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THE HORIZONTAL RIDGE AUGMENTATION USING EQUINE XENEOGRAFT AND THE CORTICAL LAMINA: A CLINICAL, RADIOGRAPHIC AND HISTOLOGICAL PROSPECTIVE STUDY

Sarah Khalil[1] | Nabil Ghosn[2] | Nadim Mokbel[1] | Carole Chakar[1,3]

Introduction: Bone regenerations are common procedures used to restore the required bone width or height for adequate implant placement. Among the wide variety of materials developed for this purpose, the soft collagenated porcine membrane known as cortical lamina (OsteoBiol® Lamina, Tecnoss®, Giaveno, Italy) has provided promising clinical and histological results. It was used along with equine-derived bone particles (OsteoBiol® Gen-Os®, Tecnoss®, Giaveno, Italy) in a series of horizontal bone augmentations to clinically, radiologically and histologically evaluate the lateral bone augmentation, and to measure the vertical gain, if present, at the site of implant placement.

Methods: Fifteen healthy patients with ridges < 4mm needing implant placement were included. The area was augmented using equine bone and the cortical lamina was immobilised using fixation screws. Six months later, at implant placement, a biopsy was taken for non-demineralised histology. Radiological superimposition of the pre and post-operative CBCT scans was made to calculate the radiological bone gain at the site of implant placement. Bone gain was also studied using histomorphometrical analysis.

Results: At 6 months, implant placement was possible in all cases except one, where a total of 26 implants were placed in 14 patients. CBCT superimposition showed that the mean horizontal width had increased significantly at the level 0 mm, 2 mm, 4 mm and 6 mm of the implant sites. Histology showed signs of bone remodelling and vital bone formation. Histomorphometric results showed a higher bone percentage in the deep part of the biopsy compared to the superficial one.

Conclusions: The porcine cortical lamina membrane used with the equine xenograft particles seems to be a promising technique for the horizontal bone augmentation. Randomized controlled clinical trials are still needed to evaluate the superiority of this membrane compared to the conventional regeneration techniques along with long term follow up of the regenerated bone.

Keywords: Cortical, Equine, Guided bone regeneration, Lamina, Xenograf

Corresponding author:
Sarah Khalil, Saint Joseph University, B.P 11-5076 – Riad El Solh, Beirut. Email: Sarahfkhalil2@gmail.com

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1. Department of Periodontology, Faculty of Dental Medicine, Saint Joseph University of Beirut
2. Digital Unit, Laboratory of Cranio-Facial Research, Saint Joseph University of Beirut
3. Biology Unit, Laboratory of Cranio-Facial Research, Saint Joseph University of Beirut
nabilghosn@gmail.com | nadim.mokbel@gmail.com | carole.chakar@hotmail.com

L'AUGMENTATION DE LA CRÊTE HORIZONTALE PAR XÉNÉOGREFFE ÉQUINE ET LA LAMINE CORTICALE : UNE ÉTUDE PROSPECTIVE CLINIQUE, RADIOGRAPHIQUE ET HISTOLOGIQUE

Introduction : La mise en place adéquate d'implants nécessite des crêtes de dimensions suffisantes. Les défauts osseux, communs et multifactoriels, sont traités avec différents matériaux et techniques de régénération visant tous à restaurer la largeur ou la hauteur requise pour la pose d'implants. Une nouvelle membrane d'origine porcine, la lamina corticale (OsteoBiol® Lamina, Tecnoss®, Giaveno, Italie), a fourni des résultats histologiques et cliniques prometteurs et semble répondre aux critères d'une régénération osseuse guidée horizontale et verticale réussie.

Méthodes : Quinze patients en bonne santé ayant des crêtes <4 mm de largeur, qui se sont présentés pour la pose d'implants, ont été inclus dans cette étude. La zone sélectionnée a été augmentée par de l'os équin (Gen-Os®) sans addition d'os autogène. La lamina corticale a été immobilisée à l'aide de vis de fixation (système de fixation de précision Pro-fix ™). Six mois plus tard, lors de la pose des implants, une biopsie a été prélevée et une histologie non-déminéralisée a été effectuée. Une superposition des CBCTs pré et post-opératoires a été faite pour mesurer l'augmentation radiologique de l'os régénéré. Les données cliniques, radiologiques et histologiques ont toutes été utilisées pour l'évaluation statistique.

Résultats : À six mois, 26 implants ont été placés au total chez 14 patients à l'exception d'un cas ou la pose d'implants s'est avérée impossible. La superposition radiographique des CBCT a montré que la largeur horizontale moyenne avait considérablement augmenté aux niveaux 0 mm, 2 mm, 4 mm et 6 mm des sites implantaires. L'histologie a montré des signes de remodelage osseux et de formation osseuse vitale. Les résultats histomorphométriques ont montré un pourcentage d'os plus important dans la partie profonde de la biopsie comparé à la partie superficielle.

