Original article

UDC: 615.46:547.962.9:616.31
doi: 10.5633/amm.2024.0403

IN VITRO FIBROBLASTS' RESPONSE TO THE TWO COLLAGEN MEMBRANES OF DIFFERENT ORIGIN

Milica Tomić[1], Milena Radenković-Stošić[1], Stevo Najman[1,2], Radmila Obradović[3,4], Sanja Stojanović[1,2]

Collagen-based biomaterials are largely used in tissue engineering and regenerative medicine. The sources of collagen for the design of those biomaterials are numerous. Although most collagens are highly biocompatible, the origin can influence the physicochemical and biological properties and guide the final outcome after implantation *in vivo*. A large number of collagen membranes are used in oral and maxillofacial surgery as barrier membranes to cover tissue defects in order to prevent connective tissue infiltration, and that is why it is crucial to examine their interaction with fibroblasts. In this study, we examined the fibroblasts' response to the two commercially available collagen membranes of different origins: porcine vs. equine, in cell culture *in vitro*. The effect of collagen membranes on the proliferation of L929 fibroblasts was examined in a direct cell culture system. Cells were seeded on the collagen membranes and incubated for seven days. The proliferation rate was assessed by the MTT test. There was a significant decrease in cell proliferation rate in the presence of both membranes with a pronounced anti-proliferative effect of the tested porcine membrane. This result speaks in favor of the application of both examined membranes as barrier membranes. Differences in examined collagen membranes may be due to the different origins of collagen although different manufacturing processes may significantly influence cell behavior *in vitro* as well. Further studies with more collagen membranes of various origins should be conducted in order to make final conclusions about the effect of collagen origin on cell behavior *in vitro*.

Acta Medica Medianae 2024;63(4):20–27.

Key words: *collagen, collagen membranes, fibroblasts, L929, in vitro, cell proliferation*

[1]University of Niš, Faculty of Medicine, Scientific Research Center for Biomedicine, Department for Cell and Tissue Engineering, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Department of Biology and Human Genetics, Niš, Serbia
[3]University of Niš, Faculty of Medicine, Department of Oral Medicine and Periodontics, Niš, Serbia
[4]Clinic of Dental Medicine Niš, Department of Oral Medicine and Periodontics, Niš, Serbia

Contact: Sanja Stojanović
81 Dr Zorana Djindjića Blvd., 18000 Niš, Serbia
E-mail: sanja.stojanovic@medfak.ni.ac.rs

Introduction

To restore cells, organs, or tissues that have been lost or damaged due to illness or trauma, regenerative medicine and regenerative dentistry aim to develop methods for creating new ones. Regenerative medicine and dentistry include techniques like tissue engineering, the construction of prosthetic organs, and the application of therapeutic stem cells (1). In scaffold-based approaches, cells, signals, biodegradable, and mechanically stable polymeric scaffolds are used to meet specific therapeutic needs and attain excellent cell survival and retention rates (2). In the field of regenerative dentistry, both soft and hard tissues can be restored and regenerated using tissue engineering methods (3, 4). It is an interdisciplinary field that integrates engineering and medical science ideas to produce biological replacements that maintain, repair, or improve tissue function. To treat a variety of tissue defects, tissue engineering combines cells, scaffolds and bioactive substances. Scaffold-based and scaffold-free treatment techniques have dramatically advanced thanks to the design of novel functionalized dental biomaterials and regenerative engineering techniques (5).

