

Evaluation and Comparison of Human Chorionic Amniotic Membrane and Platelet-Rich Fibrin in Achieving Bone Formation and Soft Tissue Healing in Extraction Sockets Indicated for Rehabilitation with Implants: A Preliminary Study

Ratima Chopra, MDS¹/Kalyani Bhate, MDS¹/Nishtha Gadkari, MDS¹/Pradnya Kakodkar²/Deepak Kulkarni²

Purpose: To evaluate and compare human chorionic amniotic membrane and platelet-rich fibrin on new bone formation and soft tissue healing in extraction sockets indicated for rehabilitation with dental implants. **Materials and Methods:** A prospective, triple blind clinical study was conducted. The inclusion criteria were as follows: patient with two extraction sites each in the same arch, intact buccal bone and soft tissue around the socket, and recommended rehabilitation with dental implants. Postextraction, the sockets were randomly placed with human chorionic amniotic membrane in one site and platelet-rich fibrin in the other site. After 3 months, a trephine drill was used to take a biopsy of the respective sites for soft and hard tissue samples. The outcome parameters that were assessed histologically were percentage of new bone formation and lymphocyte density. **Results:** After screening 80 patients, eight participants were recruited for the study. The mean percentage of new bone formation in the human chorionic amniotic membrane group was 45.71% ± 4.82%, and for the plasma-rich fibrin group, it was 41.39% ± 6.29%, showing no statistically significant difference ($z = 0.99, P = .31$). In the human chorionic amniotic membrane group, six out of eight sites had mild lymphocyte density, while the plasma-rich fibrin group had equal numbers of mild and moderate lymphocyte density. No statistically significant difference between the groups (Fischer test value = 0.60, $P = .25$) was noted. **Conclusion:** Within the limitations of the study, the results showed that there is no difference in the efficiency of human chorionic amniotic membrane compared with platelet-rich fibrin in achieving new bone formation and soft tissue healing in the extraction socket. *Int J Oral Maxillofac Implants 2021;36:341–345. doi: 10.11607/jomi.8344*

Keywords: dental implants, extraction sockets, human chorionic amniotic membrane, platelet-rich fibrin

During the first 6 months subsequent to tooth extraction, the socket undergoes bone remodeling as a part of the healing process. Remodeling changes include loss of bone volume up to 30% to 60% and a mean reduction in the crestal height (1.5 mm) and width (3.8 mm) of the alveolar ridge.¹

Bone formation in the extraction socket proceeds from the apex to the ridge along a dense network of collagen bundles, whereas the overlying epithelium is proliferating apically and regenerating at a rate faster than the bone. This results in minimal space for bone

regeneration and leads to a concave bony defect in the healing socket.

Rehabilitating the edentulous space for function or esthetics with dental implants requires good alveolar height and width for stability and osseointegration, while loss of alveolar ridge width and height postextraction proves to be an undesirable outcome.^{2,3}

The principles of guided tissue regeneration are applicable to socket healing. Wound isolation by means of an occlusive membrane prevents invagination of the oral epithelium into the healing socket. This provides undisturbed healing within the extraction socket and a greater amount of bone fill over a period of time.^{4,5} Thus, a barrier membrane can act as a mechanical separator, preventing the undesirable excessive soft tissue growth inside the socket and loss of alveolar ridge, as previously described.

For this purpose, the barrier membrane should have sufficient mechanical strength to prevent micromovements between the soft tissue and the bone during mastication, should be biocompatible, should be easy to handle, and have an extended shelf life. The available

¹Department of Oral & Maxillofacial Surgery, Dr D. Y. Patil Vidyapeeth, Pimpri, Pune, India.

²Dr D. Y. Patil Dental College & Hospital, Pimpri, Pune, India.

Correspondence to: Dr Ratima Chopra, Department of Oral & Maxillofacial Surgery, Dr. D. Y. Patil Vidyapeeth, Pimpri Pune-411018, India. Email: ratimachopra@gmail.com

Submitted February 13, 2020; accepted November 9, 2020.

