 DOI: 10.21608/ADJALEXU.2021.59378.1156

EFFECT OF COLLAGEN MEMBRANE ON HEALING OF ALVEOLAR BONE IN RATS WITH LIGATURE INDUCED PERIODONTITIS

Reem M. Salah[1]* *BDs*, Gehan M. Elba[2] *PhD*, Khadiga Y. Kawana [2] *PhD*.

ABSTRACT

INTRODUCTION: Alveolar process is thickened bone that encloses sockets of teeth in the jaws holding the teeth. Periodontitis is a prevalent condition in which the gums and deeper periodontal structures become inflamed. Inflammation can extend below gums and alongside teeth roots, initiating damage of supporting bone and periodontal ligament. Bioabsorbable (CM) collagen membranes with guided tissue regeneration (GTR) is usually performed to manage periodontal defects. Oral wounds are treated by of Resorbable collagen membranes (RCMs) xenogeneic or allogeneic origin. They are whether resorbable and non-resorbable to be easily used.

OBJECTIVE: Evaluating biological impact of collagen membrane on alveolar bone healing with a ligature made periodontitis.

MATERIALS AND METHODS: Forty-five adult male albino rats were used , each of them weighing 200-250 grams (approximately six months of age) and they were divided into three equal groups: group A; (control group), group B; (induced periodontitis), and group C: (collagen membrane) at week twelve the animals were sacrificed, and mandibles were dissected and histological sections were prepared for histological and histomorphometric analysis.

RESULTS: After 12 weeks, specimens of group B showed alveolar bone loss, while in collagen membrane group, they indicated relative restoration of alveolar bone. Histomorphometric analysis results revealed that bone percentage per field in periodontitis group was lower than collagen membrane treated group.

CONCLUSION: Placement of collagen membrane accelerates alveolar bone healing and enhances bone formation in periodontal diseases.

KEYWORDS: Collagen membrane, Guided tissue regeneration, Wound healing, Periodontitis.

RUNNING TITLE: Collagen membrane in rats with induced periodontitis.

1 Demonstrator of Oral Biology, Faculty of Dentistry, Pharos University, Alexandria, Egypt.
2 Professor of Oral Biology, Faculty of Dentistry, Alexandria University, Alexandria, Egypt.

** Corresponding Author:*
E-mail: Reem_mohamed_salah@hotmail.com

INTRODUCTION

Periodontium is the tissues that support the teeth, comprising four main components: gingiva, cementum of teeth roots, alveolar bone, and periodontal ligaments (PDLs), which are fibers tissue found between alveolar bone and cementum (1).

Bone of the alveolar process of the jaw containing teeth sockets, composed of outer cortical plate (buccal and lingual), a central spongiosa, and alveolar bone proper which covers the alveolus. Bones of the alveolar process come across alveolar crest (under cementoenamel junction level of tooth by1.5-2 millimeters) (2, 3).

Periodontitis is inflammatory disease affecting connective tissues around teeth. Periodontitis includes advanced damage of alveolar bone surrounding teeth, and without treatment, it can develop loosening and later on teeth loss (4).

Bioabsorbable collagen membranes (CM) with guided tissue regeneration (GTR) are typically performed to manage periodontal defects. Resorbable collagen membranes (RCMs) contain xenogeneic or allogeneic sources for treating extraction of sockets, sinus-lift procedures and wounds in the oral cavity, also in endodontic or periodontal operations. They are available as membranes resorbable and non-resorbable for ease of use, and the first is better recommended (5).

In medical field and dentistry, collagen materials have been used due to their high biocompatibility and ability to cure wounds. For GTR procedures, CM is equivalent to non-absorbable membranes in terms of clinical attachment advances, examining depth reduction, and bone fill percentage. Even though those membranes are absorbable, CM can avoid epithelial down growth alongside root surfaces throughout early stage of wound curing (6).

The null hypothesis in this study is that collagen membrane has no healing effect in the bone of the alveolar process in rats with ligature induce periodontitis. The aim of the present study is to investigate the biological effect of collagen membrane on healing of alveolar bone with ligature induced periodontitis in rats using light microscope and histomorphometric analysis.

MATERIALS AND METHODS

This work was performed with ethical approval for animal research by the Faculty of Dentistry at Alexandria University.

