The Relevance of Choukroun’s Platelet-Rich Fibrin and Metronidazole During Complex Maxillary Rehabilitations Using Bone Allograft. Part I: A New Grafting Protocol

Alain Simonpiéri, DDS.* Marco Del Corso, DDS,† Gilberto Sammartino, MD, PhD,‡ and David M. Dohan Ehrenfest, DDS, MS, PhD§

For the past several years, preimplant surgery has made it possible, through the use of bone grafting, to obtain a more or less total reconstitution of the alveolar walls. This first therapeutic step allows the adequate positioning of the implants and the long-term success of implant-supported reconstruction. However, these large-scale grafts often require autogenous bone taken from the patient’s calvaria (parietal bone is very often used in France) or iliac crest. It implies a rather significant operation under general anesthesia, with all of the risks associated with hospitalization and with donor site morbidity. The relative seriousness of such a procedure discourages many patients and dissuades them from using these treatments which, however, are essential to their facial rehabilitation, after many years of psychological, esthetic, and functional discomfort.

To simplify the graft protocols, bone substitutes have been considered as an alternative solution to the autogenous grafts. These substitution biomaterials can be divided into 3 overall groups: entirely synthetic products (Cerasorb, Nanobone, etc.), xenografts (lyophilized bovine bone, BioOss type, etc.), and allogeneic grafts (human bone from a tissue bank). This allogeneic bone is generally considered as an efficient and secure product, and allogeneic grafts (human bone from a tissue bank) have already been used as a reliable source. This allogeneic bone is generally considered as an efficient and secure product, and allogeneic grafts (human bone from a tissue bank) have already been used as a reliable source. Extensive bone grafting remains a delicate procedure, because of the slow and difficult integration of the grafted material into the physiological architecture. The recent use of platelet concentrates aims to improve this process of integration by accelerating bone and mucosal healing. Choukroun’s platelet-rich fibrin (PRF) is a healing biomaterial that concentrates in a single autologous fibrin membrane, most platelets, leukocytes, and cytokines from a 10 mL blood harvest, without artificial biochemical modification (no anticoagulant, no bovine thrombin). Whether used as a membrane or as fragments, PRF allows a significant postoperative protection of the surgical site and seems to accelerate the integration and remodeling of the grafted biomaterial. These properties are particularly helpful for vestibular bone grafting on the alveolar ridges. Moreover, it provides a very high quality of gingival maturation.

A small quantity of a 0.5% metronidazole solution (10 mg) can also be used to provide an efficient protection of the bone graft against unavoidable anaerobic bacterial contamination. This article describes a new technique of total maxillary preimplant bone grafting using allograft. Choukroun’s PRF membranes and metronidazole. This first part focused on the preimplant reconstructive treatment using allogeneic bone granules. PRF membranes are particularly helpful to protect the surgical site and foster soft tissue healing. This fibrin biomaterial represents a new opportunity to improve both the maturation of bone grafts and the final esthetic result of the peri-implant soft tissue. (Implant Dent 2009;18:102–111)

Key Words: bone graft, fibrin, freeze-dried bone allograft, platelet concentrate, platelet-rich fibrin (PRF), platelet-rich plasma, metronidazole, sinus-lift
these concepts. The innovative bio-
material can be defined as a platelet-
and immune-conducive constituent from a 10 mL sample of blood.

PRF was perfected in France by Choukroun et al. in 2001. Unlike other platelet concentrates developed throughout the world,24–26 this technique requires neither anticoagulants nor bovine thrombin (nor any other gelifying agent). It is nothing else than centrifuged blood without additives, conforming to all French laws related to the reimplantation of blood products. This technology requires adequate table centrifuge and collection kit (PC-02, Process Ltd., Nice, France).27

The protocol is very simple: whole blood is drawn in 10-mL tubes without anticoagulant and is immediately centrifuged at around 400 g for 12 minutes (Process protocol, Nice, France). Within few minutes, the absence of anticoagulant allows the activation of the majority of the platelets contained in the sample, in contact with the tube walls, thus triggering a coagulation cascade. The fibrinogen is at first concentrated in the upper part of the tube, until the effect of the circulating thrombin transforms it into a fibrin network. The result is a fibrin clot located in the middle of the tube and soaked with cellular plasma, with a maximum number of platelets caught in the fibrin mesh.

