Case Report

IMPLANT INSERTION AND GUIDED BONE REGENERATION IN THE ANTERIOR UPPER JAW

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ABSTRACT

Anterior maxillary atrophy is characterized by the loss of bone in the front portion of the upper jaw, specifically the alveolar ridge bone. This atrophy can result from various factors, including tooth loss, periodontal disease, trauma, or developmental abnormalities. Dental implants may be challenging to place in the atrophic anterior maxillary due to insufficient bone volume and compromised anatomical conditions. Several treatment options are available to address anterior maxillary atrophy and restore both function and aesthetics. Bone augmentation procedures, such as bone grafting or guided bone regeneration, can be employed to rebuild the deficient bone volume. This paper describes a bone atrophy of the anterior upper jaw treated with implant insertion and guided bone regeneration.

KEYWORDS: *jaw maxilla, bone, regeneration, implant, membrane*

INTRODUCTION

Anterior maxillary atrophy is a condition characterized by the loss of bone in the front portion of the upper jaw, specifically the alveolar ridge bone (1-14). This atrophy can result from various factors, including tooth loss, periodontal disease, trauma, or developmental abnormalities. As the bone in the anterior maxillary region diminishes, it determines aesthetic and functional challenges, impacting a person's facial appearance, speech, and ability to wear dental prosthetics such as dentures or implants. One of the primary causes of anterior maxillary atrophy is the loss of teeth, which initiates a cascade of events leading to bone resorption.

This can result in a loss of vertical and horizontal bone dimensions in the anterior maxillary region, creating a concave or collapsed appearance. Periodontal disease is another significant contributor to anterior maxillary atrophy. The inflammation associated with periodontal disease can destroy the alveolar bone, which surrounds and supports the teeth. In severe cases, this bone loss can extend into the anterior maxillary region, exacerbating the atrophy. Trauma to the upper jaw, such as fractures or injuries sustained in accidents, can also contribute to anterior maxillary atrophy. Trauma can damage the bone structure, leading to compromised stability and eventual resorption. Additionally, developmental abnormalities or congenital conditions may result in inadequate bone formation in the anterior maxillary region, predisposing individuals to atrophy.

The consequences of anterior maxillary atrophy extend beyond mere aesthetic concerns. The loss of bone in this region can pose significant challenges for dental rehabilitation. Traditional removable dentures may not fit securely in a resorbed maxillary ridge, leading to issues with stability, retention, and discomfort for the patient. Dental implants may also be challenging to place in the atrophic anterior maxillary bone due to insufficient bone volume and compromised anatomical conditions.

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Several treatment options are available to address anterior maxillary atrophy and restore both function and aesthetics. Bone augmentation procedures, such as bone grafting or guided bone regeneration (GBR), can be employed to rebuild the deficient bone volume.

GBR is a dental surgical technique designed to augment the volume of bone in areas where it is deficient (15-17). The primary goal of GBR is to create a conducive environment for the regeneration of bone, enabling the placement of dental implants or improving the stability of existing teeth. The procedure involves using barrier membranes, typically made of biocompatible materials, to physically separate the bony defect from surrounding soft tissues, preventing the infiltration of non-osteogenic cells. This exclusion allows space for bone-forming cells, such as osteoblasts, to populate the area and initiate the regeneration process. The barrier membrane serves as a scaffold, guiding bone growth and preventing unwanted tissue ingrowth. Additionally, GBR may involve using bone grafts or substitutes to further support and enhance the regeneration process.

Autografts (bone harvested from the patient), allografts (donor bone from another individual), xenografts (bone from another species), or synthetic bone graft materials may be utilized, depending on the specific requirements of the case. GBR has become a valuable tool in implantology, and it addresses challenges associated with insufficient bone volume for successful implant placement. The technique has demonstrated effectiveness in promoting bone growth and enhancing the long-term stability of dental implants, ultimately contributing to improved patient outcomes and satisfaction (15-17). We reported a case of bone atrophy of the anterior upper jaw treated with implant insertion and GBR.

CASE REPORT

A male patient 36-year-old presented to our dental clinic complaining about his prosthetic rehabilitation. Teeth 11 and 21 appear very disproportionate to the smile line (Fig. 1). After removing the fixed prosthetics, tooth 11 and an implant in site 22 appeared (Fig. 2).

