The horizontal ridge augmentation using equine xenograft and a collagenated porcine cortical lamina membrane:

A clinical, radiographic and histological prospective study

Study Protocol

Date: April 4, 2017

Materials and methods

1. Biomaterials

1.1. Equine xenograft

The xenograft used in this study was the Gen-Os® by OsteoBiol; as described by the manufacturer, it is a carbonated nanocrystal bone mineral and collagen of natural heterologous origin, obtained by the treatment of cortical bone tissue of equine origins. Its granulometry ranges between 0.25 to 1 mm and it is described as being slightly radiopaque.

1.2. Cortical lamina

The soft lamina (OsteoBiol®) used is a 35x35mm medium curved membrane of porcine origins. Its clinical indications as described by the manufacturer: a co-adjuvant for the reconstruction or the partial or complete recovery of lost bone portions, fillers of non-infected, non-sclerotic and well blood bedewed bone defects.

1.3. Fixation

The fixation system used was the Pro-fix™ Precision Fixation System, consisting of self-drilling membrane fixation screws of 1.5 mm x 3.0 mm.

2. Study design

Fourteen patients (1 male, 13 females) aged between 27 and 64 years old were selected from the Department of Periodontology of the Faculty of Dentistry, XXXXXX

2.1. Inclusion criteria

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) which did not allow correct implant placement. Therefore, horizontal bone augmentation procedures were proposed. All areas needing GBR, maxilla or mandible, posteriorly or anteriorly were included in this study.

2.2. Exclusion criteria

Exclusion criteria were the following: pregnant and lactating women, patients suffering from a systematic disease, patients on bisphosphonates, 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 any procedure.

Ethical approval for this study was obtained from the Scientific Research Commission (USJ- 2017- 107).

2.3. Pre-surgical preparations

Clinical and radiographic examinations were done prior to any procedure. The concerned edentulous area was examined to identify the availability of keratinised mucosa and the possibility of implant placement in the mesio-distal and inter-arch planes. Radiographic examination was completed using a cone beam computed tomography (CBCT) scan of the concerned 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. Patients were instructed to mouthrinse with chlorhexidine 0.12% gluconate mouthwash for 1 minute. Extra-oral disinfection was made using topical chlorhexidine.

2.4. Surgical procedure

Local and/or regional anaesthesia was obtained using Septanest® (Articaine hydrochloride 4% with adrenaline 1:100,000), depending on the concerned area.

A mid-crestal incision using a 15C blade (Swann-Morton®) 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, whereas palatally it was reflected to expose 3 mm of bone and lingually the flap was elevated until reaching the mylo-hyoid line, using the Buser and the Prichard periosteal elevators (Hu-Friedy®, USA). A Rhodes back action (Hu-Friedy®, USA) was used to debride the concerned crest from any fibers and connective tissue debris (Fig. 10).

Decortication was perfomed using a twist drill with a stop of 3mm of length (Meisinger® 203S) to ensure a good perfusion of the grafted site (Fig. 11).

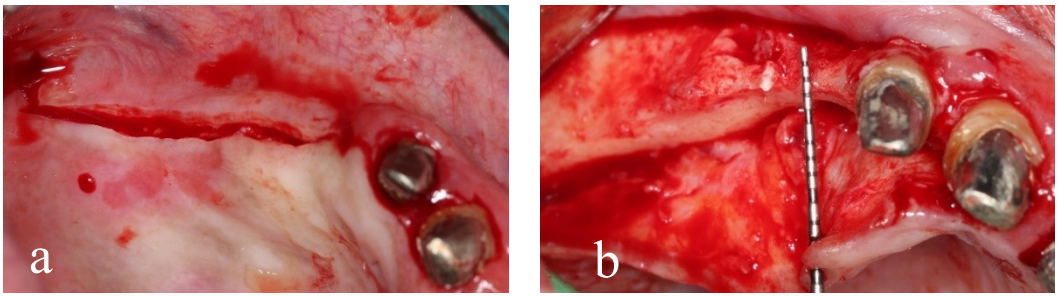


Figure 1: Incision line (a) and flap elevation (b).

