Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.11415

64 (2017) 317–323
February

Preparation of biodegradable semi-permeable membranes as 3D scaffolds for cell cultures

Aleksandra Kruk[a,*], Agnieszka Gadomska-Gajadhur[a], Paweł Ruśkowski[a], Andrzej Chwojnowski[b], Judyta Dulnik[c], Ludwik Synoradzki[a]

[a]*Faculty of Chemistry Warsaw University of Technology, 00-664 Warszawa, Noakowskiego 3 Poland, Tel. +48 22 234 54 63; Fax: +48 22 625 53 17; emails: akruk@ch.pw.edu.pl (A. Kruk), agadomska@ch.pw.edu.pl (A.G. Gajadhur), pawel.ruskowski@ch.pw.edu.pl (P. Ruśkowski); Tel. +48 22 234 55 04; Fax: +48 22 625 53 17; email: ludwik.synoradzki@ch.pw.edu.pl (L. Synoradzki)*
[b]*Nałęcz Institute of Biocybernetics and Biomedical Engineering PAS, Księcia Trojdena No 4; 02-109 Warszawa Poland, Tel. +48226599143 Ext. 143; Fax: +48226597030; email: achwoj@ibib.waw.pl*
[c]*Institute of Fundamental Technological Research PAS, Pawińskiego 5B 02-106 Warszawa, Poland, Tel. +48228261281 Ext. 211; Fax: +48228269815; email: jdulnik@ippt.pan.pl*

Received 1 April 2016; Accepted 9 August 2016

ABSTRACT

Results of the preparation of semi-permeable membranes made of biodegradable polymers membranes were presented. Among known polyesters, polylactide was selected for research. The membranes were obtained using wet phase inversion method. The influence of polyvinylpyrrolidone and polymeric nano-non-wovens as pores precursors on the structure of obtained membranes was analysed. It was shown, that utilisation of polymeric nano-non-wovens enabled preparation of semi-permeable membranes, which could be used as wide-pore 3D-type cellular scaffolds.

Keywords: Biodegradable polymers membranes; Biodegradable polyesters; Porous three-dimensional scaffolds; Inversion phase method

1. Introduction

Three-dimensional cells scaffolds are structures used for tissue cultures. They are generally in the form of semi-permeable membranes, which provide delivery of nutrients to cells and evacuation of metabolites outside the scaffold [1–3]. Synthetic polyesters such as a polylactide (PLA), poly-ε-caprolactone (PCL), polyglycolide (PGA) or copolymers are materials frequently used in the preparation of membranes [4]. They are characterised by biocompatibility and biodegradability and are well-tolerated by organisms [5–8]. Moreover, these compounds and their degradation products are non-toxic to organism cells. Additionally, they are subjected to hydrolytic decomposition, first to composing monomers, which are physiologically present in organisms of mammals, and subsequently to CO_2 and water, products of physiological metabolism, which are easily excreted from the organism. These polyesters differ in degradation time, which, for a given polymer, increases with the length of carbon chain. Due to this characteristic, it is possible to control, to some extent, degradation time of the whole scaffold by choosing appropriate polymer with a specific molecular weight [9–13].

Polyesters are easily processed and that is the reason of their often used in medicine, pharmaceutics or other industrial sectors. Nevertheless, during preparation of polyester cell scaffolds, some practical problems occur. Semi-permeable polyester membranes are characterised by their small (the order of 10 μm), practically unconnected pores, which are present inside the structure. Surfaces of those membranes are characterised by the presence of very few, small pores (range of few μm). Besides good properties of polyesters,

* Corresponding author.

Presented at the Conference on Membranes and Membrane Processes in Environmental Protection (MEMPEP 2016), Zakopane, Poland, 15–19 June 2016.

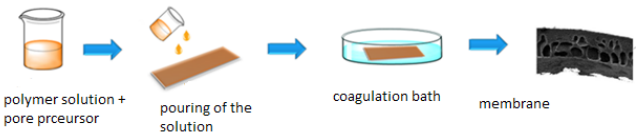

Fig. 1. Phase inversion method [22].

membranes, which are made of them, exhibit unfavourable characteristics for the culture of cells other than fibroblasts and keratinocytes (lack of possibility for cell penetration into the membrane, difficulties in migration of nutrients and metabolites) [14–17].

