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Enhancement of dental implant stability using Saccos membrane in a sheep animal model

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Abstract

Aims: The current study aimed to compare implant stability and bone density using a Concentrated Growth Factor placed before implant placement in the iliac crest of sheep. **Materials and Methods:** Four healthy local breed male sheep were enrolled in the study. All sheep served as two observation subgroups. The iliac crest of both sides of each sheep was operated on a random basis at one- and three-months intervals. Before each surgery, two 10 ml blood samples were drawn and immediately centrifuged in a specific centrifuge. After a cycle, the membrane was shredded into small pieces to be placed inside the surgical site before implant installation. Five standard osteotomy sites were created for the placement of five dental implants, two served as a control in which the implant was installed without additives. The other three osteotomy beds served as study groups in which shredded pieces of Sacco's membrane were placed before implant installation. Following implant installation, dental implant stability using the Osstell Beacon device was recorded. Following animal sacrifice, bone specimens were retrieved and secondary implant stability and bone density around dental implants using Computed Tomography and Cone Beam Computed Tomography scans were measured representing one- and three-months intervals. **Results:** For dental implant stability, the results showed no significant differences at one-month intervals between both groups yet a statistically significant difference was disclosed at 3 months favouring the tested group. **Conclusions:** Within the limits of this study, it is possible to conclude that the application of CGF to the socket walls before the placement of implants may enhance the healing of bone surrounding implants, accelerate osseointegration, and offer a choice that is both convenient and affordable for implant placement.

تعزيز استقرار زراعة الأسنان باستخدام غشاء ساكوس في نموذج حيواني للأغنام

الملخص

الأهداف: كان الهدف من هذه الدراسة هو مقارنة ثبات السن المزروع (الغرسة) وكثافة العظم حوله وذلك باستخدام عامل النمو المركز بوضعه في مكان الزرع قبل وضع الغرسة في الحافة العلوية للعظم الحرقفي للأغنام. **المواد وطرائق العمل :** تم استخدام أربعة ذكور من الأغنام المحلية السليمة في الدراسة حيث استخدمت جميعها كمجموعتين للمتابعة. أجري العمل الجراحي على الحافة العلوية للعظمين الحرقفيين في كلا الجانبين لكل شاة وقسمت بشكل عشوائي كمتابعة شهر وثلاثة أشهر. قبل كل عملية جراحية، تم سحب عينتين دم بحجم 10 مل وأدخلت في جهاز طرد مركزي وعند الانتهاء من عمل الجهاز تم تقطيع الغشاء إلى قطع صغيرة لتوضع داخل موقع الجراحة قبل تركيب الزرعة. تم إنشاء خمسة مواقع قياسية لقطع العظم لغرض وضع خمس غرسات أسنان، اعتبرت اثنتان منها كمجموعة تحكم بحيث لا يتم اضافة أي مادة قبل تثبيت السن، واعتبرت المواقع الثلاثة الأخرى كمجموعات دراسة واختبار حيث تم وضع قطع ممزقة من غشاء ساكو قبل تركيب الغرسة. بعد زرع السن، تم التقييم الأولي لثبات السن المزروعة وذلك باستخدام جهاز اوستيل بيكون. بعد ذبح الأغنام المستخدمة في الدراسة، تم استرداد عينات العظام واجراء التقييم الثانوي لثبات الغرسة وكثافة العظم حول الأسنان باستخدام التصوير المقطعي المحوسب والتصوير المقطعي المحوسب بالشعاع المخروطي لتمثل المتابعة لفترتي الشهر والثلاث أشهر. **النتائج:** بالنسبة لثبات الأسنان المزروعة لم تظهر النتائج فوارق ذات دلالة إحصائية بين المجموعتين في المتابعة بفترة شهر واحد ولكن الفوارق كانت ذات دلالة إحصائية معنوية عند 3 أشهر لصالح مجموعة الاختبار. **الاستنتاجات:** يمكن الاستنتاج ضمن حدود هذه الدراسة أن وضع عامل النمو المركز على جدران التجويف قبل وضع الغرسات قد يعزز من التئام العظم المحيط للسن ويسرع الاندماج العظمي، ويمثل خيارًا مناسباً وقليل التكلفة لزراعة الأسنان.

