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The dependence of the membrane structure on the non-woven forming the macropores in the 3D scaffolds preparation

Andrzej Chwojnowski[a,*], Aleksandra Kruk[b], Cezary Wojciechowski[a], Ewa Łukowska[a], Judyta Dulnik[c], Paweł Sajkiewicz[c]

[a]*Nałęcz Institute of Biocybernetics and Biomedical Engineering PAS, Księcia Trojdena 4. 02-109 Warszawa, Poland, Tel. +48226599143 Ext. 143; Fax: +48226597030; emails: achwoj@ibib.waw.pl (A. Chwojnowski), cwojciechowski@ibib.waw.pl (C. Wojciechowski), elukowska@ibib.waw.pl (E. Lukowska)*
[b]*Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warszawa, Poland, Tel. +48 22 234 54 63; Fax: +48 22 625 53 17; email: akruk@ch.pw.edu.pl*
[c]*Institute of Fundamental Technological Research PAS, Pawińskiego 5B, 02-106 Warszawa, Poland, Tel. +48228261281 Ext. 211; Fax: +48228269815; emails: jdulnik@ippt.pan.pl (J. Dulnik), psajk@ippt.pan.pl (P. Sajkiewicz)*

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ABSTRACT

Three types of membrane structures with wide pores were compared in this study. One of the membranes was obtained from polyethersulfone using cellulose fibers as the macropore precursors. Two of the fibers were obtained from poly(L-lactide). As the macropore precursors polyvinylpyrrolidone (1.2 MDa) and pork gelatin non-woven were used, the influence of non-woven fibers on the structure of membranes was shown. Necessity of specific membrane structure application was explained. The choice of polymers and co-polymers with a range of biodegradation times can determine the scaffold type suitable for the age of a patient.

Keywords: Polysulfone membrane; Polyester membranes; Membrane structures; Biodegradable membranes; 3D scaffold

1. Introduction

Joint diseases are ones of the most troublesome ailments of the modern human being. They are often caused by the non-physiological way of life (lack of motion) or overburdening of the joints (sports, work). However, nowadays the most severe causes of injuries are road accidents that result in severe injuries of joints, especially knee joints.

The knee is one of the largest and most complex joints in our body. It plays an essential role in movement related to carrying the body weight in horizontal (running and walking) and vertical (jumping) directions [1]. The knee joint consists of two articulations, one between the femur and tibia, and one between the femur and patella [1]. The knee is a mobile angular ginglymus or troclear, which permits flexion and extension as well as a slight medial and lateral rotation [2]. The joint is bathed in the synovial fluid, which is contained inside the synovial membrane called the joint capsule. Ligaments join the knee bones and tendons connect the knee bones to the leg muscles providing stability to the knee. Because in humans the knee supports nearly the whole weight of the body, it is vulnerable to both acute injury and chronic development of osteoarthritis. Two C-shaped pieces of cartilage called the medial and lateral menisci lie between the articular surfaces of the femur and tibia [3–5]. The menisci are shock absorbers of the load and make the articular surfaces between the femoral condyles and the tibial plateau concordant [3–5]. During flexion, the menisci slide forward and during extension they slide back [2]. The menisci are divided into outer rim, inner rim and core [3–5]. The inner rim is the most delicate part as it is not vascularized. The lateral meniscus has the form of an almost complete circle and

* Corresponding author.

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adheres to two cruciates [3–5]. The medial meniscus has the form of a half moon and is more extensive than lateral with its extremities adhering to anterior and posterior intercondylar areas. Between the two menisci, the medial meniscus is more susceptible to trauma because it is less mobile than the lateral due to presence of the semimembranous tendon and also because of the tendency to have a slight valgus during gait [3–5].

Recently, a vast expansion in biomaterial technologies, scaffolds, cell sources, and molecular and genetic manipulations took place to create functional tissue replacements to treat cartilage injuries or osteoarthritis [6–8]. Scaffolds that are most often studied in the cartilage tissue engineering area include hydrogels made from poly(ethylene glycol) diacrylate [9], collagen [10], fibrin [11], agarose, and synthetic peptides [12,13]; sponge-like scaffolds manufactured from materials such as collagen, polyglycolic acid, polylactic acid [14], and polyurethane [15]; materials with a naturally-occurring porous structure, such as coral, devitalized articular cartilage [16], and hyaluronan based scaffolds [17]. The three-dimensional scaffold provides the structural support for cell contact and matrix deposition prevents dedifferentiation of autologous chondrocytes even after long periods and promotes the expression of chondrocytes-specific markers [18].

