การศึกษาเปรียบเทียบระยะเวลาการสลายตัว การลดลงของน้ำหนัก และลักษณะโครงข่ายไฟบรินบริเวณพื้นผิว ระหว่างแผ่นเยื่อเพลตเลทริชไฟบริน ซึ่งเตรียมด้วยวิธีบีบอัดด้วยผ้าก๊อซ และวิธีบีบอัดด้วยความร้อน

The Comparison of Degradation Time, Weight Loss and Surface Fibrin Structure Between Gauze-Compression and Heat-Compression Platelet-Rich Fibrin Membrane

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บทคัดย่อ

วัตถุประสงค์: เพื่อประเมินผลของการบีบอัดความร้อนต่อแผ่นเยื่อเพลตเลทริชไฟบริน (PRF) ในระยะเวลาการย่อยสลาย การลดลงของน้ำหนัก และการตรวจสอบลักษณะโครงข่ายไฟบรินบริเวณพื้นผิว โดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM)

วิธีการ: แผ่นเยื่อเพลตเลทริชไฟบริน ที่เตรียมจากเลือดมนุษย์ (อาสาสมัครที่มีสุขภาพดีจำนวนสิบคน) จำนวน

Abstract

Objective: To evaluate the effect of heat-compression to platelet-rich fibrin (PRF) membrane in degradation time, weight loss and to examine surface fibrin structure using scanning electron microscope (SEM).

Methods: Sixty PRF membranes that were prepared from human blood (ten healthy

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60 ตัวอย่างถูกบีบอัดที่อุณหภูมิต่าง ๆ และแบ่งออกเป็นหกกลุ่ม: กลุ่มควบคุม (อุณหภูมิห้อง), 60, 70, 80, 90 และ 100 องศาเซลเซียส กลุ่มตัวอย่างเก้าจากสิบของแต่ละกลุ่มได้รับการประเมินเวลาการย่อยสลายและการลดลงของน้ำหนัก ตัวอย่างที่เหลืออีกหนึ่งตัวอย่างถูกตรวจสอบลักษณะโครงข่ายไฟบรินพื้นผิวโดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด

ผลการศึกษา: กลุ่มควบคุมที่อุณหภูมิ 90 และ 100 องศาเซลเซียส มีเวลาในการย่อยสลายที่แตกต่างกันอย่างมีนัยสำคัญ เมื่อเทียบกับกลุ่มควบคุมกลุ่มที่อุณหภูมิ 60 และ 70 องศาเซลเซียส (p <0.05) ไม่มีความแตกต่างอย่างมีนัยสำคัญในร้อยละของน้ำหนักเฉลี่ยของทั้งหกกลุ่มในแต่ละวัน (p <0.05) แต่เมื่อเปรียบเทียบระหว่างวันในกลุ่มเดียวกันกลุ่มควบคุมที่อุณหภูมิ 90 และ 100 องศาเซลเซียส มีความแตกต่างอย่างมีนัยสำคัญในช่วงแรกถึง 11 และ 6 วันตามลำดับ โครงข่ายไฟบรินในพื้นผิวในกลุ่ม 90 และ 100 องศาเซลเซียส แสดงพื้นที่ที่มีการรบกวนน้อยที่สุดและมีรูพรุนน้อยที่สุด

บทสรุป: กลุ่มควบคุมที่อุณหภูมิ 90 และ 100 องศาเซลเซียส มีเวลาในการย่อยสลายนานกว่า และมีความล่าช้าในระยะแรกของการย่อยสลาย ซึ่งอาจถูกนำไปใช้ในการผ่าตัดที่ต้องการความเสถียรของแผ่นเยื่อเพลตเลทริชไฟบรินในระยะแรก

คำสำคัญ: แผ่นเยื่อเพลตเลทริชไฟบริน ระยะเวลาการย่อยสลาย การลดลงของน้ำหนัก ลักษณะโครงข่ายไฟบรินบริเวณพื้นผิว กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด

volunteers). Samples were compressed at various temperatures and were arranged into six groups: control (room temperature), 60, 70, 80, 90 and 100°C. Nine of the ten samples from each group were evaluated for their degradation time and weight loss. One remaining sample was examined the surface fibrin structure using SEM.

Results: The 90 and 100°C groups had significantly different degradation times compared to those in the control, 60 and 70°C groups (p<0.05). There was no significant difference in the mean weight percentage among the six groups on each day (p<0.05). But when compared among the days in the same group, the 90 and 100°C groups were significantly different in the early phase to 11 and 6 days, respectively. The surface fibrin structure in the 90 and 100°C groups showed the least interfibrous space and lowest porosity.

Conclusion: The 90 and 100°C groups had significantly longer degradation times and delayed the early stage of degradation. It might be applied in surgical operations that need PRF membrane stability in the early phase.

Keywords: platelet-rich fibrin (PRF) membrane, degradation time, weight loss, surface fibrin structure, scanning electron microscope (SEM)

Introduction

At present, geriatric problems have become more of a focus and include systemic diseases, health care and social supported needs. In dentistry, a common problem is tooth loss. Several treatment are creating to restore an edentulous ridge. However, alveolar ridge resorption after tooth loss is a major issue to rehabilitate their occlusion. Schropp, *et al.* reported that the alveolar bone commonly decreases by approximately 50% in the horizontal dimension and by less than 1 mm in the vertical dimension. Alveolar ridge resorption of approximately two-thirds of all both vertical and horizontal dimension occurred in the first three months after tooth extraction.[1]

Many protocols have been explored to decrease

bone resorption rate after a tooth has been removed to preserve bone for future prostheses. After tooth extraction, immediate socket preservation with autologous or substitute bone graft, autologous platelet concentrates and resorbable or non-resorbable membrane has been suggested.[2] Previous studies have revealed that the application of bone materials with membrane[3,4] or membrane only[5] after tooth extraction reduced the bone resorption rate.

