



Original Article

Platelet-Rich Plasma Application for Acute Tympanic Membrane Perforations

Selmin Karataylı Özgürsoy, M. Emin Tunçkaşık, Fatma Tunçkaşık, Egemen Akıncioğlu, Handan Doğan, Sinan Kocatürk

Department of Otolaryngology, Ufuk University School of Medicine, Ankara, Turkey (SKÖ, MET, FT, SK)

Department of Pathology, Ufuk University School of Medicine, Ankara, Turkey (EA, HD)

Cite this article as: Karataylı Özgürsoy S, Tunçkaşık ME, Tunçkaşık F, Akıncioğlu E, Doğan H, Kocatürk S. Platelet-Rich Plasma Application for Acute Tympanic Membrane Perforations. J Int Adv Otol 2017; 13: 195-9.

OBJECTIVE: To assess the efficacy of the application of platelet-rich plasma (PRP) in the healing process of acute tympanic membrane perforations (TMPs).

MATERIALS and METHODS: Acute TMPs were made in both the ears of 12 New Zealand rabbits. Plasma gel was applied at the right tympanic membrane (TM) of the same animal until the perforations were closed. The left TM was left untreated. On days 1, 4, 7, 10, 13, 16, 21, 28, and 35, the TMs were monitored to check the closure of perforations. The days of perforation closure for the 2 groups were compared using the paired t-test. The animals were sacrificed 2 months after making the perforations. Seven histopathological parameters were reviewed by 2 blinded pathologists: acute inflammation, chronic inflammation, edema in the lamina propria, congestion in the lamina propria, sclerosis, fibroblastic reaction, and an increase in the thickness of the squamous epithelial layer. The presence or absence of each histological parameter in both groups was compared using the Pearson Chi-square test.

RESULTS: The average number of days for closure in the plasma gel group was 12 (range 8–18 days) and that in the control group was 17.7 (range 8–31 days). The difference was statistically significant ($p=0.0145$). There was no sclerosis or fibroblastic reaction in any of the specimens. No statistically significant difference was seen between the 2 groups with respect to acute inflammation, chronic inflammation, edema in the lamina propria, congestion in the lamina propria, and an increase in thickness of the squamous epithelial layer ($p>0.05$).

CONCLUSION: Platelet-rich plasma fastens TMP closure; in long term, the eventual outcome is both microscopically and macroscopically same for the control as well as study groups in a rabbit traumatic TMP model. We believe that this study will encourage the clinical use of PRP for acute TMPs and trigger clinical studies in this field.

KEYWORDS: Tympanic membrane perforation, acute, trauma, platelet rich plasma

INTRODUCTION

Tympanic membrane perforations (TMPs) usually result from infection and trauma. Most acute TMPs heal within 7–10 days^[1]. Overall, 10%–20% of TMPs do not heal within 3 months and reach a clinically chronic state^[2,3]. The traditional method of repairing chronic TMPs is myringoplasty, in which TM is reconstructed using graft materials such as temporalis muscle fascia, cartilage, and fat tissue. For cost effectiveness, avoiding the risks associated with general anesthesia, limiting the intra-operative time, and preventing the requirement of care and sick leaves after surgery, simpler therapeutic strategies are being investigated^[2]. Various biomaterials and molecules are being studied for the evaluation of their unknown safety and efficacy profiles with respect to the healing process of tympanic membranes (TMs) and in animal models before clinical trials^[1,4]. In the present study, we aimed to assess the efficacy of the application of platelet-rich plasma (PRP) in the healing process of acute TMPs.

MATERIALS and METHODS

Animal Study

Twelve (3 females, 9 males) healthy New Zealand rabbits with an average weight of 3200 g were used in the study. All animals were treated in accordance with Principles of Laboratory Animal Care formulated by the American National Society for Medical Research.

Approval for conducting this study was obtained from the Ankara University Ethical Committee for Animal Studies (2015-2-24).

Medical conditions of the animals were monitored by the veterinary and laboratory technicians to avoid any systemic disease that could interfere with the results of our study. None of the animals had otitis media or externa during the experiment. The animals were anesthetized using xylazine (5 mg/kg i.m.) and ketamine (45 mg/kg i.m.), and carprofen (1.5 mg/kg) was subcutaneously applied as an analgesic. All the surgical procedures were conducted and photographed using a 0-degree endoscope (Storz totendoscope 0 degree, Storz flowlight source Tuttlingen, Germany), camera (Dr. Camscope DCSM 102 & 103E, Sometch, Korea), and recorder (MediCap USB 200, PA, USA). Right ears constituted the experimental group because the plasma gel of the same animals was applied to these ears; left ears were left untreated and constituted the control group.

