International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Histomorphological Assessment of Biodegradation in Collagen-Hydroxyapatite and Collagen-Hyaluronic Acid Composites

Yusupalikhodjaeva S. Kh[1], Shomurodova G. Kh[2], Rajapov O.I[3], Vipova N.L[4]

[1]PhD, DSc, Associate Professor, Department of Propaedeutics of Therapeutic Dentistry, Tashkent State Medical University
[2]Doctoral Student, Department of Propaedeutics of Therapeutic Dentistry, Tashkent State Medical University
[3]Senior Researcher, Candidate of Chemical Sciences, A.S. Sadikov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan
[4]Senior Researcher, Laboratory of Pharmacology, Candidate of Biological Sciences, A.S. Sadikov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan

Abstract:
Endo-periodontal lesions represent a complex pathological process that combines endodontic inflammation with destruction of periodontal tissues and the alveolar ridge through communication between the root canal and periodontal pocket. Histomorphological evaluation of the biodegradation of collagen-hydroxyapatite and collagen-hyaluronic acid composites was conducted via subcutaneous implantation in 24 white outbred rats. The collagen–hyaluronic acid composite was completely degraded by week 3, exhibiting minimal hemorrhagic response and preserved dermal architecture. Meanwhile, the collagen-hydroxyapatite composite exhibited prolonged biodegradation, achieving complete resorption by week 4, accompanied by fibro-reparative reaction including intense vascularization of granulation tissue, active collagenogenesis, and dystrophic calcification of the mineral framework ($p<0.05$).

Keywords: endo-periodontal lesions, collagen-hydroxyapatite composite, collagen-hyaluronic acid composite.

1. INTRODUCTION

Endo-periodontal lesions (EPLs) represent a clinically complex form of tooth pathology that combines an infectious-inflammatory process in the endodontium with the spread of inflammation to periodontal tissues, the alveolar ridge, and the periapical area up to the level of communication between the root canal and the periodontal pocket [1,2,3].

In individuals with moderate to severe chronic periodontitis and inadequately treated endodontic pathology, the presence of fistulous tracts, deep periodontal pockets, and combined pyoinflammatory periapical lesions contributes to the development of endo-periodontal lesions. [4,5,6,7].

Clinical manifestations of EPLs include progressive alveolar bone loss, detachment of the attached gingiva, increased sensitivity to thermal and mechanical stimuli, and persistent dull pain, resulting in a high risk of tooth loss despite preservation of the crown and root structures [8,9]. Moreover, in EPLs a direct communication pathway exists between the root canal system and the periodontal pocket, enabling continuous bidirectional spread of pathogenic microorganisms and inflammatory mediators between endodontic and periodontal tissues, thereby aggravating disease progression. Radiographically, these lesions are characterized by vertical and horizontal bone defects along the root surface, periapical radiolucencies, and deep periodontal pockets exceeding 6 mm, which complicates differential diagnosis with primary endodontic or periodontal pathology and necessitates an integrated endodontic–periodontal therapeutic approach [3,5,10].

In recent years, the detection rate of EPLs in patients with complex periodontal and endodontic pathology has increased significantly due to advances in diagnostic imaging techniques, including CBCT, three-dimensional tomography, and high-resolution periapical radiography, as well as the growing prevalence of chronic periodontitis. Furthermore, the morphological complexity of these lesions has increased, with combined horizontal and vertical bone defects and interradicular resorption becoming more common, which limits the effectiveness of conventional treatment methods and highlights the need for bioactive osteoplastic and regenerative biomaterials, including collagen–hydroxyapatite-based composites [8,5,11].

Consequently, the development and pathogenetic justification of regenerative treatment strategies using collagen- and hydroxyapatite-based biomaterials have become particularly relevant. Recent studies have demonstrated that collagen–hydroxyapatite composites not only serve as defect fillers but also function as biocompatible osteoconductive scaffolds that promote tissue integration and stabilization of bone regeneration in endo-periodontal and periapical defects [3,5,8,10].

Clinical investigations have shown that hydroxyapatite- and collagen-containing materials enhance bone regeneration and improve tooth survival outcomes, while their combination with platelet-rich plasma further accelerates reparative osteogenesis. Additionally, emerging evidence suggests that collagen–hydroxyapatite membranes and bone substitutes can serve as carriers for antimicrobial agents and facilitate localized mineralization in endodontic and periodontal lesions [8,12].

