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Case Report

EARLY IMPLANT INSERTED IN A RECONSTRUCTED SITE WITH XENOGRAFT, HYALURONIC ACID, AND POLYNUCLEOTIDES IN NON-SYNDROMIC AGENESIS: HISTOMORPHOMETRY AND FOLLOW-UP

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ABSTRACT

The present clinical case aims to investigate how a mixture of polynucleotides and hyaluronic acid in gel form, combined with bovine-derived heterologous material, can expedite the process of bone neoformation suitable for implant placement. In this case, the biomaterials are covered with a resorbable membrane and stabilization pins to protect the grafted material. A patient with severe, non-syndromic multiple dental agenesis is described. After five months, implant placement was performed. Following a core drilling biopsy at the implant insertion site, histomorphometric investigations were conducted to assess the maturation status of the regenerated bone site. After 6 months of implant healing, prosthetic procedures were performed, followed by a subsequent radiographic follow-up. Upon return, the regenerated bone appeared well vascularized before implant placement and provided adequate primary stability.

KEYWORDS: *bone regeneration, hyaluronic acid, atrophy, polynucleotides, bone augmentation*

INTRODUCTION

Nowadays, bone regeneration remains one of the elective treatments to ensure a good three-dimensional anatomical situation of the implant site (1).

Indeed, it is now known that following dental extractions, significant bone remodeling of the alveolar process occurs massively within the first six months in both vertical and horizontal dimensions, within a range of 11-22% depending on the invasive and traumatic tooth extraction (2, 3).

Regenerative procedures are necessary to ensure the correct three-dimensional dimension of the perimplant bone tissue and to minimize perimplant resorption (4, 5). Different biomaterials are commonly used for regenerative maneuvers, including graft materials acting as scaffolds and membranes, maintaining space in the regenerating site, and stabilizing the inserted graft. The materials used as bone substitutes are of heterologous origin but are often mixed with autologous bone particulate, thus combining osteoinductive and osteoconductive properties. Moreover, the presence of morphogenetic proteins and growth factors induces and accelerates the processes of bone neoformation (6-8).

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The combined use of materials such as polynucleotides and hyaluronic acid (PNs-HA) in the form of gel seems to promote bone regeneration in horizontal alveolar defects and all the biological processes that characterize tissue regeneration (9-12).

CASE REPORT

The presence of multiple non-syndromic agenesis resulted in significant oligodontia. Agenesis is characterized by significant bone deficits, making implant treatment impossible without preliminary bone regenerative procedures.

The absence of dental elements has resulted over time in a bone deficit, especially in the horizontal component of the alveolar process. This situation is frequently described in the literature (13) (Fig. 1).

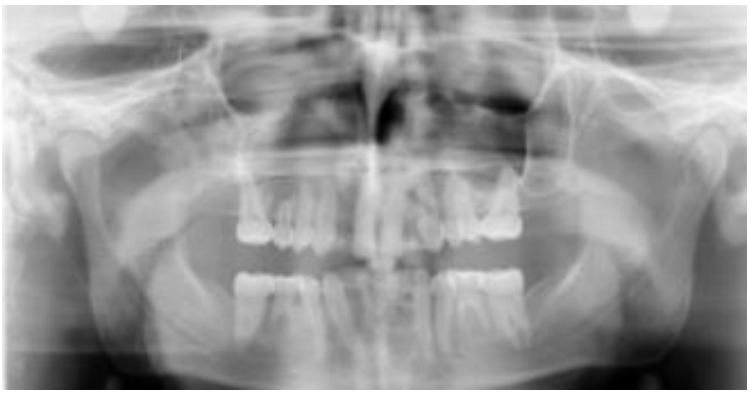


Fig. 1. *OPT X-ray showing multiple agenesis.*

The patient's decision to treat agenesis in the lower frontal group led to a preliminary study through diagnostic wax-ups to plan the implant placement following the construction of a surgical template (Fig. 2).

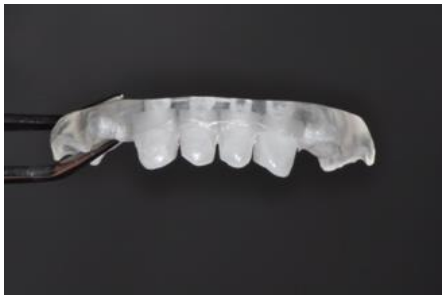


Fig. 2. *Surgical template for implant placement.*

The radiographic examination performed with Cone Beam Computer Tomography (CBCT) confirmed the need to regenerate the bone tissue in the lower frontal area to enable implant placement. The bone regeneration procedure was performed using a mixture of polynucleotides and hyaluronic acid in addition to a heterologous bovine-derived and autologous bone harvested from the surgical site.

