Antibacterial PLGA and PCL membranes, modified by magnetron sputtering method of copper target

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Abstract. The paper discusses barrier membranes which were formed from poly(lactide-co-glycolide) and polycaprolactone by electrospinning method. Then, plasma modification of membranes was carried out by magnetron sputtering of copper target in argon atmosphere. The morphology, elemental composition, mechanical and antibacterial properties of the obtained samples were investigated. Modification with copper in the magnetron discharge plasma allows maintaining the original characteristics of pristine polymeric samples and imparting antibacterial properties to them.

1. Introduction

Periodontitis is a common inflammatory disease of the periodontal tissue, which leads to jaw bone resorption and teeth loss. Barrier membranes are often used to treat such disease, which main function is preventing the penetration of fast-growing soft tissue into osseous defects in the alveolar bone and stimulation of proliferation the bone tissue in them [1]. For periodontitis treatment with barrier membranes, the guided tissue/bone regeneration (GTR/GBR) procedure is required. Nowadays, GTR/GBR membranes consist of natural and synthetic materials based on biocompatible and biodegradable polymers. Natural polymers have a high biocompatibility, but has excessively high degradation rate and low strength characteristics [2]. This can lead to membrane failure, which requires additional surgical procedures. Compared to natural polymers, synthetic membranes have higher strength characteristics and longer degradation times [2]. Most commonly used biodegradable synthetic polymer for barrier membrane manufacturing is poly(lactide-co-glycolide) (PLGA) [3]. That polymer consists of lactic and glycolic acids, which allows PLGA to have a controllable degradation time [4]. Polycaprolactone is biodegradable aliphatic polyester with extremely high elongation at break (PCL) [5]. Therefore, PCL is used like a material in different types of medical devices including barrier membranes [6].

One of the most common methods for the manufacture of polymer membranes is electrospinning, since it allows the formation of highly porous scaffolds with a developed structure, which have the ability to mimic the extracellular matrix [7].

Nevertheless, a synthetic scaffold obtained by electrospinning method doesn’t have antibacterial properties, which can increase chances of inflammatory diseases in the case of their introduction into living organism. Previously we have shown, that polymer membranes modified by magnetron sputtering of copper target suppress the growth of bacteria St. Aureus [8]. Relatively low temperature of magnetron sputtering process allows modify the surface of non-woven polymers saving the original morphology and structure without burn-through. Comparing with atmospheric plasma jet and chemical
vapor deposition, with magnetron sputtering aligned high-purity thin films can be created. Magnetron plasma modification of PLGA and PCL membranes by copper could provide the antibacterial properties to them, which could decrease the probability of surgical complications caused by the infection penetration into oral cavity.

The aim of this work is development of PLGA and PCL barrier membranes modified by copper to possess them antibacterial properties for further use in periodontitis treatment.

2. Materials and methods

2.1. Barrier membranes formation
Barrier membranes were made from 4% PLGA (85/15, Corbion Purac, Netherlands) solution in hexafluoroisopropanol ((CF₃)₂CHOH, P&M Invest, Russia) and 8% PCL (Sigma-Aldrich, UK) solution in chloroform (CHCl₃, Fisher Scientific, UK). Electrospinning of polymeric solutions was provided on installation NANON-01A (MECC Co, Japan) with regimes for PLGA membranes manufacturing: cylindrical collector 200 mm long and 100 mm diameter; solution flow rate – 4 ml/h; voltage between needle and collector – 22 kV; distance between needle and collector – 150 mm; collector rotational speed – 200 rpm. For PCL membranes, regimes were the same except: distance between needle and collector – 140 mm; collector rotational speed – 50 rpm.

2.2. Barrier membranes modification
Membranes plasma modification by magnetron sputtering of copper target (Cu – 99.95 %) was carried out using an ion-plasma installation [9] with direct current power supply (APEL-M-5PDC, JSC Apelvac, Russia) under the following technological conditions: discharge power – 600 W; current – 1.1 A; deposition time – 15.7 min; operation pressure in the chamber – 0.3 Pa; gas – argon (99.99 %).

2.3. Investigation of barrier membranes
SEM images with 1000× magnification were obtained by scanning electron microscope (SEM) JCM-6000Plus (Jeol, Japan). Elemental composition of samples was provided by method of energy dispersive X-ray spectroscopy (EDAX). The fiber diameter and porosity measurements of PLGA and PCL membranes were carried out in the ImageJ 1.48 software (National Institutes of Health, USA), using the plug-in Diameter J v1.018 (National Institute of Standards and Technology, USA).

