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Original Article

The effect of ostrich acellular dermal matrix on keratinized gingival width

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ABSTRACT

Background: Xenogeneic grafts have gained attention due to advantages in compare of autografts. This study aimed to compare Xeno (ostrich) Acellular Dermal Matrix (XADM) with the free gingival graft (FGG) to increase the width of Keratinized gingiva (KGW) in dogs.

Materials and Methods: This split mouth animal study was performed on 10 mixed breed dogs. The upper second premolar sites were randomly selected for grafting by XADM (test) or FGG (control). Measurements of KGW were recorded before surgery, 1, 3, and 6 months after surgery. Biopsies from grafted sites for histologic and histomorphometric evaluations were harvested 6 months after surgery. Data were analyzed by repeated measured, paired samples *t*-test, and Wilcoxon Signed rank test. $P < 0.05$ was considered statistically significant.

Results: KGW increased in the two study groups after surgery with no significant statistical difference between them at any time intervals ($P > 0.05$). The graft shrinkage was 23% and 21% for the test and control groups, respectively, without statistically significant difference ($P > 0.05$). Histomorphometric evaluation showed no significant difference between the two study groups. Foreign body reaction was not seen in any of the study groups.

Conclusion: Increased KWG was similar between the two study groups. With regard to FGG limitations, XADM may be assumed as a suitable alternative for FGG. It should be noted that this research was an animal study and clinical trials on human should be performed to approve the efficacy and safety of this material.

Key words: Acellular dermis, free tissue flaps, gingival diseases, gingival recession, periodontal diseases, plastic, surgery

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INTRODUCTION

Most clinicians believe that it is preferable to have keratinized gingiva adjacent to teeth to perform oral hygiene easier, resist frenum pull, reduce further recession.[1,2] Inadequate keratinized mucosa may be due to high plaque accumulation and gingival inflammation.[3] As a standard and routine technique, free gingival graft (FGG) has been used to increase the zone of keratinized gingiva.[3,4]

Despite favorable results from this technique, some disadvantages guided investigators to seek for better

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alternatives.[4] As a donor, the palatal tissue provides keratinized gingiva, however, the problems still exist in relation to the palatal phenotype, the diversity of color and texture with surrounding mucosa,[5] and the bulky appearance which seems to be an esthetic problem in anterior sites.[4-6] Most importantly, the need for a donor site adds to the patient morbidity (e.g., pain and bleeding).[4-6]

Acellular dermal matrix (ADM) allograft has been used to cover full-thickness burn wounds.[7] Not long ago, ADM has been introduced to increase width of keratinized gingiva (KGW) as an alternative to autogenous palatal graft.[4,6] ADM is made of skin in split-thicknesses with no cellular components (fibroblasts, keratinocytes, vascular endothelium, sebaceous glands, sweat glands, and smooth muscle).[8-12] ADM consists of a basement membrane complex and extracellular matrix proteins;[6] therefore, it is thought to be weakly immunogenic or nonimmunogenic. Although it is accepted well in human tissues as an allogeneic graft,[9-11] the possible risk of disease transmission, cost of commercially available human ADM, and at the same time limited availability of cadaver skin, limit the use of this material as a dermal replacement.[9] It is assumed that ADM from xenogeneic source may solve the above problems, so it will become as a favorite material to use in a broad spectrum of periodontal treatment procedures.[9,13] Recently, the use of xenogeneic ostrich skin as a new and available source for ADM in surgical procedures in skin deficit was considered.[13]

To the best of our knowledge at best, there are no published studies in dentistry showing the use of this xenogeneic ADM. Thus, for the first time, it was aimed to compare the clinical efficacy, histological structure, histomorphometric properties, and biocompatibility of the ostrich ADM with autogenous FGG, when grafted into alveolar mucosa has led to increase of width of keratinized attached tissue in dogs. Our primary outcomes were to compare the KGW and color match following Ostrich ADM and FGG, while the secondary outcomes were to analyze histological parameters.