Conclusions : La lamina corticale d'origine porcine utilisée avec les particules d'origine équine sans addition d'os autogène semble avoir des résultats prometteurs dans l'augmentation osseuse horizontale. Des essais cliniques contrôlés randomisés sont encore nécessaires pour évaluer la supériorité de cette membrane par rapport aux techniques de régénération conventionnelles et au suivi à long terme de l'os régénéré.

Mots clés : Formation osseuse, Lamina corticale, Régénération osseuse guidée, Xénogreffe equine.

Introduction

With the high prevalence of partial and total edentulism and the increasing demand for aesthetic implant therapy, bone augmentations have become common procedures aiming to provide sufficient bone support for implants of adequate dimensions and aesthetic standards [1-3].

Ridge discrepancies can result from traumatic extractions, severe periodontal disease or can be due to congenitally missing teeth, etc. They can be corrected using a variety of reliable surgical techniques such as guided bone regeneration (GBR), autogenous bone grafts, bone spreading and distraction osteogenesis. Other regenerative treatments include tissue engineering, the use of bone morphogenic proteins and growth factors [4].

GBR consists on using a barrier membrane to prevent the apical ingrowth of the gingival epithelium inside the osseous defect, hence creating a sheltered space that can be colonised by regenerative potential cells, since ingrowth of soft tissue may disturb or totally prevent osteogenesis in a defect or wound [4, 5].

An ideal membrane for GBR, whether resorbable or not, should have the following criteria, as described by Scantlebury et al. in 1993 [6]: biocompatibility, tissue integration, cell occlusivity, nutrient transfer, space making ability and clinical manageability.

Non resorbable membranes may cause some complications, including premature exposition/dehiscence which can lead to infection. Moreover, an extensive surgical procedure for their removal is always required, thus adding to patient morbidity. Consequently, the use of resorbable collagen membranes has become widespread, due to many advantageous properties like haemostasis, chemotaxis and cell adhesion functions [7]. The main disadvantage of these membranes remains their rapid resorption and collapse, not maintaining the adequate space and volume like non resorbable ones. Therefore, bone substitutes were added in order to preserve an adequate space between the bone and the membrane, to stabilise the clot and avoid the collapse of the membrane [4]. The bone substitutes normally range between autografts, allografts, xenografts or alloplastic grafting materials [5].

The collagenated cortical porcine barrier (OsteoBiol® Lamina, Tecnoss®, Giaveno, Italy) has been described to have a slow resorbability (approximately 5 to 6 months) [4], not requiring re-entry, maintaining the desired volume for bone formation due to its mechanical properties and plastic consistency thus facilitating the handling, and a second intention healing in case of exposure [8].

Its use has provided promising histological and clinical results when used with particulate porcine xenograft (OsteoBiol® Gen-Os®, Tecnoss®, Giaveno, Italy) [8] and appeared to fulfil the criteria for a successful horizontal and vertical GBR [9,10].

The primary aim of this study is to clinically, radiographically and histologically evaluate the horizontal bone regeneration of the soft cortical lamina of equine origins in procedures involving lateral ridge augmentations using an equine-derived cortico-cancellous heterologous bone mix (Gen-Os®, Osteobiol). The secondary aim of this study is to radiographically measure the vertical gain, if present, and see its statistical significance at the site of implant placement.

Materials and Methods

Fifteen patients (1 male, 14 females) aged between 27 and 64 years old were selected from the Department of Periodontology of the Faculty of Dentistry, Saint Joseph University Beirut.

Confining with the inclusion criteria, all the selected patients were systematically healthy, had good oral hygiene (FMPS and FMBS <20%). When presenting for implant placement and upon examination, the ridges had deficiencies in width (<4 mm, Cawood and Howell class IV) (Cawood & Howell, 1988) which did not allow correct implant placement. Therefore, horizontal bone augmentation procedures were proposed. All areas needing GBR, maxilla or mandible, in the posterior or anterior or anterior region were included in this study.

Exclusion criteria were the following: pregnant and lactating women, patients suffering from systematic diseases, patients on bisphosphonate, smokers (>10 cigarettes/day) and patients needing vertical augmentations.

Patients were given a thorough description of the procedure with a highlight on the risks, after which they received a consent form to sign before starting.

Ethical approval for this study was obtained from the Scientific Research Commission (FMD 141-17.10.2017).

Pre-surgical preparations

Clinical and radiographic examinations were done prior to any procedure. The edentulous area was examined to identify the availability of keratinised gingiva and the possibility of implant placement in the mesio-distal and inter-arcade planes. Radiographic examination was completed using a cone beam computed tomography (CBCT) scan of the region, where the indication for vertical augmentation was selected and excluded from the study. All the patients were given oral hygiene instructions and prophylaxis.

Each patient received 2g of Amoxicillin one hour prior to surgery. They were instructed to mouth rinse in chlorhexidine 0.12% gluconate mouthwash for one minute. Extra-oral disinfection was made using topical chlorhexidine.

