Socket Augmentation Using Atorvastatin With Or Without PRGF Derived Fibrin Scaffold
(Clinical and Histomorphometric study)

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BY

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Introduction

Approximately 50% of alveolar ridge reduction in thickness occurs over a 6–12 months period after tooth extraction ***(Johnson K. 1969).*** The severity of bone loss and the healing pattern may pose a problem for the clinician in 2 ways: it creates an esthetic problem in the fabrication of an implant-supported restoration or a conventional prosthesis; and it may make the placement of an implant challenging if not unfeasible ***(Lekovic V, et al. 1998)***. Furthermore, the placement of an implant requires sufficient alveolar bone support because lack of bone volume is correlated with poor osseointegration ***(Iasella JM, et al. 2003).***

Immediately after tooth extraction a cascade of inflammatory reactions is activated, the alveolar socket is filled by blood clot that is replaced by granulation tissue within 1 week; the epithelium migrates over the granulation tissue to cover the healing socket. Starting from the apical and lateral residual bony walls, the granulation tissue is rapidly remodeled to provisional matrix. Mineralizing processes occurs leading to the formation of woven bone that eventually is replaced by mature lamellar bone ***(Trombelli L, et al. 2008, Giorgio P, et al 2012)***

Bone depositions continue for several months but will not reach the coronal bone level of the neighboring teeth***(Schropp L, et al. 2003)***. Significant resorption of the alveolar ridge occurs after tooth extraction***(Araujo MG & Lindhe G, et al. 2005).***

The degree of residual ridge resorption is closely related to the time since tooth extraction in both maxilla and mandible***(Ulm C, et al.1992).***The reduction in width is usually greater than the loss in height,and bone resorption is significantly larger at the buccal than at the lingual side ***(Lekovic V, et al.1997).***

According to the Osteology Consensus Group 2011, ridge preservation is a general term for interventions that aim to preserve the ridge volume within the envelope existing at the time of extraction***(Christoph HF, et al 2011)***.

In order to reduce alveolar bone dimensional changes, several techniques aiming at enhancing the regeneration process in the extraction socket have been adopted' Such as filling the socket with autogenous bone graftsor bone substitutes,guided bone regeneration (GBR) with resorbable or non-resorbable barriersand the use of various bone promoting molecules such as enamel matrix derivativerecombinant growth and differentiation factorsand autologous platelet concentrates***(Darbyl I, et al. 2009, Horowitz R, et al. 2012).***

Statins (hydroxymethylglutaryl co-enzyme A reductase inhibitors) are a group of lipid-lowering drugs that are widely used to prevent cardiovascular events ***(Sotiriou CG & Cheng JW. 2000),*** they are used orally to treat hypercholesterolaemia and hyperlipidaemia with well documented safety profile ***(Guthrie RM, 2006).***

Furthermore, statins have been reported to stimulate the expression of bone anabolic factors, such as vascular endothelial growth factor and BMP-2 ***(Maeda T, et al. 2003),*** and to promote osteoblast differentiation and mineralization. In addition, it has been suggested that statins directly affect osteoclasts through mechanisms analogous to those of bisphosphonates ***(Staal A, et al. 2003, Maeda T, et al.2004).***

Statins can also modify the inflammatory cascades through pleiotropic actions at multiple levels, such as changing inflammatory mediators, altering leukocyte–endothelial cell interaction, and reducing expression of major histocompatibility complex- II ***(Terblanche M, et al. 2007).***

Among statin drugs; Atrovastatin (ATV) has been demonstrated to exhibit favorable effects in the treatment of bone remodeling disorders and bone fractures through the promotion of osteogenesis and the reduction of bone resorption ***(Wang JW, et al. 2007).***

The successful use of ATV to promote bone formation in vivo depends on the local concentration and there have been continuous efforts to find an appropriate

delivery system ***(Kishida Y, et al.1991),*** which allow localization and retention of the molecule and providing a matrix for mesenchymal cell infiltration and a substrate for cell growth and differentiation with degradation rate that does not inhibit bone growth and prevent fibrous tissue formation or fibrous encapsulation of the carrier ***(Elavarasu S, et al. 2012)***.

However, the most important limitation of local delivery vehicles is that the main bulk of the delivery vehicle or its acidic degradation products could interfere with regenerating tissues ***(Marzouk KM, et al. 2007).*** In addition, tissue-implanted materials could be surface friendly to biofilm-forming bacteria ***(Becker PL, et al. 1994).***

Plasma rich in growth factors is an autologous technology using calcium ions to activate growth factor delivery from the platelets and to develop a fibrin network with the aim of promoting tissue regeneration ***(Anitua E, et al. 2007).*** This 100% autologous preparation not only enhances tissue healing but also improves the clinical outcomes of various surgical procedures by minimizing complications like pain, inflammation and morbidity ***(Anitua E, et al. 2013a).***However, PRGF act on already differentiated cells, such as preosteoblasts and osteoblasts; however, they do not exert any effects on the stem cells present in bone tissue, whose differentiation is regulated by bone morphogenetic proteins ***(Anitua E, et al. 2013b).***

Despite the numerous studies describing the benefits of PRGF ***(Nazaroglou I, et al. 2009, Rivera C, et al. 2013)*** and Statins separately , ***(Sato D, et al.2005, Nishimura K .2008)*** there has been a lack of clinical investigation into the simultaneous use of these agents in socket augmentation. Therefore the main objective of this study is to evaluate socket bone dimensions and quality following the use of PRGF derived fibrin scaffold as a carrier for Atorvastatinin socket augmentation clinically and histomorphometrically.

