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Research Article

THE HEAT-COMPRESSED PLATELET-RICH FIBRIN PREPARATION AS A BARRIER MEMBRANE

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A B S T R A C T

Introduction: Platelet-rich fibrin (PRF) was developed to eliminate xenofactors (adding of bovine thrombin) from of platelet-rich plasma. It is mainly used as a source of growth factor for tissue regeneration. PRF in a compressed membrane-like form has also been used as a substitute for commercially available barrier membranes in guided-tissue regeneration (GTR) treatment.

Aim and Objective: To compare physical and histopathological properties of heat and gauze compressed PRF to be used as semi-rigid barrier for GTR. **Method:** A portion of the prepared PRF membrane was compressed with gauze and with an electric straightening iron. The PRF membrane was wrapped with a ultraviolet-sterilized plastic wrap (film of polyvinylidene chloride) which was compressed with the electric heat-compression device at 90 degree celsius for 15 s. Gauze and heat-compressed PRF were freshly prepared and were inserted in petri dishes. Incubated in a CO2 incubator with Hank's balanced salt solution (HBSS) supplemented with human plasmin (2µg/ml). Tensile strength was measured using Universal testing machine. Then heat and gauze compressed PRF were fixed in 10% neutralized formalin, dehydrated, embedded in paraffin block and sectioned sagittally. Sections were stained with Hematoxylin and Eosin (HE) and Masson Trichome (MT).

Results: Tensile strength of heat compressed PRF was stronger than gauze compressed PRF. The histopathologically heat compressed PRF showed increase crosslinking density among individual fibrin fibers which is expected to reduce the rate of degradation and allows it to serve as a more effective GTR barrier membrane.

INTRODUCTION

Platelet-rich plasma (PRP) was introduced as a potent source of growth factors for regenerative therapy,[1] several forms of application have been developed in many laboratories, including ours.[2,3] The most successful and widely used application for regenerative therapy is a gelation formed platelet-rich fibrin (PRF) made by an endogenous coagulation system. The primary advantage of PRF is that the gelation process does not require the addition of an animal derived thrombin.

Nonetheless, PRF is capable of functioning as a source of growth factor to facilitate tissue repair and regeneration. It is also used less often in a compressed membranous form as an absorbable barrier membrane in guided tissue regeneration (GTR) treatment.[5]

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This idea is based primarily on the chemical characteristic that PRF is composed of a biopolymer fibrin.

In addition, growth factors that can be released from the PRF membrane are expected to facilitate the tissue regeneration. However, to our knowledge, because of its rapid degradability, there has never been any published evidence demonstrating that a PRF membrane can maintain spaces for tissue regeneration for sufficient periods of time. To improve on this drawback, one option is to further crosslink the fibrin fibers within the PRF by appropriate treatments because the stability of polymer-based materials is known to be generally controlled by modifying the crosslink density among polymer fibers as described by Walker *et al* 1994[4]. Crosslinking treatments, regardless of methodology, can provide resistance against enzyme-dependent degradation while simultaneously sacrificing the bioactivity of the PRF. we hypothesized that the heat-compressed PRF membrane could be used as a GTR barrier membrane. Therefore, in this study, we employed a heat-compression technique for fibrin crosslinking and tested its degradability and physical properties in vitro.

Material and methods

PRF Preparation

All details of the study design and consent form were approved by the ethical committee .5 ml of blood was collected from healthy, nonsmoking volunteer aged 28 year (male) using VacutainerTM tubes and immediately centrifuged by a Medifuge centrifugation system at 3000rpm for 10 minutes . After the red thrombus (fraction of red blood cells) was eliminated from the PRF preparations, the resulting PRF was compressed with dry gauze for 15s.

Heat-compression of the PRF membrane

A portion of the prepared PRF membrane compressed with gauze was further compressed with an electric straightening iron for hair[1]. The surface temperature was adjusted at 90^0C; The PRF membrane preparations were wrapped with a ultraviolet (UV)-sterilized plastic wrap, which is a sealing film of polyvinylidene chloride and compressed with the electric heat-compression device for 2–15 seconds.[1]

Histological processing

Heat and gauze compressed PRF were fixed in 10% neutralized formalin dehydrated, embedded in paraffin block sectioned sagittally. Sections were stained with Hematoxylin and Eosin (HE) and Masson's trichrome**.** Hematoxylin and Eosin (HE) used for demonstration of nucleus and cytoplasmic inclusions in clinical specimens and Masson's trichrome used to evaluate type and amount of extracellular material like collagen, fibrin and muscle**.**

Physical properties

Tensile strength was measured by universal testing machine. The "universal" part of the name reflects that it can perform many standard tensile and compression tests on materials, components, and structures. The specimen was placed in the machine between the grips and an extensometer. Once the machine was started it begins to apply an increasing load on specimen. Throughout the tests the control system and its associated software record the load and extension or compression of the specimen.

Accelerated degradation in vitro test

Two types of PRF membrane disks (gauze-compressed vs. gauze and heat-compressed) were freshly prepared and were inserted in 24-well plates and incubated in a CO2 incubator with Hank's balanced salt solution (HBSS) supplemented with human plasmin (2 lg/mL).[1]HBSS was added to each well (24-well plate) by 0.5 mL and changed every 2 days.