Surgical procedure

Local and/or regional anaesthesia was obtained using Articaine

hydrochloride 4% with Adrenaline 1:100,000, depending on the treated area.

A mid-crestal incision using a 15C blade was performed and extended into the sulcus of the adjacent teeth, if present. At least one vertical releasing incision extending beyond the muco-gingival junction was made. A muco-periosteal flap of full thickness was raised and extended buccally. Palatally it was reflected to expose 3 mm of bone whereas lingually the flap was elevated until reaching the mylo-hyoid line.

Crest debridement was followed by decortication using a twist drill with a stop of 3mm of length (Meisinger® 203S) to ensure a good perfusion of the grafted site.

The soft lamina (OsteoBiol®) used is a 35x35mm medium curved membrane of equine origins.

It was soaked in saline sterile water for twenty minutes to achieve better elasticity and easier manipulation. It was then trimmed with sterile scissors and adapted to the recipient site, making sure it was not in contact with the surrounding teeth. It was fixed using self-drilling membrane fixation screws of 1.5 mm x 3.0 mm (Pro-fix™ Precision Fixation System) palatally or lingually. Cortico-cancellous heterologous bone mix (Gen-Os®, Osteobiol) which was previously hydrated in sterile saline for twenty minutes, was placed on the ridge in sufficient quantities and covered by the membrane while being adapted in the desired shape of the future ridge. The membrane was reclined and also fixed on the buccal side for better adaptability and immobilisation. In order to ensure a tension free closure, the buccal flap was advanced using a periosteal releasing incision connecting the vertical incisions thus achieving elasticity of the flap. It was followed by the brushing technique when the elasticity was not sufficient [11]. Precaution was taken to identify and carefully isolate the mental foramen from the surrounding tissues when present. The lingual flaps were advanced using a blunt instrument (e.g. Prichard) that would detach the muscular insertion of the mylo-hyoid from the lingual flap.

Once proper elasticity was achieved, horizontal mattress sutures were placed at 4 mm from the incision line using non-resorbable PTFE monofilament sutures.

They were followed by single interrupted sutures close to the edges of the flap in order to create a connective tissue-connective tissue contact, this creating a barrier to reduce the incidence of membrane exposure. The vertical incisions were closed with interrupted sutures [12].

All patients were instructed to receive an injection of betamethasone dipropionate and disodium phosphate directly after the surgery and received 2g of amoxicillin per day for a total of 7 days.

Post-operative recommendations were clearly written and given to the patient. Chlorhexidine was prescribed starting the second day after surgery and until suture removal.

Sutures were removed 14 days after the surgery, they were left for another 7 days if the healing looked compromised.

Healing was uneventful in all cases except 2 where an exposure occurred along with suppuration. They were treated with an extended dose of Amoxicillin (seven additional days) and with chlorhexidine rinsing. In both cases the membrane was not removed and re-epithelization occurred after seven days.

Implant placement

At 6 months from the surgery, a CBCT scan of the grafted area was taken and measures were made for the choice of the implant diameter and length. Based on the CBCTs, implant placement seemed possible in all of the regenerated areas.

On the day of the implant placement, local anesthesia was made, followed by a midcrestal incision and occasionally a vertical one, allowing access to the fixation screws for their removal. The crest was debrided from any soft tissue remnants and a biopsy was taken at the site of implant placement using a trephine burr of outer diameter of 3.5mm and inner diameter of 2.5mm. The trephine and bone were immersed in 10% buffered formaldehyde and fixed for histology. The implants (Straumann ®, Bone Level) were placed at the corresponding sites of the biopsies, and the choice of a cover or a healing screw was made depending on the clinical situation and the primary stability of the implant. In most of the cases, 2 mm of bone structure was established buccally while positioning the implant [13].

Implant insertion torques were noted as indicated on the implant torque wrench.

Periapical radiographs were taken, the flap was sutured and the patients received a daily dose of 2g of amoxicillin for 7 days and diclofenac potassium for pain management. They were also advised to mouth rinse with chlorhexidine 0.12% for 10 days.

In one case, implant placement was not possible. When raising the flap, the grafted bone was fibro-integrated within the flap and not to the bone. In that case, the crest was debrided and cleaned from any remaining xenograft material, and it was sutured. The patient was advised to wait for the soft tissue healing before being operated on again.

The patient was a female 38 year-old, non-smoker, and had a fixed provisional bridge covering the grafted area.

Radiological protocol

Patients were scanned with the Newtom VGI CBCT machine. For each scan, an advanced jaw segmentation technique was realized using the Blue Sky Plan software by means of threshold segmentation and contour interpolation. The result was an accurate 3D model of the jaw having the region of interest.

On the post-operative CBCT plan, virtual implants were placed in the optimal position regarding bone and prosthetic reference when present.