Nevertheless, polyesters have multiple favourable features, such as possibility of biodegradation, bioresorption and good mechanical durability. For this reason, methods utilising pore precursors, which will enable preparation of membranes with a morphology preferable for the cell cultures, are sought [18,19].

There are many techniques used for preparation of polymer membranes. One of those techniques is a method of "wet" phase inversion (Fig. 1). It is characterised by simplicity and easy operation and it does not involve the use of complex devices. For example, it is based on pouring of the membrane-forming polymer solution onto an inert base (e.g., glass plate) and on its subsequent immersion in coagulation bath consisting of non-solvent of polymer. It is important that solvent and non-solvent are miscible. After polymer coagulation, the membrane is taken out of the coagulation bath and dried. The use of pore precursor is a variant of this method. For this purpose, other polymers (e.g., polyvinylpyrrolidone (PVP), poly(ethylene glycol)) or non-organic salts with appropriate crystal diameter are mainly used. Pore precursors are washed out from the structure of already coagulated membrane. It takes place in coagulation bath with additional wash bath depending on the solubility of the precursor used [20,21].

2. Goal of studies

The goal of the studies was to obtain PLA membranes for cultures of chondrocytes with adequate morphology. According to requirements, the lower surface should have multiple, large pores with diameter about 20–80 μm. The lower surface should contain rare, small pores with a size about 5–15 μm. In the cross-section large, interconnected pores with size 20–80 μm should be present. Such the morphology allows for entry of cells inside the scaffold by upper surface and prevents of falling out by "solid" lower surface. Large, interconnected pores in the cross-section enable growth and communication of cells and migration of nutrients and metabolites.

3. Materials and methods

3.1. Materials

Poly-L-lactide (PLLA), M_n 86,000 g mol^{-1}, Nature Works NW 2003D and PVP, M_n 10,000 g mol^{-1}, supplied by Sigma Aldrich were used to prepare membranes. Chloroform, 1,4-dioxane, methanol, all produced by POCh SA, were used as solvents. Ultrapure water with 18.2 MΩcm conductivity was obtained using MiliQ device.

3.2. Polymer nano-non-wovens

Nano-non-wovens were obtained using electrospinning technique with PVP, M_n 1,300,000 g mol^{-1}, and pork gelatin.

3.3. Preparation of membrane-forming solution

Solutions of PLLA with in dioxane and in chloroform, both of with 6%$_{wt}$ concentration, were prepared. PLLA was dissolved in organic solvent after 24 h with constant stirring using a magnetic stirrer and with no heating. After complete dissolution of PLLA (minimum 24 h), polyvinylpyrrolidone was added and subsequently the stirring was continued for the next 24 h.

3.4. Preparation of membranes without polymer nano-non-woven

PLLA solution in chloroform with the addition of PVP was poured onto a glass base. Membrane was gelled in methanol. After polymer coagulation and removal of pore precursors, membrane was dried.

3.5. Preparation of membranes using PVP nano-non-woven

PLLA solution in dioxane with PVP was poured onto a glass base. Polyvinylpyrrolidone nano-non-woven was placed on the solution layer after pouring and the air was removed by applying pressure. Next, subsequent layer of solution was poured and nano-non-woven was again placed. At the end, the air removal from the formed membrane-forming solution was repeated. The membrane was gelled in water with conductivity of 18.2 MΩcm and it was dried after polymer coagulation and removal of pore precursors.

3.6. Preparation of membranes using gelatine nano-non-woven

PLLA membrane using gelatine nano-non-woven was prepared analogically to PVP nano-non-woven formation procedure. The only difference was that PLLA solution in $CHCl_3$ supplemented with PVP and methanol gelling bath were used. Washing (aqueous) bath after gelling bath was utilised in order to eliminate polymeric nano-non-wovens.

3.7. Scanning electron microscopy

Morphology of membranes cross-sections and their surfaces were analysed using Scanning Electron Microscopy (SEM) Hitachi TM1000. Samples of membranes were immersed in ethanol and then fractured in liquid nitrogen. After drying, membrane samples were coated with 7–10 nm thick gold layer using K550X Sputter Coater. Samples coated with gold were analysed in 300x and 1,000x magnifications using 15 kV acceleration voltage.