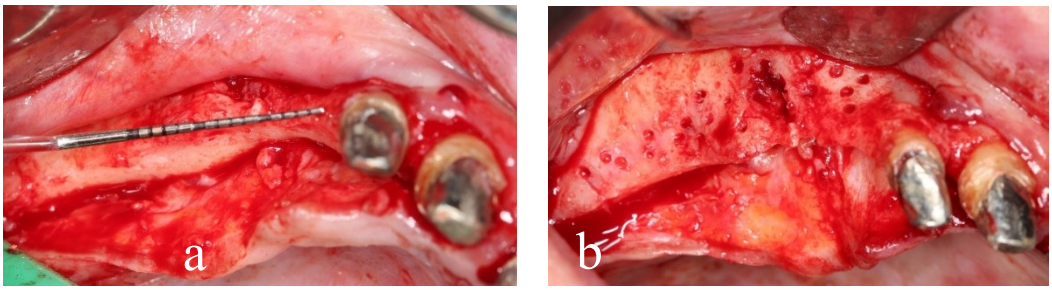


Figure 2: Measuring the area to be grafted (a) and decortication (b).

Figure 3: Twist drill used for decortication (a) and the cortical lamina soaked in saline water (b).

The soft cortical lamina had been soaked in saline sterile water since the start of the surgery in order to achieve a good elasticity and easier manipulation (Fig. 12). It was then trimmed with sterile scissors and adapted to the recipient site while making sure it was not in contact with the surrounding teeth. The cortical lamina was first fixed on the palatal side (Fig. 13). The cortico-cancellous heterologous bone mix (Gen-Os®, Osteobiol) which was previously hydrated in sterile saline for 10 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 fixated on the buccal side for better adaptability and immobilisation. Bone particles were added from the lateral sides when necessary to insure a good volume under the membrane (Fig. 14a).

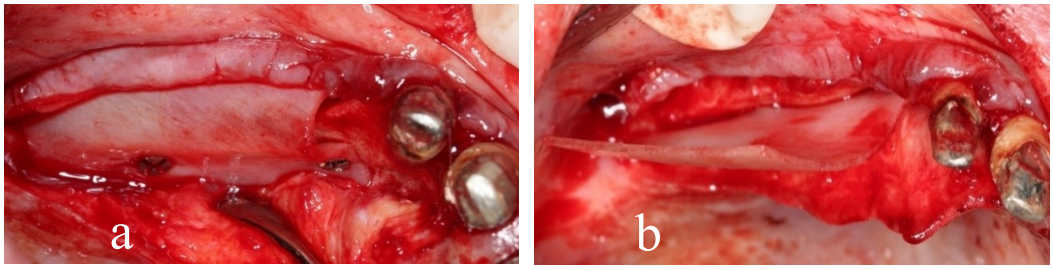


Figure 4: Membrane fixated (a) and reflected (b) palatally.

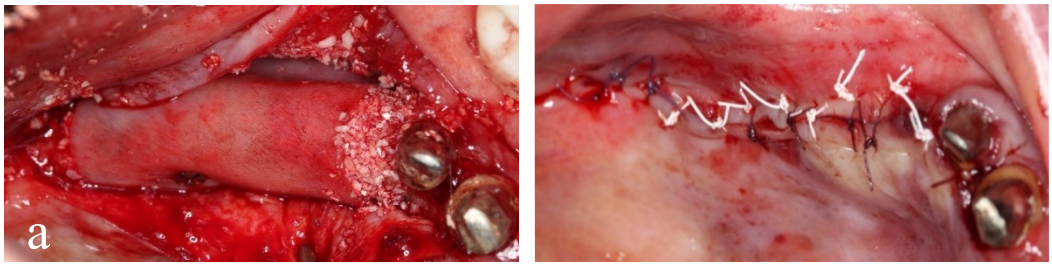


Figure 5: Membrane fixated buccally (a) and horizontal mattress and single interrupted sutures (b).

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. 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 4mm from the incision line using a non-resorbable PTFE 4/0 monofilament suture (Cytoplast™).

They were followed by single interrupted sutures close to the edges of the flap in order to create a connective tissue-connective tissue contact, thus creating a barrier to reduce the incidence of membrane exposure (Fig. 14b)

The vertical incisions were also closed with interrupted resorbable sutures (Novosyn® 5/0).

All patients were instructed to receive an injection of betamethasone dipropionate and disodium phosphate (Diprofos Injection- 2mL ampoule) 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 until suture removal.

Sutures were removed at 14 days, they were left for another 7 days when found necessary to secure the healing.

Patients were told not to wear their prosthesis when present for a month at least, after that the prostheses were relined with a soft liner and were worn only occasionally.