MATERIALS AND METHODS

Animals and presurgical procedures

Ten mixed breed male dogs, aged 1–1.5, and weighing 15–20 kg were chosen for this split-mouth animal study.

The sample size was calculated on the basis of results achieved from a preliminary sample of 5 dogs, all eligible for the subject of study.

According to the preliminary results, changes on the keratinized gingival thickness of the two treatment methods were 0.24 ± 0.15. Thus, the final necessary sample size according to paired sample size formulation is $n = 10$.

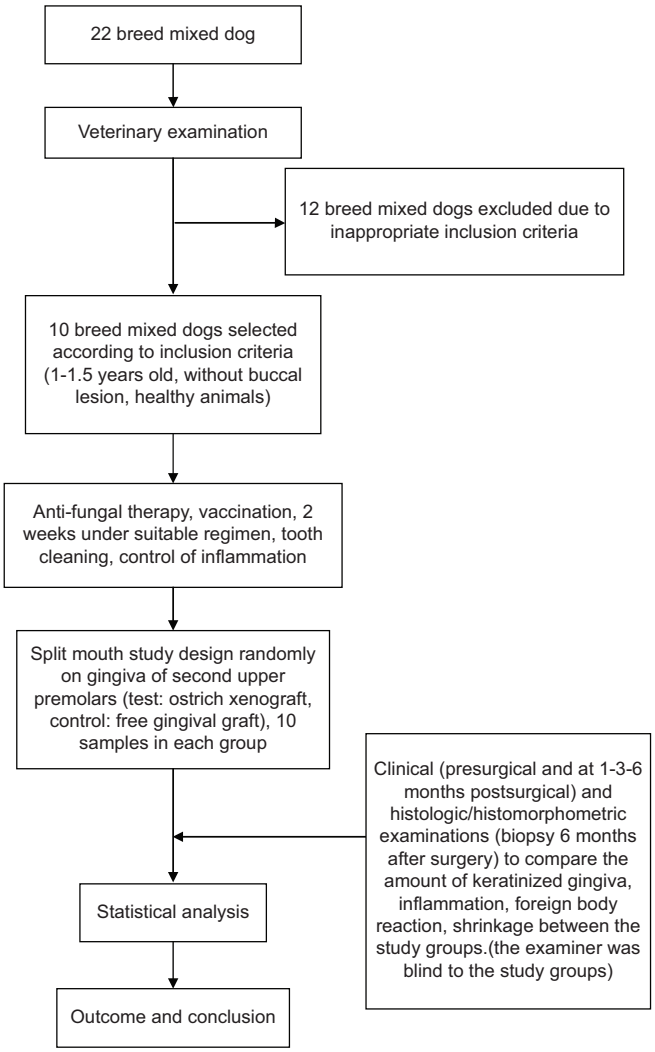
$$n = \frac{\delta_d^{\,2}\left(z_\alpha + z_\beta\right)^2}{\left(\mu_1 - \mu_2\right)^2}$$

Where $\alpha < 0.01$; $\beta < 0.05$, therefore $n = 10$

The animals were examined by a veterinarian in Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University at Tehran and vaccination was done for the animals. Oral examination was done; there were no buccal lesions in the mouth. Animals were kept in individual metal cages, provided commercial pet pad in the surgery department, science, and research center. The dogs were anestrous and kept at 25°C at normal day/light cycle. All animals provided commercial pet food and tap water. The animals were under suitable regimen for 2 weeks, during this period the dogs' teeth were cleaned carefully to control the gingival inflammation. Then, animals randomly allocated into experimental groups. The protocol was approved by the Animal Ethics Committee (no: 85-23). All experimental procedures were carried out in accordance to the *Guide for the Care and Use of Laboratory Animals to Investigate Experimental Pain in Animals*.[14] Animal handling and experimental procedures were in compliance with ARRIVE guidelines [Flowchart 1].