Therefore, these findings underscore the clinical and morphological importance of optimizing comprehensive local treatment protocols for EPLs using collagen–hydroxyapatite-based regenerative materials as pathogenetically justified therapeutic agents.

The aim of this study was to evaluate the resorption dynamics and local tissue response to collagen composites modified with hydroxyapatite and hyaluronic acid following subcutaneous implantation in rats.

2. Materials and methods

The experiment was performed using 24 outbred white rats of both sexes with a body weight of 250 ± 25 g. The animals were randomly assigned to two experimental groups (n = 6 per group). All rats were maintained under standard vivarium conditions with a natural light–dark cycle, ad libitum access to food and water, and controlled environmental parameters (temperature 22–24 °C and relative humidity 40–60%).

All experimental procedures involving animals were carried out in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and were approved by the Local Ethics Committee (Protocol No. 4, September 9, 2024).

For the first experimental group, collagen–hydroxyapatite composite samples (disks measuring 5 × 5 mm and weighing 10 mg) were implanted, while the second group received collagen–hyaluronic acid composites with identical dimensions and mass. The investigated biomaterials were produced as lyophilized white plates with dimensions of 72 × 72 mm and a thickness of 3–5 mm. Prior to implantation, sterile fragments measuring 10 × 10 × 2 mm were aseptically cut from the plates.

Subcutaneous implantation was performed in the interscapular region under general anesthesia induced by intraperitoneal administration of sodium thiamylal at a dose of 50 mg/kg. The surgical wound was closed in layers using interrupted sutures with absorbable suture material.

Animals were sacrificed at 2-, 4- and 6-weeks post-implantation (two animals per group at each time point) by anesthetic overdose in accordance with ISO 10993-2 guidelines. The implant, together with surrounding tissues and regional lymph nodes was excised for subsequent morphological assessment.

Macroscopic evaluation of the fibrous capsule (including color, presence of hematomas, and weight) was performed, followed by histological analysis. Tissue samples were fixed in 10% neutral buffered formalin for 48 hours, rinsed, dehydrated through a graded ethanol series (70%, 96%, and 100%), and embedded in paraffin using standard histological procedures. Serial sections with a thickness of 5–7 µm were prepared and stained with hematoxylin and eosin.

Histological examination was carried out using a binocular light microscope at magnifications of ×40 and ×100. Microphotographs were obtained using a digital HM-35 camera at native resolution.

3. Results

The dynamics of macroscopic changes in peri-implantational capsules are presented in Table 1. In the first 2 weeks, both groups showed a transient body weight reduction of 3–12%, followed by normalization. Capsule mass in the collagen–hydroxyapatite group was significantly higher in early periods ($p<0,05$). Lymph nodes and blood biochemical parameters showed no pathological changes. Complete resorption of collagen–hyaluronic acid composite occurred by week 3, while collagen–hydroxyapatite composite was fully resorbed by week 4 post-implantation.

Group 1 (collagen–hydroxyapatite composite) showed a pronounced fibro-reparative tissue response during all observation periods. At week 2, the implantation zone was surrounded by mature granulation tissue with numerous thin-walled vessels, perivascular edema, and macrophage and fibroblast infiltration; collagen fibers at the periphery were loosely organized (Fig. 1A). By week 4, intensified collagenogenesis led to the formation of thick coarse collagen bundles with reduced vascularity and minimal cellular infiltration, while residual implant material remained (Fig. 1B). By week 6, remodeling resulted in mature coarse fibrous scar tissue with dystrophic calcification, destruction of dermal appendages, and marked vascular reduction, indicating fibrotic healing (Fig. 1C).

Table 1. Macroscopic evaluation of capsules 2-6 weeks after implantation (M±m, n=6)

Time period	Collagen–hyaluronic acid	Collagen–hydroxyapatite
Week 2	Capsules 5,2±0.5 mg, no inflammation	Capsules 21,6±1.8 mg* + punctate hematomas
Week 4	Gray spots	Punctate hematomas
Week 6	No traces	Punctate hematomas

Note: $p<0.05$ compared to collagen–hyaluronic acid

Group 2 (collagen–hyaluronic acid) demonstrated a more organized and physiological regenerative response. At week 2, granulation tissue exhibited orderly collagen bundles and abundant functional vessels with preservation of dermal appendages (Fig. 1D). By week 4, highly differentiated connective tissue formed concentric layers of parallel collagen bundles around implant fragments, with pronounced vascularization and moderate fibroblast proliferation (Fig. 1E). By week 6, the implant was completely replaced by mature connective tissue with preserved dermal architecture, moderate vascular reduction, and absence of hyalinization, indicating favorable tissue integration and regeneration (Fig. 1F).