3.8. Membranes porosity

The porosity of membranes was calculated using Eq. (1):

$$Porosity = \frac{V_m - Vp}{V} 100\% \quad (1)$$

Both mass and volume of the prepared membranes were measured. Dividing M by the density of polymer (ρ_p) gave the volume of polymer (V_p) within membranes structure. The density of PLLA was taken from supplier characteristic sheet (1.25 g cm^{-3}).

4. Results and discussion

4.1. Morphology of membranes

The main problem in preparation of polyester membrane cell scaffolds is to obtain appropriate porosity and pore morphology. Without using pore precursors in phase inversion method, it is not possible to obtain sufficiently porous surfaces and big, interconnected pores inside the membrane. Classical pore precursors, that is, polymers, polyvinylpyrrolidone, poly(ethylene glycol) or salt crystals, which are added to membrane-forming polymer solution, and subsequently washed out from the coagulated membrane, do not give the desired effect either. Such pores are not big enough (over a dozen μm) and are unevenly distributed. Membrane for cell culture should have big (at least 20–30 μm) pores, which are distributed in a whole membrane volume and are interconnected. It is extremely important to obtain high porosity one of one of the membrane surface. In turn, the second surface should have small, not numerous pores, which will prevent cells from slipping out of the polymer scaffold.

Polymer nano-non-wovens obtained using electrospinning method are characterised by the morphology of pores being dependent on the material used for spinning, on the solvent properties and other parameters of the process (Figs. 2 and 3).

Hence, they can be accepted as non-classical pore precursors, which give the possibility to obtain structures with a morphology defined by the nano-non-woven. Its advantage, in contrast to conventional pore precursors (polymeric and non-organic), is that during preparation, they are located in the whole volume of the membrane. It guarantees even distribution of pores, which are present not only inside the membrane, but also at one of its surfaces.

Photomicrographs of PLLA membrane obtained conventionally, that is, where PVP was used as a pore precursor and added to the solution of membrane-forming polymer, are presented in Figs. 4 and 5. Lower surface, located on the glass plate side, used as a base, contained multiple, oval pores of diameter ranging from 5 to 15 μm (Fig. 4). Upper surface contained more pores. Moreover, they were bigger – their size was in the range from about 3 to 25 μm (Fig. 4). Other pores on the same surface were covered with thin coating layer.

Membrane cross-section was characterised by numerous pores of 5–15 μm in diameter (Fig. 5). There were other bigger pores present between them, of 20–30 μm in diameter. It was important that on the walls, especially of the bigger pores, there were also smaller pores (below 5 μm). It was a significant

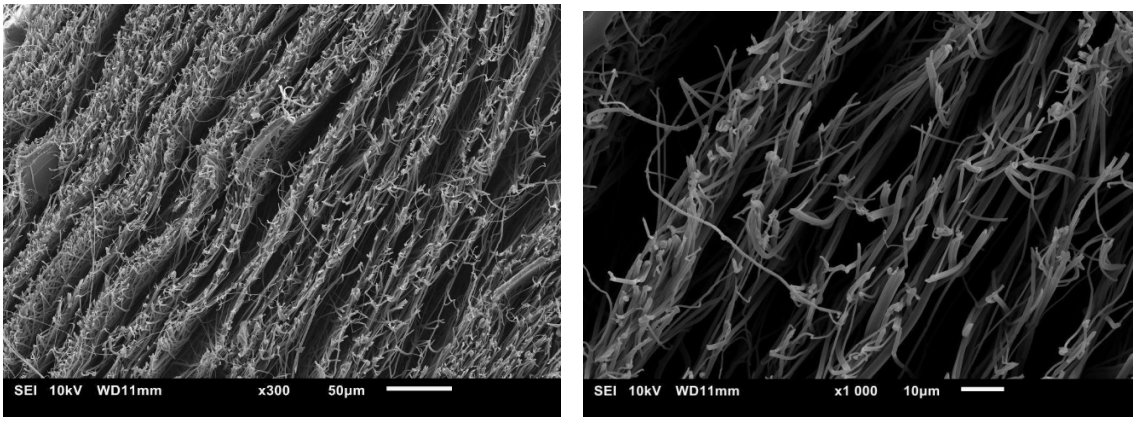

Fig. 2. SEM photomicrographs cross-section of gelatine nano-non-woven: Magnification 300x (left) and 1,000x (right).

