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Histological evaluation of effectiveness of platelet-rich fibrin on healing of sinus membrane perforations: A preclinical animal study

Ceyhun Aricioglu [a], Dogan Dolanmaz [b], Alparslan Esen [c, *], Kubilay Isik [c], Mustafa Cihat Avunduk [d]

[a] Konya Oral and Dental Health Hospital, Parsana Mh. Beyhekim Cd. No:3, 42070 Selcuklu, Konya, Turkey
[b] Selcuk University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Alaeddin Keykubat Yerleskesi, 42250 Selcuklu, Konya, Turkey
[c] Necmettin Erbakan University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Ankara Cd. No:74, 42050 Karatay, Konya, Turkey
[d] Necmettin Erbakan University, Faculty of Medicine, Department of Medical Pathology, Yunus Emre Mh, 42080 Meram, Konya, Turkey

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ABSTRACT

The aim of this study was to evaluate the effectiveness of platelet-rich fibrin (PRF) in repairing of Schneiderian membrane perforations in rabbit maxillary sinus. A total of 42 female New Zealand rabbits were randomly divided into two groups. Symmetrical bony defects were created 1 cm in diameter and the sinus membranes were exposed. The Schneiderian membranes were elevated in both sinuses and each membrane was perforated with a 1 cm incision. No treatment was applied to the right perforations in both groups. Left-sided perforations were closed with collagen membrane in the first group and PRF membrane in the other group. Seven animals randomly selected from each group were sacrificed at weeks 1, 2 and 4 in order to be able to examine the amounts of lymphocytes, fibroblasts, veins, and collagen fibers in the area where the membranes were applied. Histological analyses showed that there were no statistically significant differences between the collagen membrane and the PRF membrane in the healing of sinus perforation area. PRF may be considered as an alternative application to collagen membrane in sinus membrane perforations.

1. Introduction

Dental-implant-supported restorations have gained wide popularity over the last two decades (Moreno Vazquez et al., 2014). However, in many cases, vertical bone height is limited in the posterior maxilla (Tajima et al., 2013). To overcome this problem, sinus floor elevation or sinus lift procedures have been developed (Tatum, 1986). Sinus lift procedure is a safe and predictable technique, but some complications may still occur, such as postoperative wound infection, maxillary sinusitis development, loss of the graft material, edema, bleeding, and perforation of the Schneiderian membrane. These complications can lengthen both the procedure and the healing process. They can also lead to the need for additional surgery (Moreno Vazquez et al., 2014). Schneiderian membrane perforation is the most common intraoperative complication, with a frequency of 10–20%, and even 56% (Pjetursson et al., 2008; Chiapasco and Zaniboni, 2009; Toscano et al., 2010). Inadequate thickness of the membrane and variations in the sinus morphology can make elevation of the membrane more difficult and increase the risk of perforation (Wen et al., 2015; Zijderveld et al., 2008). It can also lead to graft contamination or migration, and postoperative sinusitis. A variety of materials and techniques have been proposed for repairing perforations of the Schneiderian membrane, such as buccal fat pad, connective tissue, resorbable collagen membranes, fibro-mucosal grafts, and amnion–chorion barriers (Holtzclaw, 2015; Biglioli et al., 2010).

Platelet-rich fibrin (PRF) is a natural and simple blood product. It is prepared by centrifugation of the patient's blood without any manipulation. During centrifugation, the coagulation cascade starts and the blood is divided into three parts in the tube: serum, PRF clot and red blood cells, from top to bottom (Tajima et al., 2013). Recently, PRF has been used as an autologous grafting material

* Corresponding author. Necmettin Erbakan Universitesi, Dis Hekimligi Fakultesi, Ankara Cd. No:74/A, 42050, Konya, Turkey. Fax: +90 332 2200045.
E-mail address: dtaesen@hotmail.com (A. Esen).

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because of its ability to accelerate physiological wound healing and new bone formation (Zhao et al., 2015).

The purpose of this study was to evaluate the effectiveness of PRF in repairing Schneiderian membrane perforations in a rabbit model.

2. Material and methods

2.1. Study design

This animal study was performed with a comparative, randomized, and prospective design. The study was supported by Coordinatorship of Scientific Research Projects and ethical approval was obtained from the University Experimental Medicine and Practice Center, Experimental Animals Ethics Committee, number 2011-002.

2.2. Animal model and animal selection criteria

We used 42 female New Zealand rabbits, aged 6–12 months, and weighing 3500–4000 g. We divided the animals randomly into two groups: the positive control group (group 1, $n = 21$); and the experimental group (group 2, $n = 21$). While the perforated sinus membranes of the animals in the experimental group were closed with PRF membrane, the collagen membrane was applied to the positive control group. In both groups, nothing was applied to the perforated membranes on the left side of the animals. The rabbit model was chosen as the experimental animal because the air pressure measurements of the nasal cavity and maxillary sinus with patent ostium are similar to those reported in humans for absolute pressures and synchronicity with the respiratory cycle (Scharf et al., 1995). The rabbit model was also preferred because of ease of care, the size of the maxillary sinuses, easy access to the surgical site, and low cost.