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INTRODUCTION

Dental implants might be loaded immediately or early with more predictability if the osseointegration process could be shortened in terms of speed. There are various ways to improve the osseointegration of dental implants, which is the process by which the implant fuses with the surrounding bone and becomes securely anchored. One approach is to alter the topography, or physical features, of the implant surface. Another approach is to alter the chemical composition of the implant [(1)]. Modulating the healing process following implant placement is another strategy for boosting osseointegration rates. That's where a class of bioactive compounds known as growth factors (GFs) comes in [(2)]. The use of platelet concentrates has become more common as a means of hastening recovery from wounds. Platelet concentrates are increasingly being used as surgical adjuncts or solutions for topical treatment in the area of regenerative medicine. Autologous blood products are a type of stem cell therapy in which healthy blood cells are implanted into a patient to speed up recovery from an injury. Enhancing recovery is an ongoing concern for surgeons across the board [(3)]. As of 2006, Sacco has developed concentrated GF (CGF) [(4)]. By centrifuging venous blood, a fibrin matrix rich in GFs and leukocytes forms a gel layer in which the platelets are concentrated [(5)]. Platelets' alpha granules are degranulated by CGF, allowing the platelets to begin their critical early wound healing function [(6)]. In comparison to platelet-rich fibrin (PRF) and platelet-rich plasma (PRP), concentrated growth factor (CGF) has been reported to have a higher concentration of GFs, and it also retains its GFs content for a longer period after application [(7)]. CGF has been shown to have favourable effects in stimulating bone regeneration surrounding implants, according to research conducted in vitro [(8 & 9)]**.** In this technique of CGF preparation, with the use of a specifically designed centrifuge (Medifuge; Italy) the blood specimens which have been collected in non-anticoagulant tubes, will rapidly be centrifuged in a pre-programmed centrifugation cycle and as follows: an acceleration cycle of x 30", 2,700 rpm x 2', 2,400 rpm x 4', 2,700 rpm x 4', 3,000 rpm x 3' followed by a deceleration down to x 36" and end of cycle [(4)]. This centrifugation method generates a fibrin matrix that is rich in growth factors and has a stronger regeneration ability than previous platelet concentrates, this owing to the high density, complex three-dimensional architectures [(4 & 10)].

The current conducted study aimed to examine post-surgery dental implant stability and mean bone density surrounding dental implants augmented with concentrated growth factor (CGF) at three different time intervals during three months using resonance frequency analysis and cone beam computed tomography (CBCT) respectively.

MATERIALS AND METHODS

The Oral and Maxillofacial Surgery Research Committee of the University of Duhok's College of Dentistry gave their stamp of approval to this study's protocol. The practical work was performed from January until May 2022 in the Animal House / College of Veterinary Medicine/ University of Duhok. A total of four male local breed sheep were involved in the research, all of which were healthy and between 40 and 45 kilograms (mean=42.5kg), all from the same farm. Their health and feeding were regularly supervised by an attending veterinarian. Animals were acclimated for two weeks before any procedure and general state inspected to ensure the absence of general or infectious disease. To overcome operator-mediated errors, all surgery was conducted by the researcher. All four sheep were to be operated on whereby each sheep model served as two observation subgroups.

The iliac crest of both sides of each sheep was operated on a random basis at a one- and three-months interval period between each surgery. Before surgery and from each sheep, two – 10 ml blood samples were drawn from the jugular vein and immediately centrifuged in a specific centrifuge (Medifuge lab. / Italy) according to the pre-programmed protocol for preparing the Sacco's membrane. The methodology of blood collection and centrifugation protocol was as follows; Each sheep was restrained from the head and rear portion of the body and kept in a standing position. Before the blood is drawn, any wool in the area of the blood collection site was removed for adequate visual and mechanical access and to avoid needle contamination. Two 10-ml blood samples were taken from each sheep and centrifuged immediately. The blood samples were collected in plastic tubes with no coatings and were made of glass and with no anticoagulants. The blood tubes for CGF are centrifuged (Medifuge, Silfradent, Sofia, Italy) with a one-step centrifugation protocol: 30sec -acceleration, 2min - 2700 rpm, 4min - 2400 rpm, 4min - 2700 rpm, 3min - 3000 rpm, 36sec – deceleration and stop. After centrifugation has been completed, separation of the newly formed CGF (the platelet-rich side i.e. proximal to the red end) from the bottom layer and then cut into small pieces by a surgical scissor so it can be placed inside the surgical site before implant installation.