Clinical presentation

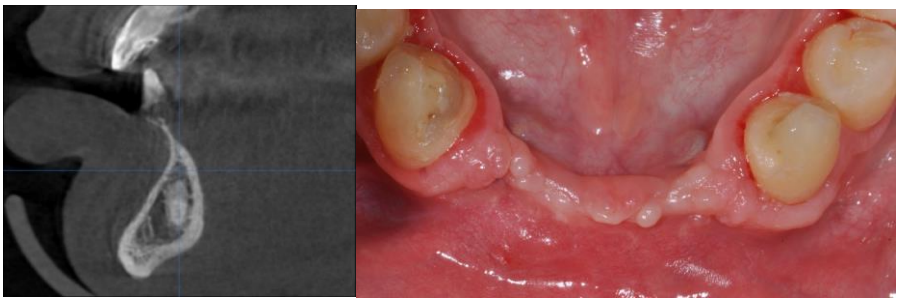
The patient came with a partial intercalated edentulism in the lower frontal area. The treatment plan, established in agreement with the patient, focused on the lower area. The patient had a temporary prosthesis placed a few years earlier at another facility.

Following the radiographic examination (Cone Beam CT), the surgical procedure that was established involved a staged implant approach, with implant surgery performed after bone regeneration.

According to the literature, dental agenesis results in bone loss, especially in the horizontal dimension of the edentulous site. In this case, the defect correction was performed using deproteinized bovine bone (Bio-Oss, Geistlich Pharma, Switzerland), autologous bone harvested from the surgical site, and a mixture of polynucleotides and hyaluronic acid (Regenfast, Officina Biofarmaceutica Mastelli Srl, Sanremo, Italy), subsequently covered with a resorbable collagen membrane (Biogide, Geistlich Pharma, Switzerland). The use of the mixture of polynucleotides and hyaluronic acid in gel form appears to boost tissue regeneration, reducing clinical time (14-17).

After the necessary period for bone integration, implants were placed. Preparation of the regenerated ite involved a 2 mm bone biopsy, the same diameter as the initial drill of the implant surgical kit. Subsequently, the implant preparation site was completed with the remaining drills of larger diameter for implant insertion. The need to start with the frontal group is due to the patient losing the deciduous teeth that supported the lower fixed prosthesis following a traumatic event.

In the initial analysis, a CBCT scan was performed to assess bone thickness. After evaluating the CBCT, the bone reconstruction procedure was scheduled as the thicknesses were unsuitable for implant insertion (Fig. 3).



a b

Fig. 3. ***A)****: Parasagittal section of horizontal bone atrophy;* ***B)****: intraoral view of bone resorption.*

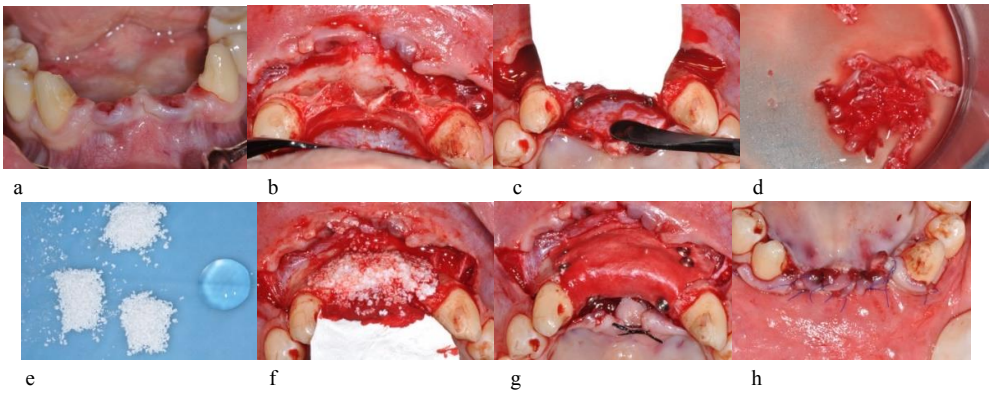
Surgical technique

Following local anesthesia with articaine with adrenaline 1:100.000 (Septanest, Saint-Maur-des-Fossés Cedex, France), a trapezoidal flap was performed with a mid-crestal incision and two distal release incisions adjacent to elements 33-43. Once the alveolar process was exposed with a full-thickness flap and after removing residual fibrous tissue, the clinical picture of severe loss of the horizontal component became evident. At this point, shaping and adaptation of the resorbable membrane to the surgical site were carried out, with fixation of the membrane using pins in the lingual area (Fig. 4a-c).