Collagen is the most important polymer in bone and soft tissue engineering (6). It is the most prevalent protein of the extracellular matrix (ECM) in the mammalian body and it makes up one-third of all proteins found in different tissues. Collagen is biocompatible, biodegradable and is neither cytotoxic nor immunogenic (4, 6). Those properties make collagen a gold standard for use in regenerative medicine and tissue engineering. There are different forms of collagen found in mammals, but the most abundant is collagen type I. Collagen can be utilized as a scaffold, membrane, gel or hydrogel, in liposomes, etc (2, 6, 7). The literature describes many resorbable collagen membrane types (2). In tissue engineering, collagen-based membranes are primarily categorized by species: porcine, bovine, equine; and tissue origin: dermis, peritoneum, pericardium, etc. (8, 9). The clinician chooses the most appropriate membrane depending on their characteristics and desired outcome. In addition to supporting wound healing for soft tissue augmentation, collagen-based membranes can serve as a physical barrier to stop connective and epithelial tissue ingrowth into the defect site so that defects can heal properly without forming scarring tissue (10). The foundation of guided tissue regeneration (GTR) is the idea that placing physical barriers inhibits the flap's epithelium and connective tissue cell ingrowth and creates an isolated area for the inward migration of periodontal ligament cells and to impart resistance to bacterial contamination (11).

Collagen-based membranes can differ by added additives and manufacturing procedures in addition to variances in indication and origin. Collagen, as part of the ECM, is naturally degraded by the group of endopeptidases, specifically matrix metalloproteinases (12). Various pathogens, especially periodontal bacteria such as *Porphyromonas gingivalis* and *Treponema denticola* also produce collagenases and may affect the degradation time of collagen membranes when implanted in the oral region (13, 14). That is important in periodontal, oral and maxillofacial surgery because pathogens can jeopardise the treatment by premature degradation of the membrane. Many cross-linking methods are used to improve the physicochemical properties of collagen and to achieve control of collagen biodegradability time. Chemical cross-linking with agents such as aldehydes improves the mechanical strength and prolongs the time of degradation while physical cross-linking treatment with irradiation or biological using biological agents (transglutaminase and horseradish peroxidase) are nonchemical manufacturing techniques that lead to the control of biodegradability (2, 10, 15). However, it has been shown that modification of collagen by cross-linking techniques can lead to partial cytotoxicity (16–18). Additionally, the origin of the collagen membrane was reported to influence the physicochemical behaviour of the collagen membrane (19).

The aim of this study was to analyse and compare the *in vitro* biocompatibility and fibroblasts' response to the two collagen membranes of different species origin, porcine and equine.

Material and Methods

Collagen membranes

In this study, two commercially available collagen membranes of different species origin, porcine and equine, were analyzed:

- 4BONE RCM (MIS Implants Technologies Ltd., Israel) (membrane labeled as PM in the study) is a resorbable collagen membrane made from porcine skin collagen types I and III. According to the manufacturer, this membrane has a prolonged time of resorption achieved by a chemical cross-linking technique using formaldehyde and can be used in GTR as an effective barrier for a period of 4–6 months.
- PARASORB RESODONT® (RESORBA Medical GmbH, Germany) (membrane labeled as EM in the study) is a collagen membrane of equine origin, which contains 2.8 mg of collagen fibrils per 1 cm². According to the manufacturer, the production procedure involves a cross-linking technique without chemical additives. The membrane is completely absorbable with no need for secondary intervention for removal.

Cell culture

L929 mouse fibroblasts were used in this study. The cells were cultured in complete DMEM (low glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 2 mM stable glutamine and 1% antibiotic-antimycotic solution, all purchased from Capricorn, Germany), in standard cell culture conditions, in a humidified atmosphere at 37 °C with 5% CO_2.

