCNT carbon nanotubes and

the assessment of their biocompatibility. The viability of cells incubated with extracts from the assessed biomaterials was not less than 76.9% for fibroblast cells. The extracts of the tested biomaterials showed no toxic effect on the tested cell line.

Keywords: biocompatibility, polycaprolactone, carbon nanotubes.