The grafting material was harvested using a safe scraper from the autologous bone portion in the mental symphysis area (Fig. 4a-d). The hyaluronic acid and polynucleotide gel was mixed with deproteinized bovine bone in a 1:3 ratio (Fig. 4e), following the manufacturer's instructions, adding the biomaterial to the biogel gradually to hydrate the granules progressively, without creating a non-homogeneous solid mass. Once the graft was inserted into the defect, the membrane is adapted vestibularly, putting it under tension and securing it with pins according to the technique described by Urban and colleagues (Fig. 4f-4g) (18-21).

An attentive periosteal release facilitated proper flap passivation for the correct horizontal mattress and interrupted sutures. Subsequently, a resin Maryland-type structure was placed as a provisional prosthesis during the healing period (Fig. 4h).

The supportive pharmacological treatment included antibiotic therapy with 2 grams of amoxicillin per day starting from the day before surgery, appropriate wound care and cleansing with chlorhexidine-based rinses (22-24), and pain management with as-needed nonsteroidal anti-inflammatory drugs (600mg Ibuprofen).



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e f g h

Fig. 4. ***A)****: frontal view of the surgical site with loss of deciduous elements;* ***B)****: skeletonization of the surgical site using a mucoperiosteal flap;* ***C)****: lingual fixation of the membrane;* ***D)****: autologous bone harvested with safe scrapers;* ***E)****: adequate preparation in ratio of 3:1 between gel and deproteinized bone;* ***F)****: graft adapted to the defect once mixed;* ***G)****: membrane fixed vestibularly with fixation pins;* ***H)****: suturing and passive adaptation of the flap.*

After 5 months of bone regeneration, a CBCT scan was performed to plan the implant placement and analyze the bone reconstruction. Radiographic images revealed an increase in the horizontal portion, allowing for implant insertion (Fig. 5).

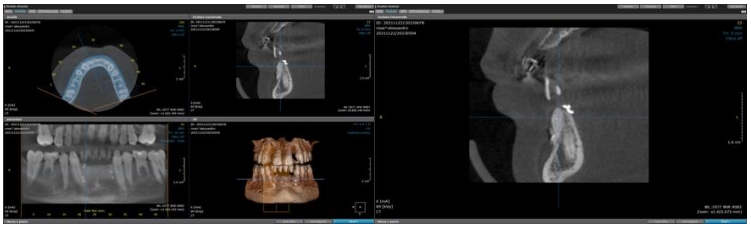

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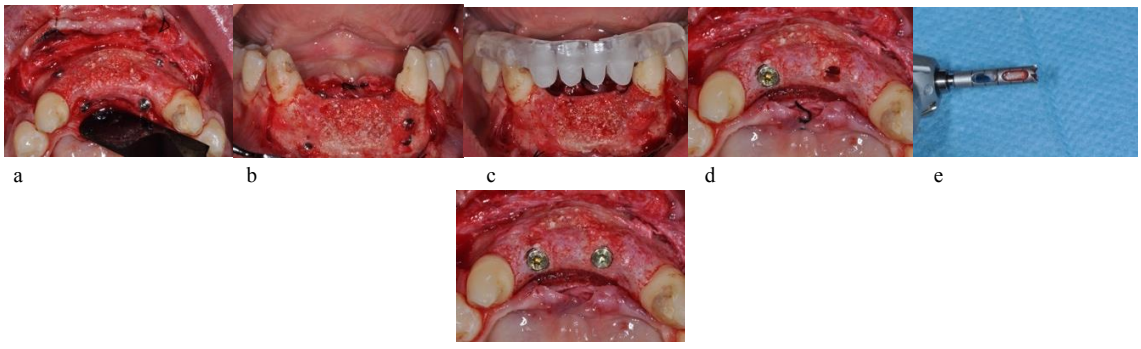
Fig. 5. ***A)****: CBCT Image;* ***B)****: the sagittal section shows evident regeneration of the horizontal dimension.*

The planning of the implant sites was carried out according to prosthetic requirements (23). The selected implants (Neodent®, Straumann Group, Switzerland), sized 3.5x11.5, were placed at 31 and 41. The implant surgery procedure included local anesthetic infiltration (1:100,000 Septanest, Saint-Maur-des-Fossés Cedex France), a crestal incision, and release incisions distal to elements 33-43. Once the full-thickness flap was detached, the reconstructed area was visible, allowing for the removal of the stabilization pins placed during the bone regeneration procedure.