In order to compare directly the pre and post-operative models, the pre-operative bone model was loaded into the post-operative plan an n-point registration technique was used for the superimposition of the two models. The outline of each model was visible in a unique color for comparison.

A vestibulo-lingual implant centric section perpendicular to the panoramic curve and parallel to the long axis of the simulated implant was used to make all the measurements as follow:

Horizontal bone width measurements

For each implant site, pre and post-operative horizontal bone width were measured at 4 levels. Bone width was calculated from the distance between the most buccal and most lingual bone points at each level while being parallel to the simulated implant platform. (Figure 1).

- H0-T1 and H0-T2: Pre and post-operative horizontal bone width at implant platform level
- H2-T1 and H2-T2: Pre and post-operative horizontal bone width at 2mm apically to implant platform
- H4-T1 and H4-T2: Pre and post-operative horizontal bone width at 4mm apically to implant platform
- H6-T1 and H6-T2: Pre and post-operative horizontal bone width at 6mm apically to implant platform

Vertical bone gain/loss measurements

For each implant site, pre and post-operative vertical bone gain were measured at 3 levels. Vertical bone gain/loss was calculated from the distance between the most coronal pre-operative bone point.

Histological protocol

Samples were taken at six months after regeneration and were treated with non-demineralized histology.

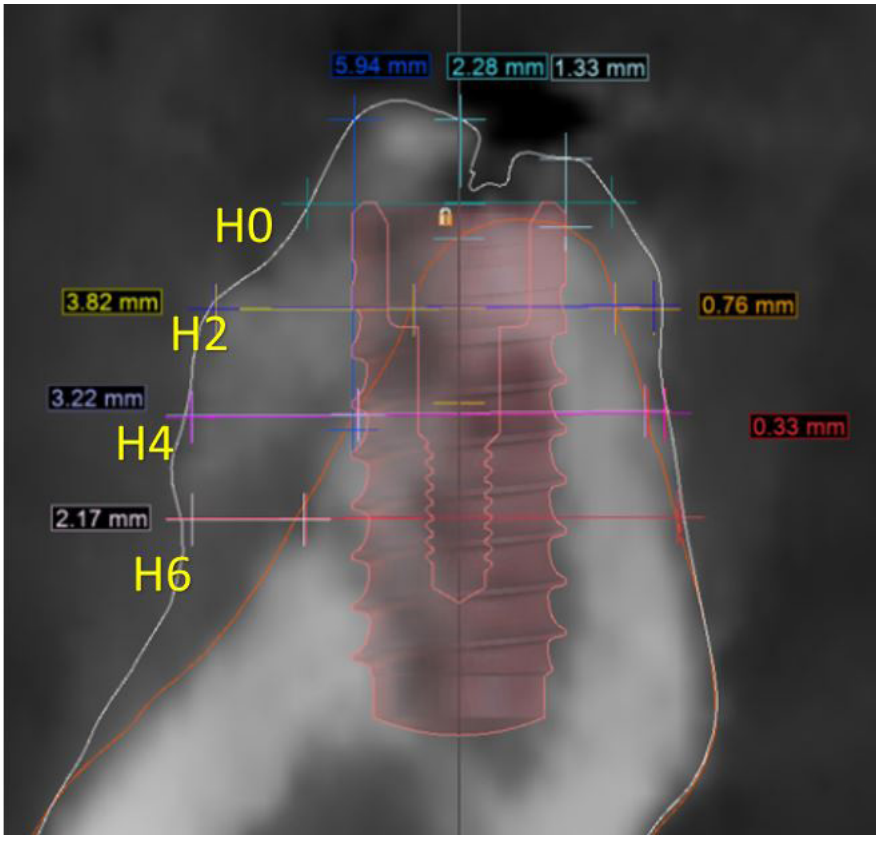

Figure 1. Radiological bone superimposition and measurements at the site of implant placement.

The sections were stained with Giemsa-Paragon and basic fushin. Giemsa gives the cells and nuclei the colour blue, and Paragon stains the bone in red. The cuts were separated into S (superficial), M (median) and P (profound) cuts, the superficial ones being the cuts facing the periosteum.

Histomorphometry

The sections were observed under an optic microscope (Olympus BX 60, Olympus Corporation, Tokyo, Japan), connected to a digital camera (Olympus E330). The software Image J/ Fiji [14] was used for histological quantification.

The total area of the each section was measured. The bone and osteoid volume were then quantified using the Bone Volume Mask and by using the 'wand tool' to select all the black areas. The percentage of bone and osteoid matrix in each section was consequently calculated [15].