Proliferation assay

Prior to cell seeding, membranes were trimmed under sterile conditions to a square with dimensions 1 x 1 cm and one square membrane was placed per each well of the 24-well culture plate (Greiner Bio-One, Germany). Confluent culture of L929 cells was harvested using Trypsin-EDTA solution (Capricorn, Germany), centrifuged, washed in buffer solution and the number of cells was determined by Trypan blue dye exclusion method. Cells were plated out at density 10^4/well/mL and were directly seeded on examined collagen membranes in 24 well plates in complete DMEM. The cells were incubated on the membranes in standard cell culture conditions for seven days. Cells seeded in wells without membranes, in complete DMEM, incubated for seven days under the same conditions, served as a control culture. Each membrane, as well as the control culture, was examined in triplicates. Cells were microscopically analyzed under phase contrast and images were acquired on an inverted light microscope Axio Observer.Z1 equipped with the Axio Cam HRc camera and ZEN software, blue edition (Carl Zeiss, Germany). Cell proliferation was assessed by the MTT test. Cell medium was removed, cells were washed with phosphate-buffered saline and 500 µL of MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide)) in concentration 1 mg/mL was added per each well. The cells were incubated with MTT solution for the next three hours. MTT is reduced by the mitochondrial dehydrogenases of the living cells and as a product purple formazan is formed. The amount of formed formazan is in direct correlation with the number of viable cells. Formazan was dissolved with 100% 2-propanol, and absorbance of the resulting solution was measured at 540 and 650 nm wavelengths on a multichannel spectrophotometer (Multiskan Ascent plate reader, ThermoLab Systems, Helsinki, Finland). The mean absorbance values were calculated for each tested membrane, as well as for the control. The cell proliferation rate was calculated according to the following formula: % cell proliferation = (absorbance value of cells incubated with membrane/absorbance value of control cell culture) × 100.

Statistical analysis

The results of the MTT test were statistically processed and the mean percentage values were calculated according to the above-mentioned formula and presented with relative standard deviations. To determine the statistically significant differences between membranes and control culture, one-way analysis of variance (ANOVA) test was performed. As statistically significant values were considered those for which $p < 0.05$.

Results

Fibroblasts' proliferation on both examined membranes, assessed by the MTT test, is shown in Figure 1. There was a noticeable difference in the cell proliferation rate among equine-derived (EM) and porcine-derived (PM) collagen membranes as well as compared to the control cell culture.

The interaction of cells with collagen membranes and proliferation pattern were monitored microscopically and images under the phase contrast were made at the end of the incubation period, prior to the MTT test, which is shown in Figure 2.

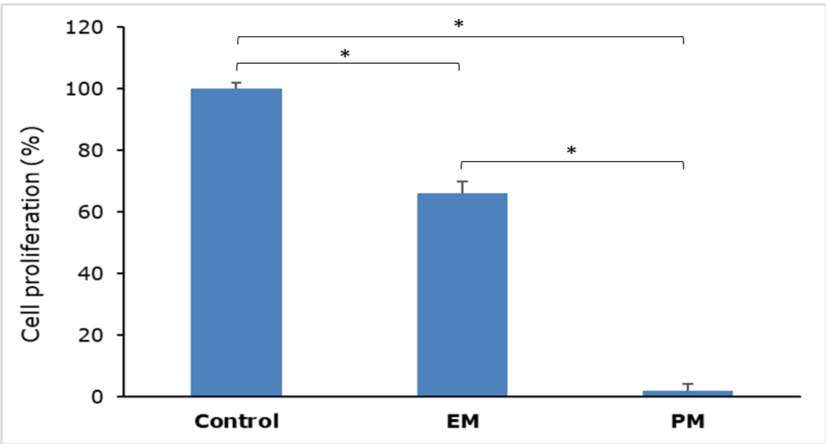

Figure 1. Proliferation of L929 cells in control cell culture, on EM and PM membrane; (*) $p < 0.001$

Figure 2. Morphological appearance of L929 cells in control cell culture (A), L929 cells cultured on EM collagen membrane of equine origin (B) and L929 cells cultured on PM collagen membrane of porcine origin (C); phase contrast, objective magnification 10x

Both examined membranes showed an anti-proliferative effect and significantly influenced cell proliferation compared to the control cell culture. In addition, the PM membrane showed a higher anti-proliferative effect than the EM membrane. Rare, elongated cells close to, onto and below the PM membrane were noticed, while a significantly higher number of cells, without significant morphological changes, were noticed in close proximity to, onto and below the EM membrane.