©2021 by Quintessence Publishing Co Inc.

membranes can be classified as resorbable; nonresorbable; and first, second, and third generation.⁶ The popular second-generation barrier membranes with growth factors are platelet-rich fibrin and human chorionic amniotic membrane.⁶

Platelet-rich fibrin is a platelet concentrate made up of platelets and leukocytes in a complex fibrin matrix, which is autologous in nature. Platelets contain growth factors that play a role in hard and soft tissue repair.¹ However, it is not an occlusive membrane, but it serves as a resorbable membrane in guided bone regeneration and guided tissue regeneration.⁷ A socket with platelet-rich fibrin membrane shows rapid soft tissue healing, less crestal bone loss, and no associated foreign body reaction that adversely affects the amount of bone formation.⁸

However, platelet-rich fibrin poses some disadvantages as follows: membrane preparation is technique sensitive and requires a glass-coated tube to achieve clot polymerization; success of the graft depends mainly on blood collection time and its handling; and possibly refusal of treatment by patients because of the need to puncture veins.⁹

Due to this limitation, other membrane options need to be explored. A fetal allograft comprised of amnion and chorion,¹⁰ called the human chorionic amniotic membrane, can be worth comparing.

One of the general methods for obtaining fresh human chorionic amniotic membrane is deriving it from the human placenta following a caesarian section after obtaining informed consent from the patient (tested seronegative for syphilis, HIV, human T-cell lymphotropic virus type 1, and hepatitis B and C viruses).¹¹ For the preparation procedure, the collected amnions are washed with sterile phosphate-buffered saline without removing the epithelial cell layers and then dried under consecutive far-infrared rays and microwaves at temperatures lower than 60°C using a hyperdrying device. Thereafter, the amniotic membranes are cut into adequate sizes and are vacuum-packaged. Further, the packages are irradiated with rays (25 kGy).¹¹

It has properties such as nonimmunogenicity, anti-inflammatory, promotion of angiogenesis, and epithelialization effective for wound healing.¹⁰ The best-known applications of human chorionic amniotic membrane are ocular surface reconstruction,¹² biomaterial in burn wound dressing,¹³ and tissue engineering.¹⁰ In dentistry, it has been used for furcation defects, infrabony defects and gingival recession coverage,¹⁴ vestibuloplasty,¹⁵ oral submucous fibrosis,¹⁶ and reconstruction after resection of oral cancerous or precancerous lesions.¹⁷ Further, it has been used widely as an adjuvant to bone grafts for alveolar ridge preservation, but the bone regenerative potential of this membrane remains unexplored. Hence, it would be interesting to evaluate the

osteogenic property and also compare it with platelet-rich fibrin as a replacement membrane.

The aim of the present preliminary study was to evaluate and compare human chorionic amniotic membrane against platelet-rich fibrin in achieving new bone formation and soft tissue healing in extraction sockets indicated for rehabilitation with dental implants.

The null hypothesis tested was that there would be no difference in the new bone formation and soft tissue healing in the extraction socket filled with human chorionic amniotic membrane and platelet-rich fibrin.

MATERIALS AND METHODS

This prospective, triple blind (patient, evaluator, and statistician were blinded) clinical study was conducted in the implant center of a dental institute after obtaining scientific and ethical committee clearance (DYPDCH/IEC/1262/11/18).

Patient Selection

The inclusion criteria for participant selection were as follows: patients older than 18 years of age requiring extraction of a minimum two teeth in the same arch (maxilla or mandible) with indication of rehabilitation with dental implants without the need for bone augmentation. Extraction sockets with intact buccal bone and soft tissue were included. Patients with any debilitating systemic diseases that would affect socket healing were excluded from the study. Apart from these, any patients who were contraindicated for the use of platelet-rich fibrin were excluded.

In total, up to 80 patients indicated for dental implant rehabilitation were scheduled for assessment, and final selection would be made as per the inclusion and exclusion criteria. Each participant would receive human chorionic amniotic membrane in one site and platelet-rich fibrin as the control in the other extraction site.

Membrane Procurement

The human chorionic amniotic membrane used in this study was commercially procured from Tata Memorial Hospital, Mumbai, India. It was 2 × 2 cm in dimensions. It was a translucent membrane that maintained its morphogenic property until it was wetted with saline. This membrane was sterilized by gamma irradiation.

Platelet-rich fibrin was freshly prepared at the source. Ten milliliters of the patient's venous blood was taken with a 20-gauge needle secured to a 10-mL syringe using a scalp vein of 20G (with anticoagulant).⁹ The whole blood was transferred to a 10-mL glass tube, which was centrifuged at 3,000 rotations per minute for 10 minutes in a REMI Machine (R-8C BL, "G" force 5070).⁹

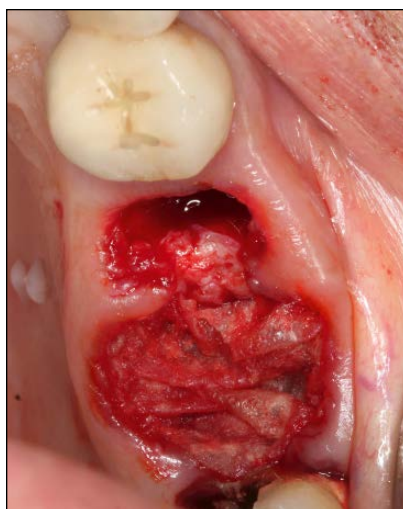


Fig 1 Placement of human chorionic amniotic membrane on first molar.