Additionally, in patients with alveolar bone resorption, various regenerative surgical techniques have been suggested for the regeneration of periodontal tissues, including bone graft operation and guided bone regeneration (GBR) with bone graft materials and membrane.[6]

Membrane is a crucial factor for successful regenerative surgical techniques.[7-11] It was first introduced as a barrier in guided tissue regeneration (GTR)[12] to restore the loss of periodontal tissues from periodontal diseases. There are two type of membrane including non-resorbable and resorbable membrane. The resorbable membrane is more frequently used than non-resorbable membrane because it does not require the second operation to remove it. It is widely applied in several surgical procedures, such as socket preservation, GTR or guided bone regeneration (GBR), block bone graft and sinus augmentation.[13] It can help promote the bone healing process by preventing a defective area or surgical site from an ingrowth of epithelial cells and connective tissue cells of the gingiva. Moreover, it can stimulate the regeneration of osseous defects.[14] However, membranes have an unpredictable degradation time and are expensive.

Currently, Platelet-rich fibrin (PRF) is a popular biomaterial that is used in oral surgical procedures. Its preparation is simple, its cost is reasonable and it is easy to use.[15] Furthermore, many studies[2,15-17] reported that PRF improved the wound-healing and regeneration process because it released growth factors, cytokines and leukocytes. It is mainly used in 3 forms, as follows: 1) Clot form for filling in extraction sockets[18]; 2) liquid or injectable form, combined with bone materials, to simplify manipulation and accelerate new bone formation[19,20]; and 3) membrane form as a barrier membrane to provide stability and space for tissue regeneration, stimulate soft tissue and periosteum healing, and avoid the penetration of soft tissue into a surgical site.[21-23] Unlike other natural membranes, PRF is derived from the patients' own blood without exogenous supplements, so there is no risk of immunologic response.[24,25]

Nonetheless, a gauze-compression PRF membrane has a rapid degradation time. It is resorbed within 2 weeks or less after the operation. Therefore, it cannot be maintained for a sufficient time in the surgical site, in which periodontal tissue regeneration and integration requires 3-4 weeks.[14,26] Accordingly, the aim of this study was to evaluate and compare the degradation time and surface fibrin structure of a gauze-compression PRF membrane and a heat-compression PRF membrane that was heated to various temperatures. This investigation will provide beneficial information about the delayed degradation time of the PRF membrane and the effect that heat-compression has on the surface fibrin structure of a PRF membrane.

Materials and Methods

This experimental study was approved by the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University. (No. 04/2017) Informed consent was obtained from all volunteers.

The samples were PRF membranes that were prepared from human blood. Ten volunteers, age

ranging from 20-30 years old, healthy, no systemic disease, non-smoking and have no history of any drug intake in the previous month, were required for this study. Blood samples (30 ml) were collected from each volunteer. The blood sample was separated into 6 glass tubes (5 ml of blood per tube) and immediately centrifuged.

Ten samples were arranged for each experimental group. Nine of the ten samples were evaluated for their degradation pattern, and only one sample's surface fibrin structure was evaluated using scanning electron microscopy (SEM) (JSM-5910LV, JOEL Peabody, MA, USA). The distribution of samples is shown in Table 1.

ตารางที่ 1 *จำนวนของแผ่นเยื่อเพลตเลทริชไฟบริน ในหกกลุ่มการทดลอง (ชิ้น)*

Table 1 *The number of PRF membranes in six experimental groups (piece)*

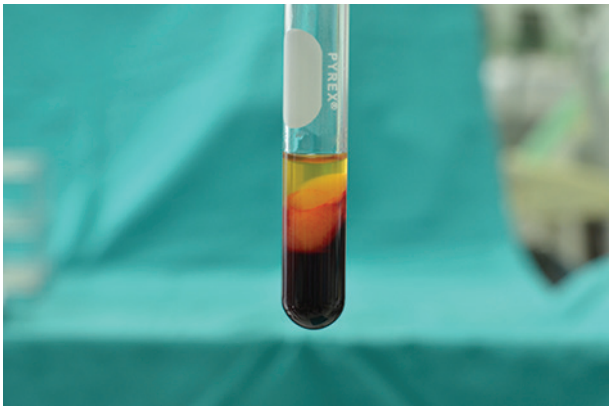
Experimental groups	Number of PRF membranes (piece)	
	Evaluation of degradation	Evaluation of surface structure
1. Control	9	1
2. 60°C	9	1
3. 70°C	9	1
4. 80°C	9	1
5. 90°C	9	1
6. 100°C	9	1

PRF preparation

A medical technologist collected 30-ml blood samples from the median cubital vein of each volunteer using a 50-ml disposable syringe and 21-gauge needle. The blood sample was immediately centrifuged by the centrifuge machine (DM0412, Scilogex, Berlin, CA, USA) (Figure 1) at 400 g for 10 minutes.[24] The blood in the glass tube was arranged into 3 layers as follows: the bottom layer was red corpuscle; the middle layer was platelet rich fibrin; and the top layer was platelet poor plasma. Only the middle layer was used for PRF membrane preparation. (Figure 2)

