Evaluating Osteogenic Cell Differentiation Efficacy in the Presence of Polylactide (PLA) Plates With Varied Compositions for Jaw Bone Grafting: An In Vitro Study

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INTRODUCTION

Bone reconstruction of jaws is the most complex area in modern dentistry and maxillofacial surgery.
Bone defects can be caused by various factors such as traumatic tooth extraction, injuries, tumor processes, infections, or birth abnormalities. Repairing these defects is a difficult challenge requiring special techniques and materials.

According to current statistics, approximately 2.2 million bone augmentation operations are performed annually. Despite the large number of techniques, the problem of jaw atrophy remains relevant, and the number of such operations increases by 13% annually.[1]

There are many world-known methods of bone augmentation, such as guided bone regeneration (GBR), augmentation with collagen bioresorbable membranes, titanium meshes, polytetrafluoroethylene (PTFE) membranes, augmentation with autoblocks, and others.
However, the main disadvantage of using ready-made titanium scaffolds is the intraoperative stage of the membrane adaptation by the individual dimensions of the patient defect.
CAD/CAM technology can be used to plan each case individually and create personalized titanium scaffolds for bone augmentation.

INDIVIDUAL TITANIUM MESHES

1. Individual product design and creation, unique for each clinical case
2. Surgery time minimization (on average 2 times)
3. The thinnest individual product (only 250 microns)
4. Less risk of complications and surgeon mistakes
5. AI-automatization: the ability to produce products accurately and quickly

BIODEGRADABLE

The placement of titanium membranes implies an additional surgical step—removing the membrane after bone maturation before implantation.

To improve the quality of maxillofacial surgery, materials with good mechanical properties and non-toxic and favorable biological and chemical characteristics are required to manufacture membranes for bone repair. Therefore, we decided to pay attention to individual but biodegradable membranes. Using bioabsorbable materials may be a new step in jawbone augmentation[2]

MATERIALS

Potential advantages:

1. No surgical mesh extraction step
2. Maintains framework for 3 to 9 months based on defect volume
3. Individual product design
4. Lack of metallic corrosion-related tissue reaction
5. Absence of artifacts on CT

THE AIM

To investigate the influence of different polymer compounds of individual scaffolds for bone repair on the processes of proliferation and osteogenic differentiation in vitro

MATERIALS & METHODS

Polylactic acid (PLA) is a bioresorbable polymer with high mechanical strength. This is the most studied material that we took as a basis.

But PLA has a prolonged degradation period: high molecular weight PLA may require from 2 to 5.5 years for complete resorption.

So we also decided to include copolymers in the study. They have the advantages of each polymer, along with significantly improved degradation times. The most promising copolymer is polylactic acid with polyglycolic acid (PLA/PGA) because its strength and resorption rate can be adjusted based on the ratio of PLA and PGA.

For example, the degradation time of copolymer with PLA/PGA concentration 85/15 is from 6 to 8 months, while with concentration 60/40 is from 5 to 6 months. So on the one hand, it could be suitable for the manufacture of scaffolds in oral surgery.[3]

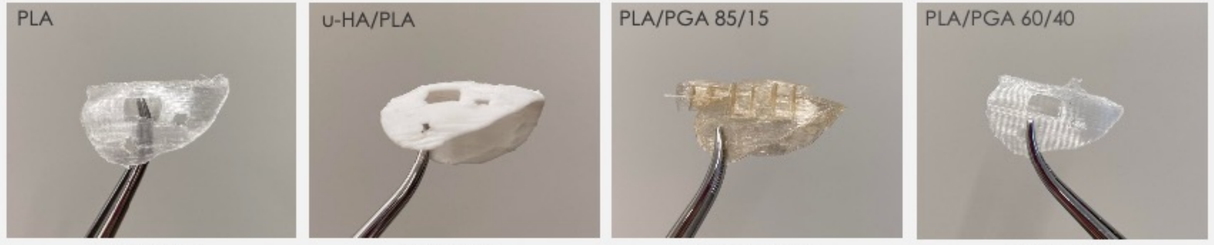


Fig.1 Individual bioresorbable membranes made of different materials (PLA, PLA/HA, PLA/PLGA 85/15, PLA/PLGA 60/40)

MATERIALS & METHODS

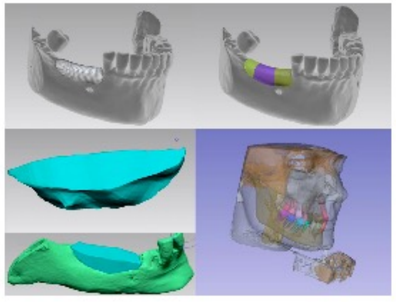


Fig. 2 Modeling and printing of an individual membrane

Prusa 3D printer

Membranes were modeled in the ZBush program and then manufactured on a Prusa 3D printer by FDM printing followed by sterilization.

Samples were sterilized by gamma radiation (a method of radiation sterilization using particle flows capable of ionizing a substance). The average dose during radiation sterilization is 25 kGy (2.5 Mrad).[4] The choice of this value is based on microbiological tests in accordance with GOST R ISO 11737-1.

