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POLYCAPROLACTONE FIBER-BASED BOVINE DENDRITIC CELL DEVELOPMENT AND THREE- DIMENSIONAL CULTURE *IN VITRO*

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ABSTRACT

Two-dimensional (2D) cell culture systems are normally used for *in vitro* experiments. These systems have some drawbacks which affect results of *in vitro* experiments because cultured cells have different morphology and physiological properties comparing to *in vivo* models. Therefore, there are current technique to develop three-dimensional (3D) cell culture systems which are closer to the environment in real tissues. In our study, we used polycaprolactone fiber scaffold for culture of bovine monocytes to develop dendritic cells (DCs) from those cells. Cultured monocytes in the polycaprolactone nanofiber scaffold differentiated to DCs. The differentiated DCs showed a 3D structure in the scaffold. These results suggest that this method is suitable for the development of DCs in a manner of three-dimension. These methods are perspective for bovine cell culture and has advantages compare to conventional 2D culture systems.

Keywords: scaffold, three-dimensional cell culture, monocyte, dendritic cell

INTRODUCTION

Dendritic cells (DCs) are antigen-presenting cells which are the connection between innate and adaptive immunity through engulfing antigens and presentation of that antigen fragment on MHC molecule to T lymphocytes (**Song *et al.*, 2018; Soto *et al.*, 2020**). There are many studies of human and mice DCs but lack of studies focused on bovine DCs (**Kratochvilova *et al.*, 2019; Kratochvilova and Slama, 2020; Cuncha *et al.*, 2022**). *In vitro* studies of DCs are influenced by many factors and conditions of cultivations. One important condition of cell incubation is to be so close to real microenvironment like in the real tissue. Studies focused on DCs is usually based on DCs developed from blood monocytes. Subsequent studies based on DCs are usually done in two-dimensional (2D) culture systems in plastic wells or plastic flasks. These DCs have limited functions as movement and phagocytosis (**Lee *et al.*, 2021; Sayde *et al.*, 2021**). Therefore, results from this 2D culture experiments may show signs of devaluation. It could be difficult to apply and compare those results with results from *in vivo* experiments. For these reasons, it is necessary to create methods for cell culture which would be similar as in the tissue. This is also very important for the methodology within there are development of dendritic cell-based vaccines which is one of the possibilities how to use bovine DCs against mastitis (**Kratochvilova *et al.*, 2023**) that is one of the most important diseases causing high economic losses in all of the world (**Cobirka *et al.*, 2020; Zigo *et al.*, 2021**). The principal of dendritic cell-based vaccines is already used in cancer therapies (**van Willigen *et al.*, 2018; Wang *et al.*, 2020; Yu *et al.*, 2022**).
Three-dimensional (3D) cell culture systems can be very good substitution of *in vivo* experiments. There are more types of 3D culture systems, but we can divided it into two group: scaffold-based or scaffold-free systems (**Sayde *et al.*, 2021**). One of the methods which belongs to the scaffold-based systems is using of nano or microfibers scaffolds. This method is using various materials for development of fibers: polycaprolactone (PCL), polyvinyl alcohol, silk fibroin etc. (**Park *et al.*, 2010; Rockwood *et al.*, 2011; Lee *et al.*, 2021**). Each material has different properties that is important for development of scaffolds with various characteristics which is suitable for specific cells.
The aim of this study was to evaluate if it is possible to use PCL fiber scaffolds for development and cultivation of bovine DCs.

MATERIAL AND METHODS

Preparing of PCL fiber membranes

PCL fiber membranes were prepared in accordance to previously published procedure by electrospinning (NanoNC, Seoul, Korea) (**Kim *et al.*, 2016; Oh *et al.*, 2020; Lee *et al.*, 2021**). Two-nozzle spinnerets were used with the flow rate of approximately 8 µL/min, using a syringe pump. Each nozzle had an inner diameter of approximately 210 µm (27G). Fibers were collected onto a rotating metallic mandrel at 100 rpm at ambient temperature for 4 h. The nozzle tip-to-collector distance was set at 20 cm, with an electrical potential of 17.5 kV. The structure of fabricated fibers was observed using scanning electron microscopy (SEM) (MAIA3, Tescan Ltd., Brno, Czech Republic). The electrospun PCL-NMs were sterilized by soaking in a solution of 70% ethanol for 12 h and dried under UV exposure for 12 h.