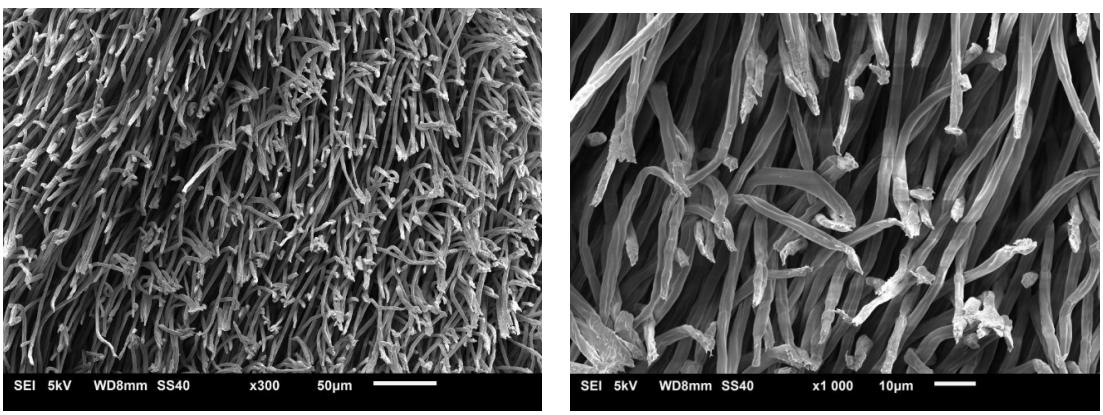

Fig. 3. SEM photomicrographs cross-section of polyvinylpyrrolidone nano-non-woven: Magnification 300x (left) and 1,000x (right).

