Tissue engineering using collagen I matrix for treatment of oral mucosal defects: Experimental study in rabbit

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Abstract- The loss of tissue by disease or trauma and congenital malformation is the most dangerous complication worldwide. It may involve the craniofacial area and cause physical and psychological morbidity to the individual. Treatment of such defects to the optimal esthetic and function is necessary. This new trend need synthetic resorbable or natural matrix that favors tissue formation .In addition modern molecular technologies to restore tissue function.

Objective of the study:

To clinically and histologically evaluate the soft tissue reaction following implantation of collagen sponge in rabbit oral mucosa.

Material and Method:

The study involve 200 Newslandy rabbit, aged 3-6 month, weight(0.5-075 kg).The study conducted in Al-Amrya animal house (2015-2016).180 rabbit was anesthetized byintraperitoneal injection of ketamine 10mg with XYL-M2 solution 20 mg xylazine base. For collagen sponge implantation, an incision (in rabbit oral mucosa of the mandibular anterior region), perpendicular to the bone surface, by the use of scalpel.

The incision was made 3 mm from the gingival margin, 10 mm mesio-distally. A full thickness periosteal flap was raised, with periosteal elevator, and a collagen sponge (lyophilized hydrolyzed collagen sponge (Hemospon) Brazil 1×1×1 cm, previously hydrated in sterile saline solution, was inserted between the connective tissue and the bone .The area was kept under pressure. The area is sutured with 3/0 black silk suture. The animals were followed up for 15-30 day .The animal were sacrificed at 30 day. The specimen was placed in 10%formalin and send for histopathological examination. The remaining 20 rabbit is control group. The thickness of epithelium is measured clinically (mm) and also measured after taking the biopsy during examination under microscope.

Results:

The histopathological examination of the specimen show increases in the thickness of the rabbit oral pithelium and tissue neoformtion in comparison with the control group.The statistical results indicate significant increase in mean thickness of epithelium in experimental group (2.5731+\- 0.72201) in comparison with control group (3.8031+\-0.20748).There is significant difference in mean thickness of epithelium between the two groups (P>0.05).

Conclusion:

The lyophilized collagen Type I matrix induced a significant increase in the thickness of oral mucosa.

Index Terms- Grafting, Lyophilized hydrolyzed Collagen Type I matrix, SubepithelialConnetive Tissue, Tissue Engineering

I. INTRODUCTION

The search for high esthetic and harmony of the face, lips, gum and good arrangement of teeth are essential for pleasant smile and hence it is important factor of beauty. Treatmentof gingivalrecession, owing to increase need by patients is become frequent cosmetic procedures.It is not only a cosmetic procedure but an exposed root is associated with pain, sensitivity and hence poor oral hygiene. [12]

Soft tissue defects is treated by grafting from oral mucosa or spilt-thickness skin .However; complication of donor site morbidity are results.Also the limited supply of oral mucosa and the associated skin appendages and keratinization associated with vascularized skin flap which make it to be infected easily in wet environment of oral cavity and heal by scar. The reconstruction of maxillofacial defects by microvascular free tissue transfer i.eosteocutaneous radial forearm flap is used.[16]

The field of tissue engineering is to reconstructlost tissues and organs.The success of these procedures is depend of the development of new natural materials and synthetic biomimetic scaffolds that aim to restore the craniofacial tissue defects and the disorders associated disease and injuries.[22]

The principle of wound healing, essential growth factor involved, development of biomaterials which able to deliver growth factors to the regenerate site, and the new oral and periodontal tissue engineering procedures and regenerative medicine are addressed.[9]

In medicine collagen used as wound dressings.And matricesfor tissue regeneration. The collagen is a main structural protein of the bony skeleton, its properties and application in dentistry which depend on the structure of amino acid sequence. [6]The availability of collagen in various forms i.e,films,powders,sponges and gels. The collagen is a biomaterial of nineties with essential biological propertiesi.e:high tensile strength, orientation of fibers, semipermeability,low-antigencity,hemostatic and wound healing capacity.[11]