With the aid of the surgical template, implants were inserted in positions 32-41 (Fig. 6c). The final prosthetic design included a cantilever element in position 42. This choice was determined by studying radiographic images that highlighted an excessive mesialization of the root of element 43. Placing the implant in position 42 would have caused conflicts with the root of element 43. While preparing the implant site in position 41, a biopsy was performed using a trephine bur for subsequent histomorphometric analysis of the regenerated bone. Subsequent steps dictated by the surgical insertion protocol involved the correct placement of implants in positions 32-41. After implant placement, the flap was sutured with interrupted stitches. The same pharmacological support therapy prescribed at the time of bone grafting months earlier was done again.



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f

Fig. 6. ***A)****: healing of graft at 5 months, occlusal view;* ***B)****: frontal view of graft healing at 5 months;* ***C)****: surgical template for implant placement;* ***D)****: excision with a 2mm trephine bur in position 41;* ***E)****: histological biopsy;* ***F)****: implants inserted.*

Prosthetic rehabilitation

Approximately 30 days after healing, healing screws were placed, and an impression was taken for the fabrication of provisional crowns (26-28). Fifteen days after tissue healing, temporaries were modeled and prepared according to the concepts expressed in the BOPT technique, as described by Loi and colleagues, regarding convergent prosthetic shapes and the adaptation of soft tissues to these structures (Fig. 7) (29).

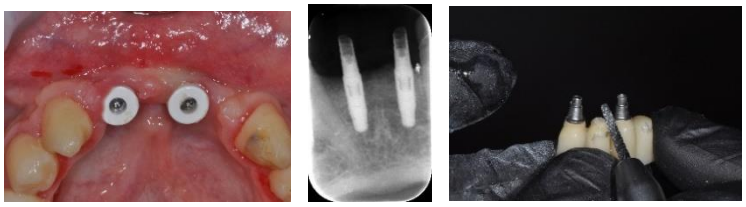

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Fig. 7. ***A)****: healing screws placement after implants osteointegration;* ***B)****: radiographic control during provisional crowns placement;* ***C)****: preparation of provisional crowns according to the Loi technique.*

After 45 days, there was evidence of tissue healing around the provisional crown, a suitable situation for the preparation of the final crown. Once the provisional crown was removed, the good amount of keratinized mucosa around the implant was evidently ready to receive the permanent one (Fig. 8).

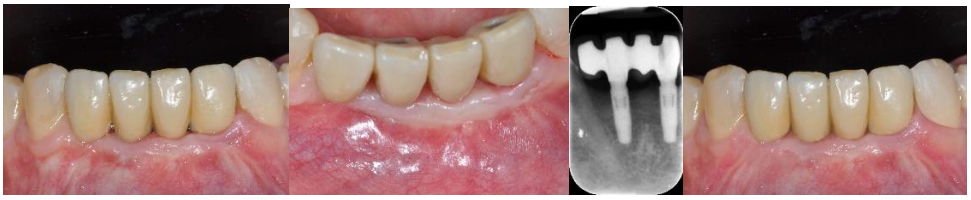

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Fig. 8. ***A)****: provisional crowns placement after the healing period;* ***B)****: detail of the evident increase in keratinized mucosa at the time of placement of the definitive prosthesis after 6 months;* ***C)****: radiographic control after 6 months from placement of provisional crowns with definitive one;* ***D)****: definitive crowns placement.*

Tissue processing

Immediately after harvesting, bone samples were immersion fixed in 4% formalin/0.1M phosphate buffer saline (pH 7.4) and processed for histological analysis without prior decalcification. Employing the technique elucidated by Donath & Breuner (1982), the biopsy specimens were systematically dehydrated in ascending concentrations of ethanol (ranging from 70% to 100%), infiltrated under agitation and vacuum, and embedded in Kulzer Technovit 7200 VLC (Bio-Optica, Milano, Italy). Subsequently, each block underwent longitudinal sectioning via a diamond saw (Micromet Remet, Bologna, Italy). The two central sections were grounded, polished to a final thickness of 90 µm, and stained with hematoxylin and eosin (H&E). Each section was viewed and photographed at different magnifications: 500x, 200x, 100x, 20x, and 10x (Fisherbrand Serie AX-500, Fisher Scientific, Milan, Italy). Immunohistochemistry was performed for KP-1 (Ventana, mouse), actin (Ventana, mouse, clone 1A4), and SATB2 (Santa Cruz, mouse, clone G-11).