Statistical analysis

The statistical package software for social sciences (SPSS for Windows, Chicago, IL, USA, version 25.0) was performed for statistical analysis of the data. The alpha error was set at -p-value<0.05. Frequency and percentage were utilized to describe categorical variables. Mean and standard deviation were used for continuous variables. Repeated measure analysis of variance (Anova) followed by Bonferroni post hoc tests were used for factors Time (baseline and six months), Distance (0mm, 2mm, 4mm and 6mm) and Level (buccal, median, lingual) to compare the mean bone level between groups, the mean horizontal gain and the mean vertical gain respectively. One sample t tests were used to compare the mean vertical gain with a theoretical value "0'' that supposed the absence of gain. Student t tests were performed to compare continuous variables between two groups. Pearson correlation coefficients were calculated to assess

the relationship between continuous variables, and finally Kruskal-Wallis tests were used to compare histomorphometric measurements between different levels.

Results

Clinical results

Implant placement was possible in all cases except one, where the grafted bone was embedded within the flap and not onto the bone. A total of 26 implants were placed in 14 patients (13 females and 1 male) aged 48.2 ± 11.7 years (Range: 27- 64 years) where 6 (42.9%) participants were light smokers. All implants were placed within the osseous walls and did not need any additional bone augmentations except one, where placing the implant in the ideal prosthetic position left the buccal wall too thin (1 mm) and equine particulate bone graft was needed for volume enhancement.

The implants were Straumann® bone level cylindrical with SLA surface.

12 implants were of 3.3mm, 14 of 4.1mm, and 1 of 4.8 mm of diameter. A cover or a healing screw were placed depending on the clinical situation. (Figure 2)

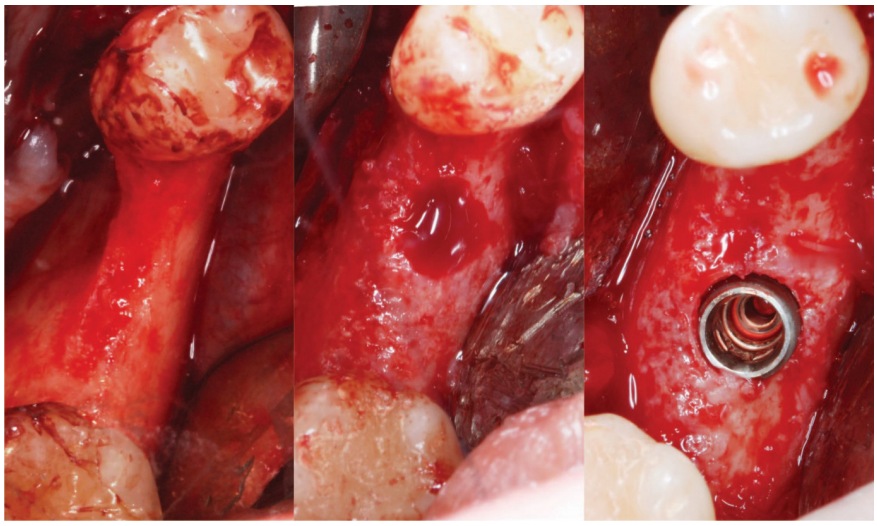

Figure 2. Views of the crest after bone graft and implant placement in the mandible.

Radiological results

Horizontal bone gain

The mean horizontal width has increased significantly after 6 months at the level 0 mm (-p-value<0.001), 2mm (-p-value<0.001), 4mm (-p-value<0.001) and 6mm (-p-value<0.001). The mean horizontal gain was significantly different within implant levels (-p-value=0.004). It was significantly smaller at 6 mm. No significant difference was found between 0 mm, 2mm and 4 mm (-p-value>0.05) (Table 1, Figure 3)

Table 1: Mean horizontal gain.

	0 mm (N=26)	2 mm (N=26)	4 mm (N=26)	6mm (N=25)	-p-value
Baseline	2.989 ± 1.612 [a]	5.160 ± 1.166 [b]	6.784 ± 1.347 [c]	8.032 ± 1.746 [c]	<0.001*
6 months	5.248 ± 0.939 [a]	7.434 ± 1.128 [b]	8.548 ± 1.499 [c]	9.514 ± 1.899 [c]	<0.001*
-p-value	<0.001*	<0.001*	<0.001*	<0.001*	
Gain (mm)	2.274 ± 1.571 [b]	2.274 ± 1.015 [b]	2.028 ± .790 [b]	1.442 ± .929 [a]	0.004*