So far, the polyethersulfone scaffold has been the only real three-dimensional (3D) type of scaffold for chondrocytes cultivation that was made from a synthetic non-polyester polymer and thoroughly tested in an animal model (rabbit) [19–21]. In contrast to other commercially available and researched scaffolds, it has the structure that ensures easy and non-limited permeation of cells in its interior. At the same time, the internal structure of the scaffold guarantees uncurbed growth of chondrocytes, their multiplication and in effect creation of cartilage. It is typical for this scaffold to have a long resorption time in a living organism. The resorption time can amount to over 6 months and is remarkably longer than in other types of scaffolds. It is undoubtedly an advantage in the regeneration of massive defects of cartilage in adults, especially with mature joints. However, it can prove to be a certain deficiency in the therapy of younger humans in formative years as the joint and the cartilage are still growing. In such cases, it is expected that scaffolds with a shorter degradation period are more beneficial. In order to test the correlation between the regeneration of defects of cartilage and the degradation period, an array of polyester scaffolds has been prepared at a laboratory scale with a real 3D structure. The assembled scaffolds differed in respect of the ingredients of the membrane and the macropore precursor [22]. All these scaffolds had a set of similar characteristics resulting from the manufacturing method used. The aim of this study was to show differences and similarities of scaffolds depending on which non-woven fabric used in membranes preparation.

2. Materials and methods

All reagents and chemicals used were of the analytical grade. PSE 6020 was purchased from BASF PVP 10 kDa, poly(L-lactide) were purchased from the Chemistry Department at Warsaw Technical University, Pluronic® F 127, N-methyplirolidone (NMP), tetrahydrofurane (THF) 1,4-dioxane were purchased from Merck. Chloroform and methanol were purchased from POCh. The deionized water (18,2 MΩcm) was produced using Mili-Q apparatus (Milipore).

All the below-presented scaffolds have been obtained using the same method. The non-woven fabric was soaked in a solution or a membrane-forming mixture to allow both sides of the fabric to form a skin layer. Next, on the "over" side of the soaked fabric a subsequent layer of the same fabric was overlaid and, instantly, the membrane was gelled through dipping in a non-solvent of the membrane-forming polymer. If the non-solvent of the membrane-forming polymer is at the same time a solvent of the fabric we can obtain the ready-made membrane at once, which is a scaffold. However, if the fabric has not been subjected to dissolving, it is necessary to rely on careful dissolution of the fabric not to harm the structure of the already-created membrane and avoid dissolution of the membrane material. For obtaining scaffolds, we have used natural materials, including cellulose (filtration tissue paper, Fig. 2(a)), nano-micro fabrics made from polyvinylpyrrolidone with an average particle mass 1.2 MDa (Fig. 2(b)) and pork gelatine (Fig. 2(c)). Those fabrics were obtained by an electro-spinning method which allows strict control of fabrics' morphology. In consequence, pores in the membrane obtained using these fabrics have strictly defined structure, size and shape corresponding to the morphology of the fabrics.

As a membrane-form mixture for soaking the cellulose fabric polyethersulphone in N-metylpyrrolidone with an addition of the polyvinylpyrrolidone (MW 10 kDa) as supporting pores precursor was used. For soaking the swine-originated fabric a solution of poly(L-lactide) in chloroform was used. For soaking the fabric with polyvinylpyrrolidone poly(L-lactide) in 1,4-dioxane was used. In both cases, we also used the polyvinylpyrrolidone of MW 10 kDa as a supporting precursor of the pores. The soaking process for both the gel fabric and the polyvinylpyrrolidone fabric has been performed in lowered temperatures.

The Hitachi TM1000 scanning electron microscopy (SEM) was used for imaging the membrane surface and cross-sectional morphologies. Membrane samples were first immersed in ethanol and then fractured in liquid nitrogen. The samples were coated with a 7–10 nm layer of gold using K550X Sputter Coater apparatus. Coated samples were examined at different magnifications at an acceleration voltage of 15 kV.

3. Results and discussion

The method developed by us designed procedure allows for obtaining scaffolds with an authentic 3D structure that has some unique features. In all presented cases, the membranes that form a scaffold are asymmetric membranes possessing a skin layer.

An essential feature of these membranes is the presence of two different types of skin layers (Fig. 1 A1 lower skin and A2 left-top). One of these skin layers has compact structure. In case of polyethersulphone scaffolds, it is a layer with the cut off ranking 20kD. Therefore, it constitutes a barrier not only for cells but also for the majority of protein particles, including proteolytic enzymes which can potentially threat

the cells. However, in the case of polyesters the layer is more porous while also being impermeable for cells. It means that in all cases, it protects from "falling out" of the cells from the scaffold (Fig. 2).

The second skin layer ("the over layer") is perforated on the entire surface. The perforation has the size of the holes enabling free penetration of the cells into the scaffold's interior (Fig. 3).

Photomicrographs presented in Figs. 2 and 3 have shown dissimilarities between both types of skin layers. Overall, the highest mechanical resistance of the membrane is obtained in the presence of two skin layers. Perforation allows for introducing the cells into the interior of the scaffold. This can lower only to a certain degree the resistance of the membrane as a whole. If the perforation is lacking, the permeation into the scaffold's interior can proceed only on the verges of the scaffold, which in the case of defects exceeding 5 mm in diameter would be insofar insufficient.