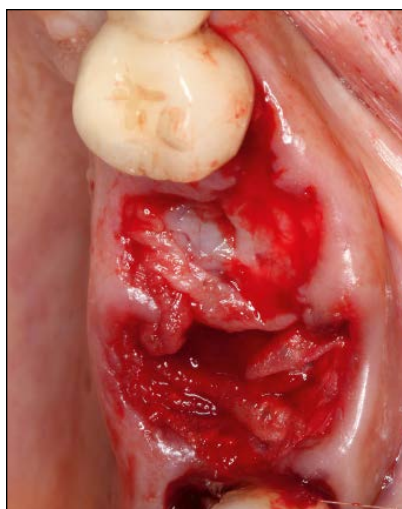


Fig 2 Placement of platelet-rich fibrin on the second premolar.

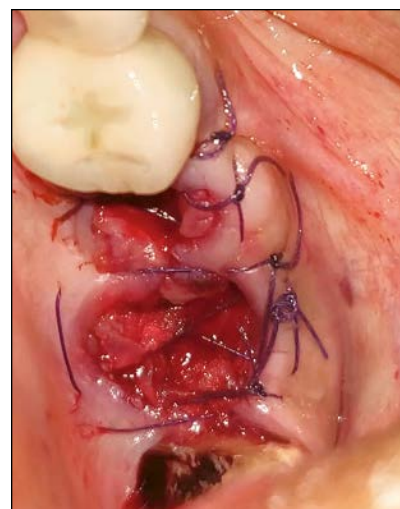


Fig 3 Closure.

The fibrin clot was obtained in the middle of the centrifuged sample. This platelet-rich fibrin was compressed with the help of a platelet-rich fibrin box to obtain the shape of a membrane.

Procedure

A detailed case history was taken. Preoperative routine blood investigations such as hemoglobin, random blood sugar level, bleeding time, and clotting time estimation were done. Radiographs such as CBCT and radiovisiography were taken, and study models were made. The patients were informed about the study protocol, and written informed consent was taken. All procedures in the study were performed by the same operator (R.C.). Extraction was done as atraumatically as possible under 2% lignocaine with 1:200,000 adrenaline. Careful examination of the buccal cortical bone was done to verify its integrity and ensure no perforations. The random allocation for placement of platelet-rich fibrin or human chorionic amniotic membrane in the respective site was decided by the Sequentially Numbered Opaque Sealed Envelope (SNOSE) technique. Human chorionic amniotic membrane (Fig 1) and platelet-rich fibrin (Fig 2) were adapted to the bone after reflection of soft tissue and secured with 4-0 Vicryl sutures (Fig 3) in hidden X suture fashion in the respective sites.

Patients were recalled to check the general healing of the wound after 24 hours. At the 3-month recall visit, a biopsy of the soft tissue and bone at the operated sites was taken with the help of a trephine drill of 3-mm diameter. Following the biopsy, the standard procedure of implant placement was performed.

Histologic Evaluation

The soft tissue was fixed with formalin for 3 days and later stained with hematoxylin and eosin. The outcome variable evaluated was lymphocyte density for soft tissue healing and was qualitatively evaluated as mild, moderate, or severe grade.¹⁸

The bone obtained in the trephine was decalcified using a decalcifying solution (5% nitric acid for 2 to 3 days) and was evaluated quantitatively for the percentage of new bone formation.

Both the soft and hard tissue samples were observed with a research microscope using an image analyzing system (Leica DM2500) and image analyzer software (QWin Plus). Each section was observed under five different fields at total magnification of 400 \times . Two blinded observers performed the evaluation to eliminate bias.

Statistical Analysis

Between-group comparison for lymphocyte density was done by Fisher Exact test, and for comparing the percentage of new bone formation, the Mann-Whitney *U* test was used. The level of significance was fixed at $P < .05$. The statistical analysis was performed using the SPSS 21.0 package.