The techniques of root coverage procedures, the important of which is subepithelial connective tissue graft (STG), by Langer and Langer of high effectiveness and predictability.[8]

One of several advantages of STGs it provides similar margins and minimal postoperative discomfort. However; it is of high esthetic results, it requires donor surgery.[14]

Hemospon is lyophilized hydrolyzed collagen sponge with high porosity .It is totally resorbed within 15 days. Indentistryi.e.

tooth extraction sockets, it has high hemostatic and healing ability .It may or may not be withdrawn after a period of time.[2] Collagen has unique biological properties, no other synthetic substitutes has. Because of the low antigenicity, hemostatic, chemotactic properties and availability by many natural sources, make the collagen I of wide clinical application recently.[4]

Collagen is the main structural protein, but collagen type I is predominant one occupying 90% of tissue. It is used as wound dressing since 85 years. It is manufactured in films, powders, sponges and gels. The commercialize process is very slow because of, cost preparation, handling and storage difficulties and suitable cost of application. [15]

One of important problem to be addressed in oral tissue engineering is the modulation of the excessive host response to microbial contaminate of the periodontal tissue and wound. [19] For success of oral and periodontal procedures,dentists need to test the delivery of anti-infective agents and modifiers to have good results. The Future discoveries in this field is continue, it combine engineering, dentistry field, medicine specialty and specialties in infectious disease to repair the complex environment of periodontal wound. [20]

Although the promising approaches in TE, it clinical use is limited. The experimental studies provide a chance to test interactions of cells,biomatrix and molecules and host factors that cannot be search. In vitro human studies can decrease the need for long,costy, and conflicting animal studies that is misleading because of species molecular and physiological differences.[7]

II. Material and Method

Materials: Hemostatic sponge made of Lyophilized Hydrolysed Collagen(Gelatin),Brasil.

Method:

The study involve 200Newslandy rabbit, aged 3-6 month, weight(0.5-0.75 kg).The study conducted in Al-Amrya animal house ,(2015-2016).180 rabbit was anesthetized byintraperitoneal injection of ketamine 10mg with XYL-M2 solution 20 mg xylazine base. For sponge implantation, an incision (in rabbit oral mucosa of the mandibular anterior region), perpendicular to the bone surface, by the use of scalpel

. 3 mm incisioinfrom the gingival margin, 10 mm in length was made. A full thickness periosteal flap was raised, with periosteal elevator, and 1×1×1cm collagen sponge (lyophilized hydrolyzed collagen sponge (Hemospon) Brazil, previously hydrated in sterile saline solution, was inserted between the connective tissue and the bone .The area was kept under pressure. The area is sutured with 3/0 black silk suture. The animals were followed up for 15-30 day .The animal were sacrificed at 30 day. The specimen was placed in 10%formalin and send for histopathological examination. The remaining 20 rabbit is control group. The thickness of epithelium is measured clinically (mm) and also measured after taking the biopsy during examination under microscope.

III. Results

1-Clinical examination: Clinical examination show increase in the thickness of epithelium in the treated animal in compared with control table (1).

2-Histopathological examination: The hitopathological examination of the biopsy specimen show increase in the thickness of the tissue in comparison with the controlled group. There significant difference in the numbers of inflammatory cells and fibroblasts in subcutaneous tissue, and in the density of blood vessels, before and after sponge placement.

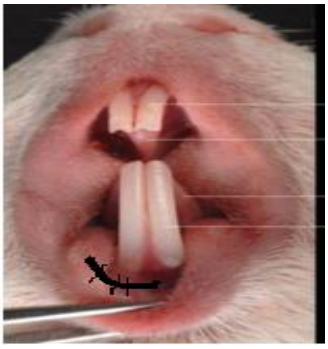

Figure (1): The site of incision and suture.

Figure(2):showing the collagen sponge hydration in normal saline.

Figure (3): The biopsy specimen.