รูปที่ 1 *เครื่องหมุนเหวี่ยงมีความเร็วที่ปรับได้ตั้งแต่ 300 ถึง 4500 รอบต่อนาที แรงเหวี่ยงสัมพัทธ์ (RCF) ตั้งแต่ 100 g ถึง 2,490 g เวลาทำงานตั้งแต่ 30 วินาที ถึง 99 นาทีและมีความจุหลอดขนาด 10 มล. จำนวน 12 หลอด.*

Figure 1 *The centrifuge machine has an adjustable speed ranging from 300 to 4500 rpm, a relative centrifugal force (RCF) ranging from 100 g to 2,490 g, a running time ranging from 30 seconds to 99 minutes, and a capacity of twelve 10-ml tubes.*



รูปที่ 2 *ตัวอย่างเลือดที่แยกตัวเป็นสามชั้นหลังจากการปั่นแยก มีดังนี้ ชั้นล่างเป็นชั้นของเม็ดเลือดแดง ชั้นกลางคือเพลต-เลทริชไฟบริน และชั้นบนสุดเป็นเพลตเลทพัวร์พลาสมา*

Figure 2 *The three layers of PRF after centrifugation were as follows: the bottom layer was red corpuscle; the middle layer was PRF; and the top layer was platelet poor plasma.*

PRF membrane preparation

Six PRF samples obtained from each volunteer

were divided into six groups according to the preparation technique and compression temperature used:

1) Control group: Gauze-compression technique at room temperature

2) 60°C group: Heat-compression technique at 60°C

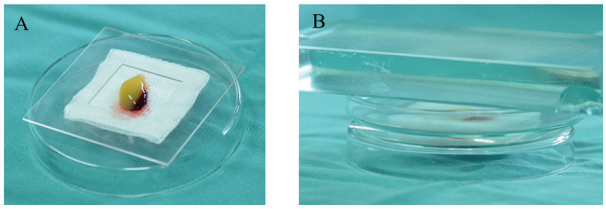
3) 70°C group: Heat-compression technique at 70°C

4) 80°C group: Heat-compression technique at 80°C

5) 90°C group: Heat-compression technique at 90°C

6) 100°C group: Heat-compression technique at 100°C

All samples were compressed with dry gauze into 1-mm-thick PRF membranes. A 1-mm-thick acrylic mold was used to control the PRF membrane thickness when the membranes were compressed. They were condensed under the same conditions (500 grams of force for 1 minute) (Figure 3).



รูปที่ 3 *เพลตเลทริชไฟบรินที่ถูกบีบอัดเป็นแผ่นเยื่อ ที่หนา 1 มม. (A: ใช้อะคริลิกแม่พิมพ์หนา 1 มม. เพื่อควบคุมความหนาของแผ่นเยื่อเพลตเลทริชไฟบริน B: เพลตเลทริชไฟบรินถูกบีบอัดที่ 500 กรัมเป็นเวลา 1 นาที)*

Figure 3 *PRF was compressed into a 1-mm-thick PRF membrane. (A: 1-mm-thick acrylic mold was usedto control PRF membrane thickness, B: PRF was compressed at 500 grams for 1 minute.)*

The samples in the 60, 70, 80, 90 and 100°C groups were then wrapped with a polyethylene wrap that could resist heat without disintegrating. An electric hair straightening iron was used to heat-compress the PRF membranes at the different temperatures for 5 seconds.[26] The surface temperature of the electric straightening iron had a 0.9°C reduction rate in 5 seconds, and it was confirmed with an infrared thermometer at 60, 70, 80, 90 and 100°C before the PRF was compressed.

Evaluation of degradation

The PRF membranes were placed on a stainless-steel mesh and put into 54 petri dishes that contained 3 ml of Hanks' balance salt solution (HBSS) and human plasmin (2 µg/ml). They were incubated in a CO_2 incubator. The PRF membranes and stainless-steel mesh were removed from the petri dishes, photographed and weighed every 24 hours until complete degradation or by 4 weeks. The millimeters' scale length was attached at the vertical and horizontal plane while taking a photo as a reference. The mean degradation time and mean weight percentage of all samples was calculated before analysis.

Statistical analysis was performed using SPSS version 17 (SPSS Inc., Chicago, USA). Data were tested for normality and presented as the means ± SD. A one-way analysis of variance (ANOVA) and multiple comparison test were applied to compare the differences in degradation time and mean weight percentage between the 6 experimental groups. The results were considered significant at $p<0.05$.

Evaluation of surface fibrin structure

One PRF membrane from each group was immersed in 2.5% glutaraldehyde in 0.1 mol/liter phosphate buffer, fixed for 24 hours at room temperature and post-fixed in 1% osmium tetroxide for 1 hour. Subsequently, the samples were dehydrated by serial transfers in ascending concentrations of ethanol before drying with critical point drying. Eventually, the specimens were made

electrically conductive by being mounted on stubs, attached to conductive carbon tape and sputter coated with gold. Finally, the specimens were examined using SEM and photographed at 15 kV using 1,000x magnifications.

Results

Degradation time

The degradation time of compressed PRF membranes ranged from 9.56±2.13 to 17.00±4.82 days. The control group had the shortest degradation time and degraded completely after 9.56 days of incubation. The 90°C group had the longest degradation time and degraded completely in 17 days. The means and standard deviations of all degradation times are shown in Table 2.