Forty-five Sprague–Dawley adult male rats of 6 months age and with weigh range from (200-250 grams) were involved in this study. Animals were obtained from the animal house of Faculty of Dentistry, Alexandria University. They were

maintained in the animal house under similar nutritional and environmental settings.

Rats were randomly divided into three equal groups, (15 rats in each group)

Group A rats : Control group.

Group B rats: Ligature induced periodontitis.

Group C rats: Ligature induced periodontitis with collagen membrane settlement

Induced periodontitis preparation (7)

Animals of B & C groups were subjected to ligature placement under general anaesthesia using a mixture of ketamine and xylazine anesthesia (Nikon Instruments Inc., NY, USA), and a 4-0 silk ligature (Roboz Surgical Instrument Co., MD, USA) was secured at gingival sulcus level of mandibular right first molar (M1) of all animals. After 14 days of silk ligature removal, periodontitis signs were evaluated clinically through bleeding and gingival inflammation. The gingival tissues became swollen, formed pocket, followed by debris accumulation and ulceration.

Placement of collagen membrane (8, 9)

1- Incision design: Vertical incision was connected to the midcrestal incision.
2- Elevation of a full-thickness flap and allowing access to the buccal side of bone.
3- Placement of (Osteo-Biol type) small pieces of collagen membrane with a width equivalent to the mesiodistal width of the rat first molar was cut from the original membrane sheet (20×20), placed and adapted to the tissue, and the flap was then repositioned to its original site.
4- Membrane was protected, tighten up, and knot was placed inside flap with membrane stabilizing sutures 4-0.
5- Flap was adjusted for tension-free primary closure.
6- Final closure of flap after horizontal mattress suture was placed.
7- Rats were given antibiotic (amoxicillin/clavulanic acid) (brand name: clavamox) 6.25 mg/lb -15 cc/lb for 10 to 14 days and nonsteroidal anti-inflammatory drug (NSAID) diclofenac or nitrofenac at 12-hour intervals.

Scarification

- The animals were subjected to euthanization using thiopental 20 mg/kg (0.5 g Thiopentax, Cristaília, Saõ Paulo) (10).
- Animals were sacrificed after 12 weeks from ligature removal. Each rat's mandible was dissected, followed by separation from muscles as well as soft tissues, maintaining the attached gingiva intact with bone. Only mandibular molar teeth segment with surrounding alveolar bone was ready for histological evaluation.

Method for histological examination (11)

- After specimens preparation, they were amenable to examination using a light microscope. Segments of molar area of specimens were embedded in neutral buffered formalin (10%). After biopsies fixation, a series of treatments were performed, including washing, decalcification, dehydration, clearance, infiltration, fixing in paraffin wax, cutting into 5 µm thick sections, mounting and stains (hematoxylin and eosin) preparation.

Histomorphometric analysis (12)

Morphometric evaluation of surface area percentage of the formed alveolar bone was carried out. The surface area value was assessed in different groups using (Image J 1.46) software.

Steps of measuring the percentage of surface area of the formed bone

- The three sections obtained from different standardized depths from each block were used to quantify each sample.
- Three photographs were taken from each section using the same magnification power.
- Extending from the apical boundary to coronal edges of alveolar bone.
- A rectangle with identical measurements was drawn on chosen standardized areas to be evaluated via image J program.
- A surface area of carefully chosen area was evaluated via choosing region of interest manager (ROI), obtained by summation of total surface areas.
- This surface area filled with marrow spaces and other tissue spaces were selected using the wand tracing tool, and dimension was documented.
- Two verified dimensions were withdrawn to get surface area filled with bone only, and its ratio to whole area selected was measured.
- The dimensions from three photographs were noted, and their means were estimated for each of the three sections obtained from each specimen.

Statistical analysis of the data

- Quantitative data were computerized and analyzed using mean and standard deviation for normally distributed data using using IBM SPSS software package version 20.0 .
- For normally distributed data in our study, we used **F-test (ANOVA) test** for comparison between more than two population. Followed by **Post Hoc test** "by Tukey method" to detect the level of significant between each two groups. **One way analysis of variance (ANOVA)** was tested for comparison between more than two groups.

RESULTS

Results of Light Microscopic Examinationof H&E sections

Control rats group

Examination of H&E stained histological sections revealed normal architecture of alveolar bone. Regular bone surface facing the periodontal ligament, healthy and continuous PDL fibers were inserted in the alveolar bone. Normal thickness of bony trabeculae with normal size, number and distribution of osteocytes were seen, and well-defined resting lines with osteoblasts lining narrow bone marrow cavity could be observed, **Figure (1)**.