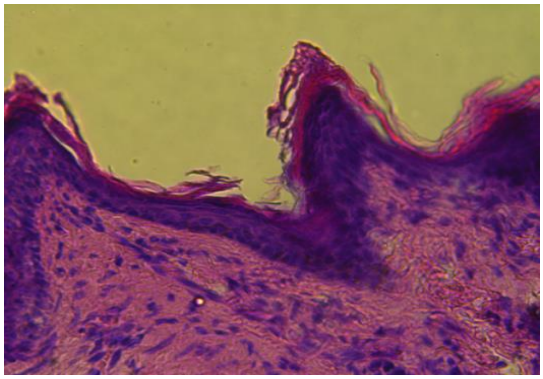

Figure (4): The normal epithelium of rabbit oral mucosa.

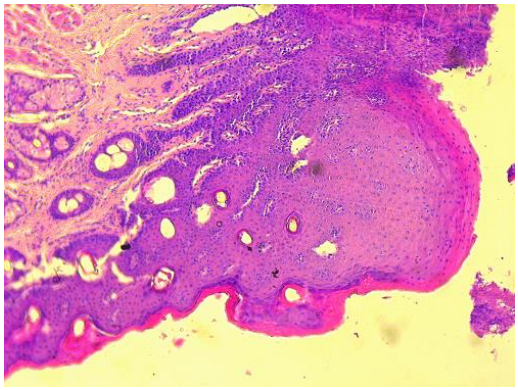

Figure (6): The increase in the thickness of epithelium.

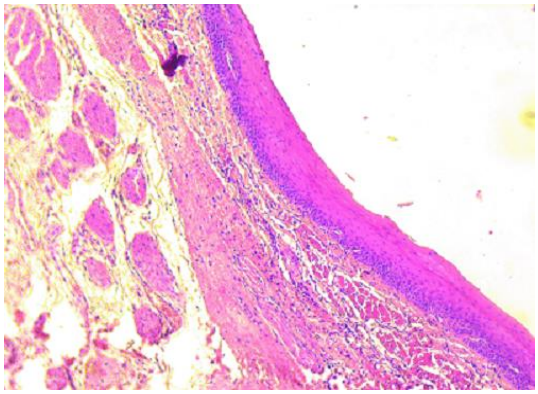

Figure (5): The mitosis of the basal cell layer with increased number of inflammatory cells in subcutaneous tissue.

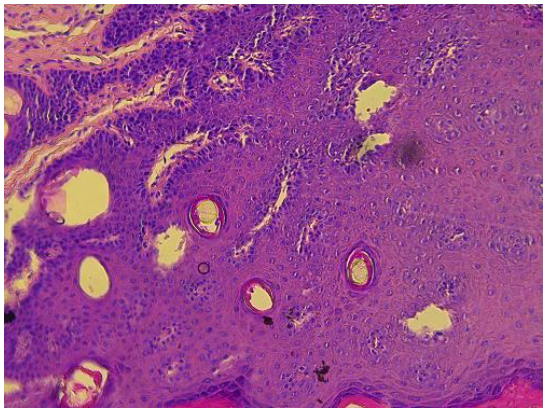

Figure (7): The high power view.

IV. STATISTICS

Table (1): Show the mean thickness of epithelium in (cm) among the study groups.

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	cont	2.5731	16	.72201	.18050
	Expre	3.8031	16	.20748	.05187

Table (2): Show the correlation and level of significance among the treated groups.

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	cont&Expre	16	.109	.686

Table (3):t- test for the level of significance.

Paired Samples Testing (difference is significant among the groups at 0.05 and 0.01)