The CBCT scans unveiled newly formed radiopaque hard tissue seamlessly integrated with the surrounding bone, devoid of any indications of radiolucent fibrous encapsulation. This augmented bone volume facilitated the precise placement of implants in alignment with prosthetic requirements, ensuring optimal primary stability. Therefore, five months after implant surgery, the radiographic images demonstrated excellent healing of inserted implants (Fig. 9). Therefore, planning the second surgical phase and the subsequent prosthetic procedure were carried out.

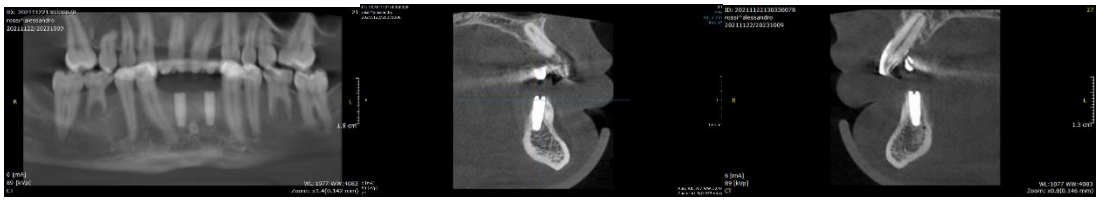

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Fig. 9. ***A)****: CBCT, frontal view: implants inserted in the regenerated site;* ***B, C)****: CBCT sagittal view: clear increase in bone thickness.*

The clinical image shows a surgical site with a reduction in keratinized mucosa (Fig. 10a). The reopening surgery of the implant was planned with a free gingival graft (Fig. 10b) harvested from the palate to increase the height of the keratinized mucosa (24, 25).

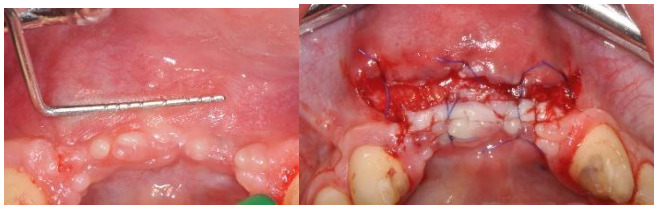

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Fig. 10. ***A****): Evidence of the surgical site with poor keratinized mucosa;* ***B****): Free gingival strip graft inserted in the surgical site and sutured with interrupted stitches onto the periosteum.*

No supplementary regenerative procedures were required concurrent with implant insertion, underscoring the favorable outcomes in localized volume augmentation. Indeed, the newly formed tissue exhibited tactile resistance, showing a good level of bone density from a macroscopic perspective.

RESULTS

Histological analysis

At low magnification, lamellar bone represented about 60% of the entire tissue volume (star), the inorganic bone matrix about 25% (arrows), and the remaining 15% interstitial spaces. (dashed arrow) (Fig. 11).

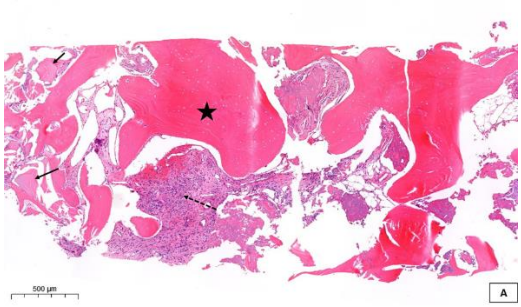

Fig. 11. *hematoxylin and eosin, Bar=500 µm*.

At higher magnification, osteoblast-like cells were easily found beneath lamellar bone fragments (arrows), with very few cells with histologic features of osteoclasts (circle) (Fig. 12).

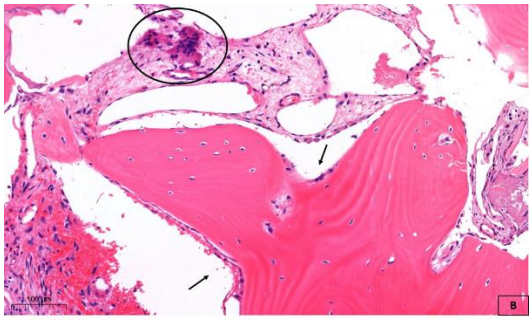

Fig. 12. *Hematoxylin and eosin, Bar=100 µm.*

Interstitial spaces comprised vessels, fibrosis, a great amount of fibroblasts, and myofibroblasts with their spindle appearance and few lymphocytes and monocytes (Fig. 13).