Aim of the study

This study will be performed to:

Compare 8 weeks postextraction augmented sockets using Atorvastatin loaded in Plasma rich in growth factors derived fibrin scaffold (PRGF/ATV) versus direct application of Atorvastatin (ATV) gel using:

1) Histomorphometric analysis of bone quality.
2) Clinical measurments of dimensional changes after extraction.

Subjects and methods

Thirty patients will be selected from the outpatient clinic of Oral Medicine, Periodontology, and Oral Diagnosis department, Faculty of Dentistry, Ain Shams University

Inclusions criteria:

1. Healthy adult patients as evidenced by Burkett's oral medicine health history questionnaire.
2. Both sexes.
3. Age from 20 –50 years old.
4. Having at least one hopeless tooth indicated for extraction.
5. Patient should agree to sign a written consent after the nature of the study will be explained.

Exclusion criteria:

1. Smokers.
2. Pregnant and breast feeding females.
3. Prisoners and handicapped patients.

Patients met the eligibility criteria will be randomly allocated using sequentially numbered sealed envelopes into 3 treatment modalities groups:-

Group I (PRGF/ATV): Will include 10 patients undergoing single tooth extraction followed by socket fill with PRGF loaded with Atorvastatin then suturing the socket.

Group II (ATV): Will include 10 patients undergoing single tooth extraction followed by socket fill with Atrovastatin gel then suturing the socket.

Group IIIEmpty socket(control) : Will include 10 patients undergoing single tooth extraction then suturing the socket.

All patients will receive implants after taking the bone biopsy after 8 weeks for histomorphometric analysis.

This proposal will be reviewed by the research ethics committee, the procedure will be fully explained to the patients and they will sign an informed consent.

Surgical procedure and clinical measurements:

1- Patients will undergo atraumatic extraction under local anesthesia for the involved tooth, to preserve bone and soft tissue followed by socket curettage.

2- Prior to surgery baseline clinical measurements (bone height measured by periodontal probe and bone width by bone caliper) will be taken for the alveolar ridge.

3- Socket will be filled with one of the assigned augmentation modalities;

Preparation of PRGF/ATV :

9ml venous blood will be collected from the same patient from peripheral circulation The blood will be deposited in 3.8% w\v sodium citrate 1:9v\v. Platelet-rich in growth factors will be separated by centrifugation at 580 g for 8 minutes at room temperature. After centrifugation blood will be separated into plasma and cells, fractionation of plasma into 3 fractions:

Platelet very rich in growth factors - F3

Plasma with growth factors -F2

Plasma poor with growth factors - F1

To produce the (PRGF)(F3) loaded with Atorvastatin we will obtained by carefully pipeting a 1ml fraction of plasma immediately above the red blood then mixed with a weighted amount of Atrovastatin powder to produce 1.2% (PRGF /ATV) formulation, then 10% calcium chloride (50 µL for each 1 ml plasma) will be added and incubated for 20 minutes in 37 c to produce a semi-solid mass ***(Anitua E, 2001).***

Preparation of 1.2% (ATV) gel:

Atrovastatin methylcellulose gel will be prepared, by adding the required amount of biocompatible solvent to an accurately weighed amount of methylcellulose heated to obtain a clear solution then a weighed amount of ATV will be added to the above

solution and dissolved completely to obtain a homogeneous phase of polymer with a concentration 1.2% as described by ***Pradeep AR, et al. 2013.***

4- Socket will be sutured.

5-Postoperative medications will include (Amoxicillin 500 mg t.d.s, Flagyl 500 mg twice/day and antiseptic mouth wash for 1 week).

6- Post operative instruction will be given to the patient and Suture will be removed after 1-2 weeks post operatively

7- Patient will be instructed to avoid wearing any prosthetic restoration.

8-After 8 weeks patient will come for postoperative clinical measurements and for taking biopsy and implant placement.