Preparation of acellular dermal matrix

Fresh skin was obtained from normal and young (1–1/5 year) male ostrich abdomen under aseptic condition. Cleaning and excision of subdermal fatty tissues were done in clean room Class B (Tissue Bank research center). The ostrich skin was then washed with phosphate-buffered saline (PBS) (PBS-Gibco-UK) and kept in Antibacterial media (Mixed of Gram positive and negative antibiotic) for 24 h. The samples were washed out 5 times entirely by Distilled water (DW) and soaked in Triton X-100 (Sigma-UK) at 4°C temperature for 24 h. The processed skins were washed again in DW and kept in sodium dodecyl sulfate (SDS) (SDS, Merck, Germany) for the next 15 h in 4°C temperature. On the next step, the samples



Flowchart 1: Study flow diagram.

were washed in DW and kept in SDS for another 10 h. Finally, the samples were washed in PBS. All solutions which were used in ADM preparation were filter-sterilized, and the procedures were performed aseptically. To prevent microbial growth, Sodium Azide, (0.02 gr/100 ml) (Merck, Germany) was present at all times in the extracted solutions.[13] At the end, big samples were divided to 40 equal square shape derms (20 mm × 20 mm). The samples were washed for 10 times and freeze derided by lyophilized Zirbus-Germany machine. The samples were packed in double sealed bristles and sterilized by 25 k gray Gamma irradiate (Atomic Research Center).

Scanning electron microscope evaluation

A random freeze dried sample selected for scanning electron microscope (SEM) evaluation [Figure 1]. A sample dimension about 3 mm × 3 mm was cut and attached on SEM disc then coated by Gold. The disc was placed in SEM chamber (JEOL 1000, USA). SEM result showed rough surface without cells and ostrich feather.

Surgical procedure

Maxillary second premolars were selected in our study [Figure 2a]. Regions were divided randomly into two groups: Xeno (ostrich) Acellular Dermal Matrix (XADM) (test) [Figure 2b] and FGG (control) [Figure 2c] groups.

The animals were not fed at the night before surgery. On the day of surgery, the animals were anesthetized after primary sedation, by intramuscular administration of ketamine hydrochloride (15 mg/kg), and xylazine (2 mg/kg).

Surgical site preparation was done by 0.12% chlorhexidine digluconate (CHX) and anesthetized by subcutaneous infiltration of 2% lidocaine with 1:100,000 epinephrine. A horizontal incision was then made by blade no. 15 (Ansen, Frankfurt, Germany) on the buccal side, apical from the mucogingival junction in the first and second premolar site (approximately 15 mm in length). Subsequently, oblique mesial and distal relaxing divergent incisions were made in the apical direction beyond mucogingival junction (length of vertical dissection was approximately 7 mm). A partial-thickness flap was elevated by sharp dissection as close as possible to the periosteum [Figure 2d]. For test sites which were allocated randomly, ADM was rehydrated for 10 min in sterile saline normal solution, then the graft was placed on recipient tissue and sutured to the periosteum with 5-0 Vicryl bioabsorbable sutures (SupaBon) [Figure 2e].

In control group, following recipient preparation similar to the test group, keratinized tissue was obtained by use of # 15 scalpel blade from the region between canine and first premolar of hard palate. A strip of absorbable oxidized regenerated cellulose was placed on the donor wound surface and fixed in place with No. 4-0 silk sutures to control bleeding. The prepared graft with dimensions 15 mm × 7 mm and 2 mm thickness (ranged between 1 and 3 mm) was placed and stabilized on the recipient site with simple interrupted 5-0 Vicryl suture (SupaBon) [Figure 2f].

The donor sites of FGG and the recipient sites of both study groups were covered with periodontal dressing (COE-Pac, GC, USA).

Postoperatively, the dogs were kept on a soft diet and antibiotics (20,000 IU penicillin and

erythromycin (Erythrocin), 0.01 g/kg body weight, were administered. Sutures were removed after 7 days. Once a week until the time of biopsy preparing (6 months), the dogs were sedated to perform precise prophylaxis with ultrasound and topical application of 0.12% CHX solution.