4. Discussion

This study elucidates the resorption behavior and local tissue response to collagen composites modified with hydroxyapatite and hyaluronic acid, assessed as bioactive matrices for regenerative applications. As shown by Maiborodin et al. (2019), biodegradation of collagen membranes in soft tissues primarily occurs within 4 weeks subcutaneously in animal models [13].

Resorption of the collagen sponge occurs through biodegradation processes. This process begins with the release of matrix metalloproteinases (MMPs) by cells in the surgical area into the wound during healing, followed by infiltration and colonization of the matrix by fibroblasts and capillaries, leading to remodeling of the collagen framework and its replacement by newly synthesized extracellular matrix [12]. It has been established that the duration of collagen composite preservation plays a key role in the success of regenerative procedures. According to the obtained data, biodegradation of modified collagen preparations occurs at different times depending on the modification.

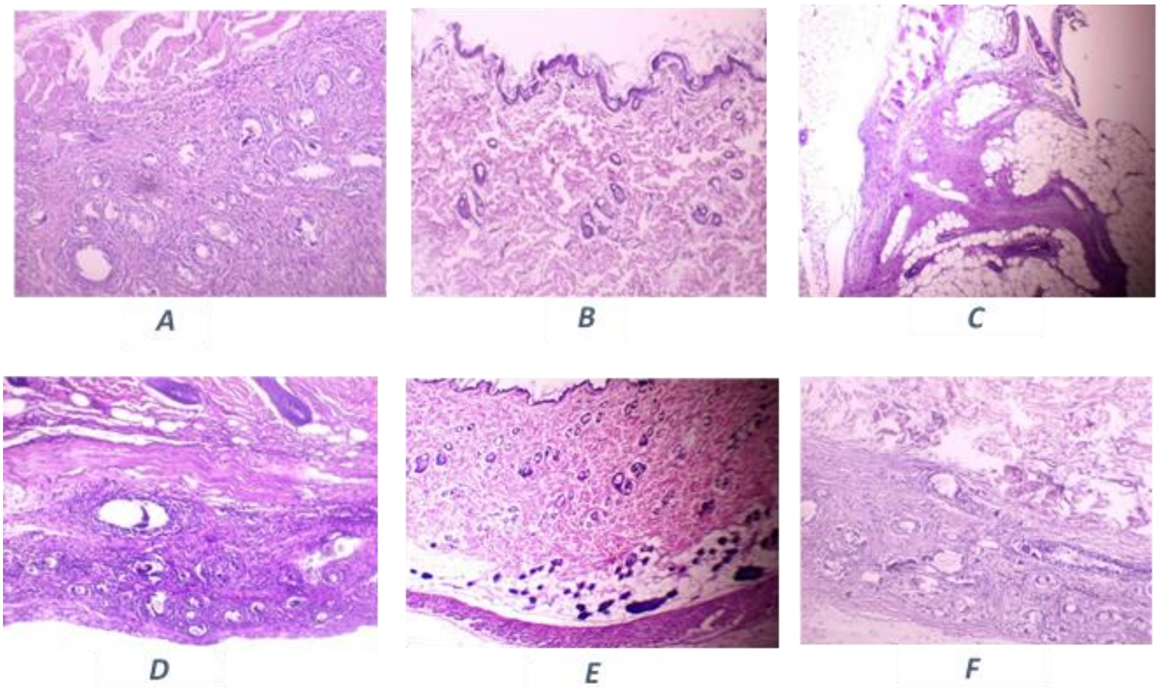

Figure 1. Histological dynamics of tissues after implantation.

A – 2 weeks, group 1; B – 4 weeks, group 1; C – 6 weeks, group 1;
D – 2 weeks, group 2; E – 4 weeks, group 2; F – 6 weeks, group 2.

Collagen–hyaluronic acid was completely resorbed by week 4 with minimal hemorrhagic manifestations, consistent with data on high biodegradability of pure collagen, whereas collagen–hydroxyapatite composite demonstrated prolonged resorption (4 weeks) due to the mineral framework providing osteoconductivity, but accompanied by a more pronounced local hemorrhagic reaction. Studies by Gürbüz et al. (2023) also demonstrated that collagen-hydroxyapatite-polycaprolactone membranes promote increased volume of regenerated bone tissue and improved clinical prognosis for tooth retention in endoparodontal lesions and periapical defects [14].

