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Biocompatibility and Bioresorption of 3D-Printed Polylactide and Polyglycolide Tissue Membranes

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We studied biocompatibility and bioresorption of 3D-printed polylactide and polyglycolide tissue membranes. Ultrasound microscopy and histological examination showed that membranes fabricated of a copolymer of lactic and glycolic acids in a mass ratio of 1:9 are bioresorbed and have good biocompatibility with soft tissues (connective tissue, adipose tissue, and epithelium). An important feature of the copolymer membranes, which differs them from pure polylactide membranes, is the formation of a thin fibrous capsule that did not interfere its destruction by the mechanism of hydrolytic resorption.

Key Words: *barrier membrane; guided bone regeneration; 3D printing; polylactide; polyglycolide*

Morphological and functional changes in the alveolar bone that develop after tooth loss are classified in the ICD-10 as atrophy of the edentulous alveolar region (K08.2), and a large number of methods have been described and used in clinical practice for its reconstruction. However, the method of volume reconstruction of the alveolar bone by the method of guided bone regeneration (GBR) is of greatest interest, as it is more effective in comparison with transplantation of bone blocks [4].

The success of GBR is ensured by the physical and biological properties of the scaffold [9] that limits the reconstruction area and determines its volume. Scaffolds can be partially resorbable (cross-linked xenocollagen) and non-resorbable (titanium, polytetrafluoroethylene) [3]. The main problem of all scaffolds is high risk of their exposure at the stage of wound healing, which leads to infection and loss of bone regenerate [11]. Moreover, removal of non-resorbable scaffolds is associated with additional trauma and repeated violation of the blood supply and nutrition of the alveolar bone in the area of dental implantation.

In this regard, synthetic membranes made of biocompatible polymers are of great interest, because they are characterized by sufficient mechanical properties at the early stages of healing and bioresorbability, which eliminates the need for their removal before the installation of dental implants. These polymers include polyesters of hydroxycarboxylic acids: synthetic polylactide, polyglycolide, and their copolymers [1,7], as well as poly-3-hydroxybutyrate and its copolymers obtained by a biotechnological method [2].

A promising method for fabrication of tailored barrier membranes is layer-by-layer 3D printing. This

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process implies melting of the polymer bar in the extruder of a 3D printer and layer-by-layer building of the membrane of a desired 3D configuration. The technology for modeling and printing of the prototypes of individualized membranes made of non-biological plastics has been previously described [4].

However, many questions regarding the use of biocompatible and biodegradable polymers melted during 3D printing for the fabrication of barrier membranes during reconstructive surgery in the oral cavity remain unanswered. The biocompatibility and biodegradation of these implantable structures are also poorly studied. Problems are presented to study and this article is devoted.

Our aim was to assess and compare the biocompatibility and bioresorption of 3D-printed polymer membranes made of polylactide (PLA) and a polylactide/polyglycolide copolymer (PLA/PGA).

MATERIALS AND METHODS

The microstructure of the initial samples was analyzed by light microscopy and scanning acoustic microscopy [6]. The latter was also used to assess the rate of *in vivo* biodegradation of the original membranes. The biocompatibility of polymers was assessed by histological methods.

For the study, polymer membranes made of pure PLA (Purac, Corbion) and a copolymer of PLA (10%) and PGA (90%) (Purasorb 1017, Corbion) were prepared.

A 3D model of the membrane in the form of a cylinder with a diameter of 8 mm and a height of 0.3 mm was prepared using the FreeCAD 0.18 3D editor (https://www.freecadweb.org). G-code for printing was generated in Slic3R 1.3.0 (https://slic3r.org). All samples were printed at 210°C in a single layer with a height of 0.3 mm on a 3D printer Prusa Mendel 2.0 using the method of layer-by-layer deposition.