2.3. Anesthesia, animal care, and sacrifice

The animals were anesthetized with i.m. injection 35 mg/kg ketamine HCl (Ketalar®, Pfizer) and 5 mg/kg Xylazin HCl (Rompun®, Bayer). All surgeries were performed under proper antiseptic conditions. After surgery all animals received postsurgical antibiotics with intramuscular penicillin (İecilline®, Flacon 400.000 IU, IE Ulagay, Turkey) for 2 days. For pain control, intraperitoneal 5 mg/kg tramadol hydrochloride (Contromal® 100 mg ampoule, Abdiibrahim, Turkey) was injected. The rabbits were fed with basal quantities of ready-made special pellet feed, containing normal city water and protein. All rabbits used in the study were housed in animal care rooms, with humidity and temperature control. With daily inspections, the general health status of the rabbits, feeding, and conditions related to the chambers in which the rabbits were housed were checked. At the end of the experimental phase, the subjects were sacrificed by administration of high-dose Pentothal sodium. In both groups, seven animals randomly selected from each group were sacrificed after 1, 2 and 4 weeks.

2.4. Surgical procedures

In all animals, we shaved the skull and exposed the skin. We then made an antero-posterior, mid-sagittal incision in the middle of the skull, starting 1 cm above from the nose and extending to eye level. We elevated a full-thickness flap and approached both maxillary sinus walls by using steel round burs, along with copious irrigation. We created symmetrical bony defects that were 1 cm in diameter and revealed the sinus membranes. Entering through these openings, we elevated the Schneider membranes in both sinuses and perforated each membrane with a 1 cm incision (Fig. 1).

In the experimental group, we obtained 8 ml of autologous blood from the central ear vein. The blood was drawn into glass tubes that did not contain anticoagulant or other substances. The tubes were immediately centrifuged at 2700 rpm for 12 min to separate the blood into its components: acellular plasma, PRF, and the red blood corpuscles, from top to bottom. The PRF was removed using fine forceps and placed in a metal tray. The PRF was then gently pressed using a sterile glass slab against the perforated metal tray to form a membrane.

In both groups, we allocated the perforations created in the Schneiderian membranes of the right sinuses as controls without any treatment. On the left sides, we repaired the perforations with resorbable collagen membrane (20 × 20 mm) (Osteobiol® HCI, Tecnoss, Italy) in group 1, and with PRF membranes in group 2 (Fig. 2). After the surgical procedures were completed, the disrupted muscles were sutured with 4-0 resorbable suture (Vicryl, Surgicryl®, Huntingen, Belgium) and the skin was sutured with 3-0 silk suture (Troge®, Hamburg, Germany).

2.5. Histological and immunohistochemical analysis

Tissue samples were fixed in 10% formalin solution for 24 h and washed in tap water for 15 min, and then decalcified in 20% formic acid solution for 72 h. The tissues were embedded in paraffin blocks, sliced into 5-μm-thick specimens, and stained with standard hematoxylin eosin and Masson's trichrome stains. The preparations were evaluated with a light microscope (Nikon Eclipse, Tokyo, Japan). During this evaluation, preparations were photographed with a camera (Nikon Coolpix 5000, Tokyo, Japan), which was attached to the light microscope. While taking the photographs, the micrometer slide images (Nikon Stage Micrometer MBM11100) were digitally recorded. The images were analyzed by Clemex Vision Lite 3.5 Image Analysis Program (Clemex Technologies, Quebec, Canada). In this phase, length calibration was first performed with a Nikon micrometer microscope slide image. Then, 51178.7 μm^2 areas covering the same regions were selected for each

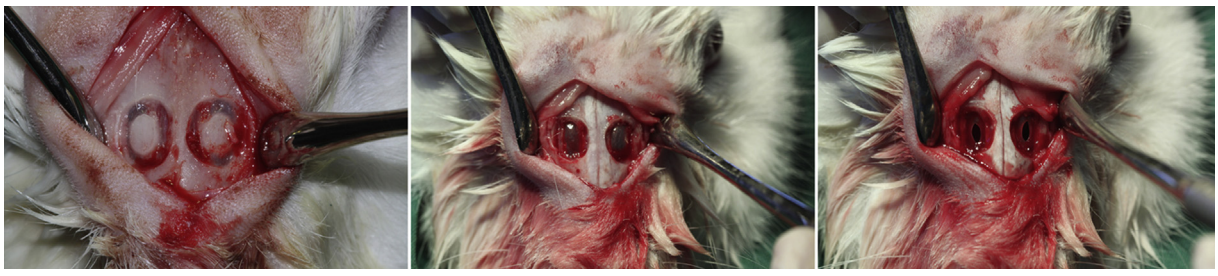

Fig. 1. Symmetrical defects were created and the Schneiderian membranes were elevated in both sinuses. Then, each membrane was perforated with a 1 cm incision.

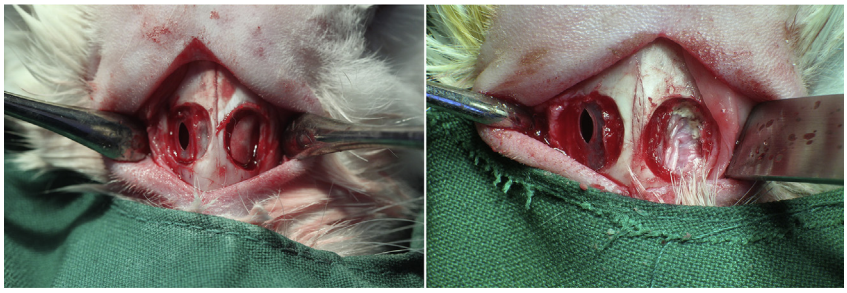

Fig. 2. Whilst the left perforations were covered with collagen membrane in the first group, they were covered with PRF membrane in the second group.

preparation and all operations were carried out in these areas. Lymphocytes, fibroblasts, veins, and collagen fibers were marked in these areas with the Clemex Vision Lite 3.5 Image Analysis Program (Clemex Technologies, Quebec, Canada) (Fig. 3). The damaged cells were not included in the evaluation. Marked cells were counted with the same image analysis program mentioned above.