A scaffold with only one skin layer is relatively simple to obtain; yet, its mechanical resistance is decidedly too low in the intended applications. The resistance of the membrane with two skin layers enables its fixing in the joint with surgical stitches or through studding. In other cases, fixation with tissue glue is used but this type of fixation is not always sufficient. Moreover, the implanted membrane that exhibit high resistance enables a "quicker putting into motion" of the operated joint, which is essential in therapy. So far, low resistance of the gel scaffolds was the greatest obstacle to overcome as the pressure on the joint was resulting in the gel scaffold to be misplaced out from the joint. Consequently, patient's joint had to be immobilized over a longer period not to burden the joint in a static manner.

For biomedical applications membranes' pore size and porosity are critical factors to consider. For cultivation of chondrocytes, the membrane should have pore size between 20 and 80 μm in diameter. This size of pores enables free proliferation of chondrocytes and in consequence creation of reclaimed cartilage. The interior of the scaffold obtained by our method is built of macropores and the density of packing of the fibers removed from the membrane structure (Fig. 4). Therefore, through the choice of fabric, it is possible to control the size of the macropores and the thickness of the walls between macropores. The walls in between the macropores are also porous, which ensures access of nutritious substances and removal of metabolic products from the interior of the newly created articular cartilage (Fig. 5).

Macropores in precursors have a significant influence on the membrane structure. The size of membrane pores is mainly determinated by the size of pores in precursor fibers. The largest pores are obtained using cellulose, whereas the smallest pore diameters have pork gelatin fibers. Moreover, fibers' material has the influence on the membrane structure. In the case of polyethersulfone membranes, the structure of macropores is identical to cellulose fibers. In the case of membranes from poly(L-lactide), where PVP and pork gelatine non-woven fibers were used, the structure of macropores is not exactly the same as the structure of used fibers. Despite similar non-woven structure (Fig. 4(b) and (c)) of PVP and pork gelatine, differences in membranes' structures are significant.

The use of PVP non-woven fibers results in more composite macropores. On the contrary, pork gelatin non-woven fibers create more uniform pores shapes. It is clear that the selection of the non-woven material as a pore precursor determines the shape of pores in the final product.

PVP and pork gelatin non-woven fibers are obtained by the electrospinning method. This method enables obtaining fibers of different thickness and arrangement, which gives a possibility of the pore size control according to desired type of cells. In case of cellulose fibers, possibilities of size control are very limited. Only several cellulose fiber types with varied diameters are available. On the other hand, viscose fibers with a wide range of diameters can be used. In this study, the electrospinning method was not available for viscose fibers.

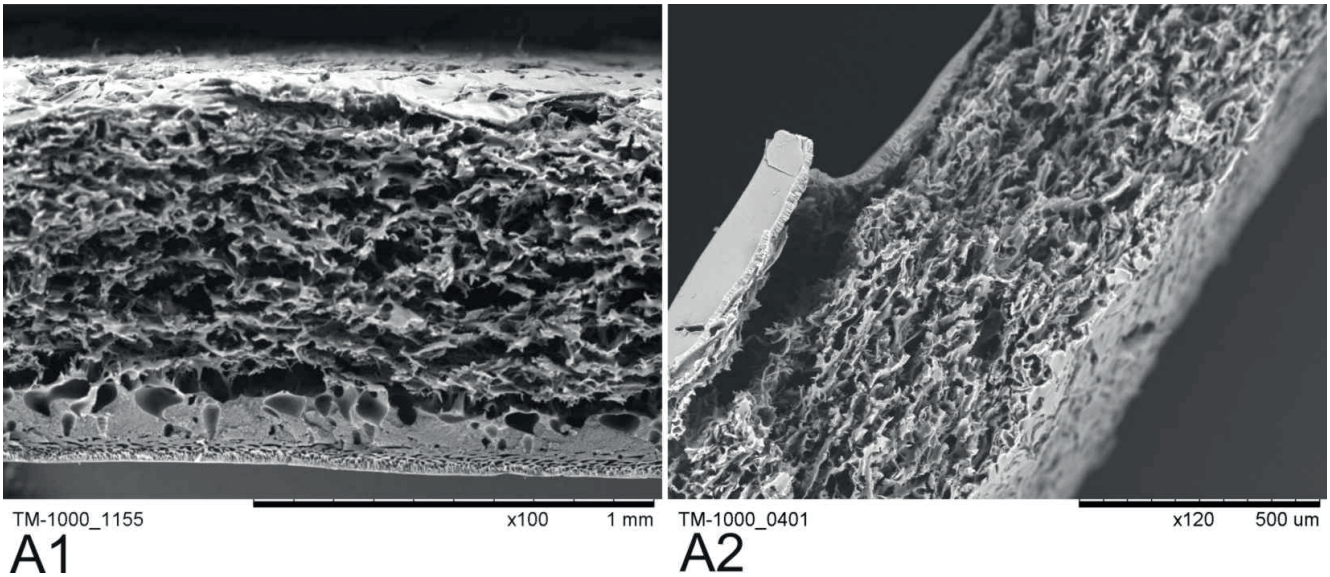

Fig. 1. Example of a 3D membrane scaffold. Photomicrographs of cross section 3D polyethersulfone scaffold for chondrocytes cultivation: Magnification x100 (left) and x120 (right).