The patient presented moderate bone atrophy in the anterior maxilla. In agreement with the patient, it was decided to replace the fixed prostheses with implant-prosthetic rehabilitation. The patient underwent a cone-beam computed tomography scan and orthopantomography. Before surgery, the patient was informed about the operative risk and complications, and written consent was obtained from the patient for publication of this case report and accompanying images. Rehabilitative surgical treatment was planned and assessed with the use of heterologous bone, reinforced membrane, and insertion of the implants in the atrophic maxilla to achieve bone height and width.

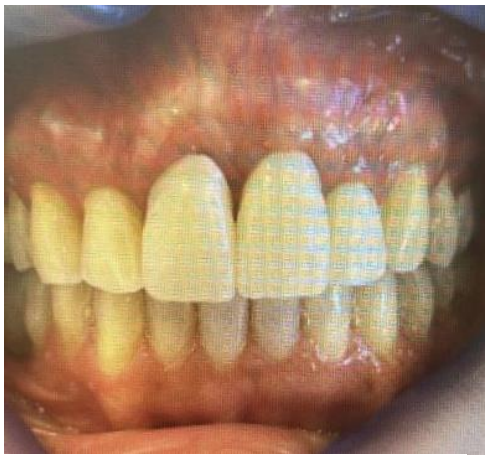


Fig. 1. *Teeth 11 and 21 appear very disproportionate to the smile line.*

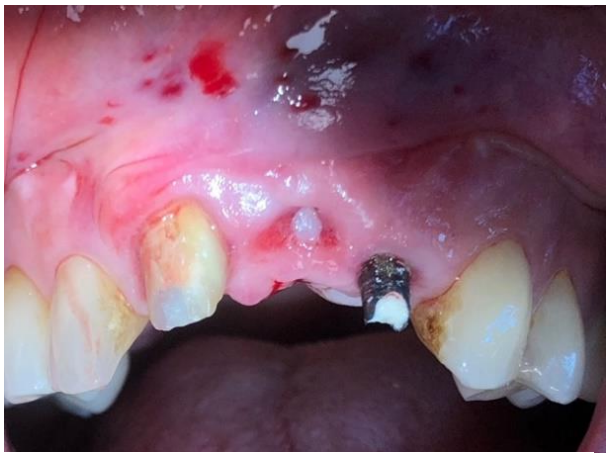


Fig. 2. *After removing fixed prosthetics, tooth 11 and an implant in site 22 appeared.*

After local anesthesia with articaine, the mucosa was incised and detached. Subsequently, 2 implants were positioned in sites 11 and 21 (Fig. 3). Heterologous bone (Geistlich Bio-Oss® Thiene VI, Italy) was then placed around the implants (Fig. 4). A resorbable membrane (Geistlich Bio-Guide® Thiene VI, Italy) covered bone graft and was fixed with mini-screws (Fig. 5). The resorbable membrane was then fixed palatially, after detaching the mucosa of the palate (Fig. 6). Thereafter a connective pedunculate flap was raised from the right side of the palate in the area of molar and premolar and rotate anteriorly (Fig. 7). It was stitched medially to cover the resorbable membrane (Fig. 8, 9)