RESULTS

Out of the 80 screened patients, only 8 patients (7 men and 1 woman, with a mean age of 40 ± 10.23 years) were selected as per the eligibility criteria. These patients underwent two extractions each in the maxilla. Sixteen sites from eight patients (left first and second

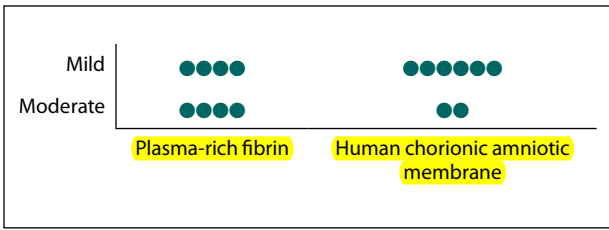


Fig 4 Dot plot showing the lymphocyte density.

Table 1 Percentage of New Bone Formation in Groups A and B as Observed Histologically

	Group A	Group B
	43.1	48.3
	48.01	41.07
	39.6	32.2
	38.2	48.2
	48.1	43.3
	49.09	44.02
	48.04	32.04
	51.6	42.04
Mean ± SD	*45.71 ± 4.82	*41.39 ± 6.29
*Z = 0.99, P = .31		

*Mann-Whitney U test calculation value.

premolar; left first and second premolar; right first premolar and left second premolar; right first and second molar; left first molar and second molar; right first molar and left second molar; left second premolar and first molar; and right second premolar and first molar) were included in the study.

Figure 4 shows the density of lymphocytes of individual patients in both groups. The density of lymphocytes in the human chorionic amniotic group was mild in the majority of the sites, while in the plasma-rich fibrin group, an equal number of sites were graded mild and moderate, respectively, and the intergroup difference was not statistically significant (Fischer exact test = 0.60, P = .25).

Table 1 shows the percentage of new bone formation. The mean percentage of bone formation in the human chorionic amniotic group was 45.71% ± 4.82%, and for the plasma-rich fibrin group, it was 41.39% ± 6.29%, and there was no statistical difference between them (z = 0.99, P = .31).

The agreement between the two examiners was assessed by kappa statistics, and the result was 92%, indicating a good accord between them.

DISCUSSION

The null hypothesis of the study was accepted. There was no difference in the soft tissue healing and new bone formation when human chorionic amniotic membrane was compared with platelet-rich fibrin. In the present study, the lymphocyte density was comparable between the groups. These results seem to demonstrate the characteristic involvement of acute inflammatory response mediated by T lymphocytes in the collagen membrane remodeling process¹⁸ and also that healing has been completed with graft placement before implant placement. Six out of eight sites showed mild lymphocytic reaction with human chorionic amniotic membrane, inferring that it is anti-inflammatory in nature. Studies by Hao et al¹⁹ and Buhimschi et al²⁰ demonstrated that the amniotic tissue cells expressed microbial peptides such as interleukin (IL)-10, tumor necrosis factor-alpha, and interferons, which are known to inhibit inflammation. However, the results are not statistically significant, and thus, it cannot be concluded that human chorionic amniotic membrane is better than platelet-rich fibrin in reducing inflammation.

A study by Zhang et al²¹ has shown increased bone formation with platelet-rich fibrin. In the present study, a majority of the sites had a larger amount of bone formation with human chorionic amniotic membrane, which confirms its osteogenic potential. However, compared with platelet-rich fibrin, the difference was not statistically significant.

The additional observations noted by the investigator in this study favor human chorionic amniotic membrane over platelet-rich fibrin. The former does not require a centrifugation machine for preparation, which is compulsory for platelet-rich fibrin; handling of human chorionic amniotic membrane was far more superior than platelet-rich fibrin; it was easier to suture human chorionic amniotic membrane than platelet-rich fibrin; the operator experienced that the human chorionic amniotic membrane adhered well to the socket, whereas platelet-rich fibrin was difficult to secure; and finally, patients with a fear of needles were uncomfortable with the process of venipuncture, but they were cooperative with human chorionic amniotic membrane. Clinically, it can be reported that human chorionic amniotic membrane is better than platelet-rich fibrin. Nevertheless, further confirmation is required with a larger sample size.

The sockets preserved in this study belonged only to the maxilla, and given the difference between the maxilla and mandible, a further study will be needed to evaluate these effects in the mandibular sockets. The increased vascular supply in the maxilla makes the healing process faster compared with the mandible, while the maxilla exhibits a faster and increased amount of resorption compared with the mandible.²²

This is a preliminary study conducted on a small sample size, and the results may be underpowered. However, it can be considered as baseline data for establishing a sample size for future large studies.