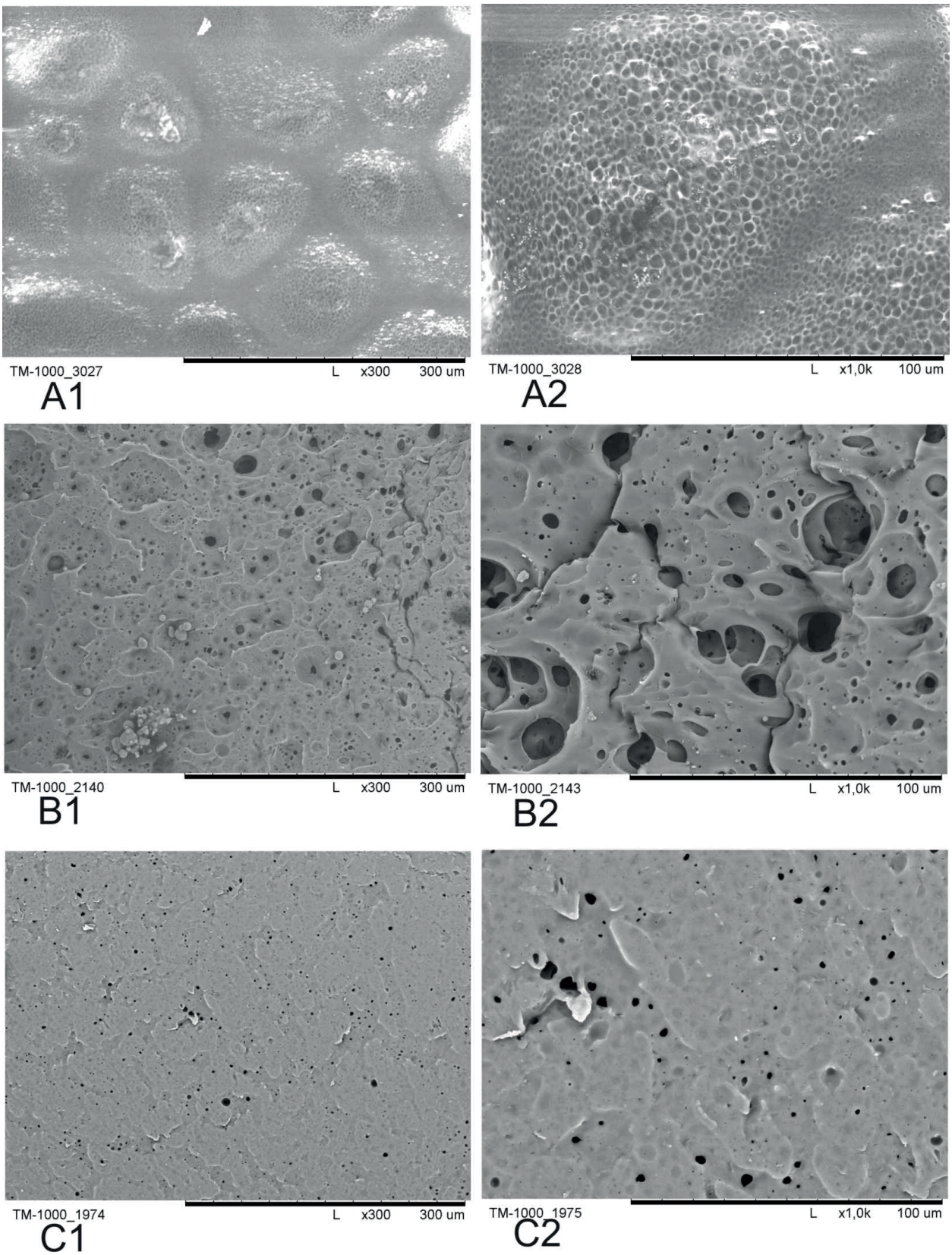

Fig. 2. Photomicrographs of dense skin layer. (a) polyethersulfone scaffold after removal of the cellulose; (b) poly(L-lactide) scaffold after removal of the PVP non-woven; (c) poly(L-lactide) scaffold after removal of the pork gelatine non-woven: Magnifications: left x300, right x1000.