*Statistically significant at p<0.05

a,b,c: *Different letters indicate a significant difference between groups.*

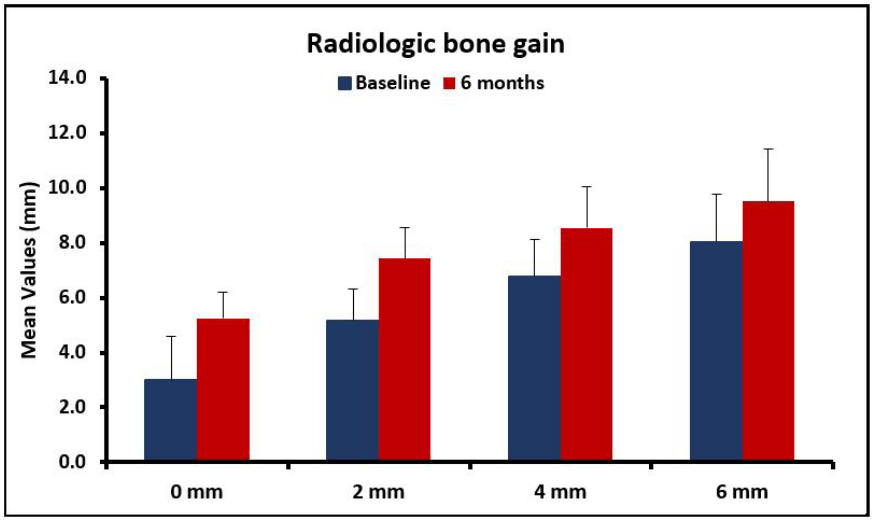

Figure 3. Mean bone gain within level of bone height at the site of implant placement.

Vertical bone gain

The mean vertical gain was significantly different from 0 value which means that the vertical gain was present (-p-value<0.001). (Table 2)

The mean vertical bone gain was significantly different within implant levels (-p-value=0.002). It was significantly elevated at buccal level. No significant difference was found between median and palatal levels (-p-value=1.000). (Table 3)

The vertical gain was significantly associated with the presence of the membrane at 6 months for the median (-p-value=0.006) and buccal levels (-p-value=0.002). (Table 4)

The vertical gain was significantly associated with the membrane exposure at 6 months for the median level (-p-value=0.037).

Histological results

Parts of the lamina were fixed alone using Giemsa-Paragon and basic fushin staining in order to study the cortical nature of the membrane.

At x500 magnification, osteons, being the primary anatomical and functional units of cortical bone, are visible (O). The lamellae surround the osteocytes lacunae (L), showing the cortical nature of the membrane (Figure 4).

The histological observations of the biopsies from the implant sites at six months showed variable results.

Loose connective tissue was present especially in the most coronal aspects, along with vessels and connective fibrous tissue. There was no fibre interposition between the particles and the newly formed bone. Regions of resorption were present along with osteons showing an active process of bone resorption and apposition. The presence of giant multinuclear cells and a newly formed non mineralized matrix were signs of ongoing bone remodelling.

In the non-active areas some bone bridges were formed between

Table 2: Mean vertical gain

	Test Value = 0					
Vertical gain	t	df	-p-value	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Buccal	5.675	24	.000	2.7756	1.7662	3.7850
Median	4.490	24	.000	0.8124	.4389	1.1859
Lingual	4.520	24	.000	0.7344	.3991	1.0697

Table 3: Mean vertical gain within distance from crest

	Buccal (N=25)	Median (N=25)	Lingual (N=25)	-p-value
Gain (mm)	2.776 ± 2.445 [b]	0.812 ± 0.905 [a]	0.734 ± 0.812 [a]	0.002*

*Statistically significant at p<0.05

a,b: *Different letters indicate a significant difference between groups.*

Table 4: Association between vertical gain and presence of membrane at 6 months.

	Membrane at 6 months	N	Mean	Std. Deviation	-p-value
Horizontal gain at 0mm	Yes	22	2.4018	1.67301	.062
	No	4	1.5675	.44888	
Horizontal gain at 2mm	Yes	22	2.3327	1.02783	.502
	No	4	1.9525	1.01513	
Horizontal gain 4 mm	Yes	22	2.1045	.81358	.255
	No	4	1.6075	.53206	
Horizontal gain at 6mm	Yes	21	1.425	1.0120	.841
	No	4	1.530	.2439	
Vertical gain Buccal	Yes	21	3.1405	2.49735	.002*
	No	4	.8600	.66187	
Vertical Gain Median	Yes	21	.9290	.94024	.006*
	No	4	.2000	.24345	
Vertical Gain Lingual	Yes	21	.7552	.75760	.776
	No	4	.6250	1.19726	

*Statistically significant at p<0.05

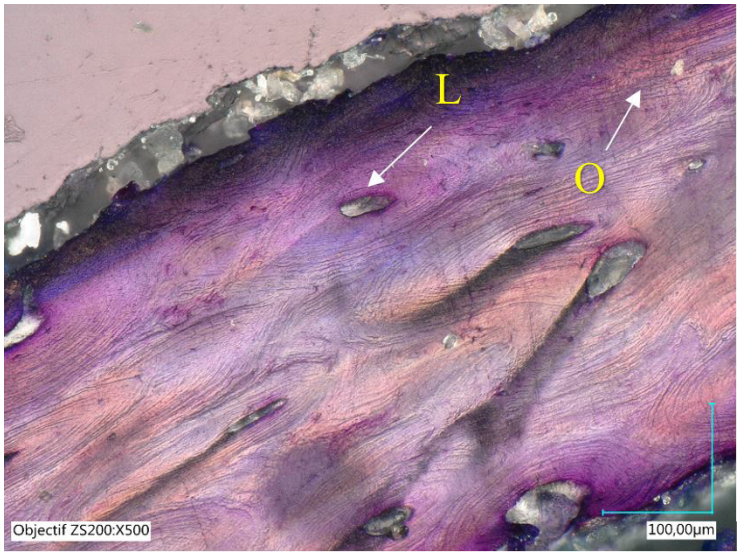

Figure 4. Aspect of the cortical lamina when fixed alone at x500 magnification. O: Osteons. L: Lamellae