CONCLUSIONS

Despite the limitations of the study, the results showed that there is no difference in the efficiency of human chorionic amniotic membrane compared with platelet-rich fibrin in achieving bone formation and soft tissue healing in extraction sockets indicated for rehabilitation with dental implants.

ACKNOWLEDGMENTS

The authors would like to show their gratitude to the department of Oral and Maxillofacial Surgery, Oral Pathology and Implant center at Dr D. Y. Patil Dental College & Hospital, Pune, India, for supporting them in their study. The authors reported no conflicts of interest related to this study.

REFERENCES

1. Van der Weijden F, Dell'Acqua F, Slot DE. Alveolar bone dimensional changes of post-extraction sockets in humans: A systematic review. *J Clin Periodontol* 2009;36:1048–1058.
2. Amler MH. The time sequence of tissue regeneration in human extraction wounds. *Oral Surg Oral Med Oral Pathol* 1969;27:309–318.
3. Amler MH. Age factor in human alveolar bone repair. *J Oral Implantol* 1993;19:138–142.
4. Rowe DJ, Leung WW, Del Carlo DL. Osteoclast inhibition by factors from cells associated with regenerative tissue. *J Periodontol* 1996;67:414–421.
5. Lekovic V, Kenney EB, Weinlaender M, et al. A bone regenerative approach to alveolar ridge maintenance following tooth extraction. Report of 10 cases. *J Periodontol* 1997;68:563–570.
6. Madhuri SV. Membranes for periodontal regeneration. *Int J Pharm Sci Invent* 2016;5:19–24.
7. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;101:e37–e44.
8. Hori J, Wang M, Kamiya K, Takahashi H, Sakuragawa N. Immunological characteristics of amniotic epithelium. *Cornea* 2006;25(suppl 1):s53–s58.
9. Borie E, Oliví DG, Orsi IA, et al. Platelet-rich fibrin application in dentistry: A literature review. *Int J Clin Exp Med* 2015;8:7922–7929.
10. Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian AM. Properties of amniotic membrane for potential use in tissue engineering. *Eur Cell Mater* 2008;15:88–99.
11. Arai N, Tsuno H, Okabe M, et al. Clinical application of a hyperdry amniotic membrane on surgical defects of the oral mucosa. *J Oral Maxillofac Surg* 2012;70:2221–2228.
12. de Rötth A. Plastic repair of conjunctival defects with fetal membranes. *Arch Ophthalmol* 1940;23:522.
13. Stern M. The grafting of preserved amniotic membrane to burned and ulcerated skin surfaces, substituting skin grafts. *JAMA* 1913;60:973–974.
14. Holtzclaw D, Toscano N. Amnion-chorion allograft barrier used for guided tissue regeneration treatment of periodontal intrabony defects: A retrospective observational report. *Clin Adv Perio* 2013;3:131–137.
15. Samandari MH, Yaghmaei M, Ejlali M, Moshref M, Saffar AS. Use of amnion as a graft material in vestibuloplasty: A preliminary report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;97:574–578.
16. Lai DR, Chen HR, Lin LM, Huang YL, Tsai CC. Clinical evaluation of different treatment methods for oral submucous fibrosis. A 10-year experience with 150 cases. *J Oral Pathol Med* 1995;24:402–406.
17. Saluja H, Dehane V, Mahindra U. Platelet-rich fibrin: A second generation platelet concentrate and a new friend of oral and maxillofacial surgeons. *Ann Maxillofac Surg* 2011;1:53–57.
18. Pellegrini G, Rasperini G, Obot G, Farronato G, Dellavia C. Soft tissue healing in alveolar socket preservation technique: Histologic evaluations. *Int J Periodontics Restorative Dent* 2014;34:531–539.
19. Hao Y, Ma DH, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea* 2000;19:348–352.
20. Buhimschi IA, Jabr M, Buhimschi CS, Petkova AP, Weiner CP, Saed GM. The novel antimicrobial peptide beta3-defensin is produced by the amnion: A possible role of the fetal membranes in innate immunity of the amniotic cavity. *Am J Obstet Gynecol* 2004;191:1678–1687.
21. Zhang Y, Ruan Z, Shen M, et al. Clinical effect of platelet-rich fibrin on the preservation of the alveolar ridge following tooth extraction. *Exp Ther Med* 2018;15:2277–2286.
22. Humphries S, Devlin H, Worthington H. A radiographic investigation into bone resorption of mandibular alveolar bone in elderly edentulous adults. *J Dent* 1989;17:94–96.