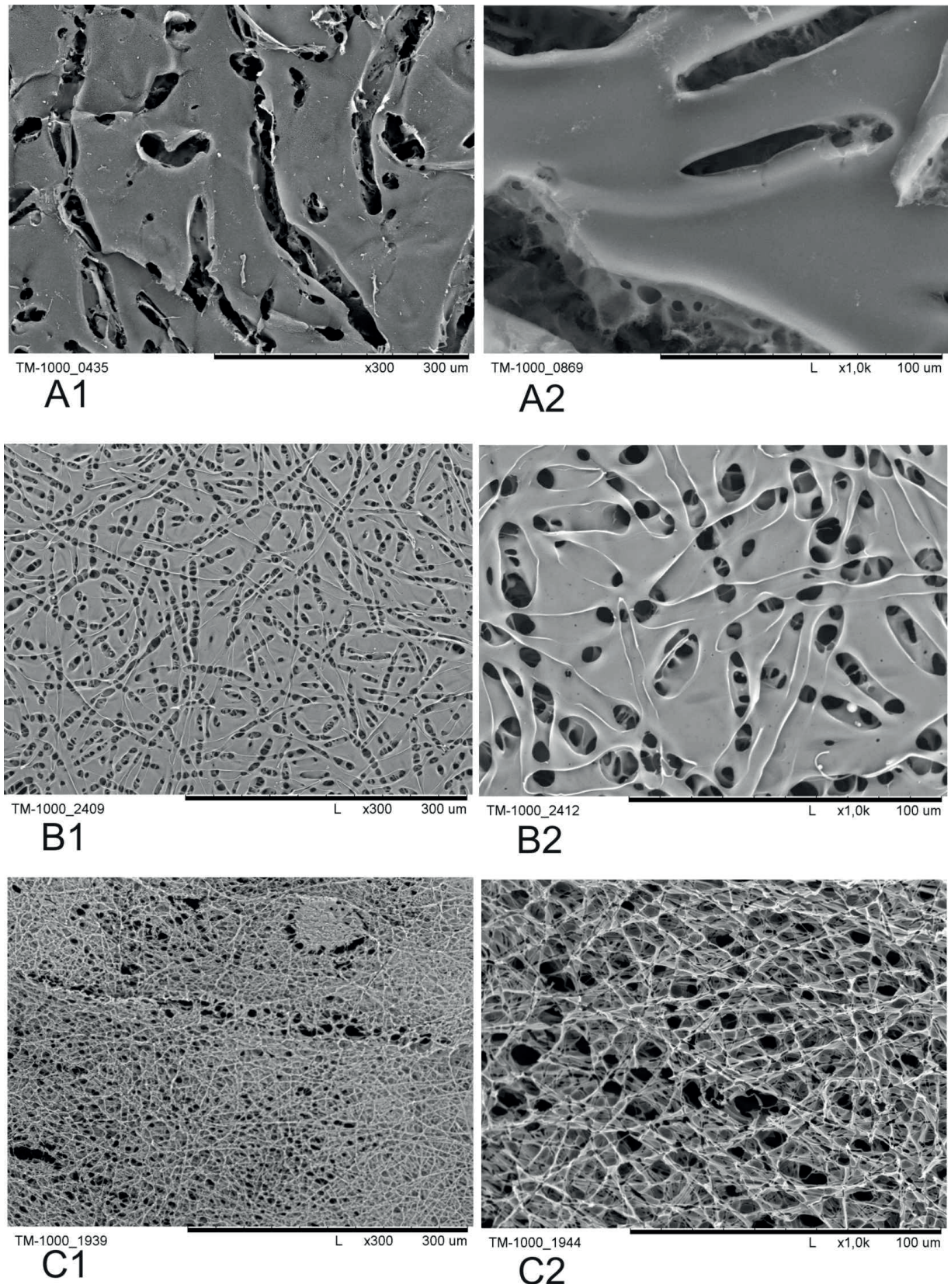

Fig. 3. Photomicrographs of upper perforated skin layer of 3D scaffolds: (a) polyethersulfone scaffold after removal of the cellulose; (b) poly(L-lactide) scaffold after removal of the PVP non-woven; (c) poly(L-lactide) scaffold after removal of the pork gelatine non-woven: Magnifications: left x300, right x1000.