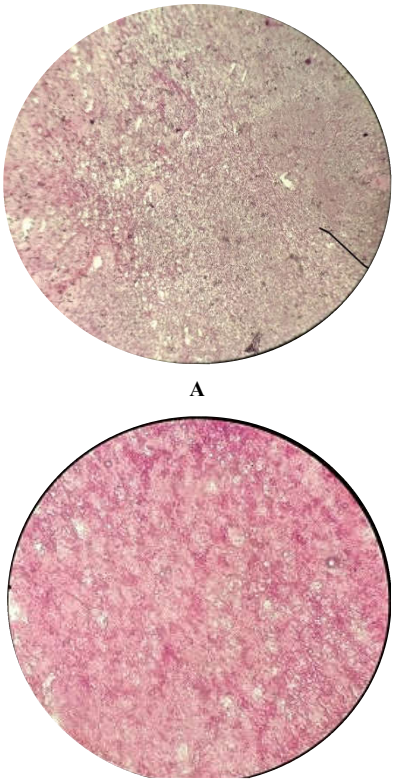
RESULTS

Histopathologically gauze compressesd platelet rich fibrin showed sparse crosslinking density among individual fibrin fibers, where heat compressed showed increased crosslinking density among individual fibrin fibers. The tensile strength of heat compressed PRF was greater than gauze compressed PRF.

The gauze-compressed control PRF was degraded in a timedependent manner and virtually completely digested at 6 days of incubation. In contrast, the heat-compressed PRF initially turned the HBSS cloudy (4 days), but thereafter the heat-compressed PRF did not show appreciable degradation for at least 10 days of incubation.

Table I Tensile strength of PRF membranes

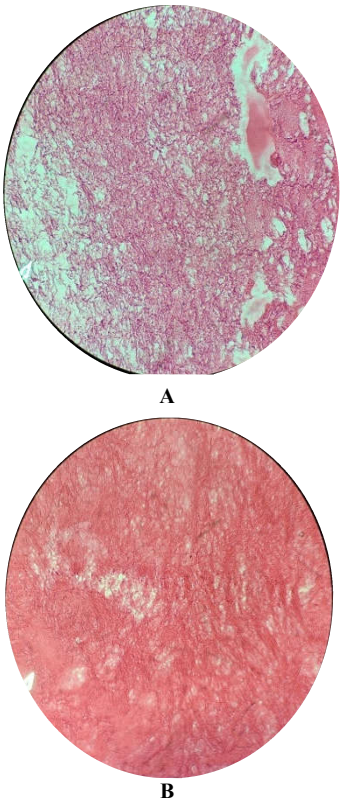
Types of PRF Membranes	Tensile strength
Gauze compressed PRF	0.02 N/mm^2
Heat compressed PRF	0.05 N/mm^2



A

B

Figure I Representative microscopic findings of PRF membrane preparations (A) The gauze-compressed PRF. (B) The heat-compressed PRF(Massion's Trichrome Stain)



A

B

Figure II Representative microscopic findings of PRF membrane preparations (A) The gauze-compressed PRF. (B) The heat-compressed PRF(Hematoxylin And Eosin Stain)

DISCUSSION

The Primary Advantage of PRF is that it serves as a source of growth factors for enhancing regenerative procedures as well as PRP. Furthermore, fibrin and fibrinogen in PRF preparations are expected to function as cell-scaffolds and adhesives. Recently, several clinical investigators have proposed the use of its membranous form as a substitute of commercially available GTR barrier membranes in a clinical setting.

It is generally thought that a barrier membrane should be preserved at the implantation site for 3–4 weeks to enhance periodontal tissue regeneration and integration. 15 Among absorbable membranes, those made of synthetic polymers such as polyglycolic acid and polylactic acid copolymer, demonstrate a slow degradation rate (=12 months), whereas collagen-based membranes degrade faster and have been reported to remain stable for 16–38 weeks without significant degradation.16 However, noncrosslinked collagen membranes lose their structural integrity in 7 days. The present in vivo animal implantation study demonstrated that the PRF could degrade as fast as noncrosslinked collagen-based membranes. Therefore, it is possible that increased crosslinking density among individual fibrin fibers within a PRF could prolong the preservation of the PRF at the implantation site and allow it to serve as a more clinically optimal GTR membrane.

We propose that short-term mild to moderate heat induces an increased crosslinking density among individual fibrin fibers. This increased crosslinking density would be expected to reduce the rate of degradation of the PRF membrane and allow it to serve as a more effective GTR barrier membrane.

Therefore, even though the heat compression further crosslinks the fibrin fibers within the PRF, it can be speculated that the heat-compression reduces the enzymatic degradation rate primarily through modifying microstructure of the PRF surface.

CONCLUSION

Our heat-compression technique successfully delayed the degradation of the PRF membrane preparation and also increased tensile strength which would be compatible with periodontal tissue regeneration. In addition, the protocol of this compression technique is very user-friendly from a clinical perspective. Therefore, we believe that the heat-compressed PRF membrane has the potential to be widely applied as a barrier membrane in periodontal GTR therapy.

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