When used as a membrane, PRF enables the protection of operative sites from outside aggression and serves as a matrix to accelerate the healing of wound edges,28 much like a fibrin bandage.22,22 When mixed with the graft material, the fibrin clot functions as a biological connector between the different elements of the graft, and as a matrix which favors neo-angiogenesis, the capture of stem cells, and the migration of osteoprogenitor cells to the center of the graft.22,22 The addition of PRF to graft material could therefore become a serious opportunity to develop new therapeutic procedures by improving the integration of bone substitution materials during preimplant grafting.29–32 The clinical case that follows is a detailed illustration of this concept.

**Clinical Illustration**

**The Initial Situation**

A 52-year-old female patient consulted on December 4, 2002. Because of a series of infections and a severe periodontal disease which was not immediately diagnosed, she had for many years been wearing a partial removable dental prosthesis. The 3 remaining teeth (17, 26, 27), secondarily infected, had induced a polypoid reaction in the mucous membrane of the right sinus. Given her young age, thisoption was, for her, a source of permanent psychological suffering and a symbol of the mutilation of her face. Under the prosthesis, the maxillary alveolar ridges had already undergone significant centripetal resorption, both in height and—especially—in thickness. It was thus impossible to place axial implants without a preimplant reconstruction of the maxillary using bone grafts. This diagnosis was confirmed by the computed tomography scan examination, which clearly showed a subcranial bone height of 3 mm and anterior ridges measuring less than 3 mm thick (Fig. 1). It was decided to first eliminate all remaining maxillary and mandibular sources of infection. Then, a complete removable maxillary prosthesis and a partial mandibular prosthesis were temporarily used.

After being clearly and openly informed on the different therapeutic options available to her, the patient accepted a multiphase treatment plan combining preimplant allografts and complete implant-supported maxillary rehabilitation.

**Graft Surgery**

During the preimplant phase, it was necessary to reconstruct resorbable maxillary bone structures. Indeed, the remaining architecture was unsuitable for adequate implantation. The entire surgical site was first opened, then the medullar bone along the vestibular side of the anterior alveolar ridges was stimulated, to facilitate proper vascularization and integration of the graft.
Fig. 2. First, the entire surgical site was opened (A). The medullar bone along the vestibular side of the anterior alveolar ridges was stimulated (B). In the posterior area, the 2 sinuses were opened to be filled (C and D).

Fig. 3. The mixture of human lyophilized bone (Phoenix freeze-dried bone allograft, TBF, France) and PRF, soaked in metronidazole, was used to fill the sinuses and to considerably thicken the vestibular side of the anterior and central alveolar ridges (A). PRF membranes were placed to cover the entire grafted area (B). After suturing, the PRF membranes were protected under the flap (C). One day after the graft surgery, healing of the incision line seemed already satisfactory for the protection of the graft underneath.

were obtained using the standard protocol described in the scientific literature.18,19

To avoid pressure on the grafted material (it could induce mobility and fibrosis of the graft), the patient was not allowed to wear a temporary removable prosthesis for the 40 days after the surgery. This waiting period is considerably shorter in the case of autogenous onlay bone grafts fixed with screws, because the allograft biomaterial is constituted of bone granules and has not a rigid architecture. The initial healing time should be long enough to allow the bone graft to become compact, before functional forces are applied.

Ten weeks (75 days) after the surgery, a preimplant computed tomography scan was performed. There was a radiological homogeneity between the grafted bone and the remaining alveolar walls (Fig. 5). The graft appeared to be integrated.

DISCUSSION
PRF and Metronidazole for Bone Grafting
Choukroun’s PRF is a matrix of autologous fibrin, in which are embedded a large quantity of platelet and leukocyte cytokines during centrifugation.14-21 The intrinsic incorporation of cytokines within the fibrin mesh implies their progressive release over time, as the network of fibrin disintegrates. At one of its extremities, each PRF clot also concentrates most platelets and leukocytes collected in its 10 mL tube of blood.

The use of this platelet and immune concentrate during bone grafting offers the following 4 advantages:

First, the fibrin clot plays an important mechanical role. Although it does not possess significant adhesive properties (unlike fibrin glues or platelet-rich plasmas), the strength of PRF membranes enables a biomaterial to be maintained and protected against moderate parasitic forces.22 Moreover, mixed with the graft, PRF fragments serve as a biological connector between bone particles. Soaked in serum, they favor the adhesion of allogeneic bone chips and constitute a biological cement between these fragments. This cohesion gives to the graft a biomechanical strength which is crucial during the first steps of healing, particularly on a site as exposed as the vestibular surface of a maxillary alveolar wall.