ตารางที่ 2 *ค่าเฉลี่ยและส่วนเบี่ยงเบนมาตรฐานของเวลาการย่อยสลาย (วัน) ของแผ่นเยื่อเพลตเลทริชไฟบรินที่ถูกบีบอัดในที่ที่มีสารละลายพลาสมินของมนุษย์*

Table 2 *Means and standard deviations of degradation time (days) of compressed PRF membranes in the presence of human plasmin solution*

Group	N	Mean	Standard Deviation	Minimum	Maximum
Control	9	9.56	2.13	7.00	14.00
60°C	9	9.89	1.27	8.00	12.00
70°C	9	9.89	2.42	6.00	15.00
80°C	9	10.33	4.06	6.00	20.00
90°C	9	17.00	4.82	12.00	24.00
100°C	9	15.67	6.73	7.00	25.00
Total	**54**	**12.06**	**4.92**	**6.00**	**25.00**

The degradation time of PRF membranes differed significantly among the six groups based on the one-way analysis of variance (ANOVA) (p<0.05). The comparison of degradation time between the groups using the multiple comparison test is shown in Table 3. The PRF membranes in the 90 and 100°C groups had significantly different degradation times compared to those in the control, 60 and 70°C groups (p<0.05). However, only the PRF membranes in the 90°C group had a significantly different degradation time compared to that in the 80°C group (p<0.05).

ตารางที่ 3 *การเปรียบเทียบเวลาในการย่อยสลายระหว่างกลุ่มโดยใช้การเปรียบเทียบพหูคูณ*

Table 3 *Comparison of degradation time between groups using multiple comparison*

Group	Compared group	P-value
Control	60°C	1.000
	70°C	1.000
	80°C	0.998
	90°C	0.004**
	100°C	0.026*
60°C	70°C	1.000
	80°C	1.000
	90°C	0.006**
	100°C	0.041*
70°C	80°C	1.000
	90°C	0.006**
	100°C	0.041*
80°C	90°C	0.012*
	100°C	0.072
90°C	100°C	0.981

* Statistical significance was considered to be p<0.05.

Weight of PRF membranes

The mean weight percentage of PRF membranes in all groups decreased gradually until complete degradation. The one-way ANOVA did not reveal a significant difference in the mean weight percentage of PRF membranes among the six groups on each day (p<0.05). In comparing the mean weight percentage of PRF membranes among the days in the same group using repeated-measures ANOVA, the mean weight percentage of the PRF membranes in the 90 and 100°C groups were significantly different in the early phase. This result indicated that heat-compression at 90 and 100°C could reduce the rate of weight loss or degradation in the early phase

ตารางที่ 4 *เปรียบเทียบค่าเฉลี่ยน้ำหนักร้อยละของแผ่นเยื่อเพลตเลทริชไฟบริน ระหว่าง 6 กลุ่มในแต่ละวันโดยใช้การวิเคราะห์ความแปรปรวนแบบทางเดียว*

Table 4 *Comparison of mean weight percentage of PRF membrane among six groups at each day using one-way ANOVA*

Day	Control	60°C	70°C	80°C	90°C	100°C	*p*-value
1	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	
2	91.87±4.30[b]	92.98±4.02[b]	91.03±6.88[a]	88.95±8.52[a]	91.26±4.37[b]	90.99±7.13[a]	0.832
3	85.31±7.44[b]	86.16±4.19[c]	86.14±8.25[b]	82.62±8.77[b]	82.68±8.74[b]	82.65±12.40[a]	0.855
4	79.83±7.87[c]	79.28±7.42[d]	78.64±10.36[c]	78.09±10.18[c]	75.98±11.32[b]	74.84±18.62[a]	0.934
5	74.84±7.23[cd]	74.99±8.18[de]	75.35±10.44[d]	74.70±9.30[c]	71.55±13.50[b]	72.93±19.81[a]	0.983
6	64.58±14.20[d]	68.61±9.94[e]	60.82±24.32[d]	67.29±6.01[d]	66.65±16.08[b]	68.40±22.55[a]	0.924
7	51.86±24.57[d]	63.64±9.34[f]	49.45±28.40	42.43±22.65	60.79±20.35[b]	61.82±28.81[a]	0.609
8	44.55±26.44	55.18±19.78[f]	43.16±26.23	29.08±26.08	56.92±22.86[b]	56.87±30.14	0.343
9	29.44±28.08	39.63±29.09	31.52±30.67	24.82±27.76	54.29±23.58[b]	49.84±27.23	0.409
10	15.39±29.73	27.12±32.53	21.82±30.33	16.98±28.16	44.78±23.26[b]	39.89±30.89	0.975
11	12.98±33.83	18.11±31.61	5.04±18.47	7.58±21.28	30.18±20.61[b]	29.84±25.97	0.245
12	4.94±25.66	1.75±9.10	2.78±14.43	3.18±8.53	17.97±13.53[b]	22.60±25.06	0.472
13	0.41±0.00	0.00	1.80±0.00	1.20±7.62	10.70±11.53	16.30±18.18	0.322
14	0.35±0.00		1.23±0.00	0.96±0.00	7.45±8.31	11.38±10.93	0.402
15	0.00		0.77±0.00	0.90±0.00	5.96±7.91	8.56±11.20	0.759
16			0.00	0.87±0.00	4.83±6.56	6.12±9.67	0.582
17				0.58±0.00	3.94±5.88	4.77±6.92	0.627
18				0.39±0.00	2.93±5.12	3.70±6.34	0.316
19				0.23±0.00	2.22±3.98	2.80±3.35	0.298
20				0.01±0.00	1.85±2.75	1.87±2.52	0.272

* Statistical significance was considered to be $p<0.05$.
Values representing the mean ± SD followed by similar letters in a column indicate a non-significant difference using repeated measures ANOVA.