2.6. Statistical analysis

SPSS 15.0 software program (Statistical Package for Social Sciences, Chicago IL, USA) was used for the statistical analyses. Since our data did not meet the criteria for normal distribution, non-parametric tests were applied. To compare the two independent groups, a Mann–Whitney *U* test was performed. For three or more groups, a Kruskal Wallis test was used. The significance level was accepted as $p < 0.05$.

3. Results

None of the 42 animals was excluded from the study. No signs of infection or wound dehiscence were found postoperatively. The average numbers of lymphocytes, fibroblasts, veins, and collagen fibrils are given in Table 1. Histological images of the lymphocytes, fibroblasts, veins, and collagen fibrils are shown in Figs. 4–7.

When the control and experimental sides were compared for the collagen membrane group (group 1), there was a statistically significant difference ($p < 0.05$) between the lymphocyte values at weeks 1, 2 and 4. For the PRF group (group 2), there was also a statistically significant difference ($p < 0.05$) between the control and experimental sides at weeks 1, 2 and 4 in terms of lymphocyte numbers. In both groups, it was found that the number of lymphocytes in the experimental side increased in each period compared with the control side. When the control sides of group 1

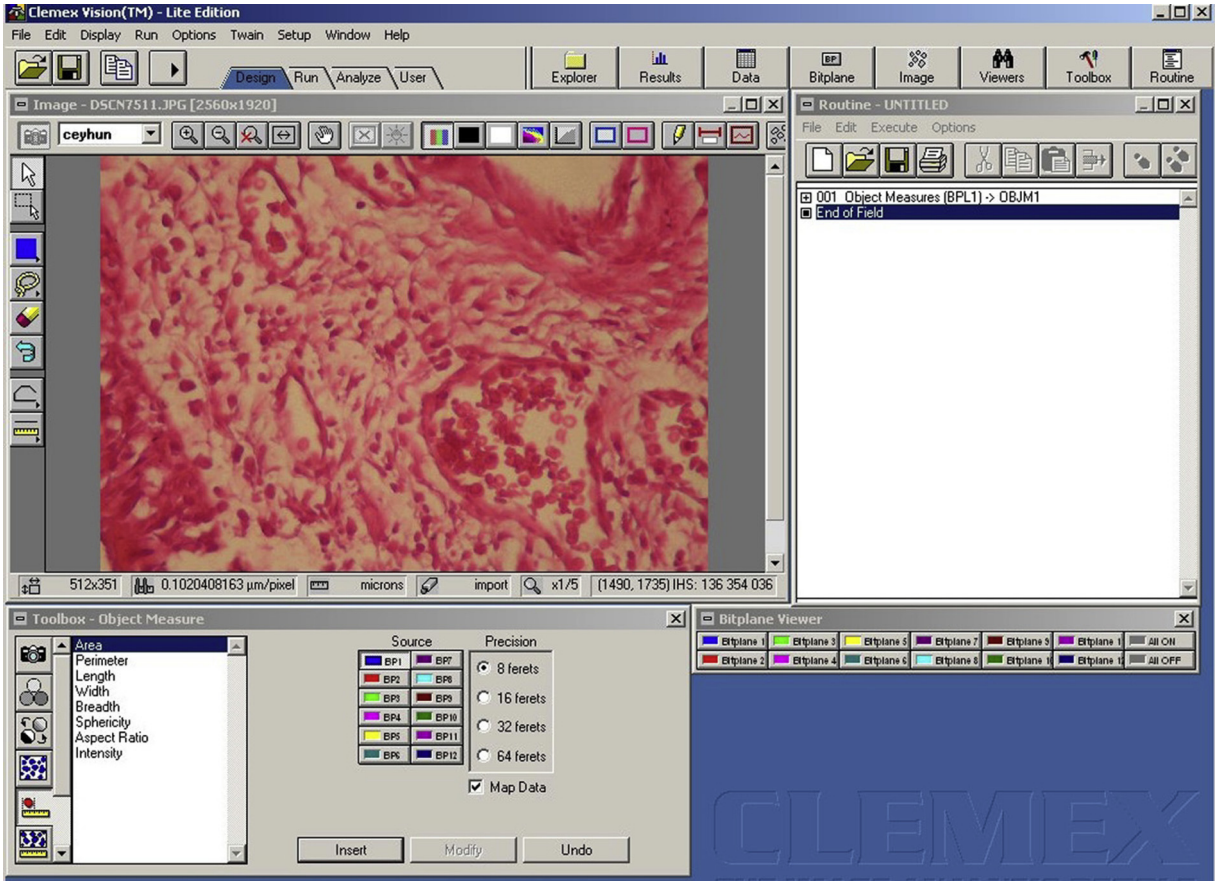

Fig. 3. The lymphocytes, fibroblasts, veins and collagen fibers were marked using the Clemex Vision Lite 3.5 Image Analysis Program.

Table 1
Mean numbers of lymphocytes, fibroblasts, vessels and collagen fibrils among the groups.

	Group 1 (Collagen membrane)						Group 2 (PRF membrane)					
	Right side (control)			Left side (experimental)			Right side (control)			Left side (experimental)		
	Weeks			Weeks			Weeks			Weeks		
	1	2	4	1	2	4	1	2	4	1	2	4
Lymphocytes	23	22	20	**42**	**39**	**37**	24	22	17	**41**	**39**	**37**
Fibroblasts	9	11	13	**18**	**22**	**27**	8	11	12	**19**	**23**	**27**
Vessels	2	3	3	**5**	**6**	**6**	2	3	4	**5**	**6**	**6**
Collagen fibrils	3	2	3	**8**	**11**	**13**	3	3	3	**9**	**12**	**13**

PRF: platelet-rich fibrin.