Surgical Procedure:

All four sheep were operated on whereby each sheep model was to serve as two observation subgroups according to a planned sacrifice timetable. Before the day of surgery, each animal was allowed to graze freely and given free access to drinking water. Utilizing scissors and an electric hair clipper, all coarse fleece was cut away from the surgical site. Surgical procedures were performed under general anaesthesia and sterile conditions. For general anaesthesia (induction and maintenance), an intramuscular injection of a mixture of Ketamine hydrochloride

(10mg/ml/kg) and Xylazine (2mg/ml/kg) sedative was given. Following anaesthesia, 10% povidone-iodine was used to clean off the surgical area before and after the procedure. Before making an incision, the surgical site was infiltrated with local anaesthetic containing epinephrine at a 1: 80,000 concentrations to prevent bleeding. The edges of the iliac crests were exposed through a skin incision of 15 cm in length. The bone was then carefully exposed. Under copious irrigation using cooled 0.9% normal saline, five standard osteotomy sites (at least 0.5 cm apart) were created for placement of five dental implants (diameter 3, length: 8) B&B -slim line system- (Italy) following the manufacturer's instructions. With the use of tweezers and curate, the defects were filled in the following order: from top to button orientation (Figure 1).

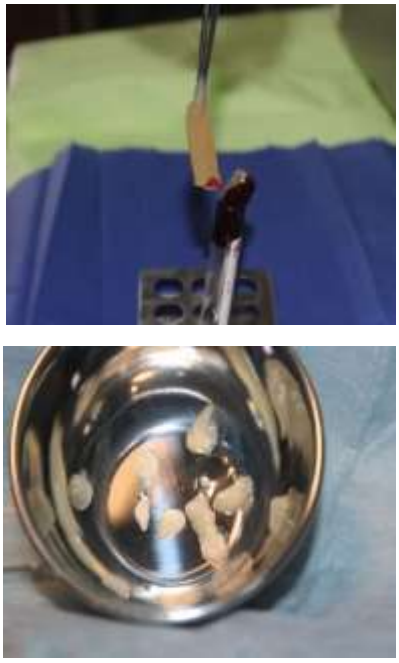

Figure (1): Handling of Concentrated Growth Factors (CGF), Using a set of sterile metal scissors, the clot's adhering red base was carefully scraped off.

Two implant beds served as a control in which the implant was installed solely without additives. The other three osteotomy beds served as study groups in which shredded pieces of Sacco's membrane were placed before implant installation (Figure 2).

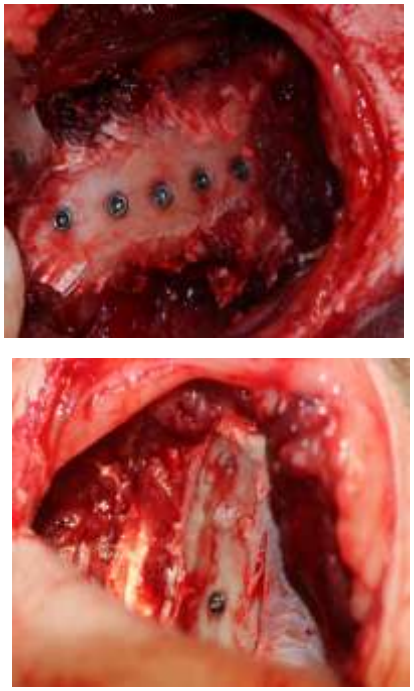

Figure (2): Installation of implant with / without CGF inside the socket.