The architectonics of the initial polymers was studied at the N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences. The surface and microarchitectonics of the initial membranes were analyzed using a Leica DM LM/P light microscope (Leica Microsystems). The surfaces of PLA and PLA/PGA membrane were uneven and rough and were formed by linear structures in the form of protrusions and depressions.

Acoustic microscopy of copolymer samples was carried out using a pulsed acoustic microscope (SIAM-2018, Institute of Biochemical Physics, Russian Academy of Sciences) at an operating frequency of 100 MHz for the original biopolymers and 50 MHz for biopsy samples. The pulse generator generated a short probe signal with a duration of 20-40 nsec and an amplitude of 30 V. The acoustic lens was moved with precision engines with interval of 15 μ.

For *in vivo* study, 18 PLA membranes and 18 PLA/PGA membranes were implanted subcutaneously (in the region of the right ear) to 36 male Chinchilla rabbits aged 12-18 months and weighting 2.5-3.0 kg (each animal received one membrane). The surgery was performed under general and local anesthesia. During the postoperative period, ofloxacin (2 ml) was injected intramuscularly to the animals daily for 3 days. The animals were kept in a vivarium at 22°C (daylight for 15 h). The experiment corresponded to the recommendations of the Local Bioethical Committee of the Institute of Biochemical Physics, Russian Academy of Sciences and was carried out in accordance with international recommendations for biomedical research using animals and Order No. 199n of the Ministry of Health (On Approval of Rules for Good Laboratory Practice, August 23, 2010).

In 7, 14, and 28 days after surgery, ear fragments with the implanted membrane and 5 mm of surrounding intact tissues were excised from 6 animals of the PLA and PLA/PGA groups under anesthesia. The obtained samples were placed in 10% formalin in phosphate buffer, examined under an ultrasound microscope, and then sent for histological examination.

Histological examination was carried out at the Experimental Laboratory of the N. N. Burdenko Main Military Hospital, Ministry of Defense of the Russian Federation. Biopsy specimens were fixed in 10% formalin in phosphate buffer, dehydrated in ascending concentrations of ethanol and embedded in paraffin. Tissue sections (~4 μ thick) were mounted on glass slides, dewaxed, and stained with hematoxylin and eosin.

RESULTS

Acoustic images of subcutaneously implanted PLA/PGA membranes at different terms after implantation are presented in Figure 1. The main criteria for evaluating bioresorption of the material was the integrity of implanted membranes on days 7, 14, and 28. At the early stage of bioresorption, uneven membrane fragmentation into macroscopic elements with a linear size of 0.5-1.5 mm was observed. On day 14, fine fragmentation of the membrane occurred that results in a considerable decrease in acoustical contrast (Fig. 1, *b*). On day 28, solitary traces of the biopolymer implant were detected on the acoustic images and the contrast was reduced to a minimum in comparison with the previous terms (Fig. 1, *c*).

On day 7 after implantation of the PLA membrane, a thin connective tissue capsule was formed around the polymer in the dermis, under which a large number of macrophages accumulated; moderate dif-