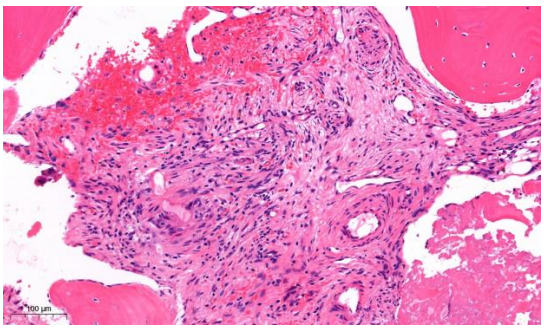

Fig. 13. *hematoxylin and eosin, Bar=100 µm.*

Immunohistochemistry for SATB2, CD68, and actin was performed to highlight these different components of the sample.

SATB2 (Special AT-rich sequence-binding protein 2) has a role in osteoblast differentiation and osteogenesis, whereas actin identifies pericytes, smooth muscle cells, and myofibroblasts. In the sample, numerous myofibroblasts positive for actin were also positive for SATB2 (circles) (Fig. 14).

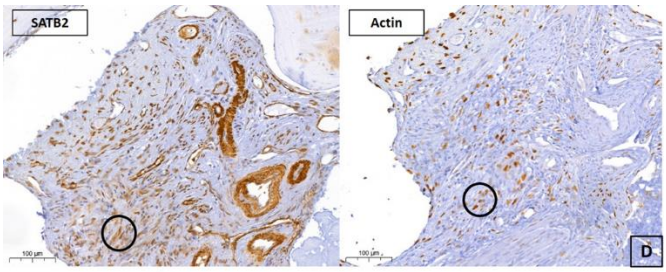

Fig. 14. *SATB2 and Actin IHC, Bar=100 µm*.

CD68 is a lysosomal marker identifying histiocytes/macrophages and monocytic cells, highlighting inflammatory components. Few macrophages were found in interstitial spaces (circles) (Fig. 15).

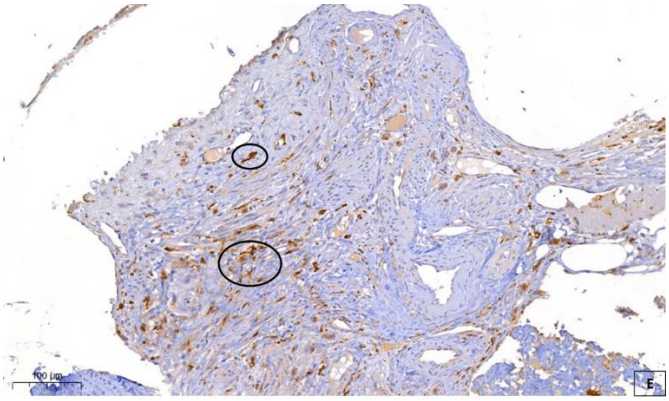

Fig. 15. *CD68 IHC, Bar= 100µm.*

DISCUSSION

In this patient, correct execution of the guided bone regenerative technique led to hard tissue augmentation, which allowed subsequent insertion of standardized osseointegrated implants in a position where, because of the marked atrophy, the presence of important anatomical structures would have made the preparation of the implant area impossible. Moreover, the use of a mixture of hyaluronic acid and polynucleotides, in addition to autologous and heterologous bone, appears to facilitate the handling of bone grafts, improve osseointegration characteristics and promote bone regeneration and repair (30, 31). As confirmed by histomorphometric analysis, the use of these biomaterials has a boosting effect on

tissue healing. With this surgical technique, a good functional outcome can be obtained by inserting standard-size implants and manufactured prosthetic products with adequate implant crown ratios in adduction to achieve a more pleasing rehabilitative solution.

Hard tissue augmentation was carried out in combination with gingival plastic surgery in which a free epithelial-connective graft was used; this resulted in a remarkable increase in the band of peri-implant keratinized gingiva leading to an objective improvement in rehabilitation esthetics and easier maintenance of soft tissue health thanks to simplified oral hygiene (32-37).

CONCLUSIONS

We can, therefore, conclude that the techniques used for tissue regeneration have allowed for implant placement in cases of severe atrophies, achieving soft tissue architecture suitable for ensuring adequate resistance to the peri-implant complex and a satisfying aesthetic outcome.

Conflict of interest
The authors declare no conflicts of interest.

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