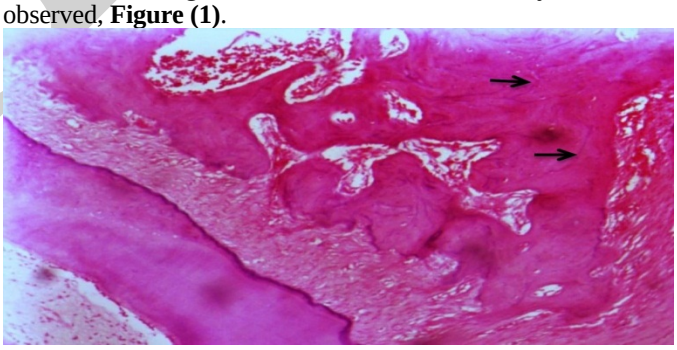

Figure (1): Light photomicrograph of interdental bone (control group)of rats showing regular bone surface lined by osteoblasts, dense bone trabeculae. Note: parallel deeply stained resting lines (arrows) with well-organized periodontal ligament fibers attached to both bone and cementum. (H&E X100).

periodontitis rats group

Microscopic examination of this group showed destruction of alveolar bone and further extensive damage in bone height with a detachment of PDL fibers, wide bone marrow spaces associated with fatty tissues infiltration, and irregular resorbed alveolar bone surface. Empty osteocytes and osteocytes with pyknotic nucleus were also observed, **Figure (2,3)**.

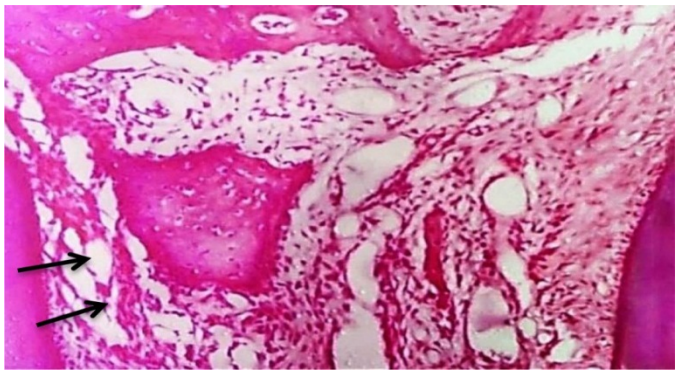

Figure (2): Light photomicrograph of interdental bone in periodontitis group (group B) of rats showing irregular alveolar bone resorption, wide bone marrow spaces and areas of detachment of PDL fiber (arrows), inflammatory cell infiltration. (H&E x100)

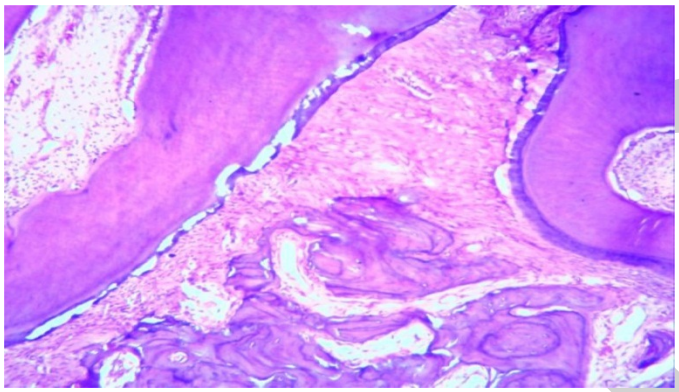

Figure (3): Light photomicrograph of interdental bone in periodontitis group (group B) of rats showing shifted crest apically, destruction of the alveolar bone, empty osteocytes and reversal line. (H&E X100)

Collagen membrane rats group

The result was almost similar to the control group. The group showed dense bone trabeculae with relatively regular and smooth boundary, normal size and distribution of osteocytes. Well-defined resting line, normal orientation of PDL fibers could be detected, **Figure (4,5)**.