Discussion

There are reports in the literature that the origin of collagen may influence the physicochemical properties of collagen membranes and cell behaviour *in vitro*, but most of these studies were focused on comparing porcine and bovine membranes that are largely used in clinical practice. To the best of our knowledge, there are only a few studies with a comparative analysis of collagen membranes of porcine and equine origins, with a focus on defining the influence of the origin on their effects on cell culture *in vitro* (14, 19, 20).

Regarding the species origin of collagen, there are some concerns and questions about the risk of collagen use and expected tissue response. For instance, the implantation of collagen-based biomaterials of bovine origin carries a risk of transmission of zoonoses such as bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE), or virus-associated diseases, while porcine originated collagen membranes can be rejected due to religious constraints (21, 22). On the contrary, the collagen of equine origin is not associated with virus disease transmission and there is no possibility of rejection due to ethical reasons (23). Furthermore, collagen originating from various species and tissue sources can differ in amino-acid sequence and consequently in its biostability (17, 24). Thus, in this study, we examined two types of collagen membranes of different species origin: porcine vs. equine. We conducted *in vitro* cytocompatibility testing on L929 cell line. *In vitro* testing of biomaterials is the first step when developing new biomaterials. It gives necessary guidance for the *in vivo* testing that comes afterwards. It is a less expensive method, experimentally controllable, repeatable and it does not raise any legal or ethical questions. The biggest disadvantage is that it cannot test chronic effects or pharmacokinetics (8, 10). In our study, the proliferation of L929 fibroblasts was tested after 7 day-cultivation period on two collagen membranes of different species origin. EM membrane demonstrated significantly higher proliferation potential than the PM membrane. Kasaj et al. (20) tested three collagen membranes and EM also showed the highest proliferation potential among tested collagen membranes. Compared to the positive control, the proliferation of cells on the tested membranes in our study was significantly lower compared to the control, which is also in accordance with the results obtained in the above-mentioned study. Data from the literature, also, indicate that the membrane of equine origin can be more suitable for cell proliferation compared to other applicable membranes for the concept of GTR. Kasaj et al. (20) demonstrated the highest rate of human gingival fibroblast (HGF) proliferation on the TutoDent® membrane of bovine origin followed by the EM membrane examined in our study as well, in an observation period of one hour and 48 hours, compared to the resorbable membrane of porcine origin and three other non-resorbable membranes. In that study, the resorbable collagen membranes, including EM, induced a significantly higher number of cells in all examined periods compared to the non-resorbable membranes in the periodontal ligament fibroblasts (PDLF) cell line (20). Authors suggested that different patterns of cell proliferation can be caused by a difference in surface topography and characteristics as well as in pore sizes (20). The above-mentioned findings about the influence of surface topography were confirmed in the study of Willershausen et al. (25) as well, where it was shown that the proliferation rate of HGF was higher on the compact layer of two examined native biomaterials of porcine origin, followed by different growth pattern, compared to spongy layer, during observation time of 48 hours. In the study of Toledano et al. (14), a difference in the biodegradation process analysed *in vitro* between membranes of different origin (porcine vs. equine) was shown, but also different results in degradation tests were obtained between two membranes of equine origin. Through the three different degradation tests, the equine collagen membrane covered with equine bone particles was more susceptible to the degradation process in comparison with other membranes, derived from the porcine dermis and equine pericardium tissue (14). The authors assumed that different biodegradation findings in this study can be related not only to different species and tissue origin but also, to the manufacturing process, in this case, the lyophilizing treatment which influenced the 3D architecture of collagen (14). Furthermore, the scaffold based on native equine collagen (PARASORB Sombrero, RESORBA), the same collagen materials and producer as the EM membrane examined in our study, was evaluated as more suitable for Human-Periosteal Cells (hPCs) proliferation than inorganic scaffolds based on PLGA alone or in combination with Hydroxyapatite (HA) (26).