As in Figure (3) a smart peg for the dental implant system used was carefully fixed in the implant and the primary stability of each dental implant was recorded (mean of four readings) using Osstell® Mentor (Guttenberg / Sweden). The higher the number of ISQs the higher stability achieved. After readings, healing screws were placed, and before wound closure (in layers) and to ensure guided tissue regeneration, absorbable collagen membranes were placed over the implant sites.

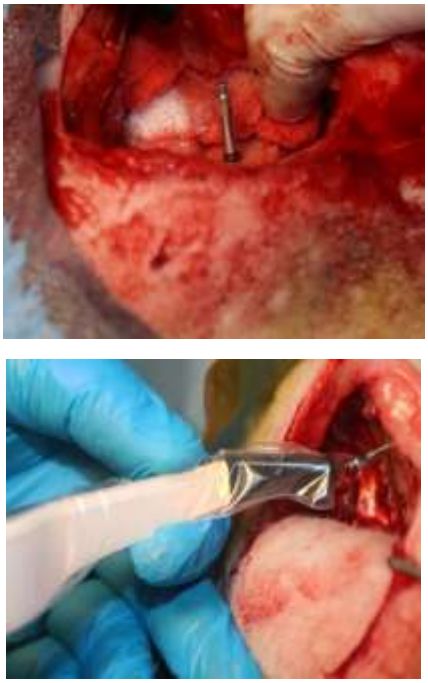

Figure (3): Smart peg fixed to record primary stability through the Osstell Beacon Device.

After the surgery, absorbable and non-absorbable sutures were used to close the wound. Before placing a fresh bandage over the wound, a generous amount of oxytetracycline antibiotic spray in an aerosol form was applied to the affected area. Immediate post-operative care for an animal after surgery involved administering medication; an intramuscular injection of Oxytetracycline at a dosage of 20 mg per ml per 10 kg of body weight. All sheep underwent post-operative monitoring until sacrifice and were free to graze and drink water. After 10 days, the sutures were removed.

Sacrification and Secondary Dental Implant Stability:

Sacrification was conducted at the end of a three-month interval where one iliac crest represented a three-month time interval and the other iliac crest represented a one-month interval. A bone specimen of at least 15 cm of iliac crest was retrieved for radiographical assessment, and resonance frequency analysis (RFA) using the same protocol for primary implant stability and representing one month and three months post-surgery, and as shown in Figure (4).

Figure (4): A- Smart peg fixed in position, B-

Osstell Beacon device in action.

Statistical analysis:

An electronic database was created from the data. Commercially accessible statistical software was used for all analyses (SPSS Version 27.0). The following tests were employed since all of the independent variables were assumed to be non-parametric:

1. Descriptive analysis showed minimum, maximum, means, and standard deviations.
2. Mann-Whitney test to show significance within groups for dental implant stability.
3. Wilcoxon signed ranks test to show significance between groups Statistical difference was set at $p < 0.05$.

RESULTS

Post–Surgery Healing Period

Besides a few instances of post-surgical limping, all animals tolerated the surgery, and the healing of wounds was uneventful. The research used a total of four local breed male sheep that were healthy, ranging in age from 1.5 to 2 years and weighing between 40 and 45 kilograms (mean = 42.5 kilograms), all from the same farm. The surgical site operated on in each sheep was the iliac crest on both sides (right and left). On each side, five dental implants were installed with a total of ten implants in each sheep and a total of 40 dental implants in all 4 sheep. All implants were stable at the time of evaluation.

Assessment of Dental Implant Stability

Comparisons within groups:

Descriptive statistics for primary and secondary stability for all groups are shown in (Table 1).

Table (1): Descriptive statistics for primary and secondary stability for all groups.

	Groups	Mean	Confidence intervals 95% Lower bound	Confidence intervals 95% Upper bound	Median	SD
Control	**Primary stability**	64.40	61.18	67.62	65.34	5.067
	1 Month Secondary stability	64.13	60.76	67.50	65.34	5.303
	3 Month Secondary stability	67.48	64.77	70.20	66.17	4.278
Sacco' s Membrane	**Primary stability**	64.50	62.54	66.45	64.75	3.082
	1 Month Secondary stability	66.58	64.79	68.36	66.50	2.810
	3 Month Secondary stability	73.87	72.21	75.53	74.50	2.612

Mann-Whitney test results for ISQ values in the control group showed no significant difference when comparing primary stability at the time of operation and one-month interval and when comparing ISQ values between primary stability and one-month interval with three months interval. In the test group, Mann-Whitney test results showed no significant difference when comparing primary stability at the time of operation and one-month interval yet a significant difference between primary stability and three-month interval and when comparing between one-month and three months intervals as shown in Table (2).