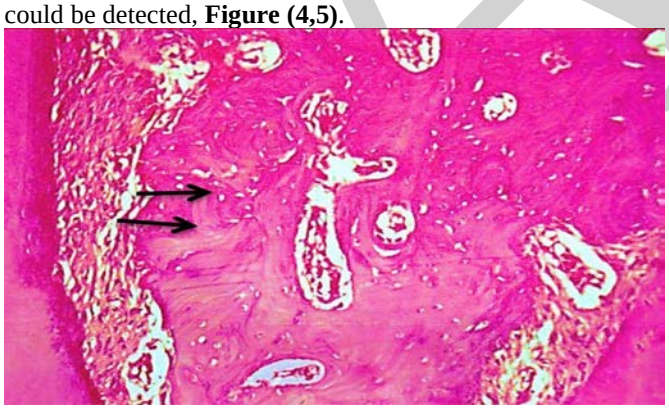

Figure (4): Light photomicrograph of interdental bone of collagen group (group c) of rats showing regular bone surface lined by osteoblasts. Dense bone trabeculae comprising well-vascularized bone marrow spaces continues well oriented periodontal ligament fibers with remodeling resting lines (arrows). (H&E X100)

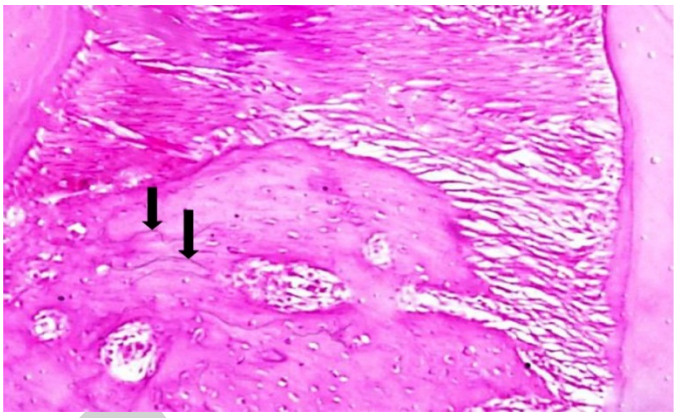

Figure (5): Light photomicrograph of interdental bone of collagen group (group c) of rats showing a relatively regular bone surface facing the periodontal ligament with parallel resting lines (arrows), normal size and distribution of osteocytes. (H&EX100)

Results of Histomorphometric Analysis

Comparing the three studied groups (control group A baseline, periodontitis group B and collagen membrane treated group C) regarding percentages of bone per field was conducted. The percentage in group B (rats with periodontitis) was lower than in group A (control rats) and group C. On the other hand, there is no statistical significance between group A and group C. Moreover, the difference between group C and group B was significant, Figure (6).

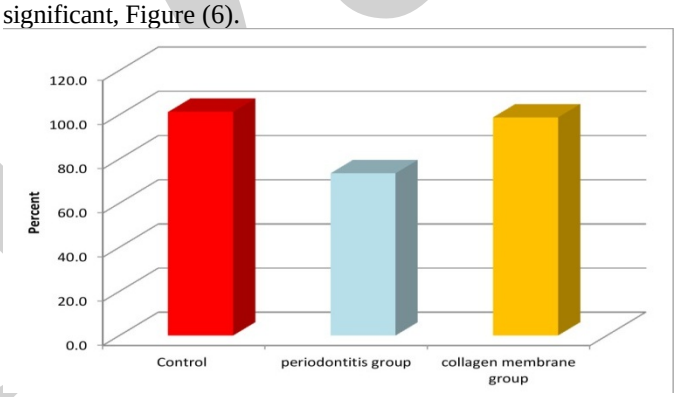

Figure (6): Comparison between three studied groups of rats regarding mean percent of bone formation.

DISCUSSION

Periodontal disease refers to any disease of connective tissues around teeth, involving diseases of supporting structures and gingival diseases. Periodontal diseases are a set of oral inflammatory diseases influenced by host–response factors. Two focal forms of periodontal disease are gingivitis, which involves only gums, and periodontitis, which showed apical migration of periodontal ligament attachment, destruction of connective tissue and alveolar bone, which preserve the teeth (8, 13).

Periodontitis healing was a main task that was considered clinically, traditionally, and experimentally pointing to assist this phenomenon positively and confirming it by variable methods, such as biomechanical measurements, surgical and influences of many factors and medications on healing of periodontal pockets were noted as well (14, 15).