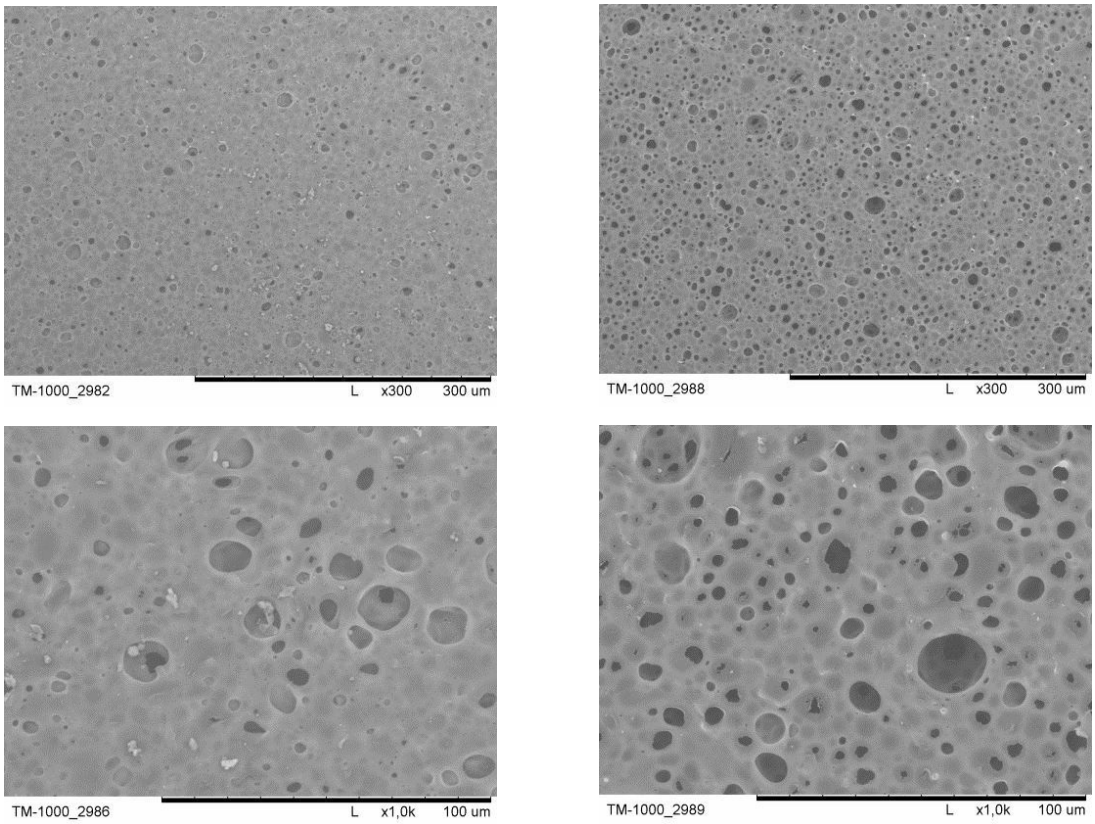

Fig. 4. SEM photomicrographs of PLLA membranes obtained with addition of PVP. Lower (left) and upper (right) surfaces: Magnification 300x (top) and 1,000x (bottom).

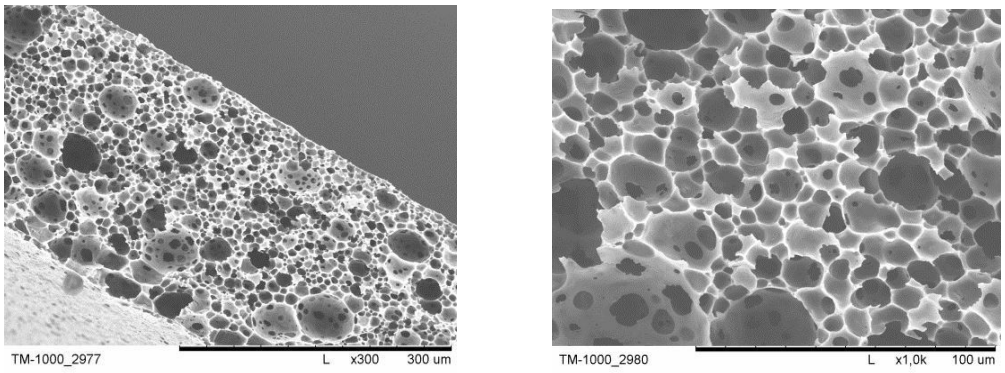

Fig. 5. SEM cross-section photomicrographs of PLLA membranes obtained with addition of PVP: Magnification 300x (left) and 1,000x (right).

feature considering cells, because perforation of inner pore walls enabled migration of nutrients and metabolites.

Morphology of the discussed membrane, despite having a few advantages, was still not preferable for the cell cultures. Upper layer contained insufficient amount of pores, through which cells could migrate. Similarly, pores, which were present in membrane cross-section were in most cases too small to contain cells.

In Figs. 6 and 7, photomicrographs of PLLA membrane obtained using gelatine nano-non-woven as a pore precursor are presented. Distinct differences in structure of both surfaces (Fig. 6) can be observed. Lower surface contained few pores with 5–10 μm in diameter. Upper surface had absolutely different characteristic. It contained numerous pores of 20–40 μm, between which there were smaller ones of 5–15 μm. On this surface, nano-non-woven "imprints" were also visible, the implementation, pressing and washing out of which led to the destruction of the covering layer formed.

Cross-section of membrane obtained using gelatine nano-non-woven characterised with the presence of very big, numerous pores of 20–70 μm and even 100 μm in diameter (Fig. 7). Shape of pores was quite irregular, yet similar