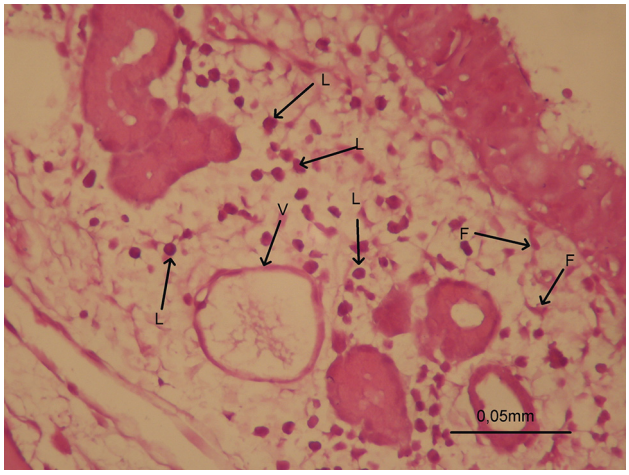

Fig. 4. Histological section of the control side – F: fibroblast, L: lymphocyte, V: vein (HE).

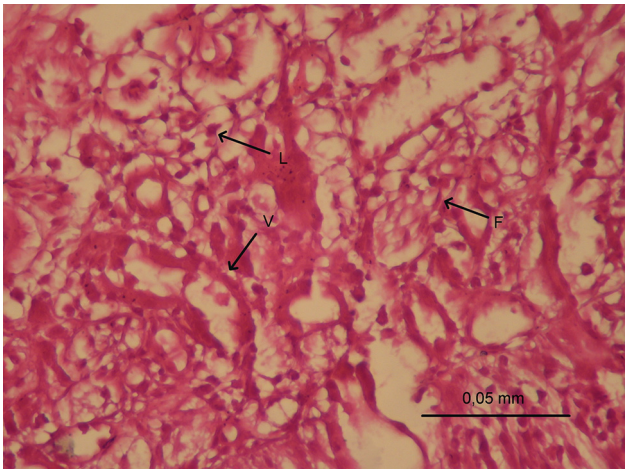

Fig. 6. Histological section of the PRF membrane group at 4 weeks – F: fibroblast, L: lymphocyte, V: vein (HE).

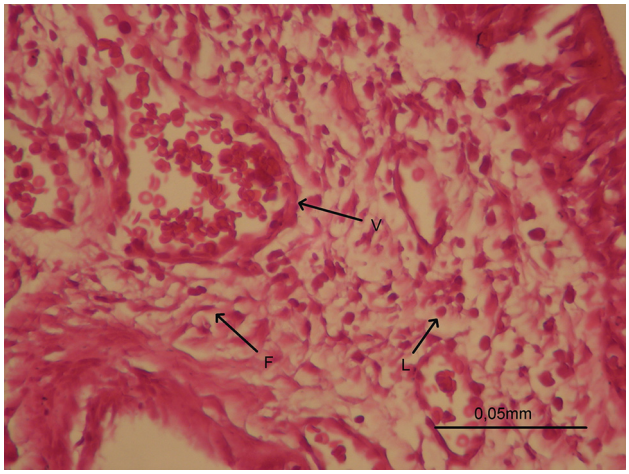

Fig. 5. Histological section of the collagen membrane group at 4 weeks – F: fibroblast, L: lymphocyte, V: vein (HE).

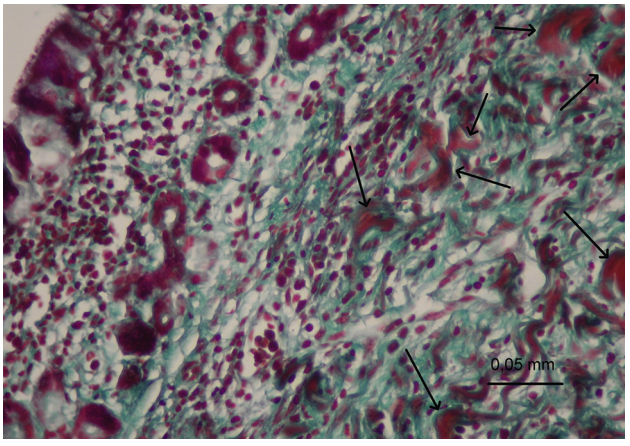

Fig. 7. Histological image of collagen fibrils at 4 weeks. Arrows indicate collagen fibers (Masson's trichrome stain).

and group 2 were compared, it was found that the values were similar and there was no statistically significant difference between the two groups ($p > 0.05$). Moreover, there was no statistically significant difference between the experimental sides of group 1 and group 2 ($p > 0.05$) (Table 2).

There was a statistically significant difference between experimental and control sides for both group 1 and group 2 at weeks 1, 2 and 4 ($p < 0.05$). In both groups, it was observed that the number of fibroblasts in the experimental side increased in each period compared with the control side. However, statistical analyses showed that there were no significant differences between the experimental sides of group 1 and group 2 at weeks 1, 2 and 4 ($p > 0.05$) (Table 3).