- Plates 10 x 10 x 0.5 mm were prepared from polylactide (PLA), polylactide with the addition of 15 wt%. hydroxyapatite nanoparticles (PLA/HA), copolymers of polylactide with glycolic acid 60:40 and 85:15 (PLGA 60:40 and 85:15).
- We use gingival fibroblasts to assess the influence of experimental samples on the processes of proliferation and osteogenic differentiation of cells, they were cultured in a low-glucose nutrient medium, then samples were added.
- Osteogenic differentiation was induced by the addition of osteofactors.
- Cell cultures were stained with alizarin red and the intensity of extracellular matrix calcification was measured using a spectrophotometer.

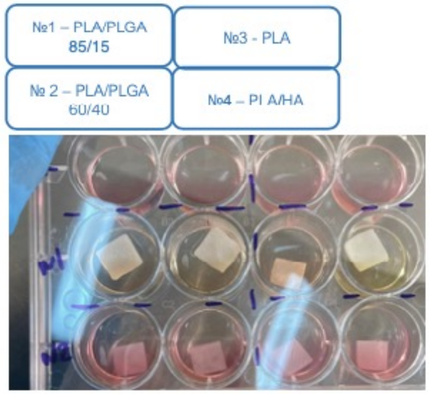


Fig.3. Gingival fibroblasts cultured with PLA composite samples No. 1-4 after cell adhesion

RESULTS

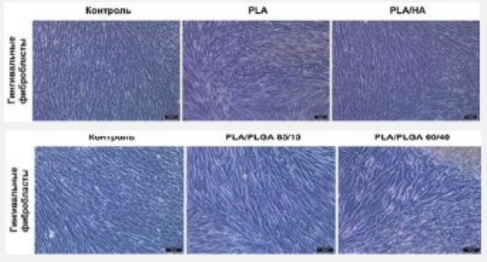


Fig. 4 Gingival fibroblasts. In-life observation. (A) PLA, PLA/HA , (B) PLA/PLGA 85/15, PLA/PLGA 60/40

During the culture process, we observed active proliferation of gingival fibroblasts at the matrix edges, indicating that the plates are not toxic and do not cause cell death

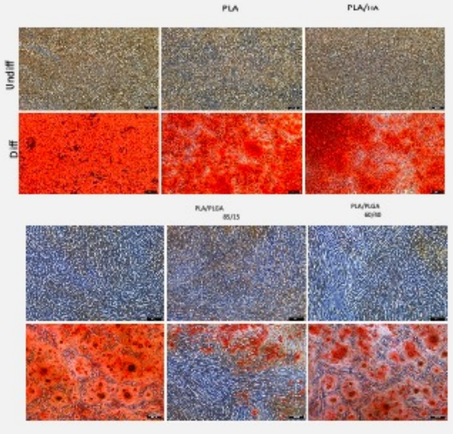
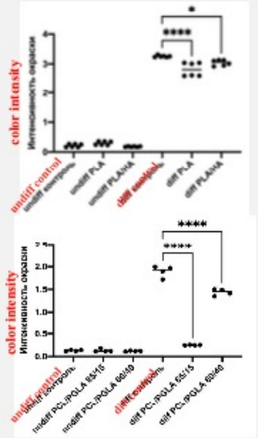


Fig. 5 . Evaluation of the efficiency of osteogenic differentiation of gingival fibroblasts in the presence of (A) PLA, PLA/HA, (B) PLA/PLGA 85/15, PLA/PLGA 60/40



As a result, it can be observed that gingival fibroblasts that were cultured under osteogenic conditions with samples No. 1, No. 2, No. 3, and No. 4 are capable of osteogenic differentiation (Fig. 4 A,B). But we did not find an increase in the induction of osteogenic differentiation in the presence of plates, regarding the control cells that were cultured in the medium with osteogenic differentiation factors without composite materials. However, when comparing samples No. 1 and No. 2 relative to each other, we found that the intensity of alizarin red staining in sample No. 2 was higher than in sample No. 1, indicating greater calcification of the extracellular matrix (Fig. 6. A). It was found that matrices supplemented with hydroxyapatite particles increase calcified extracellular matrix formation in gingival fibroblasts.

However, when comparing samples No. 3 and No. 4 relative to each other, we found that the intensity of alizarin red staining in sample No. 4 was significantly higher than in sample No. 3, indicating greater calcification of the extracellular matrix (Fig. 6. B). We suggest that a change in the percentage of PLGA relative to PLA in the plate composition may cause an increase in calcified extracellular matrix formation in gingival fibroblasts.

CONCLUSION

1) In conclusion, it can be stated that modified composite matrices made of PLA, PLA/HA, PLGA 85/15, and PLGA 60/40 are promising medical devices that could be used for bone tissue augmentation procedures in the oral cavity.

2) The prototypes of membranes from the presented compositions are non-toxic and do not promote cell death.

3) Plates with added hydroxyapatite facilitate the osteogenic differentiation of cells compared to other samples; however, they do not achieve the same level of proliferation and extracellular matrix synthesis as the control cells.

4) The results of comparing PLGA copolymer samples with different concentrations of acids (60/40, 85/15) suggest that altering the ratio of glycolic acid to lactic acid in the matrix composition may influence the degree of calcified extracellular matrix formation.

5) Further research in this area may focus on optimizing the composition of membranes to achieve maximum effect in stimulating osteogenesis and studying the biodegradation time of materials in in vivo conditions.

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