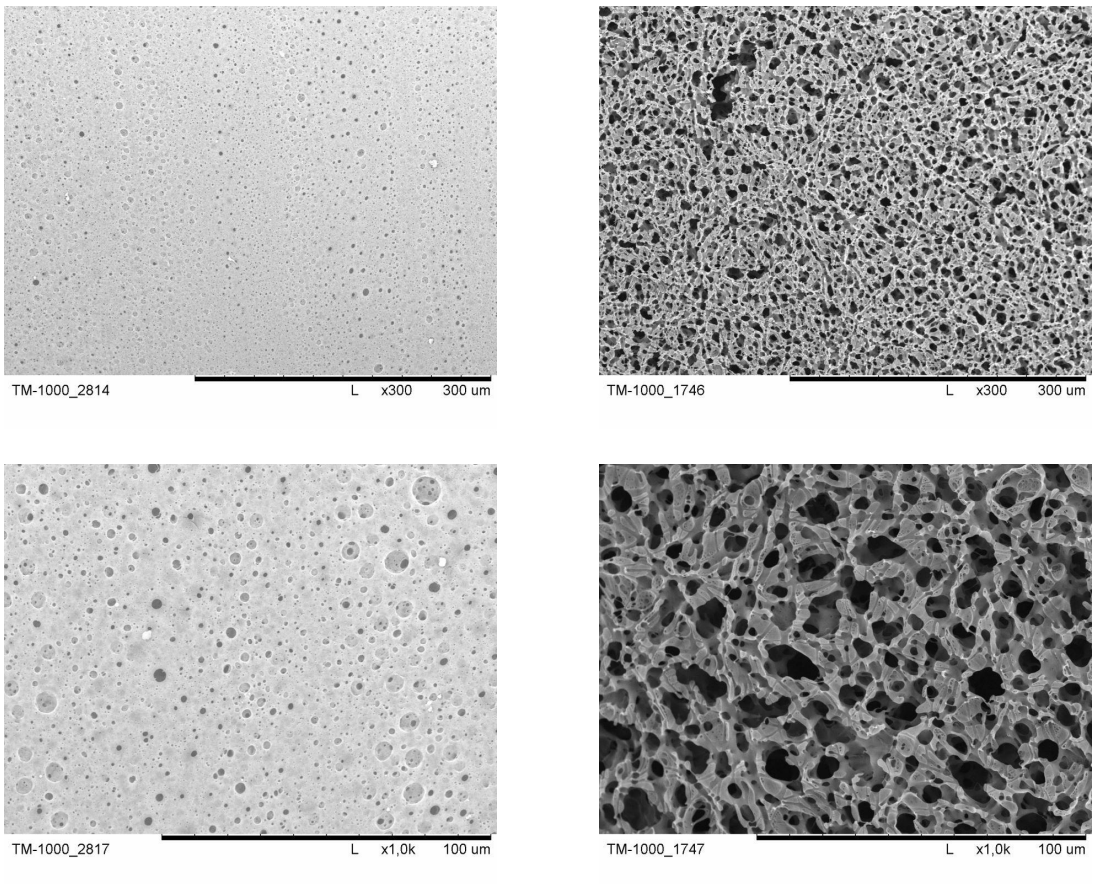

Fig. 6. SEM photomicrographs of PLLA membranes obtained with addition of gelatine nano-non-woven. Lower (left) and upper surface (right): Magnification 300x (top) and 1,000x (bottom).

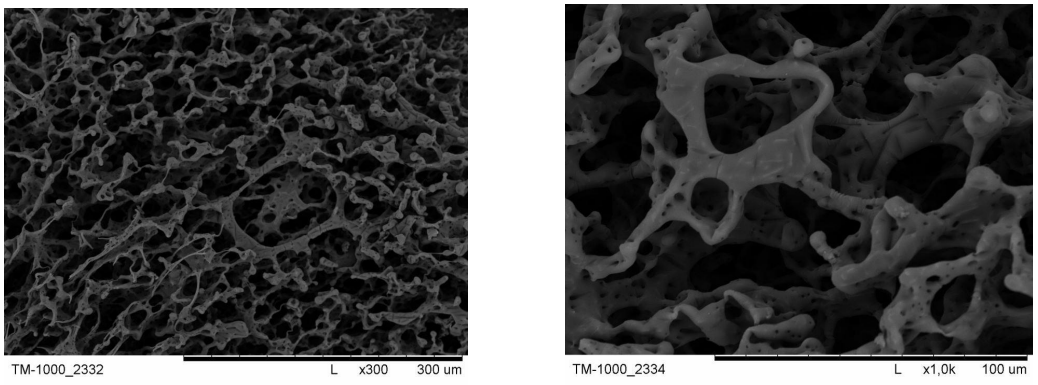

Fig. 7. SEM cross-section photomicrographs of PLLA membranes obtained with addition of gelatine nano-non-woven: Magnification 300x (left) and 1,000x (right).

to oval. Walls of pores were thin, their thickness, in most cases, was of a few μm (below 5 μm). It was essential that they formed a network of interconnected pores. Besides, in the walls of those pores, there were also much smaller pores (about 1 μm). Moreover, walls of the pores had imprints of nano-non-wovens like in upper layer.