Based on a comparison of data from our studies and other mentioned findings, we can assume that equine-based collagen material may be a good basis and environment for cell growth but it depends on the type of cells which is going to be seeded, as well as its 3D architecture. Thus, Raimondi et al. (27) showed that native, non-cross-linked collagen type I from equine Achile tendon (commercially available sponge Antema®) is not suitable to support human chondrocyte survival *in vitro* during the observation period of two weeks, even newly synthesized collagen was detected (27). On the other hand, Masci et al. (28) reported that a collagen scaffold of the same origin, is a convenient scaffold for the proliferation, migration, and adhesion of murine fibroblasts (NIH 3T3), through extended filopodia and macrovesicles shedding (28). There was no literature data about previous PM membrane testing. In our study, the cell proliferation rate on the PM membrane was significantly lower than that on the EM membrane. Previous studies of porcine collagen membranes (25) showed that they caused decreased cellular proliferation and higher cytotoxic effects compared to the collagen membranes of other origins. Also, the porcine membrane was shown to lead to increased production of proinflammatory mediators by mononuclear cells at 4 and 12 h of incubation and decreased cell viability compared to the bovine membrane (29). Behring et al. (17) suggested that not only the origin of membranes is important, but also the manufacturing process. There is data in the literature about connections between the prolonged period of biodegradation caused by cross-linking modification, with a reduction in biocompatibility (30, 31). Chemical cross-linkers that are frequently used in the production of natural polymer-based biomaterials are shown to significantly influence the biocompatibility of biomaterials, making the biomaterials cytotoxic for cells (32–34). Naturally derived chemical cross-linkers are a much better solution for the cross-linking process in polymer-based biomaterials production which was shown in the case where EDC-NHS was compared with genipin for cross-linking of wound dressing material based on alginate and chitosan (35). In our study, the production of EM membrane involves a cross-linking technique without chemical additives (information provided by the manufacturer), while in the production process of PM membrane, a chemical cross-linking method was used (information provided by the manufacturer), which could cause pronounced anti-proliferative effect of PM membrane compared to EM membrane. A study by Schorn et al. (36) showed that not only origin, collagen type and modification process such as cross-linking can affect the proliferation rate, attachment, and cytotoxicity rates, but also adding other substrates on the membrane. Results from that study showed higher cell proliferation and cell viability of osteogenic cell lines on Bio-Gide® membrane of porcine origin and RESODONT® membrane of equine origin than the other membranes tested. On the other hand, the GENTA-FOIL resorb® membrane of equine origin, with added gentamycin, showed the highest cytotoxicity rate (36). Authors of the same study assumed that the rough surfaces of the RESODONT® and Bio-Gide® membranes might be one of the reasons for their high rates of cell attachment (36).

We must mention the limitations of our study. It cannot provide us with precise information regarding the tissue response to these membranes because it was only carried out on one cell line under controlled *in vitro* cell culture conditions. It merely provides us with the appropriate direction regarding what ought to be anticipated while conducting an *in vivo* study, which is the following stage in the research of biomaterials intended for regenerative medicine and tissue engineering.

Conclusion

Our results show that there is a significant difference in the proliferation rates between cells cultured on examined membranes, in examined conditions. The proliferation of fibroblasts was significantly reduced in the presence of the PM membrane (membrane of porcine origin), while slightly reduced on the EM membrane (membrane of equine origin). This suggests that both membranes, particularly PM, may be used as good barrier membranes to prevent connective tissue ingrowth into the bone defect site. The difference in the proliferation of fibroblasts on examined membranes could be due to the different origins of collagen membranes but also observed differences and anti-proliferative effect could be due to the differences in the manufacturing process that may significantly affect the cell growth *in vitro*.

Acknowledgements

This study was financially supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia (Contracts No. 451-03-68/2020-14/200113, 451-03-9/2021-14/200113, 451-03-68/2022-14/200113, 451-03-47/2023-01/200113, 451-03-65/2024-03/200113 and 451-03-66/2024-03/200113).