Isolation of monocytes

For isolation of peripheral blood mononuclear cells, there were used density gradient centrifugation by Histopaque solution (densitiy 1.077) (Sigma-Aldrich, USA) and then there were used isolation of monocytes with the technique based on magnetic microbeads (MicroBeads, Miltenyi Biotec, Germany). This method was previously described by **Szczotka *et al.* (2009)** and adopted by **Kratochvilova *et al.* (2019)**. Briefly, blood was obtained from four clinically healthy heifers 16 to 18 months of age. The blood was drawn from the *vena jugularis externa* in an amount of 50 mL, ethylenediaminetetraacetic acid (EDTA) was used as anticoagulant. The blood was centrifuged (2100 rpm), and then the buffy coat was collected. Histopaque was put into a test tube and softly layered over with buffy coat. These test tubes were centrifuged for 1 hour (2100 rpm). The layer of peripheral blood mononuclear cells was then taken. Then, this cell suspension was marked with microbeads (CD14 MicroBeads, human, Miltenyi Biotec, Germany). Antibodies with microbeads were attached to CD14-positive cells (monocytes) which were then acquired in magnetic separator (Miltenyi Biotec, Germany). The cell viability was measured by trypan blue exclusion test. Monocytes were checked in light microscope (Leica DMi1, Pragolab, Czech Republic).

Cultivation of monocytes on PCL fiber membranes

Before using of the PCL membranes for cultivations, there were sterilized by 70% ethanol and dried under UV exposures. CD14-positive cells were used for development of DCs. 10^6 cells were placed into wells of 24-wells cultivation plate. In each well, there were placed PCL membrane. Cells were cultivated for 7 days. In each day of incubation, cells were checked in inverted light microscope (Leica DMi1, Pragolab, Czech Republic). Cell incubation was carried out in incubator under these conditions: temperature 37 °C, 5% CO_2. The incubation medium consisted of RPMI 1640 medium, cytokines IL-4 and GM-CSF (bovine dendritic cell growth kit, Bio-Rad), antibiotics – 10,000 units penicillin, 10 mg streptomycin, and 25 µg amphotericin B per ml (Antibiotic Antimycotic Solution, Sigma-Aldrich, USA).

Scanning electron microscopy (SEM)

The morphology of PCL fibres, and monocytes and DCs attached to fibres were examined by SEM on a Tescan MAIA 3 equipped with a field emission gun (Tescan Ltd., Brno, Czech Republic). The best images were obtained using the In-lens SE detector at working distances between 4.91 and 5.34 mm and at a 1.0 kV acceleration voltage (fibres with cells) and at a 2.0 kV acceleration voltage (fibres). Images were obtained at 221–4,430-fold magnification.

RESULTS AND DISCUSSION

The goal of the study is to evaluate whether PCL nanofiber scaffold is suitable for development and cultivation of bovine DCs. Fabricated PCL nanofiber membranes were evaluated by SEM (MAIA3 Tescan, Czech Republic). Their structure and morphology met the requirements for subsequent cell cultivation and therefore that were used for cultivation of bovine monocytes. In the figures (Figure 1, 2), it is visible that preparing of the PCL membranes was successful.

We isolated peripheral blood mononuclear cells from bovine blood and then monocytes (CD14-positive cells) that were seeded on PCL nanofiber membrane in the presence of GM-CSF and IL-4. During the incubation, monocytes were able to adhere to PCL fibres as it is shown in Figure 3. After 7 days of monocyte culture, we found that monocytes were differentiated to DCs.

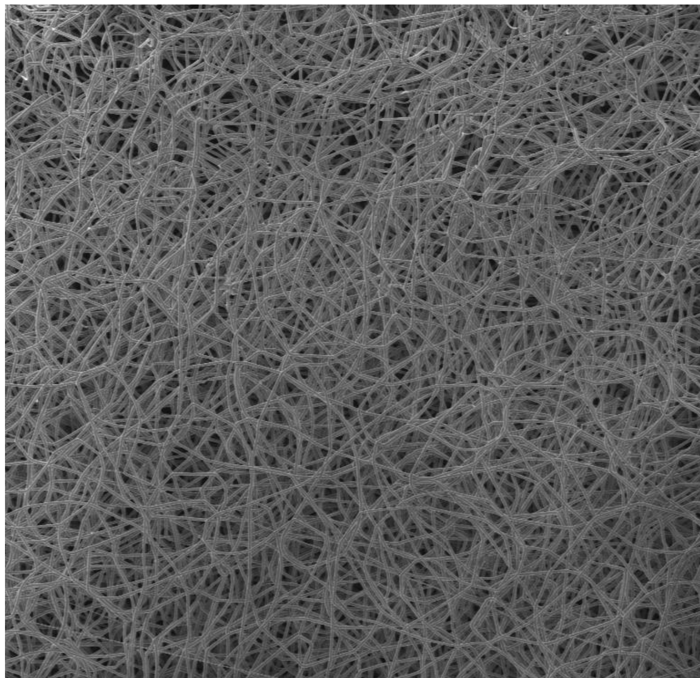

Figure 1 PCL membrane structure, SEM, magnification 221x.