Preparation of PRP

Under the effect of general anesthesia, 4 cc blood was drawn from the central ear artery of the rabbits into a sterile tube preloaded with 0.6 cc of anticoagulant citrate dextrose solution. The blood was centrifuged at 1500 rpm in a centrifugal chamber for 15 min to separate it into 3 layers: red blood cells at the bottom, white cells at the intermediate level, and plasma at the top. Upper white part was drawn to an injection syringe to readily apply on TMP.

Acute TMPs were made in both ears of all animals using a cone-shaped needle in the posteroinferior part of the pars tensa (Figure 1). Plasma gel was prepared from the blood of each rabbit on the day of perforation and applied to the right TM of the same animal (Figure 2). The left TM was left untreated. On days 1, 4, 7, 10, 13, 16, 21, 28, and 35, the TMs were monitored to check the closure of the perforations. Plasma gel obtained from the same animal was applied to its right TM on every observation day until the perforations were closed, as observed using an endoscope. The left TM was left untreated and was observed using an endoscope on the same days until no perforation was observed. The results were expressed with respect to whether the perforations were closed or not. Because the animals were not monitored daily, perforation closure observed on a particular observation day was assumed to have occurred at the middle of the time interval from the previous observation day. The days of perforation closure for 2 groups were compared using the paired t-test (SPSS, IBM, NYC, USA).

The animals were sacrificed 2 months after making the perforation; their skulls were cut into half and fixed using 10% formaldehyde solution for approximately 1 week. Before decalcification, each bone was reduced until only the mastoid bone, external auditory canal, tympanic membrane, middle ear cavity, and petrous apex remained (Figure 3). The minimized specimens were decalcified using TBD-1 Rapid Decalcifier (Shandon, Thermo Fisher Scientific Inc. NYSE:TMO, Waltham, MA, USA) solution for approximately 1 week. After decalcification, the tympanic membranes were dissected out as a whole. On macroscopic examination, all tympanic membranes were intact. Specimens were washed under running tap water for half an hour. After routine tissue processing (Leica ASP300S, Wetzlar, Germany), the tissue samples were embedded in paraffin (Leica EG1150H, Wetzlar, Germany), cut into slices of 5- μ m thickness (Leica RM2245, Wetzlar, Germany), and stained using hematoxylin-eosin (Leica Autostainer XL, Wetzlar, Germany).



Figure 1. Tympanic membrane perforations in the posteroinferior part of the pars tensa.



Figure 2. Application of platelet-rich plasma.

Two pathologists, blinded to the nature of the specimens, performed the histological analysis. Seven histopathological parameters were reviewed: acute inflammation, chronic inflammation, edema in the lamina propria, congestion in the lamina propria, sclerosis, fibroblastic reaction, and an increase in the thickness of squamous epithelial layer. Category of microscopic parameters were dichotomized as present ("1") or absent ("0") in statistical analyses. The presence or absence of each histological parameter in both groups was compared using the Pearson Chi-square test (SPSS, IBM, NYC, USA).



Figure 3. Inside view of a half skull reduced until only the mastoid bone, external auditory canal, tympanic membrane, middle ear cavity, and petrous apex remained.

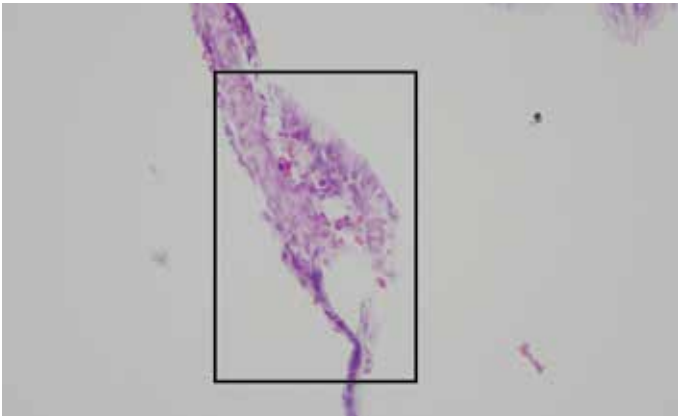


Figure 4. Histopathological view of a right tympanic membrane stained using hematoxylin-eosin under $\times 400$ magnification: the healed (thicker) part is marked with a rectangle.