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			

Pair 1	cont - Expre	-1.23000-	.72907	.18227	-1.61850-	-.84150-	-6.748-	15	.000

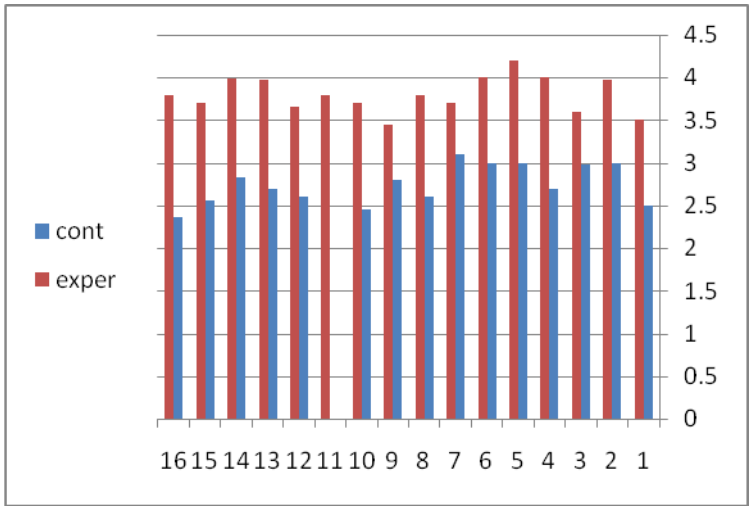

Figure (8): Show the relation between the thickness of epithelium between the control group and the experimental group.

V. Discussion

Tissue engineering (TE) oral mucosa and bone in maxillofacial field has been extensively studied. It aims to reconstruct defects associated with traumatic, pathological and congenital malformation.[1]

Studies by Rocha et al .2012 and Carnio&Hallman 2005 who make clinical and histological studies to qualitatively and quantitatively estimate the neoformed tissue in palatal donor site after placement of collagen sponge .This procedure is indicated for root coverage treatment. This procedure may involve more than one surgical procedure if the amount of tissue i.e. graft is limited. [5]

Table (1) show the mean value for the thickness of epithelium in experimental group is (2.5731+\- 0.72201) while in the control group (3.8031+\-0.20748). The statistical analysis in table (2) of our study indicate that there is significant difference in mean thickness of epithelium in the experimental group in comparison with control (P>0.05).

The success of the treatment depends on donor site evaluation before surgery.STG procedure and coronally advanced flaps is high success root coverage procedure. Scientists consider a thicker graft, of 1.5-2 mm more appropriate treatment. But because of its anatomicallimitation, hard palate does not necessarily ideal position to obtain graft of adequate size.[3]

Collagen sponges have been use in dentistry for long time, due to its hemostatic capacity and biocompatibility .It is fully resorbed by host environment without unwanted reaction .It has good wound healing effect maturation and stability because it allows initial clot formation and fibrin cross linking.[12]It is also act as a carrier foragents used periodontal surgery with no postoperative inflammatory reaction(Carino&Hallman).

Hemospon used in our study have resiliency, it restore its original form, when it implanted in to the tissue, it provide a mesh for cells involved in healing .The area occupied by material is replaced fully by folds of fibrous tissue as shown by clinical examination for the area treated by this material .[13]This tissue is adhere tightly to near bone and could not be released and limit its augmentation as a result of compression of the implanted collagen sponge.

The histological examination of our study is similar to the studied conducted by Carino&Hallmon[17].This study indicate that the area augmented by collagen had a normal structure, no inflammation or residual material ,no fatty tissue. This process takes 8 weeks for the collagen sponge to be completely absorbed and replaced by fibrous tissue.[23]

Inflammation if present is due to physical presence of collagen in the body and not related to the structure of the sponge .This delay the healing. [21]

In histological section the difference in the number of inflammatorycells, fibroblast and the density of the blood vessels seen and before and after sponge implantation is a positive response of inflammation, which is essential source for nutrient and oxygen necessary for healing. Normal fibroblast is stable but when facing inflammation i.e. implanted collagen sponge, it replicated and synthesize large volume of collagen fibers.[18]

VI. Conclusion

The results of our studies using experimental animal prove that the collagen sponge implantation under rabbit oral mucosa cause increase in the thickness of the epithelium as shown in histopathological examination of biopsy specimen with increase in number of the fibroblasts and blood vessels in lamina properia.Thisneoform tissue or tissue augmentation technique is suitable in clinical practice reconstruct the loss tissue defects in oral and craniofacial region , because these defects may require more than one donor site which unsuitable clinically.

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