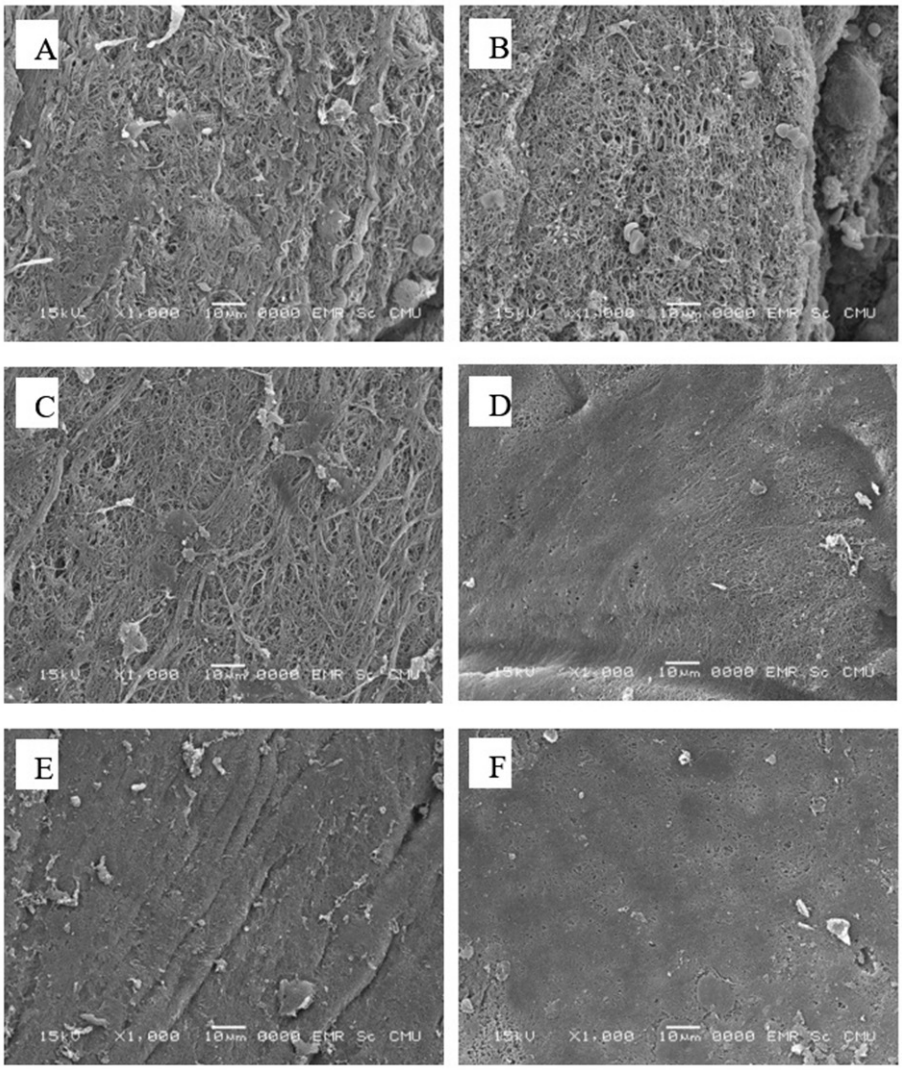
to 11 and 6 days, respectively. The PRF membranes in the control, 60, 70 and 80°C groups showed significant weight loss or rapid degradation at 3-4 days after they were immersed in human plasmin solution (Table 4).

Surface structure in Scanning Electron Microscope (SEM)

Examined at 1,000x magnification using SEM, the surface fibrin structure of PRF membranes in the control, 60 and 70°C groups showed loose interfibrous space and more porosity than those in the other groups. The interfibrous space, porosity and surface area decreased in the 80°C group. Furthermore, the surface fibrin structure of PRF membranes in the 90 and 100°C groups had the least interfibrous space and lowest porosity among the groups (Figure 4).

Discussion

The most important disadvantage of a gauze-compression PRF is rapid degradation. It is completely resorbed within 2 weeks or less, similar to non-cross-linked collagen membranes.[26,27] Therefore, PRF membranes and non-cross-linked collagen membranes cannot provide enough stability and maintain space for the graft site when it is used as a barrier membrane.[28] Several methods for modifying the cross-linking were studied to improve the mechanical stability and degradation rate of collagen membranes.[6,29-31] Chemical cross-linking



รูปที่ 4 *พื้นที่ว่าง ความพรุน และพื้นที่ผิวของโครงข่ายไฟบรินที่กำลังขยาย 1,000 เท่าโดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) (A: กลุ่มควบคุม, B: กลุ่มที่บีบอัดด้วยความร้อน 60 องศาเซลเซียส C: กลุ่มที่บีบอัดด้วยความร้อน 70 องศาเซลเซียส D: กลุ่มที่บีบอัดด้วยความร้อน 80 องศาเซลเซียส E: กลุ่มที่บีบอัดด้วยความร้อน 90 องศาเซลเซียส และ F: กลุ่มที่บีบอัดด้วยความร้อน: 100 องศาเซลเซียส)*

Figure 4 *Interfibrous space, porosity and surface area of fibrin network at 1000x magnification using scanning electron microscope (SEM). (A: Control group, B: 60°C group, C: 70°C group, D: 80°C group, E: 90°C group and F: 100°C group)*