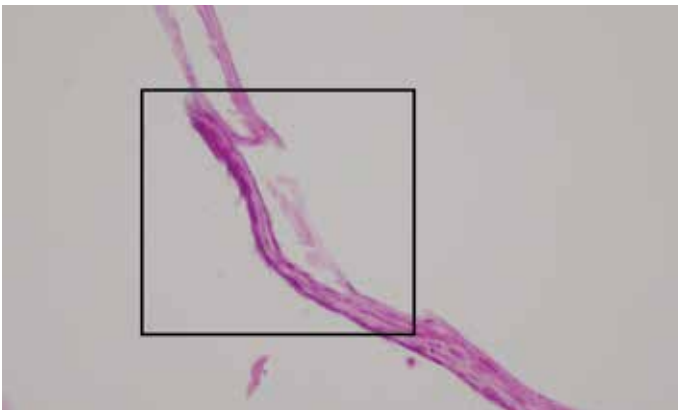


Figure 5. Histopathological view of a left tympanic membrane stained using hematoxylin-eosin under $\times 400$ magnification: the healed (thicker) part is marked with a rectangle.

Table 1. Days required for the closure of tympanic membrane perforations in each group

Animal code	Right ear (plasma gel treated)	Left ear (control)
1	18	18
2	11	24
3	8	11
4	18	31
5	8	24
6	11	11
7	11	14
8	11	11
9	11	8
10	18	31
11	8	18
12	11	11

RESULTS

None of the ears showed reperforation or otorrhea; thus, we did not have to exclude any of the animals from the study. The number of days required for TM closure in each ear is provided in Table 1. The average number of days of closure for the plasma gel group was 12 (range 8–18 days) and that for the control group was 17.7 (range 8–31 days). The difference was statistically significant ($p=0.0145$).

Based on the findings of the histopathological examination, there was no sclerosis or fibroblastic reaction in any of the specimens. When the 2 groups were compared for other 5 parameters, no statistically significant difference was observed with respect to acute inflammation, chronic inflammation, edema in the lamina propria, congestion in the lamina propria, and an increase in thickness of the squamous epithelial layer ($p>0.05$). Histological views of the healed TMs belonging to each group have been provided as Figures 4 (right ear) and 5 (left ear).

DISCUSSION

Although acute TMPs are reported to heal within 7–10 days^[1], 10%–20% of the patients have persistent TMPs that become chronic. The mechanism underlying the presence of persistent perforations is unknown. Numerous factors are considered to impede the spontaneous healing mechanism of acute TMP. In their review, Wang et al.^[4] classified the hypotheses into structural, histological, infectious, and growth-related mechanisms. In this context, the prevention of an acute TMP from becoming chronic is at least as important as trying to cure chronic TMPs to avoid the risks associated with general anesthesia, for cost effectiveness, and for limiting the time required for surgery.

Major criticisms regarding the use of acute TMP models in animal studies are as follows: TMPs mostly heal spontaneously and acute TMPs are not analogous to chronic TMPs, wherein acute repair mechanisms are deactivated^[5]. However, not all acute TMPs heal spontaneously, and today, there are no specific symptoms for a clinician to predict as to which TMP will heal with no intervention and which will

not. Thus, the authors believe that there is still a need to explore biomaterials that will help acute TMPs heal faster.

In the last decade, there have been investigations on new materials such as gelfoam patch, EGF, PGF, TGFb1, PDGF, autcollagen, egg shell membrane, estrogen, and plasminogen in human and animal studies for both acute and chronic TMPs [2, 6-11]. Although the efficacy of PRP has been investigated in wound healing [12-15], its effects on TMP healing have not yet been investigated in acute TMPs. In the literature, the only study on the effect of PRP on TMP closure is that reported by El-Anwar et al. [16], wherein they studied 64 patients with chronic TMPs who had undergone postauricular myringoplasty with underlay graft using conchal perichondrial graft. They reported that postoperatively at 6 months, the success rate (graft taking) in the case group with the use of autologous PRP (100%) was significantly higher than that in the control group without the use of autologous PRP (81.25%).

Platelet-rich plasma is a concentration of autologous platelets which release polypeptides, including PDGF, TGFb1, VEGF, and EGF, each of which have different roles in wound healing, immune reactions, angiogenesis [12], and bone regeneration [17, 18]. There have been human studies to report that PRP has a positive effect on wound healing [12-15]. In animal studies, histological examinations revealed enhanced fibroblastic and endothelial cell formation during wound healing, increased neovascularization [12, 19-22], and enhanced granulation tissue formation [12, 19, 21, 22].