Table (2): Mann-Whitney test comparing implant stability within groups at three-time intervals (primary, one month, and three months' secondary stability), shows Mann-Whitney test *p* values.

	Groups	Mann-Whitney U	Z	*p*-value
Control	**Primary vs. 1-month secondary stability**	71.00	-0.059	0.953
	Primary vs. 3 months secondary stability	50.00	-1.29	0.196
	1 month secondary vs. 3 months secondary stability	50.00	-1.26	0.206
Sacco' s Membrane	**Primary vs. 1-month secondary stability**	41.50	-1.77	0.076
	Primary vs. 3 months secondary stability	2.00	-4.05	0.0000*
	1 month secondary vs. 3 months secondary stability	5.50	-3.85	0.0001*

* Significant difference when $p \leq 0.05$.

Comparisons Between Groups

Wilcoxon signed ranks test comparisons between groups showed no significant difference when comparing primary stability between both groups, no significant difference when comparing one-month secondary stability between both groups yet a significant difference was shown when comparing three months' secondary stability between both groups with the highest mean shown in the test group as shown in Tables (3&4).

Table (3): Wilcoxon signed ranks test comparisons between groups (control vs treated), shows Wilcoxon test p values.

Groups		Z	*p*-value
Primary stability	**Control vs Treated**	-0.471	0.6378
1 month of secondary stability	**Control vs Treated**	-1.512	0.1304
3 months of secondary stability	**Control vs Treated**	-2.981	0.0028*

***** Significant difference when $p \leq 0.05$.

Table (4): Wilcoxon signed ranks test ranks table

Primary stability		**N**	**Mean rank**	**Sum of ranks**
Saccos Membrane - Control	**Negative Ranks**	7	6.43	45.00
	Positive Ranks	5	6.60	33.00
	Ties	0		
	Total	12		
1-month secondary stability		**N**	**Mean rank**	**Sum of ranks**
Saccos Membrane - Control	**Negative Ranks**	4	4.00	16.00
	Positive Ranks	7	7.14	50.00
	Ties	1		
	Total	12		
3 months of secondary stability		**N**	**Mean rank**	**Sum of ranks**
Saccos Membrane - Control	**Negative Ranks**	1	1.00	1.00
	Positive Ranks	11	7.00	77.00
	Ties	0		
	Total	12		

DISCUSSION

In dentistry, implant integration success is a primary goal of any implant treatment and is dependent on a variety of factors specific to both the technique and the patient [(11)]. Successful osseointegration, from a clinical point of view, is a parameter of implant stability following integration[(12)]. Within the context of implant therapy, there are two related concepts: primary and secondary implant stability. In the context of stability, resonance frequency analysis (RFA) is a well-established methodology for determining the primary and secondary stability of an implant while it is being evaluated in vivo [(13)]. and was adopted in the current study as a tool of measurement. When deciding on an animal species to use as a test subject, several aspects must be taken into account. Availability, cost, social acceptability, captivity tolerance, and housing convenience are further factors considered. Sheep are effective models for human bone replacement and remodelling activities, although many animal species are known to have a higher bone healing rate than humans [(14)]. Because the sheep model is a good choice for an animal model to use for evaluating biomaterials for bone regeneration and the osseointegration of dental implant systems in dentistry and orthopaedics [(15)]; so, in the current study, sheep were used as a model for evaluation of dental implant stability using concentrated Growth factor (CGF). A current trend in dentistry is using biological adjuncts to possibly accelerate both soft and

