IN VITRO EVALUATION OF POLY(L-LACTIDE-CO-GLYCOLIDE) MEMBRANE

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Abstract

The aim of this study was to prepare and evaluate the properties of a new membrane dedicated for the treatment of bone defects in periodontology according to guided tissue regeneration (GTR) technique. The first part of this study was to prepare the membrane from resorbable poly(L-lactide-co-glycolide) (PLGA) and verify its microstructure. Biological evaluation was lead using the cells interesting from the point of view of GTR, e.g. human fibroblasts and mesenchymal stem cells (hMSC). It was found that the obtained membrane has asymmetric microstructure and defined pore size. Cell culture experiments show that the membrane is biocompatible with fibroblasts and hMSC. Both types of cell proliferated well on the membrane. HMSC cultured on the membrane exhibited better osteogenic differentiation and higher mineralization as compared to control tissue culture polystyrene.

[Engineering of Biomaterials, 94, (2010), 7-10]

Introduction

Guided tissue regeneration (GTR) is new method using a barrier membrane to protect the bone defect from invasion of soft tissue [1]. Mostly used barrier membranes have asymmetric structure and are made from non-degradable or biodegradable polymers. The non-degradable membranes are produced from expanded polytetrafluoroethylene (e-PTFE – Gore-Tex®, USA). The e-PTFE is chemically and biologically stabile. This material has good barrier properties and gives good results in tissue regeneration process. However it must be removed by a secondary operation that is necessary to repair dehiscence because of natural unresorbability [2]. The second group contains resorbable polymers of synthetic or natural origin. The major benefit of bioresorbable membranes is absence of necessity of a second surgery to remove the membranes. The most commonly used natural material to produce membranes is collagen (Bio-Gide®, Bicon, BioMend, etc.). Collagen is a natural component of bone matrix and is highly biocompatible. The disadvantage of collagen materials is the possibility of disease and pathogen transfer from animals. Better solution seems to be the use of bioresorbable membranes made from synthetic aliphatic polyesters such as GoreResolut® (Goremedical, USA). All the membranes available on the market made from collagen or resorbable polyesters have asymmetric, fibrous microstructure. In this type of materials the volume fraction and size of pores are however difficult to be controlled [1-4].

Currently much research is done to work out new materials for GTR technique. Several resorbable polymers such as poly-ε-caprolacton, polylactides, polyglycolide, copolymers of lactide and glycolide and chitosan are applied to produce new generation GTR membranes [5-11]. The materials are designed in such a way to ensure biocompability, bioresorbability, optimal surface properties, and microstructure for desired cells to adhere and grow.

The aim of this study was to prepare a new barrier non-fibrous, asymmetric membrane with defined pore size and to evaluate its biological properties in vitro.

Materials and Methods

Preparation of the membrane

PLGA with a molar ratio of L-lactide to glycolide of 85:15 and molecular weights M_n=100 kDa, M_w=210 kDa was used to prepare the membrane by phase separation. PLGA was dissolved in dichloromethane (POCh, Gliwice) and 60 wt% of polyethylene glycol (PEG, Aldrich, Germany, M_w = 400 Da) was added. Mixture was slip-casted on smooth glass surface and dried in air and in vacuum, followed by leaching out PEG in distilled water. Detailed method of membrane preparation is described in patent application [12].

Microstructure

The microstructure of the membrane was studied under scanning electron microscope (SEM, Nova NanoSEM 200, FEI, USA) under magnification of 2000x and 3000x. Before the analysis, the samples were sputter-coated with a thin carbon layer to make them conductive. The atomic force microscope (Explorer, Veeco, USA) was also used to study membrane topography and average roughness (R_a). For top and bottom surfaces 3 pictures were taken at scan areas of 100 µm x 100 µm.

Biological evaluation

For in vitro studies the membrane was fixed in CellCrown inserts (Scaffdex, Finland), in such a way that top (in contact with air after slip-casting) or bottom surfaces (in contact with glass surface after slip-casting) of the membrane were exposed. The samples were sterilized by oxygen peroxide plasma (Sterrad, ASP, J&J, USA) and the cells of human origin: fibroblasts (from Universitätsklinikum „Carl Gustav Carus“ an der Technischen Universität Dresden Medizinische Klinik I) or hMSC (from Clinic for Dermatology, Venerology and Allergology, Universität Leipzig Max-Bürger-Forschungszentrum) were seeded on the membrane's surface at the density of 10000 cells/cm^2. As control the cells were cultured on tissue culture polystyrene (TCPS, Nunclon, 24-well plates) (FIG. 1). The cells were incubated at 37°C and 7.0% CO_2 atmosphere for 24 h in base medium (BM - DMEM, 10% FBS, 1% P/S, 2 mM glutamine), and for 14 and 21 days in two media: BM for fibroblasts and hMSC as well as osteogenic differentiation medium (DM) for hMSC (BM + ascorbic acid 300 mM, β-glycerophosphate 10 mM, dexamethasone 10 nM). Cell proliferation and osteogenic differentiation were studied by lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities measurements, respectively. Mineralization of hMSC was analysed by calcium concentration test. The results were expressed as means ± SD. Statistical significance was determined by t-test and the differences were regarded as significant at $p < 0.05$.