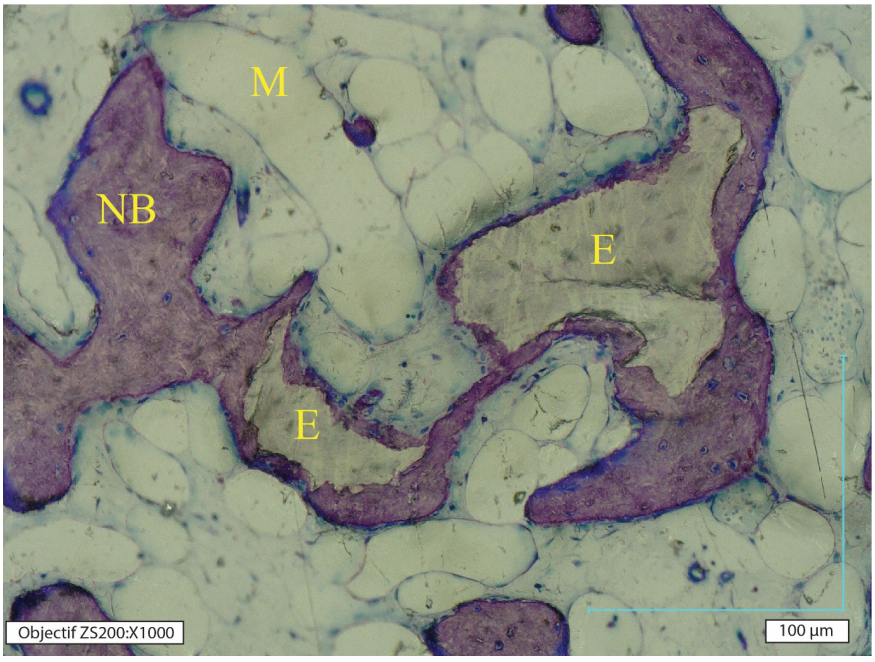

Figure 5. Equine xenograft at x1000 magnification.
E: Equine bone particle. M: Marrow Spaces. NB: Newly formed bone.

Table 5: New bone formation

	N	Mean	Standard-Deviation	-p-value
S	9	60.7811	11.62994	
M	6	73.6167	14.13625	0.021
P	7	77.2343	7.18384	
Total	22	69.5168	13.08477	

the particles showing the osteoconductive properties of the material. There were no signs of inflammatory reactions.

In figure 5, two equine bone particles are surrounded by newly formed bone and bone marrow, with one bridging starting to form between the particles. Osteoblasts line the surface of the xenograft, while bone surrounds the particle without interposition of fibrous tissue. (Figure 5)

Histomorphometry

The measurements were significantly different within levels (-p-value=0.021). They were significantly lower at the level S (superficial) and elevated at M (median) levels and P (profound) levels. The correlation coefficient was significantly elevated (r=0.625; -p-value=0.002). (Table 5)

Discussion

In this prospective study, several parameters were studied in order to assess the clinical, radiological and histological efficiency of the cortical lamina and the equine xenograft in bone augmentations. The authors' main focus was to measure the quantitative as well as the qualitative augmentation of this technique, by utilizing the radiographic CBCT superimposition and incorporating the histomorphometric analysis. A larger sample was studied, where 15 patients underwent regenerations procedures compared to eight, ten and four in similar studies using the cortical lamina [8-10]8 partially edentulous patients (6 females and 2 males.

In a study by Wachtel et al. in 2013, a collagen membrane was used as an outer layer along with the cortical lamina in order to improve the tissue integration of the membrane. Since the collagen membrane has a cell-occlusive surface, the collagen fibers of the soft tissue can interlock with the membrane and improve primary wound closure [9].

The soft cortical lamina was used alone in this study, without the addition of a second collagenated membrane. This was done to show its sole regeneration potential and soft tissue healing capacities, while avoiding the extra charges and time to the patient and the practitioner.

Although the manufacturer preconized hydrating the lamina in lukewarm sterile saline for 3 to 5 minutes, the authors have found that an extended hydration time made the handling of the membrane easier. Therefore in this study, it was left immersed in saline for approximately twenty minutes. By doing so, the membrane seemed to be more flexible and easier to stabilize, unlike the least hydrated one that had a tendency to tear when fixation pins were inserted.