In both groups, it was observed that the number of veins in the experimental side increased in each evaluation period compared with the control side. There were statistically significant differences between experimental and control sides for both groups at weeks 1, 2 and 4 ($p < 0.05$). When the experimental sides were compared, statistical analyses showed that the differences in the number of

Table 2
Statistical values for lymphocytes.

Lymphocytes	Groups	1 week		2 weeks		4 weeks	
		Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max
Control (right side)	Group 1	(23.28 ± 1.88)	[21–27]	(22.28 ± 3.98)	[16–27]	(19.85 ± 3.76)	[16–25]
	Group 2	(23.71 ± 3.49)	[19–29]	(21.85 ± 2.67)	[18–25]	(17.00 ± 2.70)	[14–22]
	p value	$p = 0.948$		$p = 0.606$		$p = 0.120$	
Experimental (left side)	Group 1	(42.14 ± 4.74)	[34–48]	(38.57 ± 3.86)	[34–44]	(37.28 ± 1.60)	[36–39]
	Group 2	(41.28 ± 2.75)	[39–47]	(38.85 ± 3.67)	[34–43]	(36.71 ± 1.70)	[35–39]
	p value	$p = 0.519$		$p = 0.948$		$p = 0.453$	

SD: standard deviation, Min: minimum, Max: maximum.

Table 3
Statistical values for fibroblasts.

Fibroblasts	Groups	1 week		2 weeks		4 weeks	
		Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max
Control (right side)	Group 1	(8.57 ± 2.29)	[5–11]	(10.85 ± 2.11)	[8–14]	(13.00 ± 1.91)	[10–16]
	Group 2	(8.28 ± 1.97)	[6–11]	(11.24 ± 1.67)	[9–14]	(12.28 ± 1.79)	[9–14]
	p value	$p = 0.697$		$p = 0.746$		$p = 0.557$	
Experimental (left side)	Group 1	(18.00 ± 2.00)	[15–21]	(21.85 ± 3.28)	[18–28]	(26.57 ± 2.63)	[24–31]
	Group 2	(18.71 ± 3.77)	[14–24]	(23.14 ± 2.34)	[19–26]	(27.00 ± 1.52)	[25–29]
	p value	$p = 0.846$		$p = 0.273$		$p = 0.516$	

SD: standard deviation, Min: minimum, Max: maximum.

veins were not significant between group 1 and group 2 at weeks 1, 2 and 4 ($p > 0.05$) (Table 4).

In both groups, it was observed that the number of collagen fibrils in the experimental side increased in each period compared with the control side. There were statistically significant differences between experimental and control sides for both groups at weeks 1, 2 and 4 ($p < 0.05$). When the experimental sides were compared, statistical analyses showed that the differences in the number of collagen fibrils were not statistically significant between the group 1 and group 2 at weeks 1, 2 and 4 ($p > 0.05$) (Table 5).

4. Discussion

Sinus lifting is a relatively easy and safe procedure. One of the possible complications that can occur during the surgery is perforation of the Schneiderian membrane. While a small perforation might not be important, large perforations need repairing. This situation is considered as a major complication and can even cause termination of the surgery, especially if the graft material is in the form of granules or chips (Becker et al., 2008; Choi et al., 2006). Although several techniques have been suggested, no guidelines have been established for the repairing of sinus membrane perforations (Ardekian et al., 2006).

It has been shown that the rabbit sinus model is valid and useful for maxillary sinus augmentation studies because the ostium anatomy that opens to the nasal cavity and the air changes in the nasal cavity are similar to those in humans (Kim et al., 2012; Kumlien and Schiratzki, 1985). The maxillary sinuses of rabbits are easy to approach and large enough to operate on. Moreover, rabbits are among the easiest animals to care for, and have sufficient blood volume for conducting such a study. Thus, the rabbit model is preferred in this study. However, we recommend using large rabbits in PRF studies because ear veins are more prominent and more blood is available.

Although the rabbit sinus structure resembles that of humans, it differs in terms of the number of platelets present. Coagulation factors in rabbit blood are more prevalent than in human blood. The concentration of platelets in normal rabbit blood is 468,000 ± 182,000/mm^3, whilst the number of normal platelets in human blood is 150,000–400,000/mm^3 (Butterfield et al., 2005). The higher concentration of platelets makes the rabbit healing pattern faster than in the human. Peleg et al. (1999) state that the healing period is 3–6 months for humans and only 6 weeks for rabbits. Moreover, studies have shown that when rabbits are used as experimental animals to obtain PRF, the amount of blood required is 6–10 ml (Jang et al., 2010; Lee et al., 2010). In our study, subjects were sacrificed at the end of week 1, week 2, and week 4 to assess early and late recovery.

Some authors (Choukroun et al., 2006) suggest to centrifuging the blood at 3000 rpm for 10 min, whilst others (Rodella et al., 2011) suggest a lower centrifugation speed (2700 rpm) and a longer processing time (12 min). In our study the latter method was preferred. Although rabbit blood differs structurally from human blood, it was possible to produce good-quality PRF in good quantities.

Table 4
Statistical values for vessels.

Vessels	Groups	1 week		2 weeks		4 weeks	
		Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max
Control (right side)	Group 1	(2.14 ± 0.69)	[1–3]	(2.57 ± 0.53)	[2–3]	(3.42 ± 0.97)	[2–5]
	Group 2	(2.14 ± 0.69)	[1–3]	(2.71 ± 0.75)	[2–4]	(3.85 ± 1.06)	[3–6]
	p value	$p = 1.000$		$p = 0.775$		$p = 0.496$	
Experimental (left side)	Group 1	(4.85 ± 1.34)	[3–7]	(5.71 ± 0.95)	[4–7]	(5.57 ± 0.53)	[5–6]
	Group 2	(5.00 ± 1.15)	[4–7]	(5.57 ± 1.27)	[4–8]	(5.71 ± 0.75)	[5–7]
	p value	$p = 0.902$		$p = 0.544$		$p = 0.775$	

SD: standard deviation, Min: minimum, Max: maximum.