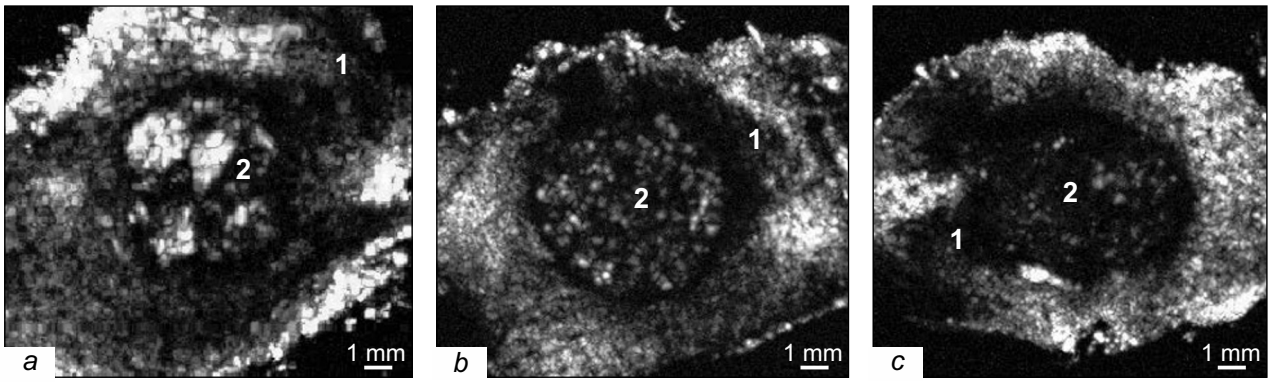

Fig. 1. Acoustic images of internal structure of PLA/PGA biopsy specimens on days 7 (*a*), 14 (*b*), and 21 (*c*) after implantation. *1*) Tissue, *2*) membrane.

fuse lymphoplasmacytic infiltration was noted. No vascular ingrowth into the polymer was detected. The structure was homogeneous and optically transparent (Fig. 2, *a*).

On day 7 after implantation of the PLA/PGA membrane, the copolymer in the dermis was surrounded by fibrous connective tissue and separated by layers of collagen with single small foci of mature granulation tissue, weak diffuse lymphoplasmacytic infiltration, and single macrophages. No vascular ingrowth into the copolymer was detected. We also observed signs of swelling and impregnation with the tissue fluid, which indicated hydrolysis of the copolymer (Fig. 2, *b*).

On day 14, foreign body giant cells (FBGC) were seen on the surface of the PLA membrane forming scalloped structures. The polymer had almost even contours except sites of scalloped resorption. On day 14 after implantation of PLA/PGA membrane, intensification of hydrolysis was observed.

On day 28, the PLA membrane was surrounded by a connective tissue capsule; numerous macrophages and moderate diffuse lymphoplasmacytic infiltration was seen inside and under the capsule. FBGC were found on the polymer surface. No vascular ingrowth into the polymer was detected. The polymer had almost even contours except the sites of scalloped resorption. The structure was homogeneous, optically transparent (Fig. 3, *a*).

On day 28 after implantation of PLA/PGA membrane, a copolymer surrounded by fibrous connective tissue and separated by wide layers of dense fibrous connective tissue was seen in the dermis. Few FBGC were located on the surface of the connective tissue capsule. The polymer was at the final stage of hydrolytic disorganization: the cavity was filled with a homogeneous pinkish content (Fig. 3, *b*).

There are different opinions on the biocompatibility and bioresorption of PLA and PGA products [8,10]. Polyglycolic acid, unlike polylactic acid, belongs to the categories of rapidly decomposing polymers with a resorption time of up to 6 months [1,2,5]; however, the strength properties decrease with increasing resorp-