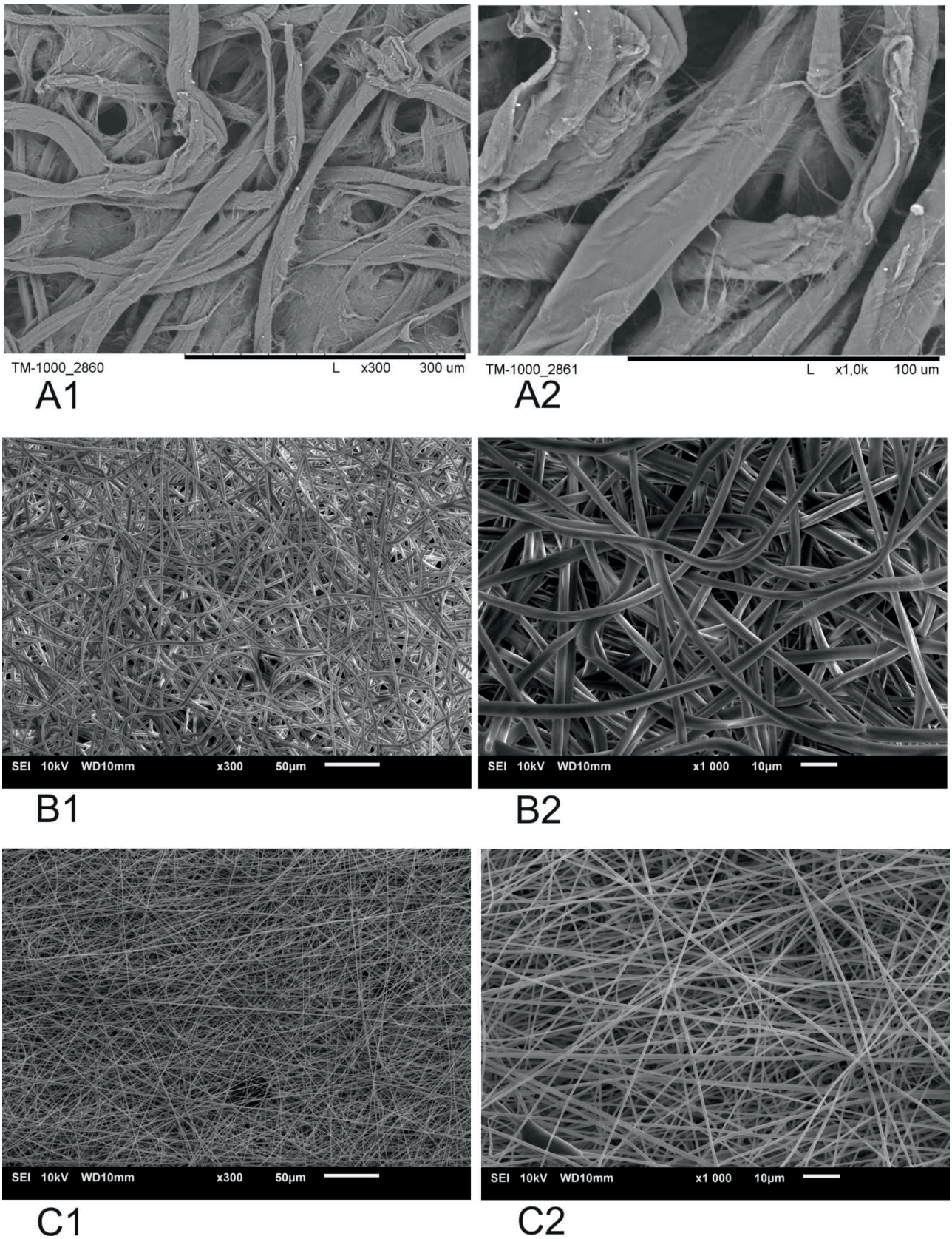

Fig. 4. Photomicrographs of non-woven fabrics: (a) cellulose; (b) PVP; (c) pork gelatin: Magnifications: left x300, right x1000.