Table 5
Statistical values of collagen fibrils.

Collagen fibrils	Groups	1 week		2 weeks		4 weeks	
		Mean (SD)	Min–Max	Mean (SD)	Min–Max	Mean (SD)	Min–Max
Control (right side)	Group 1	(2.85 ± 0.69)	[2–4]	(2.71 ± 0.75)	[2–4]	(3.00 ± 0.81)	[2–4]
	Group 2	(3.42 ± 0.97)	[2–5]	(3.42 ± 0.97)	[2–5]	(3.00 ± 0.81)	[2–4]
	p value	$p = 0.241$		$p = 0.156$		$p = 1.000$	
Experimental (left side)	Group 1	(7.71 ± 1.97)	[5–11]	(11.14 ± 3.48)	[7–16]	(13.00 ± 3.46)	[9–18]
	Group 2	(8.57 ± 2.57)	[6–14]	(12.42 ± 2.43)	[9–15]	(13.28 ± 1.97)	[10–16]
	p value	$p = 0.600$		$p = 0.511$		$p = 0.700$	

SD: standard deviation, Min: minimum, Max: maximum.

Collagen membranes have been used for repairing sinus membrane perforations and/or closing the sinus lateral window during sinus augmentation surgery (Becker et al., 2008; Van den Bergh et al., 2000a,b; Pikos, 1999; Aimetti et al., 2001). In our study, we compared the effectiveness of PRF membranes with collagen membranes because the collagen membranes have some biological advantages and are commonly used for membrane perforations in sinus lifting surgery. Additionally, in vitro studies have shown that bioabsorbable membranes made of collagen increase secretion of TGF-beta1 – a growth factor involved in bone remodeling. It is also argued that collagen membranes may promote bone regeneration through their activity on osteoblasts (Marinucci et al., 2001). In a clinical trial, the Schneiderian membrane perforations that occurred during sinus lifting operations were repaired using collagen membrane (Ardekian et al., 2006). The authors compared the success of dental implants with a non-perforated membrane group and found that there were no significant differences. In another retrospective study, it was reported that the resorbable collagen membrane was successful in sinus membrane perforations up to 10 mm (Hernández-Alfaro et al., 2008). Becker et al. (2008) repaired the sinus membrane perforations of less than 5 mm in diameter with collagen membranes and reported that there was no healing problem as long as membrane perforations were repaired during the operation. Tawil and Mawla (2001) used collagen membranes to close the lateral sinus window in their study, and observed that there was more fine bone formation on the side of the collagen membrane. While 93.1% implant success was achieved on the collagen membrane side, the success rate on the control side without membrane was 78.1%. In a histomorphometric study, Wallace et al. (2005) closed the lateral sinus window with different membranes and found that mean vital bone formation was 17.6% for collagen, 16.9% for non-resorbable membrane, and 12.1% without any membrane coverage, respectively. Furthermore, there are few studies in the literature comparing PRF and collagen membranes in which effects on bone tissue are examined. In an in vitro study, PRF and collagen membranes were examined as a matrix in periosteal tissue engineering. The researchers showed that PRF significantly increased the amount of periosteal cell proliferation (Gassling et al., 2010). In a clinical trial, the effects of two different membranes (PRF and collagen) on the vital bone formation of bone autografts in sinus augmentation when covering the lateral osteotomy site were examined and the authors found that the amount of vital bone formation was similar in both types of membranes (Gassling et al., 2013). However, there is no study evaluating the effectiveness of PRF membrane in the repair of sinus membrane perforations.

PRF is an autologous and inexpensive biomaterial that contains a strong fibrin matrix and ensures the slow release of growth factor from alpha granules of the platelets. Growth factor release increases over the first 7 days and reaches its highest level on the 14th day, continuing, but decreasing, until the 28th day (Dohan et al., 2006). So, PRF can accelerate soft and hard tissue healing and is gradually absorbed (Sohn et al., 2011). Numerous studies have demonstrated the efficacy of PRF for different procedures in oral surgery, such as dental implants, periodontal regeneration, and sinus floor augmentation (Choukroun et al., 2006; Sohn et al., 2011). PRF is easy to produce, and it has positive effects on angiogenesis and wound healing (Dohan et al., 2010). Moreover, the leukocytes and cytokines, which can be found in high concentrations in PRF fibrin networks, play an important role in controlling inflammatory and infectious processes. As the fibrin matrix is resorbed, cytokines are released from thrombocytes and promote healing. Defence against infection depends greatly on chemotactic properties and the ability of the cytokines to accelerate neovascularization. Thus, it has been stated that PRF could prevent infection. Additionally, when it is used in membrane form, it stabilizes the graft material and protects the wound (Simonpieri et al., 2009a,b). The PRF can be easily manipulated and sutured. It is stable at room temperature and, in a similar way to natural fibrin networks, allows cell migration as well (Choukroun et al., 2006).