The role of various immunological molecules on TMP closure has been investigated. The effect of local TGFb1 application has been studied on acute TMP models in rats, and repeated application of TGFb1 seems to accelerate the healing process of TMPs [7]. Early immune reaction after acute TMP was reported in a study on rats [23], wherein T cells were demonstrated to peak on day 3 and B cells on day 6 after the perforation. It was concluded that products inducing the healing process secreted by these cells should be identified to promote the closure of TMPs. Because PRP contains many growth factors with different roles in the immune mechanism, our results to show statistically faster closure of acute TMPs with local PRP application, supporting the existing knowledge.

Regarding our histopathological examination, the reason for not observing any significant difference in any of the parameters between the 2 groups could be the time at which we sacrificed the animals. It has been shown in the literature that healing process in TMP is complete by day 14 in rat models [24], and most of the histological studies on TMPs in animal models in the literature are limited to a 14-day follow-up [24, 25]. Shen et al. [2] showed that in 9 days, a single local injection of plasminogen resulted in TMP closure with a continuous but rather thick outer keratinocyte layer; however, three plasminogen injections led to a completely healed TM with a thin keratinizing squamous epithelium covering a connective tissue layer. We observed that in long term (2 months), there is no significant difference in scar indicators such as the thickness of TM and fibrosis between control and experimental groups.

Thus, PRP fastens TMP closure in ways we do not exactly know yet; however, in long term, eventual outcomes are both microscopically and macroscopically same in the study and control groups using a

rabbit traumatic TMP model. Clinical studies are needed to evaluate if PRP increases the closure rate of acute TMPs with various etiologies under variable underlying conditions.

In the present study, we demonstrated that PRP is an effective autologous material for the healing process of acute TMPs in a rabbit model. In particular, in acute TMPs such as trauma, local PRP application on TMP may be a rapid, easy, cheap, noninvasive, and safe procedure that can be repeatedly applied in outpatient settings. We believe that this study will encourage the clinical use of PRP in acute TMPs and trigger clinical studies in this field.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ankara University Ethical Committee for Animal Studies (2015-2-24).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.K.Ö.; Design - S.K.Ö., M.E.T., F.T.; Supervision - S.K.Ö., S.K.; Resources - S.K.Ö., S.K., F.T., M.E.T.; Materials - H.D., E.A., S.K.Ö.; Data Collection and/or Processing - H.D., E.A., S.K.Ö.; Analysis and/or Interpretation - H.D., E.A., S.K.Ö.; Literature Search - S.K.Ö., E.A., M.E.T., H.D.; Writing Manuscript - S.K.Ö., E.A.; Critical Review - S.K.Ö., S.K.; Other - S.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Teh BM, Marano RJ, Shen Y, Friedland PL, Dilley RJ, Atlas MD. Tissue engineering of the tympanic membrane. *Tissue Eng Part B Rev* 2013; 19: 116-32. [\[CrossRef\]](#)
2. Shen Y, Guo Y, Wilczynska M, Li J, Hellström S, Ny T. Plasminogen initiates and potentiates the healing of acute and chronic tympanic membrane perforations in mice. *J Transl Med* 2014; 12: 5. [\[CrossRef\]](#)
3. Lindeman P, Edström S, Granström G, Jacobsson S, von Sydow C, Westin T, et al. Acute traumatic tympanic membrane perforations. Cover or observe? *Arch Otolaryngol Head Neck Surg* 1987; 113: 1285-7. [\[CrossRef\]](#)
4. Wang AY, Shen Y, Wang JT, Friedland PL, Atlas MD, Dilley RJ. Animal models of chronic tympanic membrane perforation: a 'time-out' to review evidence and standardize design. *Int J Pediatr Otorhinolaryngol* 2014; 78: 2048-55. [\[CrossRef\]](#)
5. Truy E, Disant F, Morgon A. Chronic tympanic membrane perforation: an animal model. *Am J Otol* 1995; 16: 222-5.
6. Lou ZC, Tang YM, Chen HY, Xiao J. The perforation margin phenotypes and clinical outcome of traumatic tympanic membrane perforation with a Gelfoam patch: our experience from a retrospective study of seventy-four patients. *Clin Otolaryngol* 2015; 40: 389-92. [\[CrossRef\]](#)
7. Kaftan H, Herzog M, Miehe B, Hosemann W. Topical application of transforming growth factor-beta1 in acute traumatic tympanic membrane perforations: an experimental study in rats. *Wound Repair Regen* 2006; 14: 453-6. [\[CrossRef\]](#)
8. Lou Z, Yang J, Tang Y, Fu Y. Topical application of epidermal growth factor with no scaffold material on the healing of human traumatic tympanic membrane perforations. *Clin Otolaryngol* 2016; doi: 10.1111/coa.12627. [Epub ahead of print] [\[CrossRef\]](#)
9. Rössli C, von Büren T, Gassmann NB, Huber AM. The impact of platelet-derived growth factor on closure of chronic tympanic membrane perforations: a randomized, double-blind, placebo-controlled study. *Otol Neurotol* 2011; 32: 1224-9. [\[CrossRef\]](#)