Second, the integration of this fibrin network with fragments of allogeneic bone facilitates cellular migration, particularly for endothelial cells necessary for the neo-angiogenesis, vascularization and survival of the graft, as well as for mesenchymal stem cells (drifting or dose to wound site). The incorporation of a fibrin network into a grafted mass of lyophilized inactive bone would thus be very positive: paths of cellular migration would appear within the bundles of PRF that vein the graft. Moreover, this fibrin bandage acts as a healing matrix for the soft tissue around the incision and the whole wounded site. Indeed, we can systematically observe a high gingival maturational change after healing on PRF membranes, with a thickening of keratinized gingival tissues which improves the esthetic integration and final result of prosthetic rehabilitations.23

Third, the platelet cytokines, essentially platelet-derived growth factors, transforming growth factor β-1, and insulin-like growth factors, seem
gradually released as the fibrin matrix is resorbed, thus creating a perpetual process of healing. These mechanisms of gradual release are used by the organism to guide healing and connective tissue remodelling (such as bone), but such a phenomenon is difficult to reproduce synthetically. PRF, however, through physiological polymerization, seems to have this slow release property. It reproduces the elementary mechanisms of hemostasis and healing, on the scale of a fibrin clot which is large enough to be used clinically. In the middle of a bone graft, particularly with lyophilized bone, such a mid- and long-term capacity to maintain healing and remodelling would be extremely beneficial for the maturation of the graft and its overall integration over time. 

Lastly, the role of PRF in immunity seems significant, because of the presence in the fibrin clot of an important number of leukocytes activated by the centrifugation, and because of the incorporation of inflammatory and anti-inflammatory cytokines into the network of fibrin. The gradual release of these molecules would play a significant role in the self-regulation of inflammatory and infectious phenomena within the grafted material. Indeed, all clinical experiences emphasized that the use of PRF seems to reduce postoperative pain and edemas, and to limit even minor infectious phenomena. The control of inflammation and especially the risk of sepsis within a bone graft seems yet another decisive reason to use PRF during bone grafting.

Metronidazole could be another decisive surgical adjuvant. This antibiotic from the nitro-5-imidazole family is often used orally or applied locally. Incorporated into the graft material during bone graft surgery, it protects against the systemic peroperative contamination by anaerobic bacteria. However, metronidazole will never replace rigorous aseptic conditions during the operation, combined with a general antiseptic covering. This protocol is not an antibioticotherapy: 2 mL of this 0.5% solution are containing only 10 mg of metronidazole, i.e., 1/20 of a standard 200 mg oral tablet. This is just enough to limit the contamination of the biomaterial and to protect the early phases of bone construction from infection and the related inflammatory reaction. The local use of this small quantity of metronidazole thus enables the practitioner to increase the quality of maturation of the graft, and to reduce the risk of developing bone infection and, eventually, necrosis.

The combination, within the graft, of these 2 adjuvants seems to increase the radiological and histological quality of the grafted bone tissue. The use of PRF membranes likewise considerably improves the healing and maturation of soft tissue. In this sense, PRF is a healing concentrate: it contains, in a single usable membrane, most key elements of hemostasis and healing.

CONCLUSION

The use of bone substitutes during extensive bone grafting remains a delicate procedure, because of the challenge of adequate integration of the graft. The use of PRF during these interventions offers better postoperative control of the surgical site and seems to accelerate the integration and remodeling of the grafted biomaterial. Combined with existing maxillofacial implant reconstructive therapy, PRF represents a new opportunity to improve grafting procedures, keeping in mind that it is a healing biomaterial and not a "miracle" product. It just increases the potential for therapeutic success when used by a skilled practitioner.

Disclosure

The authors claim to have no financial interest, directly or indirectly, in any entity that is commercially related to the products mentioned in this article.

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Fig. 4. Three days after the graft surgery, the sutures were removed. The PRF membranes had acted as fibrin bandages, enabling quick closure of the surgical site despite the substantial volume of bone added.