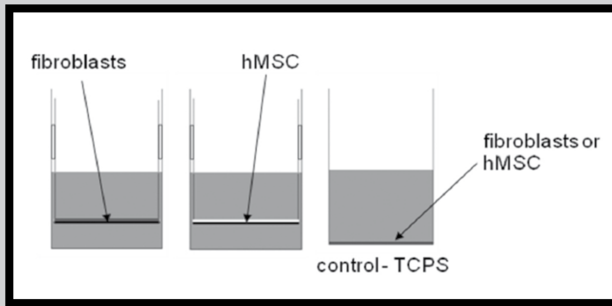

FIG. 1. Scheme of cell culture.

Results

Microscopic characterization

Microscopic evaluations showed that the membrane has asymmetric microstructure with pores in the range of 3-6 μm. The bottom side of the membrane is more rough than the top one (FIG. 2). These findings were also confirmed by AFM analysis (FIG. 3), which shows similar surface topography as registered by SEM. Surface roughness analysis shows that the top of the membrane is more smooth with R_a = 280 ± 10 nm, while the bottom is more textured and porous with R_a = 550 ± 30 nm.

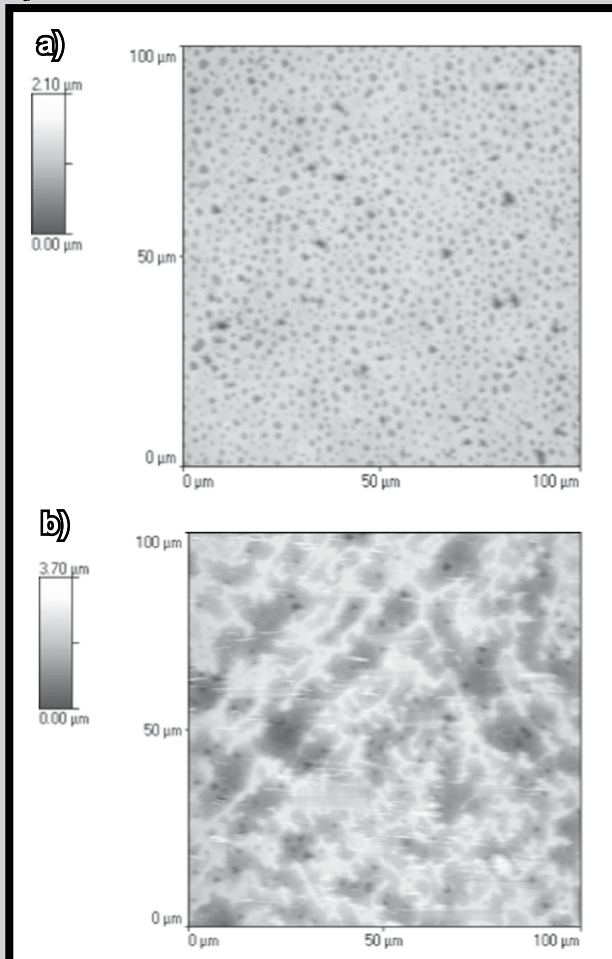

FIG. 3. AFM pictures of PLGA membrane a) top, b) bottom.