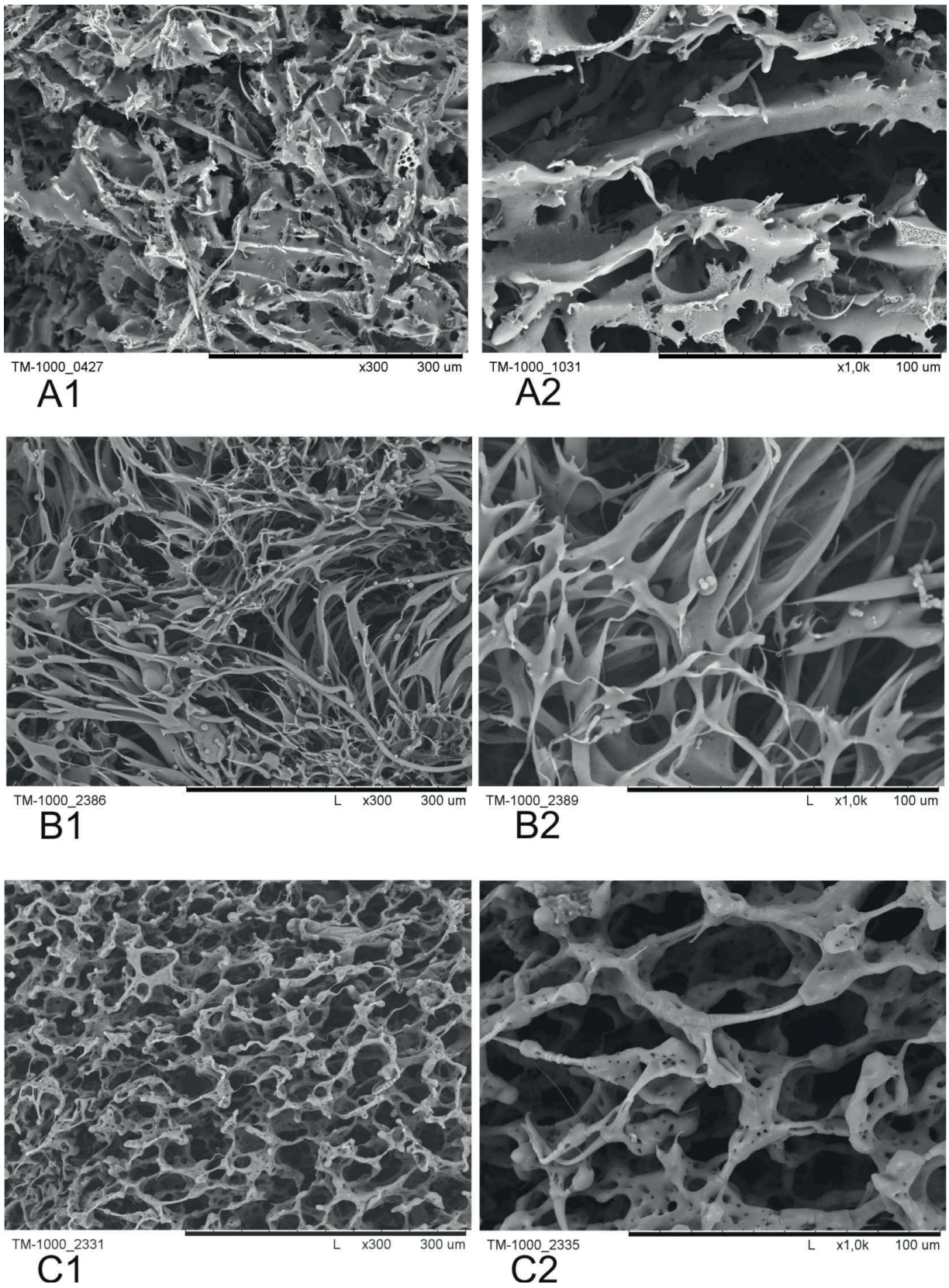

Fig. 5. Photomicrographs of the scaffolds' interior: (a) polyethersulfone scaffold after removal of the cellulose; (b) poly(L-lactide) scaffold after removal of the PVP non-woven fabric; (c) poly(L-lactide) scaffold after removal of the pork gelatin non-woven fabric: Magnifications: left x300, right x1000.

Viscose fibers cannot be used to obtain membranes from poly(L-lactide). Similarly, PVP and pork gelatin fibers cannot be used to obtain polysulfone and polyethersulfone membranes. This often implicates impossibility of selection of solvent and non-solvent for both materials of membranes and fibers. Therefore, for each material of membrane specified selection of non-woven is needed. The selection of non-woven fibers will be one of the key goals in obtaining widely porous membranes for 3D scaffolds for cells cultivation.

4. Final remarks

The structure of widely porous semipermeable membranes for cells cultivation, in particular for chondrocytes cultivation, depends on both the material of membranes and macropore precursors. However, the material used is less likely to influence the final membrane structure. Macropore precursors play a key role in determining the membrane structure. Both fiber diameter and material of the non-woven fabric are significant factors. Application of the non-woven macropore precursor enables obtaining macropores of diameters suitable for chondrocytes cultivation. Size of macropores and the membrane structure depends on both thickness of the fibers and the density of non-woven spinning. By adjusting those parameters, the structure of a membrane can be controlled.

Macropore connection is a critical feature of the membrane structure that enables penetration of the cells into the membrane interior and chondrocytes colonization of the entire membrane. This leads to creating conditions for cartilage regeneration.

The membrane preparation method tested in this study can be applied not only to poly(L-lactide) and polyethersulfone but also to other biocompatible polymers. For example, cellulose can be used for polysulfone or polyamides, PVP non-woven fabric or pork gelatin can be used for polycaprolactone, polyglicolide and its co- and terpolymers with lactide. The choice of polymers and co-polymers with a range of biodegradation times can determine the scaffold type suitable for the age of a patient.

References

[1] T.A. Blackburn, E. Craig, Knee anatomy: a brief review, Phys. Ther., 60 (1980) 1556–1560.

[2] K.L. Bennell, R.S. Hinman, A review of the clinical evidence for exercise in osteoarthritis of the hip and knee, J. Sci. Med. Sport, 14 (2011) 4–9.

[3] G. Musumeci, M.L. Carnazza, R. Leonardi, C. Loreto, Expression of β-defensin-4 in "an in vivo and ex vivo model" of human osteoarthritic knee meniscus, Knee Surg. Sports Traumatol. Arthrosc., 20 (2012) 216–222.

[4] G. Musumeci, C. Loreto, M.L. Carnazza, V. Cardile, R. Leonardi, Acute injury affects lubricin expression in knee menisci: an immunohistochemical study, Ann. Anat., 195 (2013) 151–158.