Fig. 5. Ten weeks (75 days) after surgery, a preimplant computed tomography scan showed that the graft seemed integrated. There was a complete radiological homogeneity between the grafted bone and the remaining alveolar walls.

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Repport requests and correspondence to:
Dafid M. Dohan Birenszt, DDS, MS, PhD
Department of Biomaterials
Institute for Clinical Sciences
The Sahlgrenska Academy at University of Gothenburg
Medicalsgatan 18, 41390 Gothenburg, Sweden
Phone: 02 33 6 64 72 96 40
E-mail: LoB5@mac.com or
drhondan@hotmail.com

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SCHLÜSSELWÖRTER: Knochentransplantat, Fibrin, gefriergetrocknetes Knochenallotransplantat (FDBA), Thrombozytkonzentrat, Thrombozytreiches Fibrin (PRF), Thrombozytreiches Plasma (PRP), Metronidazol, Sinusanhebung

SPANISH / ESPAÑOL

ABSTRACTO: Los extensos injertos de hueso siguen siendo un procedimiento delicado, debido a la integración lenta y dificultosa del material injertado en la arquitectura fisiológica. El uso reciente de concentrados de plaquetas trata de mejorar este proceso de integración al acelerar la curación del hueso y la mucosa. La fibrina rica en plaquetas de Choukroun (PRF por sus siglas en inglés) es un biomaterial de curación que concentra en una sola membrana de fibrina autóloga la mayoría de las plaquetas, leucocitos y citocinas de una recolectión de sangre de 10 mL sin modificación bioquímica artificial (sin anticoagulante, sin trombina de bovinos). Ya se que se use como membrana o como fragmentos, la PRF permite una protección postoperatoria significativa del lugar quirúrgico y parece acelerar la integración y remodelación del biomaterial injertado. Estas propiedades son particularmente útiles para el injerto de hueso vestibular en las crestas alveolares. Además, proporciona una maduración gingival de muy alta calidad. También se puede usar una pequeña cantidad de solución de metronidazol al 0.5% (10 mg) para proporcionar una protección eficaz del injerto de hueso contra la contaminación inevitable con bacterias anaerobias. Este artículo describe una nueva técnica para el injerto de hueso maxilar total previo al implante usando un aloinjerto, las membranas de PRF de Choukroun y metronidazol. Esta primera parte se concentra en el tratamiento reconstructivo previo al implante.
usando gránulos de hueso alogénico. Las membranas de PRF son particularmente útiles para proteger el lugar quirúrgico y apoyar la curación del tejido suave. Este biomaterial con fibrina representa una nueva oportunidad para mejorar la maduración de los injertos de hueso y el resultado estético final del tejido suave periimplante.

PALABRAS CLAVE: injerto de hueso, fibrina, aloinjerto de hueso congelado-desecado (FDBA por sus siglas en inglés), concentrado de plaquetas, fibrina rica en plaquetas (PRF), plasma rico en plaquetas (PRP), metronidazol, elevación del seno

RESUMO: O enxertamento extensivo de osso permanece um procedimento delicado, devido à lentas e dificil integração do material enxertado na arquitetura fisiológica. O uso recente de concentrados de plaquetas visa melhorar esse processo de integração acelerando a cura do osso e mucosal. A Fibrina Rica em Plaquetas (PRF) de Choukroun e metronidazol durante complexas reabilitações maxilares usando enxerto aloplástico. Parte I: um novo protocolo de enxertamento. Parte I

AUTOR(ES): Alain Simonpieri, Cirurgião-Dentista, Marco Del Corso, Cirurgião-Dentista, Gilberto Sammartino, Mestre em Odontologia, PhD, David M. Dohan Ehrenfest, Cirurgião-Dentista, Mestre em Ciência, PhD. Correspondência para: David M. Dohan Ehrenfest, Department of Biomaterials, Institute for Clinical Sciences, The Sahlgrenska Academy at University of Gothenburg, Medicinaregatan 8B, 41390 Gothenburg, Sweden. Telefone: 00 33 6 64 72 95 40, e-mail: doldohan@hotmail.com