5. Conclusion

Experimental results demonstrate differences in biodegradation rates of modified collagen implants. Disks containing collagen-hyaluronic acid were completely resorbed within 4 weeks with minimal hemorrhagic manifestations, consistent with known high biodegradability characteristics of pure collagen. The collagen-hydroxyapatite composites exhibited prolonged resorption due to the mineral framework providing osteoconductivity, but was accompanied by a more pronounced local hemorrhagic reaction.

References

Юсупалиходжаева, С., & Шомуродова, Г. (2023). Сочетанные воспалительно-деструктивные поражение пародонта: этиология, патогенез, клиника, диагностика. Стоматология,1(1), 75–79. извлечено от https://inlibrary.uz/index.php/stomatologiya/article/view/20665

2 Herrera D, Retamal-Valdes B, Alonso B, Feres M. Acute periodontal lesions (periodontal abscesses and necrotizing periodontal diseases) and endo-periodontal lesions. J Periodontol. 2018;89. S85–S102. doi: 10.1002/JPER.16-0642.

3 Oh S, Chung SH, Han JY. Periodontal regenerative therapy in endo-periodontal lesions: a retrospective study over 5 years. J Periodontal Implant S.

4 Моисеев Д.А., Румянцев В.А., Волков С.И., Кулюкина М.А., Конов А.А. Морфологические аспекты взаимосвязи тканей пародонта и пульпы зубов. - Проблемы стоматологии. - 2021; 2: 77-83. eLIBRARY ID: 46411886

5 Моисеев Д.А., Копецкий И.С., Никольская И.А., Гусева О.Ю., Михайлова Е.Г., Еремин Д.А., Патракова Н.Н., Погабало И.В., Оглобин А.А. Лечение, профилактика и исходы эндопародонтальных поражений: современный взгляд.-*Клиническая стоматология*.—2024; 26 (4): 18-28.DOI:10.37988/1811-153X_2023_4_18

6 Шомуродова Г. Х., Юсупалиходжаева С. Х. Ассоциация эндодонто-пародонтальных поражений с системными заболеваниями.

7 Lin L.M., Ricucci D., Saoud T.M., Sigurdsson A., Kahler B. Vital pulp therapy of mature permanent teeth with irreversible pulpitis from the perspective of pulp biology. - Aust Endod J. - 2020; 46 (1): 154-166. PMID: 31865629

8 Людчик Т.Б., Абдуллаев Ш.Ю., Муратова Н.Ю., и др. Роль коллагена и гидроксиапатита в составе композиционных костнопластических материалов, используемых в челюстно-лицевой хирургии (обзор литературы) // Вестник современной клинической медицины. - 2023. - Т.16, Прил.2. - С.83-90. DOI: 10.20969/VSKM.2023.16(suppl.2).83-90.

9 Ruetters M., Kim T.S., Krisam J., El-Sayed S., ElSayed N. Effect of endodontic treatment on periodontal healing of grade 3 endo-periodontal lesions without root damage in periodontally compromised patients-a retrospective pilot study. - Clin Oral Investig. - 2021; 25 (4): 2373-2380. PMID: 32948927

10 Шомуродова Г. Х., Юсупалиходжаева С. Х. Изменения метаболических маркеров ротовой жидкости и мочи при комплексном лечении эндодонто-пародонтальных заболеваний с применением лазера. Медицинский журнал узбекистана, 1(5), 7.

11 Betancourt P., Elgueta R., Fuentes R. Treatment of endo-periodontal lesion using leukocyte-platelet-

rich fibrin. A case report. Colomb Med (Cali). 2017; 48 (4): 204-207.PMID:29662262

12 Tal, H.; Kozlovsky, A.; Artzi, Z.; Nemcovsky, C. E.; Moses, O., Cross-linked and non-cross-linked collagen barrier membranes disintegrate following surgical exposure to the oral environment: a histological study in the cat. Clin Oral Implants Res 2008, 19, (8), 760-6

13 Майбородин И.В., Хоменюк С.В., Михеева Т.В. и др. Ускорение биодеградации полимера на основе коллагена после адсорбции мезенхимальных мультипотентных стромальных клеток в эксперименте. Трансляционная медицина. 2019;6(5):55–67

14 Gürbüz S, Doğan A, Karakeçili A, Toközlü B. In vivo behavior of a collagen-coated nano-hydroxyapatite enriched polycaprolactone membrane in rat mandibular defects. Ulus Travma Acil Cerrahi Derg 2023;29:1081-1090.