At baseline, months 1, 3, and 6 after surgery, the width of keratinized tissue was measured in millimeter by rolling the alveolar mucosa coronally by the side of a probe. Postoperative photography of treatment sites after 1, 3, and 6 months of healing are illustrated in Figure 3 [Figure 3a-c show test site after 1, 3, 6 months, respectively and Figure 3d-f show control site after the same months, respectively].

Histology

After 6 months of healing, biopsies with dimensions about 2 mm × 6 mm were excised from grafted areas in anesthetized dogs. Following biopsy removal, each specimen was fixed immediately in 10% formalin (Merck-Germany) for a minimum of 72 h. The specimens were processed and embedded in paraffin and serial sectioned longitudinally about 7 µm. For microscopic evaluation, the 5th, 10th, and 15th section of each specimen was stained by use of hematoxylin and eosin.

Histologic analysis

Stained specimens were evaluated individually by light microscope (Olympus-American) outfitted with a digital camera by an examiner blind to the purpose of the study. Further analysis of each section was carried out through different microscopic magnifications to study the structure and composition of the tissues. Qualitative analysis of the tissues was consisted of the evaluation of the inflammatory infiltration, foreign body reaction (presence/absence), and the kind of keratinization (non-, para-, ortho-keratinization). Inflammatory infiltration was classified as grade 0 for not detected inflammation, Grade 1 for <25 inflammatory cells/field, and Grade 2 for more than 25 inflammatory cells/field in each specimen.[9] Furthermore, the histomorphometric evaluation was performed to have quantitative analysis.

Statistical analysis

Quantitative data (KGW and the amount of shrinkage) were analyzed by repeated measured and paired samples *t*-test. Qualitative data (The amount of inflammation, foreign body reaction and keratinization) were analyzed by Wilcoxon Signed-rank test. Histomorphometric analysis results were nearly same between the study groups so not comparable. All analyses were performed by SPSS software (version 22.0, Armonk, NY: IBM corp.

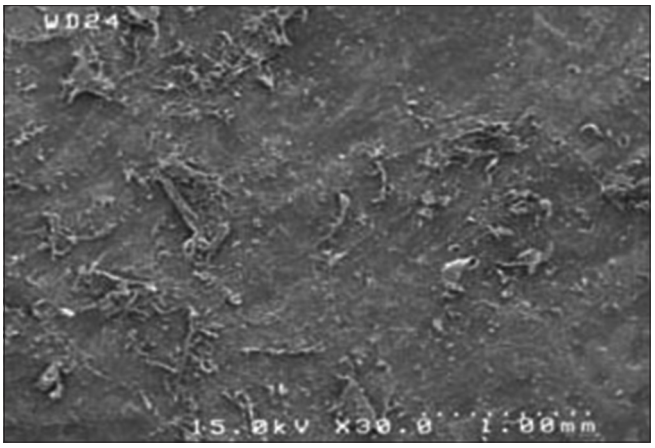


Figure 1: Scanning electron microscope view of a freeze dried sample of ostrich skin.

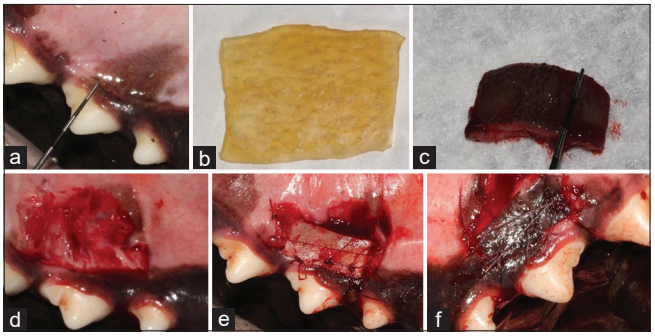


Figure 2: (a) Preoperative view of maxillary second premolar area shows 2–3 mm probing depths. The gingiva is normal and there is no bleeding on probing, (b) Xeno (ostrich) acellular dermal matrix, (c) free gingival graft, (d) Partial-thickness flap elevated, (e) Xenogenic acellular dermal matrix sutured to the periosteum and stabilized with 5-0 VICRYLsutures, (f) Gingival graft repositioned and sutured with 5-0 VICRYL suture.