Wound healing is a complex phenomenon, which consists of three stages – inflammation, proliferation, and maturation. The inflammation stage begins at the time of injury and ends within 24–48 h. During this stage there is a vascular and cellular response to the stimulation caused by the injury. The cellular response begins immediately after the occurrence of vascular changes. Neutrophils are the first cells to reach the wound area. They reach their maximum number in 1–2 days and, if there is no infection in the region, the numbers decrease after 2–3 days. The lymphocytes migrate to the wound area after neutrophils and reach their maximum number on the sixth day. Their number gradually decreases, and they are replaced with macrophages (Park and Barbul, 2004; Martin and Leibovich, 2005). In our study, it was seen that lymphocyte numbers started to decrease after the first week in both group 1 and group 2. In both groups, more lymphocytes were observed on the experimental sides than on the control ones. Since lymphocytes are associated with inflammation, the high numbers of lymphocytes in membrane regions can be explained by the presence of resorbable materials. Although PRF is an autogenous product, it caused a similar inflammatory response to the collagen membrane.

During the healing process in the wound area, macrophages provide fibroblastic proliferation and transformation, as well as mitogenic elements that stimulate angiogenesis and collagen synthesis. The proliferative phase, which is the second stage of wound healing, begins on the third day after injury and ends in the third week. The main cells present at this stage are fibroblasts and endothelial cells. The fibroblasts migrate to the wound area through the action of TGF-β, which is a chemo-attractant secreted by macrophages. In addition, collagen – the main macromolecule of connective tissue – is produced by the fibroblasts. Collagen synthesis peaks in the third week, and then the synthesis is reduced (Park and Barbul, 2004; Martin and Leibovich, 2005). In this study, the fibroblast counts were significantly higher on the experimental

sides than on the control sides at each stage of the examination in both groups. This suggests that both collagen membrane and PRF contribute positively to the proliferative phase of the healing. When evaluating collagen fibrils produced from fibroblasts, the effect on the healing of the wound was found to be similar in both groups. The numbers of collagen fibrils were higher on the experimental sides compared with the control sides, and contributed positively to wound healing.

Angiogenesis, beginning on the fourth day after injury, was the other parameter examined in our study. Capillary buds formed by proliferation of endothelial cells create new veins in the field (Martin and Leibovich, 2005). Vascularity, which is an important parameter used in the assessment of healing, was defined as the number of veins in the unit area. Similarly to the fibroblast and collagen fibril evaluations, the number of veins was significantly higher on the experimental sides of both groups during each period of examination.

Although there are many studies in the literature on PRF, there is no study evaluating the efficacy of PRF in the repair of sinus membrane perforations. PRF can be considered as an alternative material for repairing sinus perforations because it is economic, easily obtained and adapted, and does not cause an immunological reaction. The main disadvantage of PRF is that it needs specialist equipment and a specific blood intake procedure.

5. Conclusion

The histological analyses revealed that there were no statistically significant differences between PRF and collagen membrane regarding the healing process. When compared with collagen membranes, PRF leads to a similar inflammatory response and the contribution to healing is similar to that of collagen membrane. Within the limits of this study, it can be said that PRF membrane can be an alternative to collagen membranes for closing Schneiderian membrane perforations. However, clinical studies are needed to confirm our results.

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Competing interests

The authors declare that there are no conflicts of interest.

References

Aimetti M, Romagnoli R, Ricci G, Massei G: Maxillary sinus elevation: the effect of macrolacerations and microlacerations of the sinus membrane as determined by endoscopy. Int J Periodontics Restor Dent 21: 581–589, 2001

Ardekian L, Oved-Peleg E, Mactei EE, Peled M: The clinical significance of sinus membrane perforation during augmentation of the maxillary sinus. J Oral Maxillofac Surg 64: 277–282, 2006

Becker ST, Terheyden H, Steinriede A, Behrens E, Springer I, Wiltfang J: Prospective observation of 41 perforations of the Schneiderian membrane during sinus floor elevation. Clin Oral Implant Res 19: 1285–1289, 2008

Biglioli F, Pedrazzoli M, Colletti G: Repair of a perforated sinus membrane with a palatal fibromucosal graft: a case report. Minerva Stomatol 59: 299–302, 2010

Butterfield KJ, Bennett J, Gronowicz G, Adams D: Effect of platelet-rich plasma with autogenous bone graft for maxillary sinus augmentation in a rabbit model. J Oral Maxillofac Surg 63: 370–376, 2005

Chiapasco M, Zaniboni M: Methods to treat the edentulous posterior maxilla: implants with sinus grafting. J Oral Maxillofac Surg 67: 867–871, 2009

Choi BH, Zhu SJ, Jung JH, Lee SH, Huh JY: The use of autologous fibrin glue for closing sinus membrane perforations during sinus lifts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 101: 150–154, 2006

Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, et al: Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 101: e56–60, 2006

Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al: Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 101: e45–50, 2006

Dohan DM, Del Corso M, Diss A, Mouhyi J, Charrier JB: Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol 81: 546–555, 2010

Gassling V, Douglas T, Warnke PH, Açil Y, Wiltfang J, Becker ST: Platelet-rich fibrin membranes as scaffolds for periosteal tissue engineering. Clin Oral Implant Res 21: 543–549, 2010

Gassling V, Purcz N, Braesen JH, Will M, Gierloff M, Behrens E, et al: Comparison of two different absorbable membranes for the coverage of lateral osteotomy sites in maxillary sinus augmentation: a preliminary study. J Craniomaxillofac Surg 41: 76–82, 2013

Hernández-Alfaro F, Torradeflot MM, Marti C: Prevalence and management of Schneiderian membrane perforations during sinus-lift procedures. Clin Oral Implants Res 19: 91–98, 2008

Holtzclaw D: Maxillary sinus membrane repair with amnion–chorion barriers: a retrospective case series. J Periodontol 86: 936–940, 2015