One of the main advantages of the cortical lamina is its ability to allow second intention healing of the wound in case of exposure. Therefore, it was left in place in the case where exposure occurred, while being regularly maintained and cleaned by topical applications of chlorhexidine gel until complete healing was achieved. The manufacturer recommends removing it only in the case of supra-infection and not automatically when exposure occurs. As a result, the cortical lamina is particularly indicated in regenerations with high risk of exposure.

In this study, only equine bone substitutes were used without the addition of autogenous bone. This was done primarily to reduce bias and post-operative complications, where it would be difficult to ensure the 50/50 proportions equally for all the patients, without having to open a second donor site.

Secondarily, it would test the properties of the equine xenograft (Gen-Os®), whose properties were

associated to human bone in the following studies [16, 17].

In this study, the mean horizontal width has increased significantly after 6 months at the level 0mm, 2mm, 4mm and 6mm. The mean horizontal bone gain was 2.274mm, 2.274mm, 2.028mm and 1.442 at 0mm, 2mm, 4mm and 6mm of distance from the top of the planned implant, respectively. It was also significantly different within implant levels; being lesser at 6 mm whereas there was no significant difference between 0mm, 2mm and 4mm.

Mordenfeld et al. in 2014 conducted a study to radiologically and histologically evaluate the graft healing and volumetric changes after lateral augmentation with two compositions of deproteinized bovine bone (DPBB) and autogenous bone (AB). The mean horizontal bone gain was 2.9 mm and 3.5 mm for 90 : 10 and 60 : 40, respectively, 3mm from the top of the crest. They also found a significant difference in width reduction between the 90 : 10 and 60 : 40 mixtures when measured 3 mm from the top of the crest, 46.9% (2.7 mm) and 37.0% (2.0) respectively (p=0.0029) [18].

However, there was no significant difference in width reduction between the 90: 10 and 60 : 40 mixtures when measured 6 mm from the top of crest, 34.7% (2.3 mm) and 27.2% (1.8 mm) respectively (p=0.07).

Their results are in concordance with the results of this present study; this is mainly due to the anatomy of the knife-like ridges, which usually have sufficient width at 6mm of depth at the site of which the augmentation procedure does not play a significant role.

The mean vertical bone gain was also significantly different within implant levels. It was significantly elevated at the buccal level. This is also related to the knife-like ridge anatomy, resulting from the resorption of the buccal wall that normally occurs after an extraction.

These results are in harmony with the results of the study conducted by Rossi et al. in 2016, where the bone lamina technique proved to be a valid method to treat the horizontal and vertical defects similarly to other non-resorbable barriers [8,19, 20].

The findings of this study also show that the vertical gain was significantly associated with the presence of the membrane at 6 months for both median and buccal levels. In the pre-mentioned study by Rossi et al. [8]8 partially edentulous patients (6 females and 2 males, the cortical lamina was observed from both a clinical and histological point of view at 6 months for the horizontal regenerations; however it was not detected when the re-entry was performed at 12 months in the cases of the vertical regenerations. An explanation to that would be the different individual resorption rate of each patient, where a slow resorbability of the membrane led to better bone regeneration, mainly in the vertical dimension.

The histomorphometric measurements of the bone formation were significantly different within levels. The bone density percentages were the lowest in the superficial cuts where the graft particles and membranes were most likely to be found. The bone was denser in the middle and profound cuts where the bone particles were more densely packed and where the native autogenous bone was more present.

This is in harmony with the results of the study by Mordenfeld et al. in 2014 .Their qualitative observation on histologically stained sections illustrated more 'boneintegrated particles' in the graft facing the bone side, whereas the side facing the periosteum showed less integrated particles. They also found a wide individual range in all of the histomorphometric results [18].

Conclusion

This work is part of a research project aimed at studying the bone regeneration potential of the cortical lamina combined with the equine xenograft, on a clinical, radiological and histological point of view. It also aims at helping the clinician to understand the characteristics of the lamina and put into perspective its advantages and indications, so it could be used in the right situations and give foreseeable and predictable results.

By using the membrane alone without the addition of a collagenated membrane, the bone as well as the soft tissue healing potential were studied and the lamina proved to be 'soft tissue friendly' by permitting a second intention healing when membrane exposure occurred.

Moreover, the success of the use of the equine xenograft alone without the combination with autogenous bone gives the clinicians an acceptable solution in the cases where autogenous bone is deficient. However, a split-mouth, controlled clinical study comparing the use of this material alone or in combination with autogenous bone would be interesting to highlight the role of autogenous bone in this type of regeneration.

There seems to be promising results for this augmentation technique, especially in high risk cases where membrane exposure is probable. Yet randomized controlled clinical trials are still needed to evaluate the superiority of this membrane compared to the conventional regeneration techniques along with long term follow up of the regenerated bone.

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