10. Jun HJ, Oh KH, Yoo J, Han WG, Chang J, Jung HH, et al. A new patch material for tympanic membrane perforation by trauma: the membrane of a hen egg shell. *Acta Otolaryngol* 2014; 134: 250-4. [\[CrossRef\]](#)
11. Barati B, Abtahi SH, Hashemi SM, Okhovat SA, Poorqasemian M, Tabrizi AG. The effect of topical estrogen on healing of chronic tympanic membrane perforations and hearing threshold. *J Res Med Sci* 2013; 18: 99-102.
12. Sommeling CE, Heyneman A, Hoeksema H, Verbelen J, Stillaert FB, Monstrey S. The use of platelet-rich plasma in plastic surgery: a systematic review. *J Plast Reconstr Aesthet Surg* 2013; 66: 301-11. [\[CrossRef\]](#)
13. Kazakos K, Lyras DN, Verettas D, Tilkeridis K, Tryfonidis M. The use of autologous PRP gel as an aid in the management of acute trauma wounds. *Injury* 2009; 40: 801-5. [\[CrossRef\]](#)
14. Saad Setta H, Elshahat A, Elsherbiny K, Massoud K, Safe I. Platelet-rich plasma versus platelet-poor plasma in the management of chronic diabetic foot ulcers: a comparative study. *Int Wound J* 2011; 8: 307-12. [\[CrossRef\]](#)
15. Knighton DR, Ciresi K, Fiegel VD, Schumert S, Butler E, Cerra F. Stimulation of repair in chronic, nonhealing, cutaneous ulcers using platelet-derived wound healing formula. *Surg Gynecol Obstet* 1990; 170: 56-60.
16. El-Anwar MW, El-Ahl MA, Zidan AA, Yacoup MA. Topical use of autologous platelet rich plasma in myringoplasty. *Auris Nasus Larynx* 2015; 42: 365-8. [\[CrossRef\]](#)
17. Chang SH, Hsu YM, Wang YJ, Tsao YP, Tung KY, Wang TY. Fabrication of pre-determined shape of bone segment with collagen-hydroxyapatite scaffold and autogenous platelet-rich plasma. *J Mater Sci Mater Med* 2009; 20: 23-31. [\[CrossRef\]](#)
18. Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR. Platelet-rich plasma: Growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; 85: 638-46. [\[CrossRef\]](#)
19. Pietramaggiori G, Kaipainen A, Czezug JM, Wagner CT, Orgill DP. Freeze-dried platelet-rich plasma shows beneficial healing properties in chronic wounds. *Wound Repair Regen* 2006; 14: 573-80. [\[CrossRef\]](#)
20. Pietramaggiori G, Scherer SS, Mathews JC, Alperovich M, Yang HJ, Neuwald J, et al. Healing modulation induced by freeze-dried platelet-rich plasma and micronized allogenic dermis in a diabetic wound model. *Wound Repair Regen* 2008; 16: 218-25. [\[CrossRef\]](#)
21. Pietramaggiori G, Scherer SS, Mathews JC, Gennaoui T, Lancerotto L, Ragno G, et al. Quiescent platelets stimulate angiogenesis and diabetic wound repair. *J Surg Res* 2010; 160: 169-77. [\[CrossRef\]](#)
22. Takikawa M, Nakamura S, Nakamura S, Nambu M, Ishihara M, Fujita M, et al. Enhancement of vascularization and granulation tissue formation by growth factors in human platelet-rich plasma-containing fragmin/protamine microparticles. *J Biomed Mater Res B Appl Biomater* 2011; 97: 373-80. [\[CrossRef\]](#)
23. Tahar Aissa J, Hultcrantz M. Acute tympanic membrane perforations and the early immunological response in rats. *Acta Otolaryngol* 2009; 129: 1192-7. [\[CrossRef\]](#)
24. Araújo MM, Murashima AA, Alves VM, Jamur MC, Hyppolito MA. Spontaneous healing of the tympanic membrane after traumatic perforation in rats. *Braz J Otorhinolaryngol* 2014; 80: 330-8. [\[CrossRef\]](#)
25. Santa Maria PL, Redmond SL, Atlas MD, Ghassemifar R. Histology of the healing tympanic membrane following perforation in rats. *Laryngoscope* 2010; 120: 2061-70. [\[CrossRef\]](#)