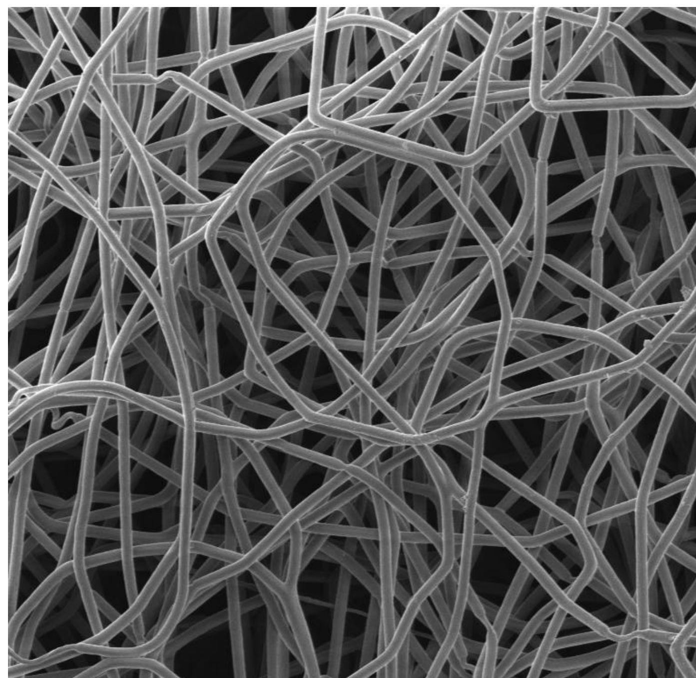

Figure 2 PCL membrane structure, SEM, magnification 871x.

Our results shown that PCL membranes seems to be applicable for the culture of bovine monocytes and differentiation to DCs. In comparison, this 3D cell culture system using PCL nanofibrous scaffold has been used for culture of mouse bone marrow-derived DCs (**Kim *et al*., 2016; Lee *et al*., 2021**). PCL nanofibrous scaffold was also used for cultivation of mouse neutrophils and macrophages (**Lee *et al*., 2021**). In our experiments, when we seeded monocytes on PCL membrane, these monocytes were able to infiltrate nanofibrous scaffold and adhere to fibres (Figure 3) without spontaneous activation. Moreover, those monocytes were able to fully differentiated into DCs by adding cytokines. The differentiated DCs also infiltrated the scaffold and adhere to nanofibers in a 3D manner.

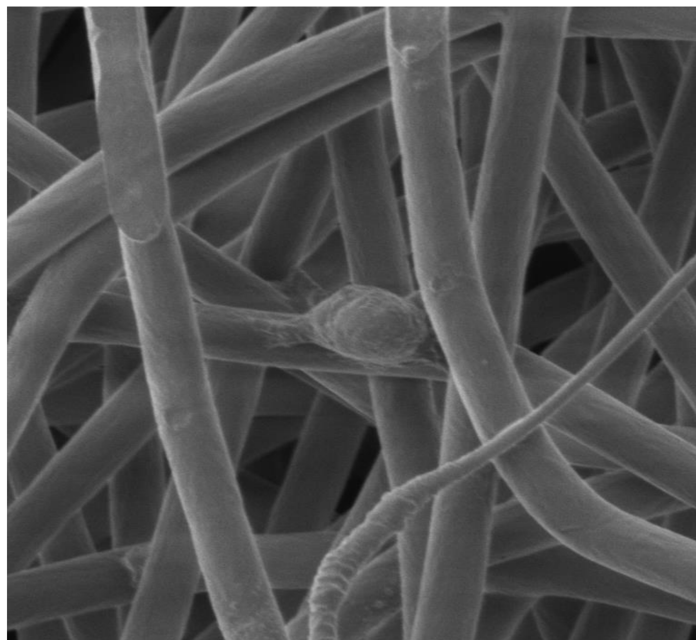

Figure 3 Monocyte attached to fibre, 2 days of incubation, SEM, magnification 4,410x.

Monocytes were able to transform into DCs on the fibres inside of the membranes. After 7 days of incubation, DCs were fully developed (Figure 4, 5).