References

1. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. J Endod 2007;33:377-90. [CrossRef][PubMed]
2. Chevallay B, Herbage D. Collagen-based biomaterials as 3D scaffold for cell cultures: applications for tissue engineering and gene therapy. Med Biol Eng Comput 2000;38(2):211-8. [CrossRef][PubMed]
3. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod 2008;34:645-51. [CrossRef][PubMed]
4. Jency G, Jegan SR, Mahija SP. Collagen and Its Therapeutical Applications in Regenerative Medicine. International Journal for Scientific Research & Development 2018;6(01):268-77.
5. Yelick PC, Sharpe PT. Tooth bioengineering and regenerative dentistry. J Dent Res 2019;98(11):1173–82. [CrossRef][PubMed]
6. Ferreira AM, Gentile P, Chiono V, Ciardelli G. Collagen for bone tissue regeneration. Acta Biomaterialia 2012;8(9):3191–200. [CrossRef][PubMed]
7. Khan R, Khan MH. Use of collagen as a biomaterial: An update. J Indian Soc Periodontol 2013;17(4):539-42. [CrossRef][PubMed]
8. Meyer M. Processing of collagen based biomaterials and the resulting materials properties. BioMed Eng OnLine 2019;18:24. [CrossRef][PubMed]
9. Bunyaratavej P, Wang HL. Collagen Membranes: A Review. Journal of Periodontology 2001;72(2):215-29. [CrossRef][PubMed]
10. Bottino MC, Thomas V, Schmidt G, Vohra YK, Chu T-MG, Kowolik MJ, et al. Recent advances in the development of GTR/GBR membranes for periodontal regeneration—A materials perspective. Dental Materials 2012;28(7):703–21. [CrossRef][PubMed]
11. Bee L, Abdul Hamid ZA. Asymmetric resorbable-based dental barrier membrane for periodontal guided tissue regeneration and guided bone regeneration: A review. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2022;110(9):2157-82. [CrossRef][PubMed]
12. Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, Ramirez-Acuña JM, Perez-Romero BA, Guerrero-Rodriguez JF, et al. The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases. Int J Mol Sci 2020;21(24):9739. [CrossRef][PubMed]
13. Sela MN, Babitski E, Steinberg D, Kohavi D, Rosen G. Degradation of collagen-guided tissue regeneration membranes by proteolytic enzymes of *Porphyromonas gingivalis* and its inhibition by antibacterial agents. Clinical Oral Implants Research 2009;20(5):496-502. [CrossRef][PubMed]
14. Toledano M, Asady S, Toledano-Osorio M, García-Godoy F, Serrera-Figallo MA, Benítez-García JA, Osorio R. Differential Biodegradation Kinetics of Collagen Membranes for Bone Regeneration. Polymers (Basel) 2020;12(6):1290. [CrossRef][PubMed]
15. Lin K, Zhang D, Macedo MH, Cui W, Sarmento B, Shen G. Advanced Collagen-Based Biomaterials for Regenerative Biomedicine. Adv Funct Mater 2019;29(3):1804943. [CrossRef]
16. Ghanaati S. Non-cross-linked porcine-based collagen I–III membranes do not require high vascularization rates for their integration within the implantation bed: A paradigm shift. Acta Biomaterialia 2012;8(8):3061–72. [CrossRef][PubMed]
17. Behring J, Junker R, Walboomers XF, Chessnut B, Jansen JA. Toward guided tissue and bone regeneration: morphology, attachment, proliferation, and migration of cells cultured on collagen barrier membranes. A systematic review. Odontology 2008;96(1):1-11. [CrossRef][PubMed]
18. Brunel G, Piantoni P, Elharar F, Benqué E, Marin P, Zahedi S. Regeneration of Rat Calvarial Defects Using a Bioabsorbable Membrane Technique: Influence of Collagen Cross-linking. Journal of Periodontology 1996;67(12):1342-8. [CrossRef][PubMed]
19. Osorio R, Asady S, Toledano-Osorio M, Toledano M, Bueno JM, Martínez-Ojeda RM, et al. Biomimetic Remineralization of an Extracellular Matrix Collagen Membrane for Bone Regeneration. Polymers 2022;14:3274. [CrossRef][PubMed]
20. Kasaj A, Reichert C, Götz H, Röhrig B, Smeets R, Willershausen B. *In vitro* evaluation of various bioabsorbable and nonresorbable barrier membranes for guided tissue regeneration. Head Face Med 2008;4:22. [CrossRef][PubMed]
21. Silvipriya K, Kumar K, Bhat A, Kumar B, John A, Lakshmanan P. Collagen: Animal Sources and Biomedical Application. J App Pharm Sci 2015;5(03):123-7. [CrossRef]
22. Flaig I, Radenković M, Najman S, Pröhl A, Jung O, Barbeck M. In Vivo Analysis of the Biocompatibility and Immune Response of Jellyfish Collagen Scaffolds and its Suitability for Bone Regeneration. Int J Mol Sci 2020;21(12):4518. [CrossRef][PubMed]
23. Wagner-Ecker M, Voltz P, Egermann M, Richter W. The collagen component of biological bone graft substitutes promotes ectopic bone formation by human mesenchymal stem cells. Acta Biomaterialia 2013;9(7):7298-307. [CrossRef][PubMed]
24. Wang Y, Hua Y, Zhang Q, Yang J, Li H, Li Y, et al. Using biomimetically mineralized collagen membranes with different surface stiffness to guide regeneration of bone defects. Journal of Tissue Engineering and Regenerative Medicine 2018;12(7):1545-55. [CrossRef][PubMed]
25. Willershausen I, Barbeck M, Boehm N, Sader R, Willershausen B, Kirkpatrick CJ, et al. Non-cross-linked collagen type I/III materials enhance cell