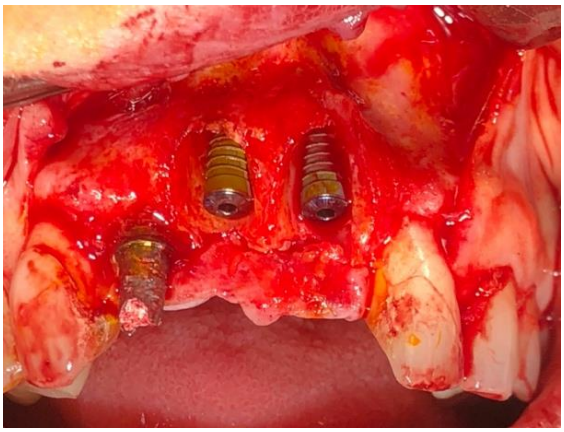


Fig. 3. *Implants positioned in sites 11 and 21.*

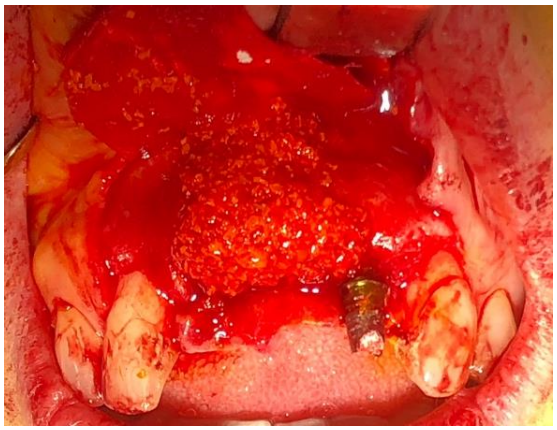


Fig. 4. *Heterologous bone placed around the implants.*

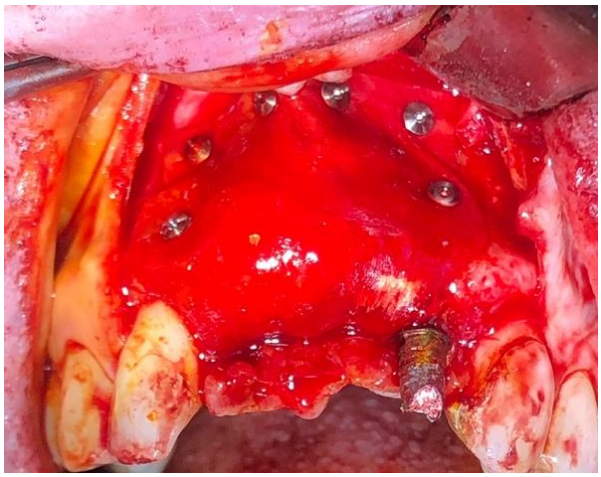


Fig. 5. *Resorbable membrane fixed on vestibulum.*

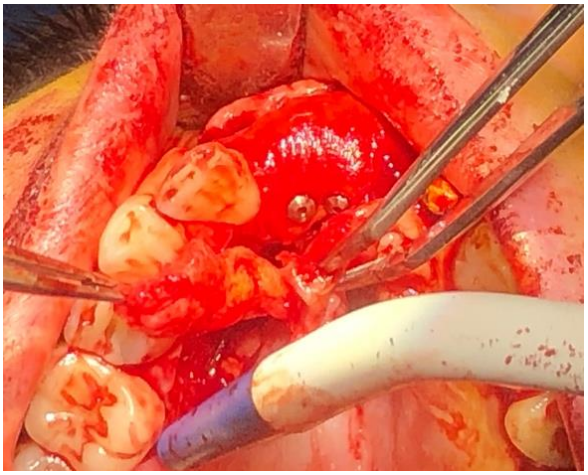


Fig. 6. *Resorbable membrane fixed on the palate with miniscrews.*

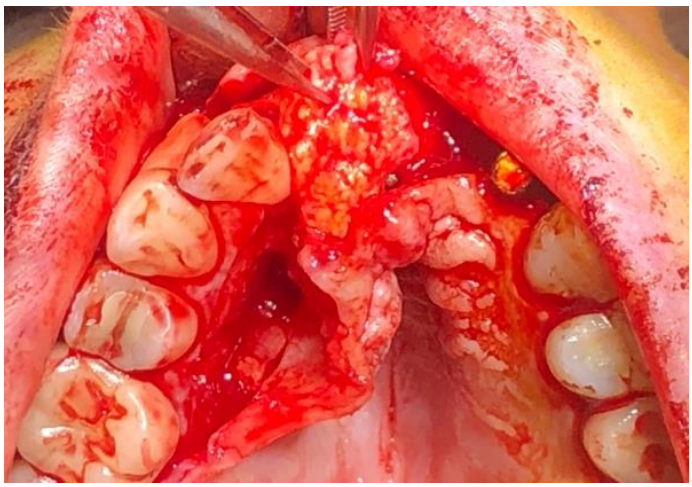
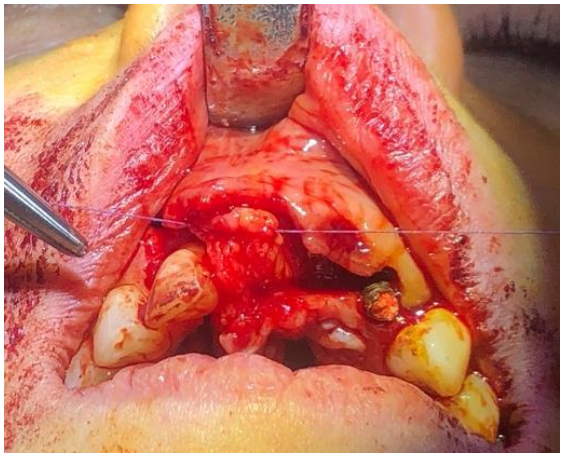