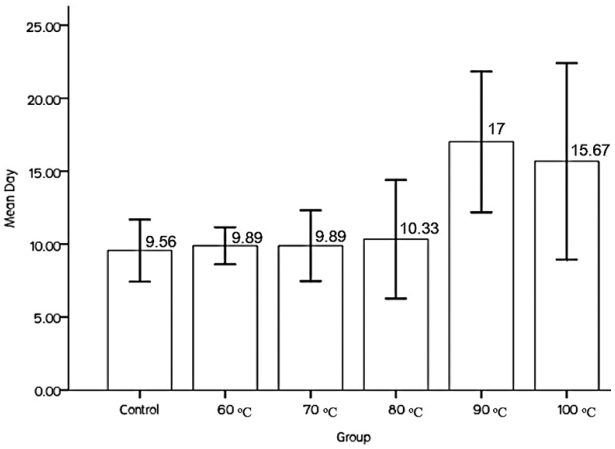
methods were commonly used, such as glutaraldehyde[32], genipin[33], polyepoxy[34], and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride.[35] Unfortunately, in an *in vivo* study[36], these methods increased cytotoxicity and caused a substantial inflammatory response. Alternative methods include physical cross-linking methods, such as heat treatment[31] and ultraviolet irradiation.[37] The results showed that cross-link modification increased the degradation time of non-cross-linked collagen membranes to 2-9 months.[31]

Only two studies[26,33] have investigated techniques for the cross-linking modification of PRF membranes, which is the same principle as cross-linked collagen membranes. In an *in vitro* study[33], genipin was used to modify cross-linking

of PRF membranes. It is a natural biodegradable molecule with low cytotoxicity. PRF membranes cross-linked with genipin have been reported to be more stable than non-cross-linked PRF membranes when they are soaked in trypsin. In 2015, Kawase, *et al.*[26] developed a heat-compression technique to modify the density of cross-linking of PRF membranes, leading to improved stability. Both *in vitro* and *in vivo* studies[26] revealed that cross-linking modification by short-term mild to moderate heat was able to delay degradation of PRF membranes. Therefore, the heat-compressed PRF membrane could be used as a barrier membrane. In their study, PRF was compressed at 90 and 120°C for 2-15 seconds and then punched out to form an 8-mm disk in the experiment. Accordingly, the PRF membrane samples only represented only parts of the whole PRF membranes.

In this study, we sought to investigate and develop a heat-compression technique using basic laboratory instruments. A heat-compression technique was selected because of its low cost and non-cytotoxicity.[26] The entire PRF membrane was used in this experiment to simulate degradation because the entire PRF membrane is usually used in daily practice. Different temperatures, including 60, 70, 80, 90 and 100°C, were chosen to compress the PRF membrane in heat-compressed groups in this study to investigate the appropriate temperature for a heat-compression technique.

The heat-compressed PRF membranes at 90 and 100°C showed complete degradation in 17 and 16 days, respectively. The degradation times of both groups were significantly longer than that in the control group, which showed complete degradation in 10 days (Figure 5). A previous *in vitro* study[26] reported the same result. It revealed that heat-compressed PRF membranes at 90°C were completely degraded over 10 days, compared with 5 days in the control group. When the heat-compressed PRF membranes were implanted in the subcutaneous tissue of nude mice, the degradation time extended to 4 weeks.[26] The variations between *in vivo* and *in vitro* studies may have been due to the different



รูปที่ 5 *ค่าเฉลี่ยระยะเวลาการเสื่อมสภาพ (วัน) ของแผ่นเยื่อเพลต-เลทริชไฟบรินจากแต่ละกลุ่ม*

Figure 5 *Mean degradation time (days) of PRF membranes from each group*

conditions. The same result is promising if further *in vivo* studies are conducted. Further investigation is needed.

Similar to the previous SEM study[26], the fibrin structure at the surface of the heat-compressed PRF membranes exhibited a significant reduction in porosity, interfibrous space and surface area. Higher temperatures were associated with less porosity, interfibrous space and surface area, especially at 90 and 100°C. There was also an obvious relationship between temperature and degradation time. At 90 and 100°C, the degradation time increased. Therefore, heat compression could modify the cross-linking of PRF membranes by making their structure denser and improving their mechanical stability and rate of degradation. Furthermore, the dense fibrin structure and the reduction in surface area decreased

degradation using human plasmin.