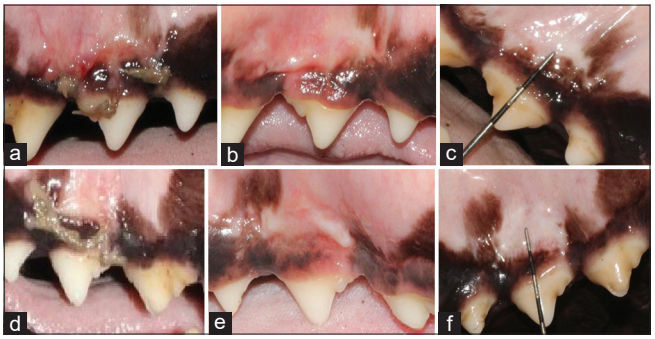


Figure 3: (a-c) Postoperative view. Clinical observation of a site treated with xeno (ostrich) acellular dermal matrix after 1, 3, and 6 months of healing, (d-f) Postoperative view. Clinical observation of a site treated with free gingival graft after 1, 3, and 6 months of healing. (a and d): 1 months; (b and e): 3 months; (c and f): 6 months.

USA. accordingly. $P > 0.05$ was considered significant statistically.

RESULTS

Pre- and post-surgical measurements were performed by a periodontist not involved in the surgeries. Measurements were made at the nearest 0.5 mm using a Marquis Color coded periodontal probe (Hufridy, USA). The mean score for each variable was calculated, and the results were used in the statistical analysis.

The mean KGW before and 1, 3, 6 months after surgery in the study groups is shown in Table 1.

The results of the study showed that the mean KGW within the study groups, after surgery and in interval examinations, had been increased significantly compare to the baseline ($P = 0.001$ in both groups), however, there was no statistically significant difference between the groups ($P > 0.05$).

After 6 months from the initiation of treatment, the mean KGWs in the control and test group were 8.15 ± 0.27 and 8.07 ± 0.24 mm, respectively. At this time, control and test groups showed 4.65 ± 0.50 and 4.8 ± 0.45 mm increase in KGW, respectively. Meanwhile, the graft shrinkage in test and control groups was 23% and 21% respectively and there was no statistically significant difference between the groups ($P > 0.05$).

There was no statistically significant difference between the two groups with respect to the amount of inflammation, foreign body reaction, and keratinization ($P > 0.05$).

Histologic evaluation revealed 8 samples with Grade 0, and 2 samples with Grade 1 inflammation in the FGG group, whereas in the XADM group, 7 samples with Grade 0 and 3 samples with Grade 1 inflammation were detected. The test and control group showed no significant difference when using Wilcoxon test ($P > 0.05$).

None of the samples in the both groups had Grade 2 inflammation, also no foreign body reactions were seen in histologic sections. Para-keratinized epithelium was seen in all specimens [Figure 4].

Based on histomorphometric results, there were two samples in the test groups and one sample in the control group with slightly inflammatory cells; and with respect to other parameters, there were no statistically difference between the study groups [Supplementary Table 1].

DISCUSSION

FGG procedure due to its limitations made clinicians' attention toward use of substitute materials. ADM has been used as dermal substitute however, there were also limitations.[9] Thus, ADM from xenogenic skin was introduced. Porcine ADM, as a temporary treatment has been used clinically in burns, mucogingival defects,[6,15] and other skin wounds.[9,16]

Recently, xenogenic skin of ostrich has offered fruitful results in the treatment of deep dermal burns.[13] The