On the other hand, last 30 years have realized advance of materials involved in regenerating periodontal tissues after guided tissue regeneration and periodontal disease, and more usage of

those materials in bone regeneration in recent times guided bone regeneration. Such materials involved membranes, bone grafts, growth factors and cell-based therapies (16).

The current study pointed to histologically assessment of alveolar bone healing with collagen membrane therapy in ligature-induced periodontitis in rats.

Animal models were involved in studying pathologic processes of infectious diseases such as periodontitis. periodontitis induced by ligature technique has been tested in primates, dogs, and rats to study factors affecting periodontitis severity (10). In most cases, as rats or hamsters, are appropriate to be used in histological evaluation of the role of micro-organisms, diet, or further factors in periodontal inflammation, given that appropriate statistical importance and preclinical relevance. In this study, rats were used as experimental animal models to assist collagen membrane role in periodontitis healing (7).

In the year **2019, Cho et al.** stated their results which are similar to the current study findings. The histological results of the current study for control group revealed normal structure and architecture of alveolar bone, characterized by a regular bone surface lined by osteoblasts, incremental lines, and Volkmann canals (17).

Cancellous bone showed normal thickness of bony trabeculae with normal cellular and well-vascularized bone marrow, in addition to normal size and distribution of osteocytes, with healthy continuous PDL fibers inserted in alveolar bone. In **2018**, **Novince et al**. mentioned the results of their study which is in agreement with the current study results (18). Also in **2019**, **Sanz-Requena et al.**, they studied the normal structure of alveolar bone in rats and concluded Similar findings (19).

Compared to control group after 12 weeks from ligature removal, the periodontitis group results showed destruction of alveolar bone, loss of its height, shifting of the crest of alveolar bone and impaired new bone formation. In **2016**, **Goudouri et al.** reported that experimental induced periodontitis by ligature significantly increased bone resorption compared to the group A and this is in accordance with our findings. They also stated that periodontitis increased the levels of IL-1 and intercellular adhesion molecule I (ICAM-1), which is participate in migration of leucocytes to the tissue (20).

The result also showed a significant infiltration of inflammatory cells. In **2018**, **Gürsoy et al**. supported our results , as they studied the effect of molecular forms and fragments of salivary MMP-8 on periodontitis healing and found that Gram-positive bacteria and lactobacilli had been isolated at five weeks of age from the oral cavity (21).

In contrast, the collagen membrane application herein inhibited alveolar bone loss and restored its normal original architecture in comparison of control rats in group A . The alveolar bone surface showed a continuous layer of osteoblasts. Several resting lines, as well as reversal lines, indicate bone remodeling. In **2020, Elgali et al.** were agreed about such findings (9,22,23).

Elgali reported that applying a membrane mediates forming bone in underlying weakness. Conversely, GBR studies usually highlighted the traditional histological analysis of new bone formed in membrane-treated defects. Conventionally the suggested elucidation depends on the passive barrier acting of the membrane for soft-tissue infiltration, instead of directly promoting sequences of biological processes, resulting in regenerating bone and defect filling, remodeled bone (9).

During application of experimental GBR technique , the membrane in a rat defect enriched an earlier and greater level of cbf-1/Runx2-positive osteoprogenitor cells and high expression of bone-formation marker (osteocalcin, calcitonin receptor, cathepsin K and RANKL) in underlying treated defect compared with untreated defect. Analogous results were mentioned in a periodontal bone defect in human during a GTR procedure. The membrane existence caused high stimulation of expression of many bone-formation-related genes, such as osteopontin, alkaline phosphatase (ALP), and bone sialoprotein, in primary defect in comparsion with non-treated defects (24).

CONCLUSION

We conclude from this work that collagen membrane placement accelerates alveolar bone healing in rats with induced periodontitis. Besides, it enhances osteoblastic activity and new bone formation. As a consequence, it can be employed as adjunct treatment in addition to traditional periodontitis treatment.

Conflict of Interest

Not found in our study

Funding

This is non fundable work.