proliferation: *in vitro* and *in vivo* evidence. J Appl Oral Sci 2014;22(1):29-37. [CrossRef][PubMed]
26. Ceccarelli G, Presta R, Lupi SM, Giarratana N, Bloise N, Benedetti L, et al. Evaluation of Poly(Lactic-co-glycolic) Acid Alone or in Combination with Hydroxyapatite on Human-Periosteal Cells Bone Differentiation and in Sinus Lift Treatment. Molecules 2017;22(12):2109. [CrossRef][PubMed]
27. Raimondi MT, Falcone L, Colombo M, Remuzzi A, Marinoni E, Marazzi M, et al. A comparative evaluation of chondrocyte/scaffold constructs for cartilage tissue engineering. J Appl Biomater Biomech 2004;2(1):55-64. [PubMed]
28. Masci VL, Taddei AR, Gambellini G, Giorgi F, Fausto AM. Ultrastructural investigation on fibroblast interaction with collagen scaffold. J Biomed Mater Res A 2016;104(1):272-82. [CrossRef][PubMed]
29. Moura CC, Soares PB, Carneiro KF, Souza MA, Magalhães D. Cytotoxicity of bovine and porcine collagen membranes in mononuclear cells. Braz Dent J 2012;23(1):39-44. [CrossRef][PubMed]
30. Parvini P, Mihatovic I, Sahin D, Becker J, Schwarz F. Lateral alveolar ridge augmentation using an equine-derived collagen-containing bone block: A prospective case series. Clin Oral Implants Res 2022;33(2):142-9. [CrossRef][PubMed]
31. Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J. Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. Clin Oral Implants Res 2005;16(3):369-78. [CrossRef][PubMed]
32. Ghorpade VS. Preparation of hydrogels based on natural polymers via chemical reaction and cross-linking. In: Chen Y, editor. Hydrogels Based on Natural Polymers. Amsterdam (Netherlands): Elsevier; 2020. p. 91–118. [CrossRef]
33. Zafar S, Hanif M, Azeem M, Mahmood K, Gondal SA. Role of cross-linkers for synthesizing biocompatible, biodegradable and mechanically strong hydrogels with desired release profile. Polym Bull 2022;79:9199–219. [CrossRef]
34. Joseph AM, George B. Cross-Linking Biopolymers for Biomedical Applications. In: Thomas S, Ajitha AR, Chirayil CJ, Thomas B, editors. Handbook of Biopolymers. Singapore (Singapore): Springer Nature Singapore; 2023. p. 1135–72. [CrossRef]
35. Hautmann A, Kedilaya D, Stojanović S, Radenković M, Marx CK, Najman S, et al. Free-standing multilayer films as growth factor reservoirs for future wound dressing applications. Biomater Adv 2022;142:213166. [CrossRef][PubMed]
36. Schorn L, Handschel J, Lommen J, VON Beck FP, Depprich R, Kübler N, et al. Evaluation of Biocompatibility of Different Membrane Surfaces Using Unrestricted Somatic Stem Cells. In Vivo 2019;33(5):1447-54. [CrossRef][PubMed]