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

2.5. Implant placement

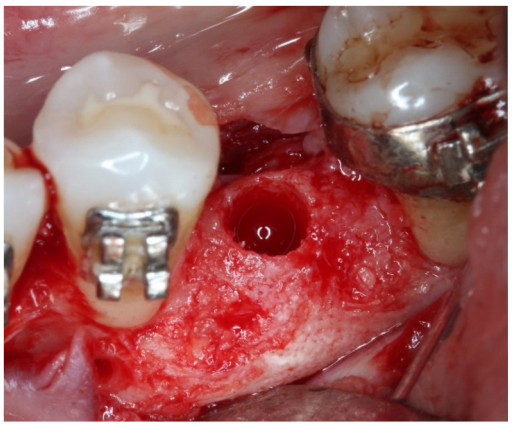
At 6 months from the surgery, a CBCT scan of the grafted area was taken and measures were made for the choice of the implant diameter and length. In all of the post-operative CBCTs, implant placement seemed possible.

On the day of the implant placement, local anaesthesia was made, followed by a mid-crestal incision and occasionally a vertical one, allowing access to the fixation screws for their removal.

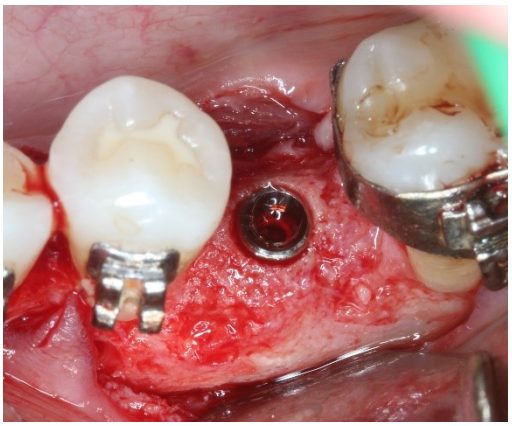
The crest was debrided from any soft tissue remnants and a biopsy was taken at the site of implant placement using a trephine burr of outer diameter of 3.5mm and inner diameter of 2.5mm (Meisinger®). The trephine and bone were immersed in 10% buffered formaldehyde and fixated for histology. The implants (Straumann®, Bone Level Cylindrical) 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, a 2 mm of bone structure was established buccally while positioning the implant.

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 (Cataflam® 50mg) inflammatory for pain management. They were also notified to mouth rinse with Chlorhexidine 0.12% for 10 days.



a



b

Figure 6: View of the crest after biopsy (a) and implant placement in the mandible (b).

3. Radiological protocol

3.1. Image acquisition

At consultation and six months after the regeneration procedure, patients were scanned with the Newtom VGI CBCT machine. Imaging conditions were: 110 kv tube voltage; 2.2 to 8.30 mA tube current; 15 x 15 cm field of view; and 0.3mm voxel size. Projection data were collected with a device rotating 360 degrees around patients over a total acquisition time of 18 seconds.

3.2. Evaluation of images

Scan data were saved in DICOM (Digital Imaging and Communications in Medicine) format and image analysis and measurements were performed using the Blue Sky Plan® (Blue Sky Bio, LLC, Grayslake, IL, USA) which provided axial, coronal and sagittal views through multiplanar reconstructions of 0.3mm slices. Axial images were reoriented to occlusal plane when present or to palatal plane as a horizontal reference. A panoramic curve was created and cross-sectional images perpendicular to that curve were reconstructed at a 1 mm interval.

3.3. Advanced jaw segmentation

For each scan, an advanced jaw segmentation technique was realized using the Blue Sky Plan software by means of threshold segmentation and contour interpolation. First the region of interest (corresponding jaw) was selected on the panoramic view. Second, several axial, coronal and cross-sectional slices equally

distributed/chosen by the software were used to draw the outline of the bone. This created a matrix to the final automatic segmentation step by the software to finalize the segmentation data and create a 3D model of the jaw. Finally, the outline of the 3D model was checked and adjusted manually on the 2D slices in all the planes in cases of over or missing contour. The result was an accurate 3D model of the corresponding jaw.

3.4. Virtual implant placement and jaw superimposition

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:

3.4.1. 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.

- 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.

3.4.2. 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 points.