REFERENCES

1. Washio K, Tsutsumi Y, Tsumanuma Y, Yano K, Srithanyarat SS, Takagi R, et al. In vivo periodontium formation around titanium implants using periodontal ligament cell sheet. Tissue Eng Part A. 2018;24:1273-82.
2. Kishor C, Mishra RR, Saraf SK, Kumar M, Srivastav AK, Nath G. Phage therapy of staphylococcal chronic osteomyelitis in experimental animal model. Indian J Med Res. 2016;143:87-94.
3. Florencio-Silva R, Sasso GR, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of bone tissue: structure, function, and factors that influence bone cells. Biomed Res Int. 2015;2015:421746.
4. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015;15:30-44.
5. Stoecklin-Wasmer C, Rutjes AW, da Costa BR, Salvi GE, Jüni P, Sculean A. Absorbable collagen membranes for periodontal regeneration: a systematic review. J Dent Res. 2013;92:773-81.
6. Bunyaratavej P, Wang HL. Collagen membranes: a review. J Periodontol. 2001;72:215-29.
7. Longo M, Gouveia Garcia V, Ervolino E, Ferro Alves ML, Duque C, Wainwright M, et al. Multiple aPDT sessions on periodontitis in rats treated with chemotherapy: histomorphometrical, immunohistochemical, immunological and microbiological analyses. Photodiagnosis Photodyn Ther. 2019;25:92-102.
8. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol. 2018;89:S159-72.

9. Elgali I, Omar O, Dahlin C, Thomsen P. Guided bone regeneration: materials and biological mechanisms revisited. Eur J Oral Sci. 2017;125:315-37.
10. O'Boyle C, Haley MJ, Lemarchand E, Smith CJ, Allan SM, Konkel JE, et al. Ligature-induced periodontitis induces systemic inflammation but does not alter acute outcome after stroke in mice. Int J Stroke. 2020;15:175-87.
11. Feldman AT, Wolfe D. Tissue processing and hematoxylin and eosin staining. Methods Mol Biol. 2014;1180:31-43.
12. Compston J, Skingle L, Dempster DW. Bone histomorphometry. In: Feldman D, Pike JW, Bouillon R (eds). 4th ed. Vitamin D. London, United Kingdom: Academic Press; 2018. pp. 959-73.
13. Manji F, Dahlen G, Fejerskov O. Caries and periodontitis: contesting the conventional wisdom on their aetiology. Caries Res. 2018;52:548-64.
14. Slots J. Periodontitis: facts, fallacies and the future. Periodontol 2000. 2017;75:7-23.
15. Kissa J, Chemlali S, El Houari B, Amine K, Khlil N, Mikou S, et al. Aggressive and chronic periodontitis in a population of Moroccan school students. J Clin Periodontol. 2016;43:934-9.
16. Liang Y, Luan X, Liu X. Recent advances in periodontal regeneration: A biomaterial perspective. Bioact Mater. 2020;5:297-308.
17. Cho HJ, Jeon JY, Ahn SJ, Lee SW, Chung JR, Park CJ, et al. The preliminary study for three-dimensional alveolar bone morphologic characteristics for alveolar bone restoration. Maxillofac Plast Reconstr Surg. 2019;41:33.
18. Novince CM, Kirkwood KL. Alveolar Bone Homeostasis in Health and Disease. In: Bilezikian JP (ed). Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 9th ed. Ch 121. Hoboken: Wiley Blackwell; 2018. pp. 933-40.
19. Sanz-Requena R, Ten Esteve A, Hervás Briz V, García-Martí G, Beltrán M, Martí-Bonmatí L. Quantitative structural analysis of trabecular alveolar bone in the mandible by multidetector computed tomography: differences according to tooth presence and type. Radiologia. 2019;61:225-33.
20. Goudouri OM, Vogel C, Grünewald A, Detsch R, Kontonasaki E, Boccaccini AR. Sol–gel processing of novel bioactive Mg-containing silicate scaffolds for alveolar bone regeneration. J Biomater Appl. 2016;30:740-9.
21. Gürsoy U, Kharzeev D, Marcus E, Rajagopal K, Shen C. Charge-dependent flow induced by magnetic and electric fields in heavy ion collisions. Phys Rev C. 2018;98:055201.
22. Ataol M, Kılınç A. Effects of collagen membrane on bone level and periodontal status of adjacent tooth after third molar surgery. 2020. Available at: https://assets.researchsquare.com/files/rs-19864/v1/64994c08-1c98-459a-9767-09b182e9cd8f.pdf
23. Liang Y, Luan X, Liu X. Recent advances in periodontal regeneration: A biomaterial perspective. Bioact Mater. 2020;5:297-308.
24. Omar O, Elgali I, Dahlin C, Thomsen P. Barrier membranes: More than the barrier effect?. J Clin Periodontol. 2019;46:103-23.