Membrane obtained using gelatine nano-non-woven characterised with a structure preferable for cell cultures. The structure of upper surface enabled infiltration of cells into the membrane. Cross-section enabled nutrients migration and contact with other cells due to the high perforation of pore walls. Lower surface eliminated the risk of cells slipping out due to the low porosity.

Photomicrographs of membrane prepared using PVP nano-non-woven as a pore precursor are presented in Figs. 8 and 9. Lower surface contained few pores, like in case of membrane, where gelatine nano-non-woven was used, its surface ranged between 5 and 20 μm (Fig. 8). Upper

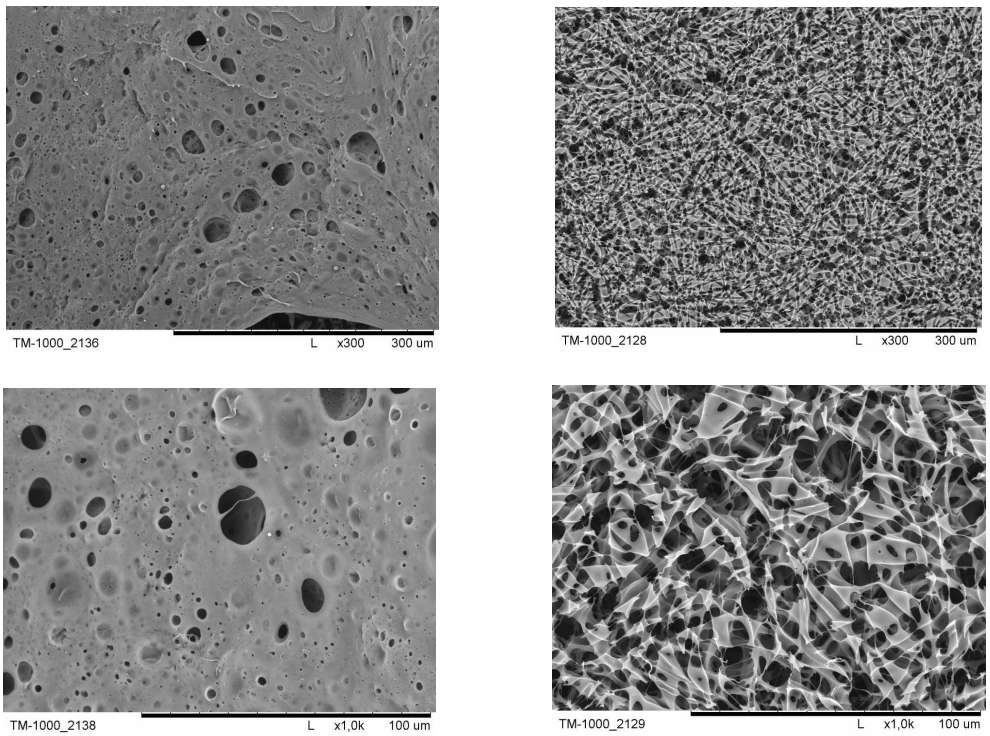

Fig. 8. SEM photomicrographs of PLLA membranes obtained with addition of PVP nano-non-woven. Lower (left) and upper surface (right): Magnification 300x (top) and 1,000x (bottom).