RELEVANCE OF CHOUKROUN’S PRF AND METRONIDAZOLE • SIMONPIERI ET AL

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ÖZET: Büyük ölçüde kemik grefflere, greft materyalinin fizyolojik mimariye ağırlı ve zor entegrasyonu nedeniyle nazik bir prosedürdür. Yakın geçmişte platelet (trombosit) konsantrelerinin kullanılımı kemik ve mukoza iyileşmesini hızlandırmak suretiyle bu entegrasyon sürecini geliştirmeyi amaçlamaktadır. Choukroun'un Plateletten Zengin Fibrini (PRF), yapay biyo-mekanik değişiklik olmadan (anti-koagülent veya bovin trombin olmadan) 10 mL'lik bir kan hasatmdan alınan çoğu plateleti, 10 kositi ve sitokini tek bir otolog fibrin membranında yoğunlaştırılarak iyileştirici bir biyo-materyaldir. Gerek membran olarak, gerekse parça olarak kullanıldığında PRF, cerrahi yerinde anlamlı derecede post-operatif koruma sağlayarak greftlenen biyo-materyalin entegrasyonunu ve yeniden şekillenmesini hızlandırmaktadır. 

Bu nitelikler, özellikle alveoler krette vestibular kemik grefflere yardımı olur. Ayrıca, bu şekilde yüksek kaliteli dış eti olgunlaşması da sağlanmaktadır. Kemik greftini kaçınılmaz anaerobik bakteri kontaminasyonuna karşı etkin bir şekilde korurak için küçük mikarda 0.5% metronidazol sıvısı (10 mg) da kullanılmaktadır. Bu çalışma, allogreff, Choukroun'un PRF membranları ve metronidazol kullanılarak gerçekleştiren yeni bir total maksiller implant oncesi kemik greftleme yontemini sunmaktadır. Bu birinci bölüm, allogenik kemik granülleri kullanın implant oncesi rekonstrüktif tedaviye odaklanmaktadır. PRF membranları, cerrahi yerinde korunmak ve yumuşak dokunun iyileşmesini teşvik etmek açıdan etkindir. Bu fibrin biyo-materyali, hem kemik grefflere olgunlaşmasını hem de peri-implant yumuşak dokunun son estetik görünüşünün geliştirilmesi açısından yeni bir fayda sunmaktadır.

ANAHTAR KELİMELER: kemik greffe, fibrin, dondurulmuş kuru kemik allogreff, plateletten Zengin Fibrini (PRF) ve metronidazol. Bölüm I: Yeni bir grefflere Protokolü. Bölüm I:
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Common research者氏名: Alain Simonpieri DDS, Marco Del Corso DDS, Giuseppe Del Corso DDS, Gilberto Sammartino MD, PhD, David M. Dohan Ehrenfest DDS, MS, PhD.

パート1

研究概要: 大規模な移植術は移植材が生理的構造へ融合するまで時間がかかる。かつ困難なために未だに微少ない処置である。最近は骨と粘膜の治癒を促進するためプロセスを改善するため、厚手血小板製剤が使用されている。コープラン多血小板組織調製（PRF）は治癒力を生かしたバイオマテリアルで、採血量10mlから分別した多量の血小板と白血球を含むサイクライドを人工化学修正（凝固剤またはシクロピロピジン）を加えて、1枚の同種移植組織成膜用プレートに製造したものである。PRFは腫瘍ならびに創部の使用法で術後部位の保険にすぐれ、また移植後のバイオマテリアルの融合と改変も促進するといわれる。

こうした特性は口腔内外の移植材にすることがわくわくであるばかりか、極めて優れたな骨形成を助けるにも役立つ。さらに数値（10mg）の5.3％メトロニダゾール溶液を使用することによって、骨移植部位を不可避な損傷性パクテリア感染か効果的に保菌することができる。

この論文はコープランPRF膜を使用した、インプラント術前処置上顎骨移植の新テクニックを説明したものである。パート1では同種移植骨移換を使用したインプラント術前再建治療に焦点をあてた。

PRF膜は術後部位の保菌に欠かす変態を示し、また新組織治癒を促進する。この組織成膜バイオマテリアルは骨移植を成熟化し、最終的にインプラント周辺組織の新系性を改善する新たな将来性を特徴づけている。

キーワード: 骨移植、組織調製（フィブリン）、ヒト冷凍保存骨（FBHA）、濃縮血小板製剤、多血小板組織調製 (PRF), 多血小板血漿 (PRP), トロニダゾール, 交差幹手術