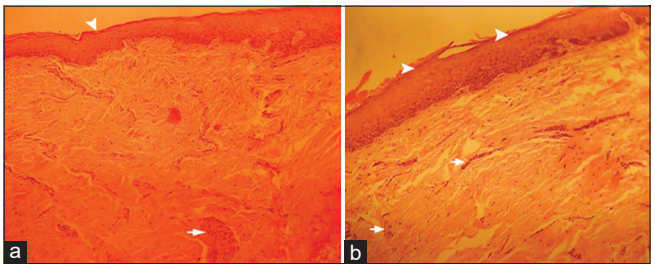


Figure 4: (a and b) Stratified para-keratinized epithelium at 6 month (arrow head), blood vessels and normal connective tissue with minimal inflammatory infiltrate and collagen fibers which oriented normally observed in all specimens of the xeno (ostrich) acellular dermal matrix and free gingival graft groups. The presence of apparent capillaries and randomly oriented collagen bundles are as signs of the connective tissue regeneration by xeno (ostrich) acellular dermal matrix as a template or scaffold; (a) = Xeno (ostrich) acellular dermal matrix(hematoxylin and eosin stain; original magnification: ×160); (b) = Free gingival graft(hematoxylin and eosin stain; original magnification: ×160)

Table 1: Keratinized gingiva width (mean±standard deviation) and tissue shrinkage (%) in Xeno (ostrich) acellular dermal matrix and free gingival graft groups at baseline and at postoperative intervals

Group	KGW (mean±SD)				Shrinkage (%)	
	Baseline	1 month	3 months	6 months	3 months	6 months
XADM (mm)	3.35±0.4	9.52±0.35	8.97±0.21	8.15±0.27	15	23
FGG (mm)	3.42±0.4	9.42±0.23	8.89±0.17	8.07±0.24	13	21
P	0.363	0.221	0.182	0.247	0.176	0.185

Significance ($P<0.05$). SD: Standard deviation; XADM: Xeno (ostrich) acellular dermal matrix; KGW: Keratinized gingiva width; FGG: Free gingival graft

results of histological studies on structure of ostrich skin showed that this material in both dermis and epidermis level, is very different from those found in other domesticated animals.[17,18] The dermis is a very dense connective tissue predominantly composed of collagen.[17] Since, collagen is a key component in healing wounds,[5] the development of dermal connective tissue makes ostrich skin highly suited for studying wound healing and skin grafting.[13,16-18] In ostrich skin, the stratum compactum is a dense layer of connective tissue that predominantly consists of collagen.[16] Moreover, there is a thin and highly vascularized layer of loose connective tissue between the stratum superficiale and stratum compactum; despite our little knowledge; authors showed that XADM has a dense but porous structure that predominantly composed of collagen. It works like a biologically compatible framework that let fibroblasts, epithelial cells and blood vessels migrate and easily adhere to it, through which a newly formed tissue appears.[16,17]

While the results of our study showed significant increase in the KGW, there was no statistically significant difference between the study groups; thus, treatment by ostrich skin could result in increase of KGW similar to FGG.

The graft shrinkage rate in the ADM site was a little greater than in the FGG site (23 and 21 percent respectively) however, there was no statistically significant difference between the two groups. It is similar to the results of Harris which showed the amount of increased keratinized tissue at the ADM sites was similar to that in FGG-treated sites (4.1 mm), albeit the mean dimension of the grafts was not reported.[5] Wei *et al*. compared the ADM and FGG and reported significant increase in width of keratinized tissue with FGG rather than with ADM, however, the graft shrinkage in ADM group was greater than FGG group (71% vs. 16%).[19] This difference between the studies might be attributed to graft location, observation period, surgical technique, and the graft extent. It was showed that the thickness of the grafts are important, the thick grafts show statistically the least shrinkage.[20-22]

Thoma *et al*., in their systematic review article on soft tissue grafting techniques revealed statistically significant less shrinkage in FGG group.[23] More shrinkage of ADM allograft in comparison to autogenous tissue may be due to the ADM fabrication process, since this material was prepared from cadaver skin after removal of epidermis and other cellular components.[15,21,23]

In our study, the mean shrinkage values in different intervals were respectively lower than those in studies done by using allogenic ADM.[19,24]