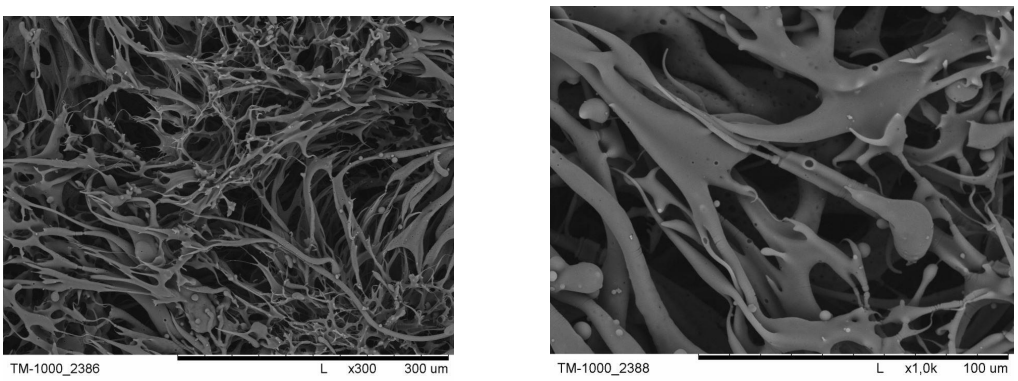

Fig. 9. SEM photomicrographs of PLLA membranes obtained with addition of PVP nano-non-woven: Magnification 300x (left) and 1,000x (right).

surface was highly porous and pores, which were present there, had elongated (10–20 μm width, 50 μm long) and oval shape (10–20 μm in diameter) (Fig. 8).

Cross-section of the membrane where PVP nano-non-woven was used contained multiple elongated pores (Fig. 9). They were characterised with large size, that is, they were 20–50 μm wide and 50–100 μm long. Walls of pores had perforations, wherein they were very small in size (smaller than 1 μm). Pore walls were 5–15 μm thick. Similarly to the case of gelatine nano-non-woven, pores present in the cross-section were interconnected.

The membrane, where PVP nano-non-woven was used as a pore precursor, exhibited a morphology, which was favourable for the cell culture. Upper surface enabled infiltration of the cells into the membrane due to its structure and lower surface prevented cells from slipping out. Cross-section contained numerous, big, interconnected pores and small perforations.

Table 1
Porosity of the membranes

Pore precursor	Porosity, %
PVP in polymeric solution	99
Gelatine nano-non-woven	98.1
PVP nano-non-woven	99.7

4.2. Porosity of membranes

In Table 1, porosity of the membranes is presented. Results show that membranes obtained with addition of PVP and gelatine or PVP nano-non-wovens as a pore precursors characterised with a high porosity. The higher value of this parameter was observed membranes obtained with PVP, which had been added to polymeric solution, but this difference was not significant. In the case of membranes, which would be used as a scaffold, the most important feature was the size of pores and their presence on the upper surface.

5. Final remarks

The conducted studies show that the utilisation of a classical pore precursor, in the form of PVP, for the preparation of semi-permeable PLLA membranes facilitated generation of small pores inside the membrane. Their size was insufficient for cell cultures leading to the formation of tissue, because it eliminated the possibility of cells infiltration into the membrane. Besides, both surfaces had the same rate of porosity and the formed pores were too small. Morphology of PLLA membranes obtained using wet phase inversion was changed, when polymer nano-non-wovens were used. First of all, numerous pores appeared at the upper surface. Cross-sections of membranes prepared using polymeric nano-non-wovens, were highly porous; however, bigger pores were observed for PVP nano-non-woven. Inner walls of pores were perforated in both cases, wherein bigger perforations were observed in the membrane, where gelatine nano-non-woven was used. Pores, which were generated as a result of the use of nano-non-woven, differ in their shape. Gelatine nano-non-wovens formed pores with irregular shape, which was similar to oval, and PVP one formed pores with elongated shape. Presence of "imprints" at the upper surface and in the cross-section proved that pores were generated thanks to nano-non-wovens. In case of lower surfaces, those "imprints" were not present (nano-non-woven was not present there, while pouring the mixture). Next significant feature of the prepared membranes was the even distribution of pores in whole volume of the developed membrane.

Morphology of the membrane, where polymer nano-non-wovens were used as precursors, was the favourable one for cell cultures. Nano-non-wovens, depending on the type of material which they were made of, facilitated formation of pores with a different shape and size. Based on that, they enabled cell culture of different cell types. Cells differed in size and shape, and thus they required different spatial conditions depending on the cell type.

Acknowledgement

Financial support of Warsaw University of Technology, Faculty of Chemistry is gratefully acknowledged.

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