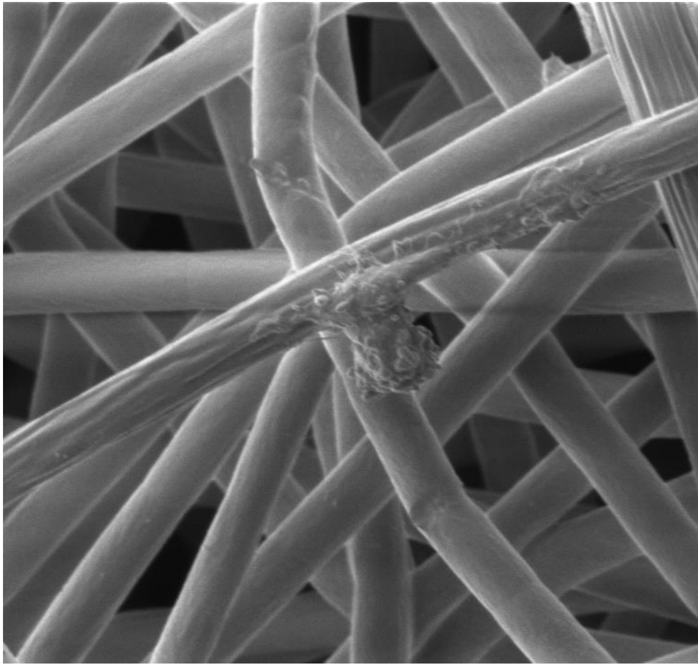

Figure 4 DC attached of PCL fibre, 7 days of incubation, SEM, magnification 4,430x.

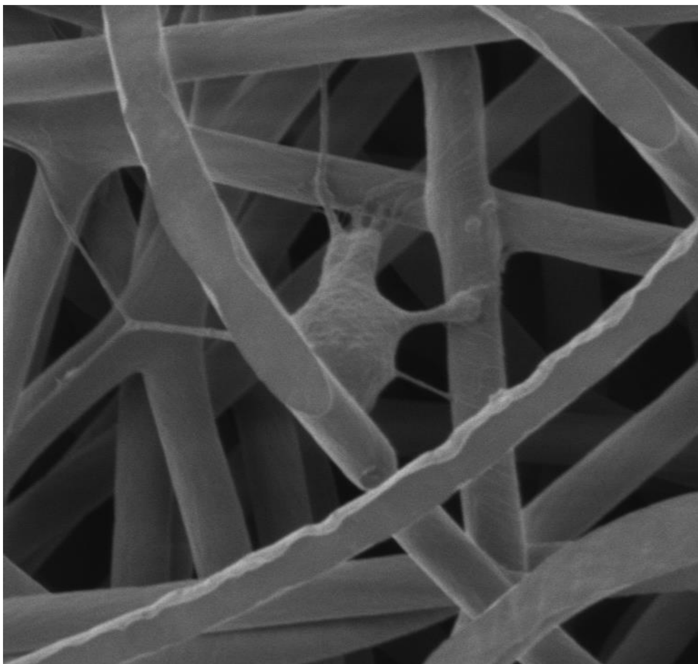

Figure 5 DC attached of PCL fibre, 7 days of incubation, SEM, magnification 4,300x.

Lee *et al.* (2021) referred that it is possible to observe the movement of neutrophils and DCs over time in the PCL fibrous membrane. Moreover, this culture system is suitable for observation of migratory behavior of neutrophils, macrophages and DCs. They found out that neutrophils and DCs become elongated on fibres. Then, these phagocytes are able to move along fibres to find the nearest bacteria.

One big advantage of PCL fibres is that they are inert and biocompatible with immune cell. PCL fibres does not influenced activation status of DCs (**Woodruff and Hutmacher, 2010; Jin *et al*., 2015**) in comparison with 2D culture in plastic wells or plastic flasks.

PCL nanofibrous scaffolds are also capable as multicellular culture model. For example, cancer and immune cells can form a microenvironment which is physiologically relevant more than normally used 2D systems (**Kim *et al*., 2016**). Nano or microfibrous scaffolds can be prepared from various materials as PCL, polyvinyl alcohol, and silk fibroin (**Park *et al*., 2010; Rockwood *et al*., 2011; Lee *et al*., 2021**) or with mixture of those materials. Combination of more materials can improve advantage of each of them. There are used mixture of PCL and silk fibroin for preparing of scaffolds. This mixture combines the mechanical benefits of PCL with biological benefits of silk fibroin. These nanofibers can be potentially promising material for better attachment of cells and for tissue regeneration (**Singh *et al*., 2020**).

CONCLUSION

3D cell culture systems are very important for obtaining relevant results which can be comparable with *in vivo* experimental works. Nano or microfiber scaffolds are suitable for cell cultivation and mimicking real microenvironment in tissues and organs. It seems that PCL fibrours membranes are convenient for cell cultivation and development including development of bovine DCs. For our next experiments, we plan to use PCL fibrous membranes to mimic bacterial infection caused by *Streptococcus uberis* and to study interactions of phagocytes with bacteria.

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