In the absence of dermal matrix, fibroblasts initially synthesize an immature matrix which subsequently are remodeled to form a hypertrophic scar or scar contracture.[21,23] The researchers suggested that after removing the cellular components (decellularization), structural and functional molecules such as collagen and sulfated glycosaminoglycans (sGAG) remain, this improve and facilitate the communication of the adjacent cells and their external environment. It was shown that inducing regeneration and delay in wound contraction were impacted by sGAG.[24-26]

Independent photographic analysis of the treated areas in our study showed XADM as a good model in increasing keratinized gingiva rather than FGG. It is also preferred in terms of tissue color match with adjacent untreated tissue and absence of scar tissue or keloid-like appearance. There was an increase of collagen in XADM sites, but the shape and distribution of collagen were similar to FGG sites, however, it was neither similar to healing process with ADM, nor with the hyalinized collagen seen in keloid scars [Figure 4]. Hence, our findings are similar to the results of Scheyer and contradict Wei studies.[4,19]

Moreover, mild inflammatory cell infiltration was observed in XADM and FGG-treated sites 6 months after treatment in histologic specimens of our study. The presence of inflammatory cells is important in continuity process of ADM with host tissues, that is, generation of a tissue more similar to the host tissue.[27]

After 6 months, blood vessel penetration could be seen in XADM, also collagen fiber bundle branches from XADM to the connective tissue were seen in all directions, XADM appeared well integrated with the host connective tissue. Histological presence of mild inflammatory cells and new vessels formation beneath ostrich acellular matrix in our study agreed with Farahany results.[13]

To the best of our knowledge, it is the first study reporting the efficacy of the ostrich acellular dermal matrix in increasing the band of keratinized tissue in a dog model study. In this animal study, a good

healing pattern and clinical behavior were seen in the matrix along with similar clinical outcomes when compared with the standard FGG. It also demonstrated acceptable increase of keratinized tissue, and good maintenance of the marginal tissue health with better color match.

Interpretation of these results was limited by certain aspects of the study design. Although the duration of this study was typically the same as for soft tissue studies,[23] the longer follow-up study would have allowed us to more precisely evaluate the durability of the result. Moreover, there were several limitations of the esthetic assessment such as the nature of the photography (as opposed to direct assessment), as well as varying degrees of consistency in the photographs (e.g. lighting, focus, global vs. close-up views).

CONCLUSION

The results showed similar outcomes after treatment with autogenous FGG and XADM in terms of increasing the width of keratinized tissue, the amount of shrinkage, and the quality of the attached tissue after 6 months' follow-up. Due to FGG disadvantages, XADM seems to be a suitable alternative for FGG. Since, there is no similar study on XADM with ostrich origin in periodontics, it is suggested:

1. More histologic emphasis on matrix characteristics including collagenous and noncollagenous proteins (e.g. tenascin, elastin,)
2. Perform human studies to approve biocompatibility and usability to use this material in periodontal plastic surgeries.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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Supplementary Table 1: Histomorphometric data of the study groups (Free gingival graft=Control group, Xeno (ostrich) acellular dermal matrix=Test group)

Index	Score	FGG1	FGG2	FGG3	FGG4	FGG5	XADM1	XADM2	XADM3	XADM4	XADM5
Epithelium Formation	Thickening of incision line (0) Migration of epithelial cells <25% (1) 25%< migration of epithelial cells <50% (2) 50% < migration of epithelial cells <75% (3) Complete epithelialization (4)	4	4	4	4	4	4	4	4	4	4
Inflammatory Cells	None (0) Slight <25% (1) 25%< slight <50% (2) 50%< moderate <75% (3) Severe >75% (4)	0	0	0	0	1	0	0	0	1	1
New vessels	None (0) Slight (around the tissue) (1) Slight (granulation tissue) (2) Moderate (3) Eminent (4)	3	3	3	3	3	3	3	3	3	3
Collagen	None (0) Slight (around the tissue) (1) Slight (granulation tissue) (2) Moderate (3) Eminent (4)	4	4	4	4	4	4	4	4	4	4

XADM: Xeno (ostrich) acellular dermal matrix; FGG: Free gingival graft