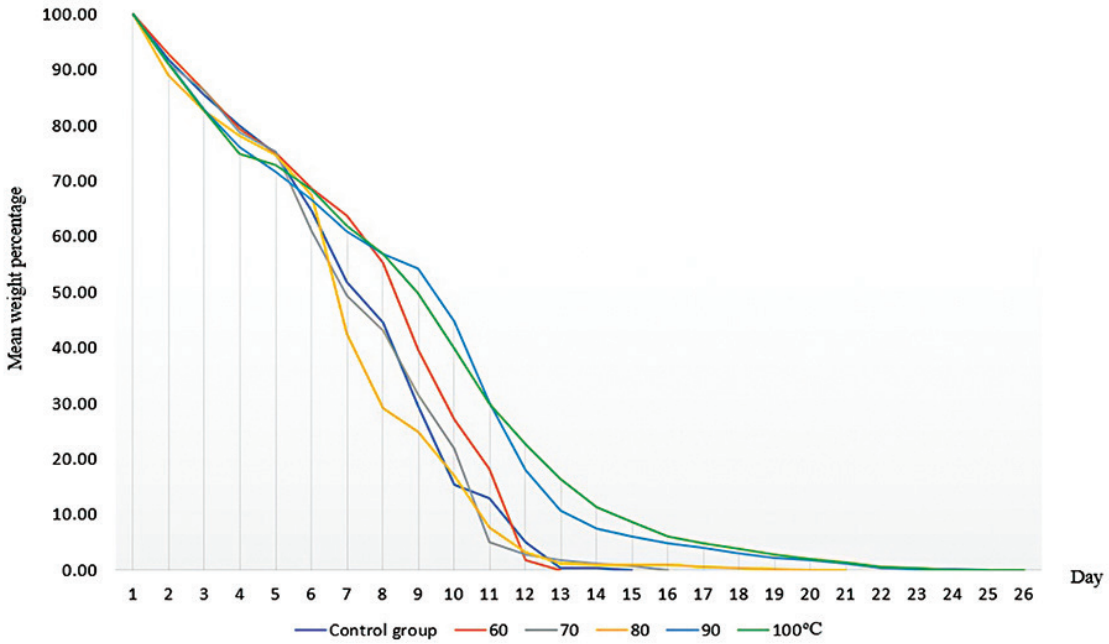
Although the heat-compression technique significantly extended the degradation time in this study, at least 4 weeks were required for periodontal tissue regeneration and integration.[14,26] However, this study revealed an approximately forty percent loss of PRF membranes weight within 1 week (Figure 6). Moreover, the weight of the PRF membranes rapidly decreased in the early phase in all groups except the 90 and 100°C groups, in which the membranes slowly degraded in the first 11 and 6 days, respectively. According to the results of the weight loss, heat-compressed PRF membranes had a slow rate of degradation in the early phase, but it was still an inadequate time to provide sufficient stability and maintain space for periodontal tissue and bone regeneration, similar to gauze-compression PRF membranes.[28]

However, heat-compressed PRF membranes can adapt to fit in some operations, such as socket preservation, closure of oro-antral communication and soft tissue wound dressing, which do not need long-term stability but need to be durable in the early phase.

For further studies, the biological and mechanical properties of heat-compressed PRF membranes should be investigated. The heat from this preparation technique may sacrifice some key components of PRF, such as growth factors (PDGF-AB and TGF-β1) and leukocytes. Therefore, PRF probably loses its regenerative ability to promote wound healing. Furthermore, the mechanical properties may change, such as mechanical strength and elasticity, which are one of important factors for a barrier membrane.

Conclusion

This study revealed that PRF membranes compressed at 90 and 100°C had significantly longer degradation times when compared to a gauze-compression group. However, weight loss did not significantly differ at each day, but heat-compression



รูปที่ 6 *ค่าเฉลี่ยร้อยละน้ำหนักของแผ่นเยื่อเพลตเลทริชไฟบรินในแต่ละกลุ่ม*

Figure 6 *Mean weight percentage of PRF membranes in each group.*

at 90 and 100°C delayed the early stage of degradation.

The surface fibrin structure at the surface of 90 and 100°C heat-compressed PRF membranes showed obvious reductions in porosity, interfibrous space and surface area compared to that of the other groups based on SEM. The heat-compression technique may modify cross-linking, improve stability and reduce the rate of degradation of PRF membranes.

In conclusion, heat-compression at 90 and 100°C using basic laboratory instruments can increase degradation time by up to 2 weeks. It can reduce weight loss in the early stage of degradation. Therefore, this preparation technique might be applied in surgical operations that need PRF membrane stability in the early phase. Further study of the other cross-linking techniques for cross-linked PRF membranes is needed.

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References

1. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *Int J Periodontics Restorative Dent* 2003; 23(4): 313-323.
2. Del Fabbro M, Bortolin M, Taschieri S. Is autologous platelet concentrate beneficial for post-extraction socket healing? A systematic review. *Int J Oral Maxillofac Surg* 2011; 40: 891-900.
3. Iasella JM, Greenwell H, Miller RL, *et al.* Ridge preservation with freeze-dried bone allograft and a collagen membrane compared to extraction alone for implant site development: a clinical and histologic study in humans. *J Periodontol* 2003; 74(7): 990-999.
4. Cardaropoli D, Tamagnone L, Roffredo A, Gaveglio L, Cadaropoli G. Socket preservation using bovine bone mineral and collagen membrane: a randomized controlled clinical trial with histologic analysis. *Int J Periodontics Restorative Dent* 2012; 32(4): 421-430.
5. Lekovic V, Camargo PM, Klokkevold PR, *et al.* Preservation of alveolar bone in extraction sockets using bioabsorbable membranes. *J Periodontol* 1998; 69(9): 1044-1049.
6. Bottino MC, Thomas V, Schmidt G, et al. Recent advances in the development of GTR/GBR membranes for periodontal regeneration--a materials perspective. *Dent Mater* 2012; 28(7): 703-721.
7. Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest 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* 2009; 18(2): 102-111.