The strength characteristics of the membranes were evaluated using an Instron 3343 instrument (Illinois Tool Works, USA) equipped with an Instron 2519-102 sensor (Illinois Tool Works, USA). The antibacterial activity was assessed in accordance with ISO 20732: 2013. Suspension with St. Aureus (ATCC 25923) was applied to the surface of 10 x 10 mm specimens.

The statistical analysis of the obtained data was performed using Mann-Whitney and One-way ANOVA tests in OriginPro v.2019b (OriginLab, USA) software. Differences were considered statistically significant at p < 0.05.

3. Results

3.1. Scanning electron microscopy and EDAX
SEM images, fiber diameter distribution histograms and EDAX spectra of PLGA and PCL membranes are shown in figure 1.

On SEM images are shown, that barrier membranes consist of polymeric fibres, which forms chaotically interlaced nonwoven structure. The EDAX spectra of control samples shows three reflections corresponding to carbon (C), oxygen (O), gold (Au), last of which was deposited to increase electrical conductivity. Plasma modified membranes have a high intensity copper (Cu) peaks on EDAX spectra.
Figure 1. SEM images, fiber diameter histogram and EDAX spectra of:
a) PLGA (pristine sample); b) Cu-PLGA (modified sample);
c) PCL (pristine sample); d) Cu-PCL (modified sample).

Mean fiber diameters and porosity of PLGA and PCL membranes are shown in table 1.

| Sample names | Fiber diameter (μm) | Porosity (%) |
|--------------|---------------------|--------------|
| PLGA         | 1.82±0.63           | 61±5         |
| Cu-PLGA      | 1.72±0.74           | 57±5         |
| PCL          | 1.75±0.77           | 56±3         |
| Cu-PCL       | 1.81±0.73           | 54±4         |

Modification of PLGA and PCL membranes with copper does not significantly affect the fiber diameters and porosity. The retention of the average fiber diameter and porosity after plasma modification indicates that the modification under the selected modes does not affect the surface morphology of PLGA and PCL membranes.

3.2. Mechanical properties
Values of the membranes mechanical characteristics are shown in table 2.

| Sample names | Tensile strength, MPa | Relative elongation, % | Young's modulus, MPa |
|--------------|-----------------------|------------------------|----------------------|
| PLGA         | 3.3±0.3               | 260±25                 | 80±10                |
| Cu-PLGA      | 3.5±0.1               | 230±10                 | 95±1                 |
| PCL          | 3.2±0.3               | 850±50                 | 6.4±0.3              |
| Cu-PCL       | 3.3±0.2               | 820±20                 | 7.5±0.2              |
Pristine PLGA and PCL membranes have a comparable values of tensile strength. PCL membrane has a ~ 3 times higher relative elongation than PLGA.

After plasma modification of PLGA and PCL membranes, values of tensile strength, elongation and Young's modulus do not change reliably. This is primarily due to the retention of the morphology and surface structure of membranes after their modification.

3.3. Antibacterial activity
Indicators of the antibacterial activity of membranes are shown in table 3.

| Sample names | Bacteria’s number (0 h), CFU×10^4/ml | Bacteria’s number (24 h), CFU×10^7/ml | Antibacterial activity (A) |
|--------------|--------------------------------------|---------------------------------------|--------------------------|
| PLGA         | 1.9±0.4                               | 6.0±1.0                               | ~                        |
| Cu-PLGA      | 1.8±0.2                               | 0.2±0.1                               | 1.51                     |
| PCL          | 1.3±0.2                               | 10.6±1.3                              | ~                        |
| Cu-PCL       | 1.6±0.4                               | 0.3±0.1                               | 1.44                     |

PLGA and PCL membranes, modified with copper have a significant antibacterial activity (A>1.0). In comparison with the number of bacteria on pristine samples after 24 hours, the colony forming units (CFU) on modified samples have decreased by ~ 97%.

4. Summary
Modification by the method of magnetron sputtering of a copper target makes it possible to impart significant antibacterial properties to barrier membranes while maintaining the surface morphology and their mechanical properties. In this way magnetron modification of PLGA and PCL materials by copper can be recommended for creating barrier membranes with ability of effective prevention of infectious complications in the oral cavity.

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