[5] G. Musumeci, R. Leonardi, M.L. Carnazza, V. Cardile, K. Pichler, A.M. Weinberg, C. Loreto, Aquaporin 1 (AQP1) expression in experimentally induced osteoarthritic knee menisci: an in vivo and in vitro study, Tissue Cell, 45 (2013) 145–152.

[6] J. Elisseeff, W. McIntosh, K. Anseth, S. Riley, P. Ragan, R. Langer, Photoencapsulation of chondrocytes in poly(ethylene oxide)-based semi-interpenetrating networks, J. Biomed. Mater. Res., 51 (2000) 164–171.

[7] R. Dorotka, U. Windberger, K. Macfelda, U. Bindreiter, C. Toma, S. Nehrer, Repair of articular cartilage defects treated by microfracture and a three-dimensional collagen matrix, Biomaterials, 26 (2005) 3617–3629.

[8] G.A. Ameer, T.A. Mahmood, R. Langer, A biodegradable composite scaffold for cell transplantation, J. Orthop. Res., 20 (2002) 16–19.

[9] G.M. Peretti, M.A. Randolph, M.T. Villa, M.S. Buragas, M.J. Yaremchuk, Cell-based tissue-engineered allogeneic implant for cartilage repair, Tissue Eng., 6 (2000) 567–576.

[10] J.D. Kisiday, M. Jin, M.A. DiMicco, B. Kurz, A.J. Grodzinsky, Effects of dynamic compressive loading on chondrocytes biosynthesis in self-assembling peptide scaffolds, J. Biomech., 37 (2004) 595–604.

[11] S. Grad, C.R. Lee, K. Gorna, S. Gogolewski, M.A. Wimmer, M. Alini, Surface motion upregulates superficial zone protein and hyaluronan production in chondrocyte-seeded three-dimensional scaffolds, Tissue Eng., 11 (2005) 249–256.

[12] L. Hangody, G. Vásárhelyi, L.R. Hangody, Z. Sükösd, G. Tibay, L. Bartha, G. Bodó, Autologous osteochondral grafting—technique and long-term results, Injury, 39 (2008) Suppl. 1: S32–S39.

[13] J.Y. Kang, C.W. Chung, J.H. Sung, B.S. Park, J.Y. Choi, S.J. Lee, B.C. Choi, C.K. Shim, S.J. Chung, D.D. Kim, Novel porous matrix of hyaluronic acid for the three-dimensional culture of chondrocytes, Int. J. Pharm., 369 (2009) 114–120.

[14] E. Filová, F. Jelínek, M. Handl, A. Lytvynets, M. Rampichová, F. Varga, J. Cinátl, T. Soukup, T. Trc, E. Amler, Novel composite hyaluronan/type I collagen/fibrin scaffold enhances repair of osteochondral defect in rabbit knee, J. Biomed. Mater. Res. B Appl. Biomater., 87 (2008) 415–424.

[15] K. Masuda, R.L. Sah, M.J. Hejna, E.J. Thonar, A novel two-step method for the formation of tissue-engineered cartilage by mature bovine chondrocytes: the alginate-recovered-chondrocyte (ARC) method, J. Orthop. Res., 21 (2003) 139–148.

[16] M.J. Stoddart, L. Ettinger, H.J. Häuselmann, Enhanced matrix synthesis in de novo, scaffold free cartilage-like tissue subjected to compression and shear, Biotechnol. Bioeng., 95 (2006) 1043–105.

[17] S. Grad, D. Eglin, M. Alini, M.J. Stoddart, Physical stimulation of chondrogenic cells in vitro: a review, Clin. Orthop. Relat. Res., 469 (2011) 2764–2772.

[18] S.J. Nho, M.J. Pensak, D.A. Seigerman, B.J. Cole, Rehabilitation after autologous chondrocyte implantation in athletes, Clin. Sports Med., 29 (2010) 267–282.

[19] K. Dudziński, A. Chwojnowski, M. Gutowska, M. Płończak, J. Czubak, E. Łukowska, C. Wojciechowski, Three dimensional polyethersulphone scaffold for chondrocytes cultivation – the future supportive material for articular cartilage regeneration, Biocybern. Biomed. Eng., 30 (2010) 65–76.

[20] M. Płończak, J. Czubak, A. Chwojnowski, B. Kupikowska-Stobba, Culture of human autologus chondrocytes on polysulfonic membranes – preliminary studies, Biocybern. Biomed. Eng., 32 (2012) 63–68.

[21] A.K. Chwojnowski, Dudziński–Widely Porous Scaffold for 3D Cell Cultivation – PL Patent 211793, Nov. 18, 2011.

[22] A. Chwojnowski, A. Kruk, A. Gadomska-Gajdhur, P. Ruśkowski C. Wojciechowski, E. Łukowska, L. Synoradzki, J. Dulnik, P. Denis, P. Sajkiewicz, The Method Preparation Polyesters Widely Porous Scaffold – The Patent Application (PL) P-414353, Dec. 15, 2015.