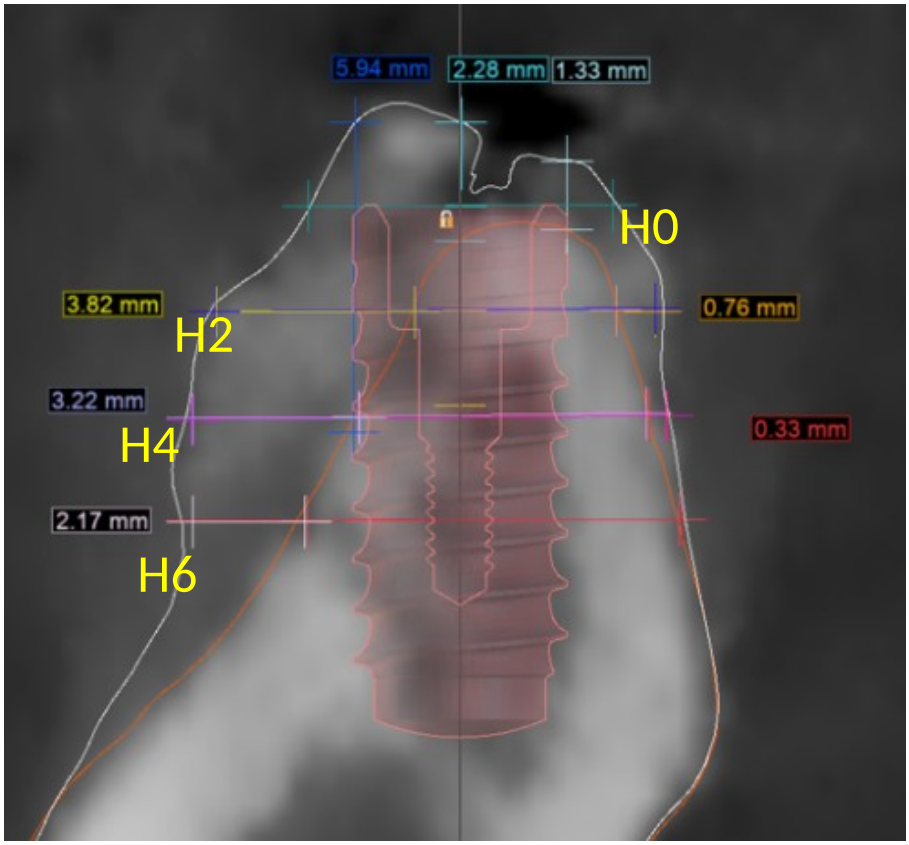


Figure 7: Bone superimposition and measurements at the site of implant placement.

4. Histology

4.1. Fixation and inclusion

Samples were taken at six months after regeneration and were treated with non-demineralized histology. At the time of sampling, the samples were fixed in LILLIE neutral formolin, diluted to 10% in buffered sodium phosphate pH 7.4. The fixation period lasted 3 weeks. The samples were then rinsed under running water for 48 hours.

The dehydration of the samples was carried out in alcohol baths of increasing concentration and for 48 hours in each bath, in ethanol 70°, 80°, 90°, 95°, 100°, 100°, 100°, then the clarification, allowing the penetration of methacrylate, in 2 successive xylene baths of 24 hours each.

4.2. Cutting technique

The blocks were cut under irrigation and at slow speed with an Exact saw (Cutting machine EXACT-APPARATEBAU Nordersted, Germany), so as to take cuts of at least 80 µm. These cuts were subsequently reduced in thickness with the Exact abrasion system. The polishing was carried out with abrasive paper discs of decreasing granulometry making it possible to reduce the thickness of the cuts automatically to the desired value. The cuts were separated into S (superficial), M (median) and P (profound) cuts, the superficial ones being the cuts facing the periosteum.

4.3. Staining

The sections were stained with Giemsa-Paragon and basic fushin.

Giemsa will give cells and nuclei the colour blue, and Paragon will stain bone in red.

5. Histomorphometry

The qualitative observation of the sections was done under a digital microscope (Keyence digital microscope VHX-6000) with normal and polarised light visualisations. For histological quantification, an optical microscope was used (Olympus BX 60, Olympus Corporation, Tokyo, Japan) connected to a digital camera (Olympus E330), along with the software Image J/ Fiji.

It was first calibrated by measuring the scale bar present on the image. The image was made into black and white (Type: 8-bit). The scale was set by measuring the length of the scale bar in pixels and by setting its known distance in millimetres. It was then set as 'Global' for all the images with the same scale.

The total area of the concerned section was measured. Then the bone and osteoid volume were 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.