Fig. 7. *A connective tissue flap from the palate was rotated anteriorly.*



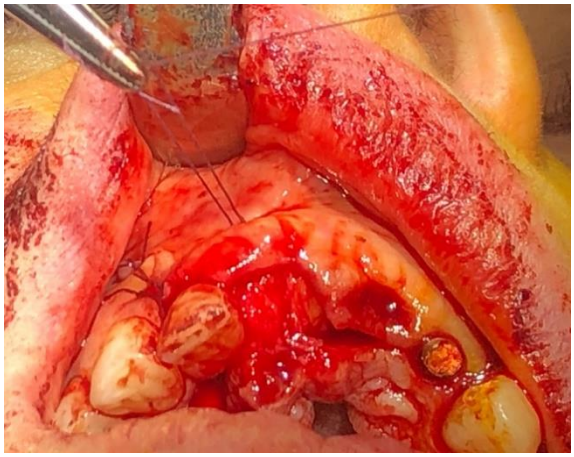


Fig. 8, 9. *A connective tissue flap was fixed to cover the resorbable membrane.*

Finally, the mucosa was completely sutured with absorbable transmucosal stitches (Fig. 10, 11).

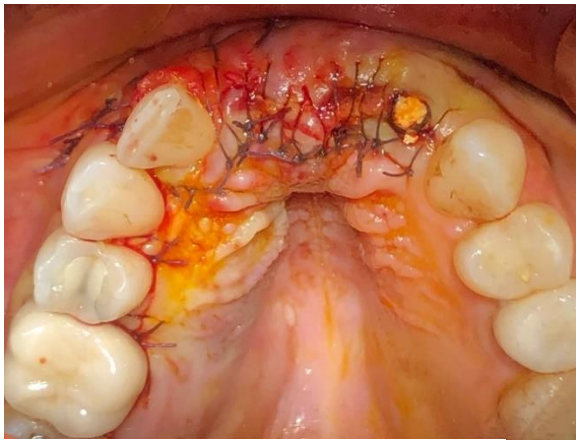


Fig. 10. *Occlusal view of the mucosa completely sutured with absorbable stitches.*

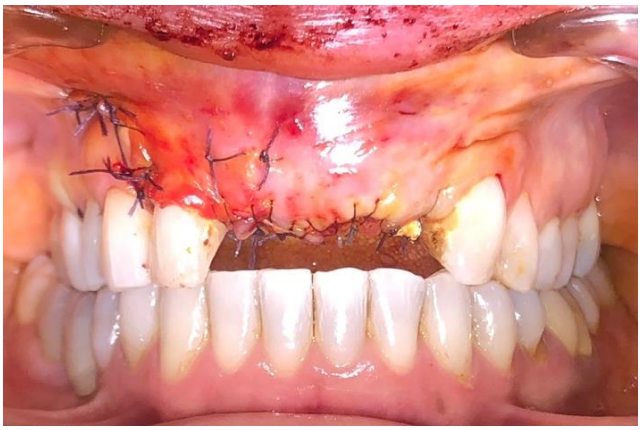


Fig. 11. *Frontal view of the sutured surgical field.*

A temporary fixed prosthesis was cemented while awaiting the healing of the soft and hard tissues (Fig. 12)

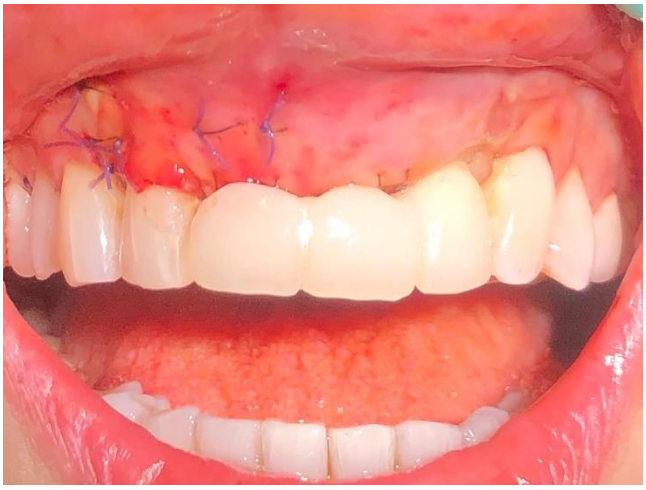


Fig. 12. *Frontal view of the temporary fixed prosthesis.*

DISCUSSION

Anterior maxillary atrophy is a condition characterized by the loss of bone in the front portion of the upper jaw, specifically the alveolar ridge bone (1-14). The loss of bone in this region can pose significant challenges for dental rehabilitation. Several treatment options are available to address anterior maxillary atrophy and restore both function and aesthetics.