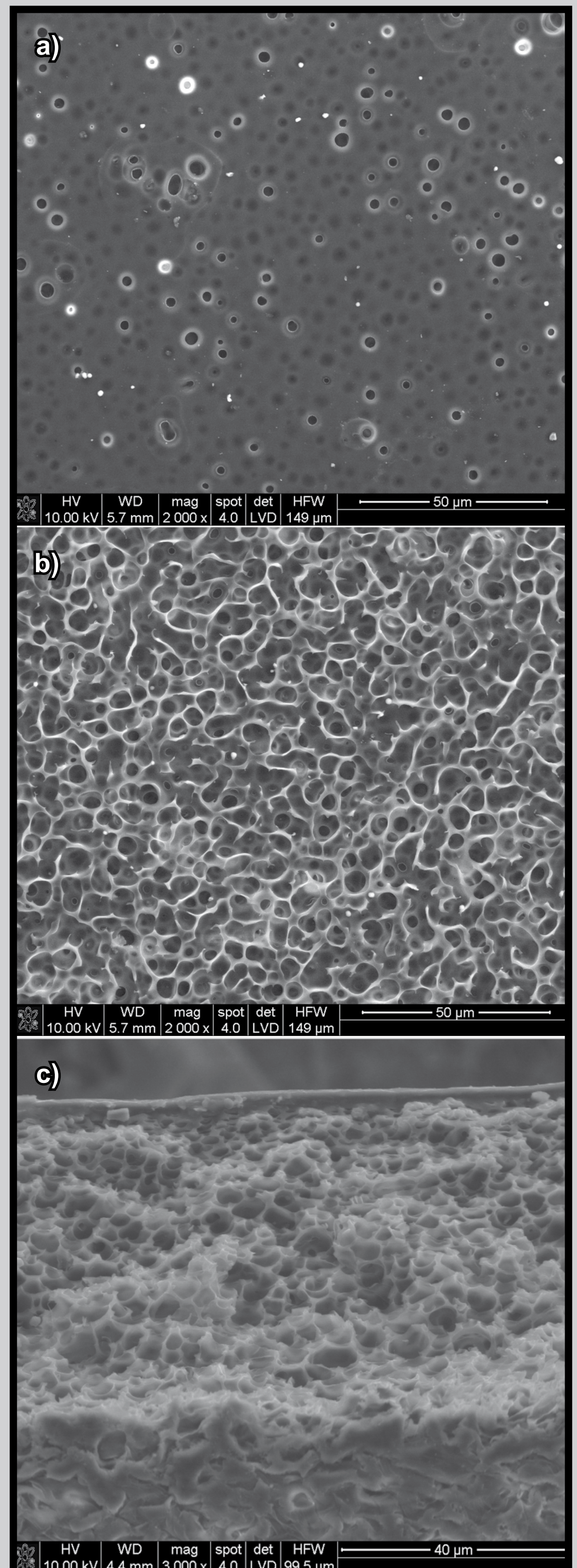

FIG. 2. SEM microphotographs of PLGA membrane a) top, b) bottom, c) cross-section.

Biological evaluation

LDH activity results show that fibroblasts and hMSC proliferation after 24 h and 14 days in BM were similar as on control material (FIG. 4a and b). LDH activity of hMSC in DM was higher than in BM, but the cells proliferated better on control TCPS than on tested membrane (FIG. 4b). The results show that fibroblasts proliferation does not depend on the side of the membrane (FIG. 4a), while hMSC proliferation on more smooth top surface tended to be higher (FIG. 4b). Osteogenic differentiation of hMSC measured by ALP activity (FIG. 5a) and their mineralization (FIG. 5b) were higher in DM than in BM. Concomitantly hMSC differentiation and mineralization on the membranes were significantly higher than for cells cultured on control TCPS (FIG. 5a,b). A tendency of higher mineralisation of hMSC on more smooth top surface of the membrane was also observed (FIG. 5b).