Originalni rad

UDC: 615.46:547.962.9:616.31
doi: 10.5633/amm.2024.0403

IN VITRO ODGOVOR FIBROBLASTA NA DVE KOLAGENSKE MEMBRANE RAZLIČITOG POREKLA

Milica Tomić[1], Milena Radenković Stošić[1], Stevo Najman[1,2], Radmila Obradović[3,4], Sanja Stojanović[1,2]

[1]Univerzitet u Nišu, Medicinski fakultet, Naučnoistraživački centar za biomedicinu, Odeljenje za ćelijsko i tkivno inženjerstvo, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, UNO Biologija sa humanom genetikom, Niš, Srbija
[3]Univerzitet u Nišu, Medicinski fakultet, Katedra Oralna medicina i parodontologija, Niš, Srbija
[4]Klinika za dentalnu medicinu Niš, Služba za oralnu medicinu i paradontologiju, Niš, Srbija

Kontakt: Sanja Stojanović
Bulevar dr Zorana Đinđica 81, 18000 Niš, Srbija
E-mail: sanja.stojanovic@medfak.ni.ac.rs

Biomaterijali na bazi kolagena koriste se u velikoj meri u tkivnom inženjerstvu i u regenerativnoj medicini. Brojni su izvori kolagena za proizvodnju ovih biomaterijala. Iako je većina kolagena izuzetno biokompatibilna, poreklo kolagena može uticati na fizičko-hemijska i biološka svojstva biomaterijala i usmeriti konačni ishod nakon implantacije *in vivo*. Budući da se znatan broj kolagenskih membrana koristi u oralnoj i maksilofacijalnoj hirurgiji u svojstvu barijerne membrane za pokrivanje defekta koštanih tkiva da bi se sprečila infiltracija vezivnog tkiva, interakcija ovih membrana sa fibroblastima predstavlja ključan faktor. U ovoj studiji ispitivan je odgovor fibroblasta na dve komercijalno dostupne kolagenske membrane različitog porekla – svinjskog i konjskog – u ćelijskoj kulturi *in vitro*. Uticaj kolagenskih membrana na proliferaciju L929 fibroblasta ispitivan je u sistemu direktne ćelijske kulture. Ćelije su zasađene na kolagenske membrane i inkubirane sa njima sedam dana. Proliferacija ćelija procenjivala se MTT testom. Došlo je do značajnog smanjenja proliferacije ćelija u prisustvu obeju membrana, s tim što je uočen izraženiji antiproliferativni efekat membrane svinjskog porekla. Ovaj rezultat govori u prilogu tome da obe ispitivane membrane mogu biti primenjene kao barijerne membrane. Razlike u ispitivanim kolagenskim membranama mogu biti posledica različitog porekla kolagena, mada treba istaći i da različiti primenjeni proizvodni procesi mogu značajno uticati na ponašanje ćelija *in vitro*. Treba sprovesti dalja istraživanja sa više kolagenskih membrana različitog porekla kako bi se doneli konačni zaključci o uticaju porekla kolagena na ponašanje ćelija u prisustvu ovih biomaterijala *in vitro*.

Acta Medica Medianae 2024; 63(4):20−27.

***Ključne reči**: kolagen, kolagenske membrane, fibroblasti, L929, in vitro, proliferacija ćelija*