hard tissue healing [16]. Platelet concentrates for topical application have evolved into surgical adjuncts or suspensions, and they are currently being employed in the field of dental implantology to promote stability and/or treat peri-implantitis. The current study investigated the possible effect of Sacco's membrane on accelerating bone healing around dental implant through a clinical approach. The results of the current study showed that using concentrated growth factor (CGF) has a positive effect on bone formation surrounding dental implants at each time interval investigated as assessed by resonance frequency analysis. Pirpir et al. showed that CGF can affect positively implant stability by accelerating osseointegration. The study included twelve implant cavities divided into two groups. The CGF membrane was used to cover the implant cavities before implant placement, Resonance frequency measurements were taken intraoperatively, postoperatively, after one week, and after four weeks. It was determined that there was a statistically significant difference between the control and test groups [17]. The present investigation confirmed previous findings from clinical and radiographic examinations by demonstrating that densitometric analysis of all Sacco's membrane samples had an increasing mean with a specified timeline schedule, the number of blood vessels, fibroblasts, and osteoid tissue all increased, indicating an increase in bone production in both clinical and radiographic findings. All of the increased osteoid tissue and fibroblast were to be the reason for the increased stability of the dental implant. To the scope of our knowledge, few types of research have been recognized dealing with Sacco's membrane either on animal models or on humans assessed by resonance frequency analysis. Moutamed [12] showed in healthy male adult mongrel dogs that CGF could enhance the healing of bone surrounding implants and accelerate osseointegration that in turn will allow for early loading of immediate implants providing a convenient and affordable choice for implant placement. In his study, the results were analysed 8 weeks postoperatively through histological analysis [18]. GE et al. also showed an animal study on rats in which a concentrated growth factor was used to enhance the osseointegration of titanium implants installed in the femora. The results showed a significant increase in the bone mineral density, percent bone volume, and intersection surface, 8 weeks after implantation using a Micro-CT scan; assuming that increased expression of genes involved in osteoblast development, such as BSP, Runx2, and ALP, may facilitate osseointegration of titanium implants treated with concentrated growth factor [19]. Honda et al., in an in-vitro study, showed that using CGF will promote more proliferation, osteogenic maturation, and mineralization of mesenchymal cells much more than those authors who used CGF mixed with bone marrow stroma cells resulting in excellent healing of critical-

sized bone defects in-vivo [20]. Durmuslar *et al.* determined the difference between using CGF alone or mixed with autogenous bone on inducing bone regeneration in a total of 40 peri-implant defects in each one of the rabbit tibiae and filled with either CGF alone (CGF), CGF and autogenous bone (CGF+AB), autogenous bone (AB) alone and some were just left empty (E) as a control group. The specimens were harvested after 8 weeks and were evaluated for histomorphometric analysis. CGF-combined implants were shown to be more effective since the new bone is formed around the dental implants in the defect areas of groups CGF, AB, and CGF+AB, while implant-to-bone contact was lowest in Group E [21]. Talaat et al. used CGF in the healing of bone defects in the mandible. The results showed much greater bone density, suggesting that this approach is a viable biotechnological option for regenerating bone in surgically created deficiencies [22]. Bonazza et al. confirmed that CGF could stimulate cell growth proliferation and metabolic activity of fibroblasts, endothelial cells, and osteoblasts which all are determinant cells in tissue healing and tissue regeneration to investigate the positive effect of CGF on these critical 3 human cells in vitro [23]. Rodella *et al.*, in an immunohistochemical study, showed that CGF is a large fibrin matrix of high density and rich in growth factors having a positive effect on stimulating cellular proliferation, matrix remodelling, and neo angiogenesis; furthermore, all biocompatibility issues are avoided because these concentrates are autologous and don't need bovine thrombin [10].

CONCLUSION

Within the limits of this study, it is possible to conclude that the application of CGF to the socket walls before the placement of implants may enhance the healing of bone surrounding implants, accelerate osseointegration, and offer a choice that is both convenient and affordable for implant placement. In current clinical scenarios, this technology could be successful in accelerating the pace of osseointegration and enabling the early loading of immediate implants. A favourable impact of CGF treatment in many areas of oral surgery has to be demonstrated in future clinical investigations.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication and/or funding of this manuscript.

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