Jang ES, Park JW, Kweon H, Lee KG, Kang SW, Baek D: Restoration of peri-implant defects in immediate implant installations by Choukroun platelet-rich fibrin and silk powder combination graft. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 109: 831–836, 2010

Kim YS, Kim SH, Kim KH, Jhin MJ, Kim WK, Lee YK, et al: Rabbit maxillary sinus augmentation model with simultaneous implant placement: differential responses to the graft materials. J Periodontal Implant Sci 42: 204–211, 2012

Kumlien J, Schiratzki H: The vascular arrangement of the sinus mucosa. A study in rabbits. Acta Otolaryngol 99: 122–132, 1985

Lee EH, Kim JY, Kweon HY, Jo YY, Min SK, Park YW, et al: A combination graft of low-molecular-weight silk fibroin with Choukroun platelet-rich fibrin for rabbit calvarial defect. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 109: 33–38, 2010

Martin P, Leibovich SJ: Inflammatory cells during wound repair: the good, the bad and the ugly. Trends Cell Biol 15: 599–607, 2005

Marinucci L, Lilli C, Baroni T, Becchetti E, Belcastro S, Balducci C, et al: In vitro comparison of bioabsorbable and non-resorbable membranes in bone regeneration. J Periodontol 72: 753–759, 2001

Moreno Vazquez JC, Gonzalez de Rivera AS, Gil HS, Mifsut RS: Complication rate in 200 consecutive sinus lift procedures: guidelines for prevention and treatment. J Oral Maxillofac Surg 72: 892–901, 2014

Park JE, Barbul A: Understanding the role of immune regulation in wound healing. Am J Surg 187: 11–16, 2004

Peleg M, Chaushu G, Mazor Z, Ardekian L, Bakoon M: Radiological findings of the post-sinus lift maxillary sinus: a computerized tomography follow-up. J Periodontol 70: 1564–1573, 1999

Pikos MA: Maxillary sinus membrane repair: report of a technique for large perforations. Implant Dent 8: 29–34, 1999

Pjetursson BE, Tan WC, Zwahlen M, Lang NP: A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation. J Clin Periodontol 35: 216–240, 2008

Rodella LF, Favero G, Boninsegna R, Buffoli B, Labanca M, Scari G, et al: Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction. Microsc Res Tech 74: 772–777, 2011

Scharf KE, Lawson W, Shapiro JM, Gannon PJ: Pressure measurements in the normal and occluded rabbit maxillary sinus. Laryngoscope 105: 570–574, 1995

Simonpieri A, Del Corso M, Sammartino G, Dohan DM: The relevance of Choukroun's platelet-rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part I: a new grafting protocol. Implant Dent 18: 102–111, 2009a

Simonpieri A, Del Corso M, Sammartino G, Dohan DM: The relevance of Choukroun's platelet-rich fibrin and metronidazole during complex maxillary rehabilitations using bone allograft. Part II: implant surgery, prosthodontics, and survival. Implant Dent 18: 220–229, 2009b

Sohn DS, Heo JU, Kwak DH, Kim DE, Kim JM, Moon JW, et al: Bone regeneration in the maxillary sinus using an autologous fibrin-rich block with concentrated growth factors alone. Implant Dent 20: 389–395, 2011

Tajima N, Ohba S, Sawase T, Asahina I: Evaluation of sinus floor augmentation with simultaneous implant placement using platelet-rich fibrin as sole grafting material. Int J Oral Maxillofac Implant 28: 77–83, 2013

Tatum H: Maxillary and sinus implant reconstructions. Dent Clin North Am 30: 207–229, 1986

Tawil G, Mawla M: Sinus floor elevation using a bovine bone mineral (Bio-Oss) with or without the concomitant use of a bilayered collagen barrier (Bio-Gide): a clinical report of immediate and delayed implant placement. Int J Oral Maxillofac Implants 16: 713–721, 2001

Toscano NJ, Holtzclaw D, Rosen PS: The effect of piezoelectric use on open sinus lift perforation: a retrospective evaluation of 56 consecutively treated cases from private practices. J Periodontol 81: 167–171, 2010

Van den Bergh JP, Bruggenkate CM, Krekeler G, Tuinzing DB: Maxillary sinus floor elevation and grafting with human demineralized freeze dried bone. Clin Oral Implants Res 11: 487–493, 2000a

Van den Bergh JP, Bruggenkate CM, Disch FJ, Tuinzing DB: Anatomical aspects of sinus floor elevations. Clin Oral Implants Res 11: 256–265, 2000b

Wallace SS, Froum SJ, Cho SC, Elian N, Monteiro D, Kim BS, et al: Sinus augmentation utilizing anorganic bovine bone (Bio-Oss) with absorbable and nonabsorbable membranes placed over the lateral window: histomorphometric and clinical analyses. Int J Periodontics Restor Dent 25: 551–559, 2005

Wen SC, Lin YH, Yang YC, Wang HL: The influence of sinus membrane thickness upon membrane perforation during transcrestal sinus lift procedure. Clin Oral Implants Res 26: 1158–1164, 2015

Zhao JH, Tsai CH, Chang YC: Clinical application of platelet-rich fibrin as the sole grafting material in maxillary sinus augmentation. J Formos Med Assoc 114: 779–780, 2015

Zijderveld SA, Van den Bergh JP, Schulten EA, Bruggenkate CM: Anatomical and surgical findings and complications in 100 consecutive maxillary sinus floor elevation procedures. J Oral Maxillofac Surg 66: 1426–1438, 2008