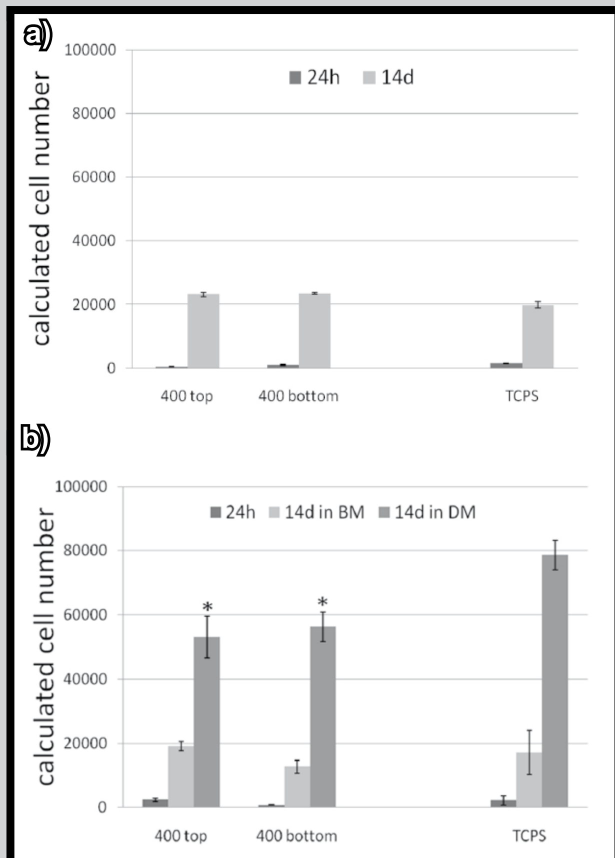

FIG. 4. Proliferation of fibroblasts (a) and hMSC (b) evaluated by LDH activity after 24 h and 14 days in BM and DM. Asterisks indicate significant difference ($p < 0.05$) compared to control TCPS within one group.

Discussion and conclusion

The results show that the elaborated method of preparation enables to obtain porous non-fibrous asymmetric PLGA membrane. The method is based on phase separation between PLGA and PEG dissolved in non-polar solvent. As a results spherical PEG domains 3-6 μm in diameter are created in a PLGA matrix. Differences in density of both compounds cause sedimentation of the component of higher density, thus resulting in asymmetry of the membrane. The membrane microstructure is different from that of typical fibrous non-resorbable and resorbable GTR membranes available on the medical market [2].

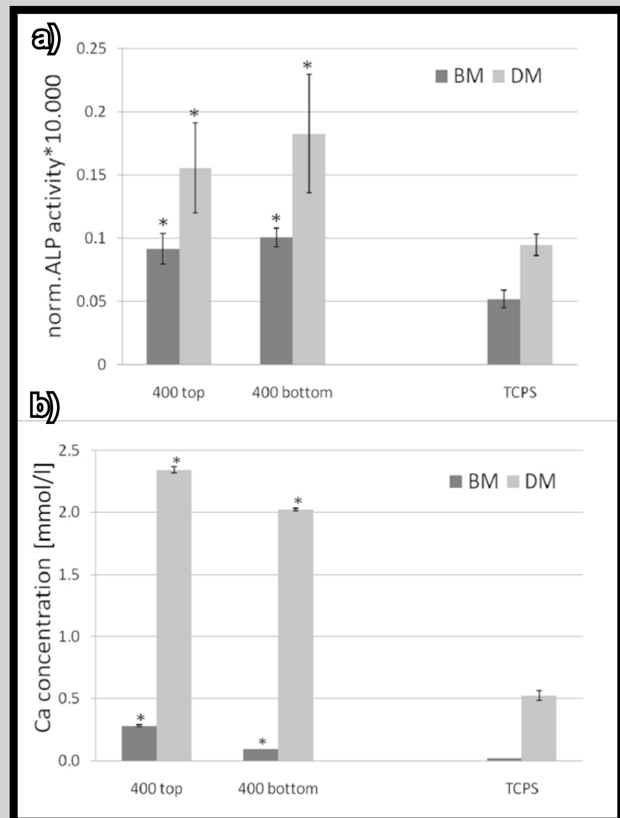

FIG. 5. HMSC differentiation after 14 days in BM and DM (a) and mineralization after 21 days in BM and DM (b). Asterisks indicate significant difference ($p < 0.05$) compared to TCPS within one group.

Biological experiments show that the membrane supports growth of fibroblasts and hMSC. Our results may be compared with the results obtained by Alpar et al. who found that collagen BioGide® membranes facilitate bone cells proliferation to the same extent as a control tissue culture polystyrene [4]. On the other hand in the same study it was found that e-PTFE and polylactic acid membranes induced slight to moderate cytotoxic reactions [4], what was not the case in our experiment. The growth of hMSC was better in osteogenic differentiation medium than in base medium, what suggests that supplementary compounds provided to the medium do favour not only cell differentiation, but also cell proliferation. As expected hMSC differentiation and mineralization in differentiation medium were higher than in base medium. Differentiation and mineralisation of hMSC cultured on the membranes were higher than for those cells cultured on control tissue culture polystyrene. It implies that the microstructure and physic-chemical properties of the elaborated material are appropriate for hMSC. Moreover hMSC show the tendency of better proliferation and mineralization on more smooth top surface of the membrane that on more rough bottom surface of the membrane. This finding shows that biological response to the material could be modulated by the microstructural parameters of the membrane.

To sum up, the results show that PLGA membranes presented in this study specifically do support stem cells adhesion, proliferation and osteogenic differentiation. It is of key importance taking into account future application of the membranes in GTR technique in periodontology.

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Piśmiennictwo

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