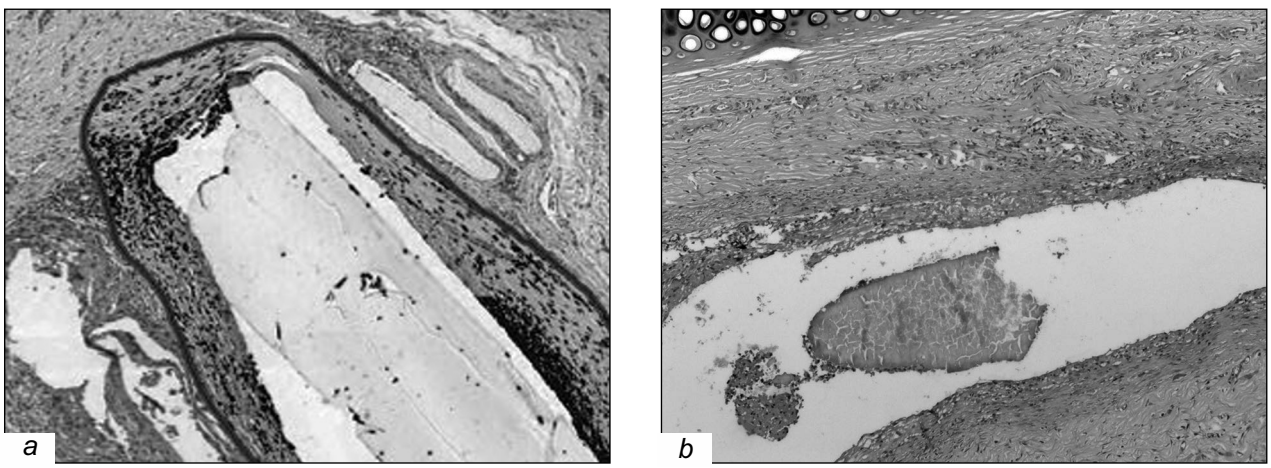

Fig. 2. Tissue reaction on day 7 after subcutaneous implantation of PLA (*a*) and PLA/PGA (*b*) membrane. Staining with hematoxylin and eosin, ×100. *a*) Activation of macrophage resorption of the polymer; *b*) hydrolysis of the polymer without visible involvement of macrophages.

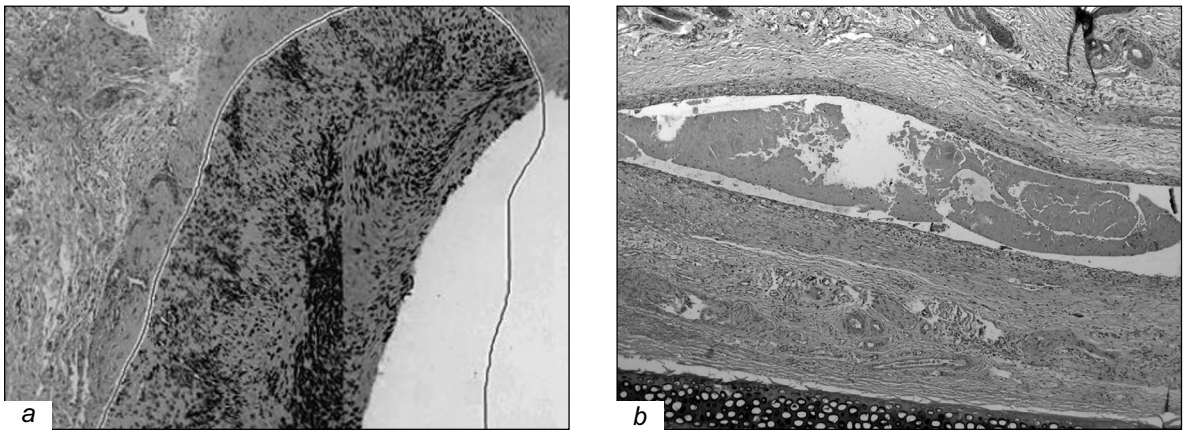

Fig. 3. Tissue reaction on day 28 after subcutaneous implantation of PLA (*a*) or PLA/PGA (*b*) membrane. Staining with hematoxylin and eosin, ×100. *a*) FBGC resorption; *b*) swelling and homogenization of the polymer.

tion. Hence, pure PGA is not suitable for fabrication of barrier membranes.

Modification of the physical properties and biodegradation terms can be achieved in copolymers. For instance, some authors have demonstrated increased terms of resorption for the PLA/PGA copolymer due to the polylactide component. The maximum decomposition time of the PLA/PGA copolymer can reach 18 months [5,8,10].

Using ultrasound microscopy, we demonstrated consistent and uneven loss of the material in living tissues, which manifested in a decrease in acoustic contrast and fragmentation. Our findings suggest that biodegradation of the PLA/PGA copolymer begins in 1-2 weeks after implantation and increases over time.