8. Simonpieri A, Del Corso M, Sammartino G, Dohan Ehrenfest 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* 2009; 18(3): 220-229.
9. Gassling V, Purcz N, Braesen JH, 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* 2013; 41(1): 76-82.
10. Dapper WR, Shubin NJ. GBR The BACKBONE of implant dentistry: PART 1. *Dental Products Report* 2006; 40(9): 82-84.
11. Dapper WR, Shubin NJ. GBR: The BACKBONE of implant dentistry: PART 2. *Dental Products Report* 2006 ;40(10): 124-125.
12. Hurley LA, Stinchfield FE, Bassett CAL, Lyon WH. The role of soft tissues in Osteogenesis: an experimental study of canine spine fusions. *J Bone Joint Surg Am* 1959; 41(7): 1243-1266.
13. Karring T. Regenerative periodontal therapy. *J Int Acad Periodontol* 2000; 2(4): 101-109.
14. Aurer A, Jorgic-Srdjak K. Membranes for periodontal regeneration. *Acta Stomatol Croat* 2005; 39(1): 107-112.
15. Hartshorne J, Gluckman H. A comprehensive clinical review of Platelet Rich Fibrin (PRF) and its role in promoting tissue healing and regeneration in dentistry. Part 1: definition, development, biological characteristics and function. *Int Dent* 2016; 6: 14-24.
16. Naik B, Karunakar P, Jayadev M, Marshal VR. Role of Platelet rich fibrin in wound healing: a critical review. *J Conserv Dent* 2013; 16(4): 284-293.
17. Choukroun J, Diss A, Simonpieri A, *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* 2006; 101: E56-60.
18. Eshghpour M, Dastmalchi P, Nekooei AH, Nejat A. Effect of platelet-rich fibrin on frequency of alveolar osteitis following mandibular third molar surgery: a double-blinded randomized clinical trial. *J Oral Maxillofac Surg* 2014; 72(8): 1463-1467.
19. Bölükbaşı N, Yeniyol S, Tekkesin MS, Altunatmaz K. The use of platelet-rich fibrin in combination with biphasic calcium phosphate in the treatment of bone defects: a histologic and histomorphometric study. *Curr Ther Res Clin Exp* 2013; 75: 15-21.
20. Miron RJ, Fujioka-Kobayashi M, Hernandez M, et al. Injectable platelet rich fibrin (i-PRF): opportunities in regenerative dentistry? *Clin Oral Investig* 2017; 21(8): 2619-2627.
21. Gassling V, Douglas T, Warnke PH, Açil Y, Wiltfang J, Becker ST. Platelet-rich fibrin membranes as scaffolds for periosteal tissue engineering. *Clin Oral Implants Res* 2010; 21(5): 543-549.
22. Del Corso M, Vervelle A, Simonpieri A, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 1: Periodontal and dentoalveolar surgery. *Curr Pharm Biotechnol* 2012; 13(7): 1207-1230.
23. Aroca S, Keglevich T, Barbieri B, Gera I, Etienne D. Clinical evaluation of a modified coronally advanced flap alone or in combination with a platelet-rich fibrin membrane for the treatment of adjacent multiple gingival recessions: a 6-month study. *J Periodontol* 2009; 80(2): 244-252.

24. 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(3): e37-44.
25. Dohan DM, Choukroun J, Diss A, 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* 2006; 101(3): e45-50.
26. Kawase T, Kamiya M, Kobayashi M, et al. The heat-compression technique for the conversion of platelet-rich fibrin preparation to a barrier membrane with a reduced rate of biodegradation. *J Biomed Mater Res B Appl Biomater* 2015; 103(4): 825-831.
27. Blumenthal NM. The use of collagen membranes to guide regeneration of new connective tissue attachment in dogs. *J Periodontol* 1988; 59(12):830-836.
28. Hartshorne J, Gluckman H. A comprehensive clinical review of Platelet Rich Fibrin (PRF) and its role in promoting tissue healing and regeneration in dentistry. Part II: preparation, optimization, handling and application, benefits and limitations of PRF. *Int Dent* 2016; 6(5): 34-48.
29. Walker KA, Markoski LJ, Deeter GA, Spilman GE, Martin DC, JS M. Crosslinking chemistry for high-perfomance polymer networks. *Polymer* 1994; 35(23): 5012-5017.
30. Rodella LF, Favero G, Labanca M. Biomaterials in maxillofacial surgery: membranes and grafts. *Int J Biomed Sci* 2011; 7(2): 81-8.
31. Wang J, Wang L, Zhou Z, et al. Biodegradable polymer membranes applied in guided bone/tissue regeneration: a review. *Polymers* 2016; 8(4):115-135.
32. Veríssimo D, Leitão R, Ribeiro R, *et al.* Polyanionic collagen membranes for guided tissue regeneration: Effect of progressive glutaraldehyde cross-linking on biocompatibility and degradation. *Acta Biomaterialia* 2010; 6(10): 4011-4018.
33. Madurantakam P, Yoganarasimha S, Hasan FK. Characterization of leukocyte-platelet rich fibrin, a novel biomaterial. *J Vis Exp* 2015; 29(103): 1-8.
34. Miyata T, Fususe M, Yamane Y, Noishiki Y. A biodegradable antiadhesion collagen membrane with slow release heparin. *ASAIO Trans* 1988; 34(3): 687-691.
35. Park JY, Jung IH, Kim YK, et al. Guided bone regeneration using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)-cross-linked type-I collagen membrane with biphasic calcium phosphate at rabbit calvarial defects. *Biomater Res* 2015; 19(1): 15-25.
36. Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J. Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. *Clin Oral Implants Res* 2005; 16(3): 369-378.
37. Lee JE, Park JC, Hwang YS, Kim JK, Kim JG, Suh H. Characterization of UV-irradiated dense/porous collagen membranes: morphology, enzymatic degradation, and mechanical properties. *Yonsei Med J* 2001; 42(2): 172-179.

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