Histological Examination :-

Bone Biopsy will be taken by a trephine bur and the implant will be inserted in the same visit , and correspond to a type 2 delay according to the International Team for Implantology classification ***(Chen ST & Buser D.2008).***

10- All the biopsy specimens will be fixed in 10% buffered formalin and demineralized in 10% ethylenediamine tetra acidic acid solution. A 7-μm-thick longitudinal section, representing the central part of each core specimen, will be stained with hematoxylin and eosin (H&E) for histologic examination. The histologic section will be examined using a research light microscope, and photomicrographs will be taken with the attached digital camera.

11- Histomorphometric measurement of the tissue image will be performed using the image analysis software.

• Total area of newly regenerated tissue including the newely formed bone above the old basal bone within the obtained core and the adjacent soft tissue .

Statistical analysis:

All the clinical and histomorphometric data will be collected, tabulated and subjected to statistical analysis.

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تدعيم مكان ما بعد الخلع باستخدام دواء الأتورفاستاتين المحمّل على هيكل الفيبرين من البلازما الغنية بعوامل النمو:دراسة إكلينيكية و تحليل هيستولوجي

مقدمة من الطبيبة

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بكالوريوس طب الفم و الأسنان

جامعة عين شمس٢٠٠٦

للحصول على درجة الماجستير في طب الفم و علاج اللثة و التشخيص

تحت إشراف

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الملخص العربي

المقدمة

تعد زراعة الأسنان من أفضل الحلول لاستبدال الأسنان التي لا علاج لها الا الخلع و ما ينتج عن الخلع من مشاكل اذا ترك مكان الخلع بدون ملئه عن طريق وضع أيا من مشتقات البلازما مثل البلازما الغنية بعوامل النمو او جزيئات العظم او دواء مثل دواء الاتورفاستاتين مما يحسّن حالة التعافي من الخلع و يهيئ المكان لاستقبال زراعة الأسنان بصورة تحسن النتائج المرجوة من جودة و سرعة العظم المتكون بعد ثمان أسابيع من الخلع بدلا من ستة أشهر و هو الوقت المتوقع لتكون عظم جديد في مكان الخلع مما يوفر الوقت و يخدم مصلحة المريض .

لذلك الادوية من مجموعة الستاتين و هي أدوية تقلل الكوليستيرول في الدم وُوجد ان لها تأثيرا إيجابيا في علاج مكان الخلع لما وجد لها من عدة فوائد مثل مضادة للالتهاب و مضادة للأكسدة وزيادة البروتينات العظمية المتعددة عن طريق تحفيز الخلايا الام على التحول لخلايا بناء العظم و تساعد على التكلس و لها تأثير يقلل نشاط الخلايا الأكلة للعظم.

البلازما الغنية بعوامل النمو باستخدام كلوريد الكالسيوم تكوّن هيكل من ألياف الفيبرين الذي شاع استخدامه كمساعد لتجديد الأنسجة و يقلل المضاعفات مثل الالتهاب و الألم.

بالرغم من الدراسات المتعددة عن أهمية كل من أدوية الستاتين ، أوهيكل الفيبرين المشتق من البلازما الغنية بعوامل النمو منفصلين ، لا توجد دراسات كافية عن تأثيره محمل علي هيكل الفيبرين و لهذا تهدف هذه الدراسة لمقارنة النتائج بعد استخدام دواء الاتورفاستاتين المحمل على هيكل الفيبرين المشتق من البلازما الغنية بعوامل

النمو، و دواء الاتورفاستاتين المحمل على جل الميثيل سيليلوز و مقارنتهم بعدم وضع اي شئ مكان الخلع و ذلك عن طريق القياسات الفعلية لطول و عرض العظم قبل الخلع و بعد الخلع و عن طريق تحليل عينة العظم المأخوذة بعد الخلع بثمان أسابيع أثناء عمليةزرع السن الجديدة .

المرضى و الطرق:

سيتم إجراء الدراسة الحالية على مجموع ٣٠شخص من الجنسين علي ان يكون لديهم اسنان ليس لها علاج الا الخلع و سيتم تقسيم هذه المواقع عشوائيا إلى ٣مجموعات. والمجموعة الاولى ستضم ١٠ مواقع بعد الخلع يتم ملئها بدواء الاتورفاستاتين المحمل علي البلازما الغنية بعوامل النمو والمجموعة الثانية ستضم ١٠ مواقع بعد الخلع يتم ملئها بدواء الاتورفاستاتين المحمل علي جل الميثيل سيليلوز و و المجموعة الثالثةستضم ١٠ مواقع سيتم الخلع بدون ملء مكان بعد الخلع باي شئ .

سيتم عمل قياسات اكلينيكية لارتفاع و عرض العضم مكان الخلع قبل العلاجات و بعد٨ اسابيع كما سيتم أخذ عينات من العظم المتكون بعد ثمان أسابيع بعد عملية الخلع و تحليلها هيستولوجيا تحت الميكروسكوب و سيتم تعويض السن المخلوعة عن طريق عملية زراعة سن بديلة في نفس الزيارة بعد أخذ العينة.

سيتم جمع النتائج و تحليلها احصائيا