According to published reports [1], biodegradation of PLA and PGA occurs via hydrolysis or macrophage cell response. In the present work, we obtained similar results. PLA retains its crystalline structure throughout the experiment, while copolymer undergoes hydrolysis by the tissue fluid by day 7 of the study, which manifested in its swelling and further almost complete resorption.

Based on the obtained data, we can conclude that the use of barrier membranes from a copolymer during bone reconstructive operations in the oral cavity is more promising than from PLA, because the PLA/PGA membrane has acceptable bioresorption periods, and hydrolytic decomposition of the copolymer is gentler for the surrounding tissues.

REFERENCES

1. Belov DA. Polylactide: a biodegradable polymer. Nauka Innovatsii. 2013;(9):21-23. Russian.
2. Ivanov SY, Bonartsev AP, Gazhva YV, Zharkova II, Mukhametshin RF, Mahina TK, Myshkina VL, Bonartseva GA, Voinova VV, Andreeva NV, Akulina EA, Kharitonova ES, Shaitan KV, Muraev AA. Development and preclinical studies of insulating membranes based on poly-3-hydroxybutyrate-co-3-hydroxyvalerate for guided bone regeneration. Biomed. Khim. 2015;61(6):717-23. doi: 10.18097/PBMC20156106717
3. Ivanov SYu, Gazhva YuV, Muraev AA, Bonartsev AP. Contemporary membrane to guided bone regeneration in dental surgery (review). Sovremen. Probl. Nauki Obrazovaniya. 2012;(3):74. Russian.
4. Muraev AA, Gazhva YV, Ivashkevich SG, Riabova VM, Korotkova NL, Semyonova YA, Metsuku IN, Faizullin RL, Ivanov SY. A novel approach to alveolar bone complex defects 3D reconstruction. Sovremen. Tekhnol. Med. 2017;9(2):37-45. Russian.
5. Nasonova MV, Hodyrevskaya YI, Nemoykina AL, Mikhaylenko MYu, Kudryavtseva YA. Optimization of physical, mechanical and degradation properties for biodegradable antiadhesive membranes. Vestn. Kemerov. Gos. Univer. 2015;(2-1):65-69. Russian.
6. Petronyuk YS, Khramtsova EA, Levin VM, Bonartsev AP, Voinova VI, Bonartseva GA, Muraev AA, Asfarov TF, Guseynov NA. Developing Techniques of Acoustic Microscopy for Monitoring Processes of Osteogenesis in Regenerative Medicine. Bull. Russ. Acad. Sci. Phys. 2020;84(6):653-656.
7. Khoninov BV, Sergunin ON, Skoroglyadov PA. Biodegradable materials application in traumatology and orthopedics (review). Vestn. Ross. Gos. Med. Univer. 2014;(1):20-24. Russian.
8. Dedukh NV, Makarov VB, Pavlov AD. Polylactide-based biomaterial and its use as bone implants (analytical literature review). Pain, Joints, Spine. 2019;9(1):28-35. doi: 10.22141/2224-1507.9.1.2019.163056
9. Gutta R, Baker RA, Bartolucci AA, Louis PJ. Barrier Membranes Used for Ridge Augmentation: Is There an Optimal Pore Size? J. Oral Maxillofac. Surg. 2009;67(6):1218-1225. doi: 10.1016/j.joms.2008.11.022
10. Khramtsova E, Morokov E, Lukanina K, Grigoriev T, Petronyuk Y, Shepelev A, Gubareva E, Kuevda E, Levin V, Chvalun S. Impulse acoustic microscopy: A new approach for investigation of polymer and natural scaffolds. Polym. Eng. Sci. 2017;57(7, Special Issue;2):709-715. doi: 10.1002/pen.24617
11. Murphy KG. Postoperative Healing Complications Associated With Gore-Tex Periodontal Material. Part II. Effect of Complications on Regeneration. Int. J. Periodontics Restorative Dent. 1995;15(6):548-561.