ご質問の宛先: David M. Dohan Ehrenfest, Department of Biomaterials, Institute for Clinical Sciences, The Sahlgrensk Academy at University of Gothenburg, Medicinaregatan 8B, 41390 Gothenburg, Sweden.
電話: 003366472 95 40 *電子メール: Lob5@mac.com または ddrdohand@hotmail.com

CHINESE / 中國語

使用異體骨移植進行複雜上顎重建期間・Choukroun的術後組織的缺陷蛋白 (PRP) 及甲磺醯的相關性・第一部分

作者: Alain Simonpieri, DDS; Marco Del Corso, DDS; Gilberto Sammartino, MD, PhD; David M. Dohan Ehrenfest, DDS, MS, PhD.

摘要:
大規模骨移植仍是一項棘手的手術，原因在於移植材料與生理架構的整合緩慢而困難。舊有術後血小板凝固液的使用，其目標是加速加速骨質與粘膜結合，改進此整合流程。Choukroun的術後組織的缺陷蛋白 (PRP) 是一個具有治療功用的生物材料，可以從10ml的血小板凝固液中收集到自體組織蛋白，大多數血小板、白血球與組織因子，有著人工化修整（凝固凝固液，靜物性物質萃取的凝血酶）。無論是作為溝流或製品，PRP都能提供手術部位疑問的術後保護，而且似乎能加速移植材料的整合與復原，這特性對於術前骨質上的術前骨移植尤其有幫助。此外，它也提供非常高品質的血漿酸化碳-使用少量的甲磺醯溶液 (0.5%, 10mg) 也可以提供有效的骨移植保護。防治難以避免的銀氰酸塩感染。

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글 형 동종이식을 이용한 임상 실험물 중 Choukroun의 혈소판 풍부 섬유소(PRF)와 메트로니다졸의 관련성 (책임성 Part I: 새로운 이식 프로토콜 Part I)

저자: 알랭 시몬피에리 (Alain Simonipecri), DDS, 마르코 델 코소 (Marco Del Corso), DDS, 잭레르도 삼마르티노 (Gilberto Sammartino), MD, PhD, 데이비드 M. 도한 에렌페스트 (David M. Dohan Ehrenfest, DDS, MS, PhD)

요약:
광범위한 글 이식은 일정의 경로함을 요하는 작업으로, 이식 물질의 생리구조 내 융합 지역과 어려움이 따른다. 류근 사용된 혈소판 동종들은 빠른 후식의 치유를 가속화함으로써 이러한 융합 과정을 개선시킬 수 있는 목적을 한다. Choukroun의 혈소판 풍부 섬유소(PRF)는, 인공적으로 생화학적 변형(혈장고체 처리 및 소(bovine)의 프로틴이 없ᆫ)을 하지 않은 10mL 혈액 재귀물의 단일 자가 섬유소막 및 대부분의 혈소판, 페혈구, 그리고 사이토카인을 동축한 치유 물질들이다. 만약 사용되거나 조각으로 사용되는 지 상관없이, PRF는 수술 후 수술 부위의 중요한 보호작용을 하며 이식한 생물제의 융합과 재활을 가속화시키는 것으로 보인다. 이러한 속성들은 특히 이식 후 일기의 정액이 이식 후 고정 끈이 염과 동시에 잦으므로 갈 상실을 해고, 소량의 0.5% 메트로니다졸 용액(10mg) 또한 글 이식 시 혈기성질 강화를 위해 경제적으로 보안하는 것을 효과적으로 보호한다.

본 논문은 Choukroun의 PRF 작과 메트로니다졸 동종이식을 이용한 임상 실험 일괄락에 전 기어식에 적용되는 새로운 기술에 대해 기술하고 있다. 제 I부에서는 동종 글 피질을 이용한 일괄락 전 실험 치료에 집중하였다. PRF는 특히 수술후 보호와 연 조직 치유 촉진에 도움이 된다. 섬유소 생물제품은 글 이식의 성장도를 함축시키고 최적으로 염증반응 수면 연조직에 상이적 효과를 이끌어내는 새로운 기여를 제공한다.

키워드: 글 이식, 냉동간조 동종이식(FDBA), 혈소판 동종물, 혈소판 풍부 섬유소 (PRF), 혈소판 풍부 혈장 (PRP), 메트로니다졸, 상용 동종술

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