First, several biomaterials were investigated. Kamadjaja et al. (1) evaluated the stability of tissue augmented with deproteinized bovine bone mineral (DBBM) particles associated with implant placement in the anterior maxilla. They verified that horizontal ridge augmentation using DBBM particles related to implant placement in the anterior maxilla produces good clinical stability. The stability appears higher in immediately inserted fixtures than those inserted in a second stage. Caramês et al. (2) focused on a composite PRF/particulate xenograft used for GBR. By using computer beam computer tomography scans obtained at pre-surgery, post-surgery, and the 12-month follow-up. Authors showed that PRF associated with a xenograft aid to promote an effective horizontal bone gain.

de Freitas et al. (3) compared the effect of recombinant human bone morphogenetic protein-2 (rhBMP-2) in an absorbable collagen sponge carrier (ACS) with autogenous bone graft for augmentation of the edentulous atrophic anterior maxilla. The alveolar ridge dimension was assessed using an analogue caliper and cone-beam computed tomography. The authors found a positive effect of rhBMP-2 for GBR. Moussa et al. (4) investigated the effect of platelet-rich fibrin (PRF) added to autologous graft on the augmentation results of autogenous palatal bone blocks. They found that autogenous palatal bone block surface resorption is significantly decreased using PRF coverage.

To cover alveolar ridge augmentation, specific flap or connective grafts were investigated. Yu et al. (5) introduced a novel method of split-thickness labial flap in maxillary anterior ridge horizontal augmentation. The authors found that the flap advancement technique facilitates clinically passive primary closure. This technique can be used successfully in both particulate and onlay horizontal graft procedures. Kirmani et al. (6) reported a single-staged ridge split approach using piezoelectric surgery with simultaneous implant placement followed by connective tissue grafting at second-stage surgery. The single-staged segmental ridge split technique reduces not only the total treatment duration but also the surgical morbidity of the patient. A subepithelial connective tissue graft is strongly advocated around implants as it is highly predictable while ensuring better esthetic results in terms of tissue color, texture, and long-term stability of the surrounding mucosa. Canullo et al. (7) presented a series of patients treated with tooth extraction and single-implant placement in the anterior maxilla. The horizontal bone deficiency was treated with beta-tricalcium phosphate and a bioresorbable polylactic acid membrane. Primary closure was obtained by a novel coronally advanced flap adapted from mucogingival techniques. Authors concluded that GBR using a bioresorbable polylactic acid membrane and resorbable beta-tricalcium phosphate bone graft in conjunction with a coronally advanced flap is a predictable procedure for horizontal bone augmentation with simultaneous implant placement in the esthetic area.

Some studies investigated implant survival/success rate and GBR bone stability. Kuchler et al. (8) did a systematic revision of clinical studies examining the survival and success rates of implants in horizontal ridge augmentation, either prior to or in conjunction with implant placement in the anterior maxilla. They found that staged and simultaneous augmentation procedures in the anterior maxilla are both associated with high implant success and survival rates. Jiang et al. (9) performed a clinical study to evaluate hard tissue volume stability during the healing stage of GBR with particulate bone graft and resorbable collagen membrane, showing that GBR partially undergoes horizontal volume reduction during the healing stage. Chen et al. (10) evaluated the buccal bone thickness of immediate implant placement with buccal bone augmentation in patients with a thin buccal plate in the esthetic zone. A clinical trial was done on eighteen consecutive patients requiring a single tooth replacement. Authors found that simultaneous buccal bone augmentation may maintain a predictable buccal bone thickness for immediate implant placement in the maxillary anterior sites with a thin buccal plate (<1 mm) at 1-year follow-up after final restoration. The tentpole procedure has also been positively applied for anterior maxillary regeneration. Guillen et al. (11) compared horizontal bone augmentation in the anterior maxilla associated with two types of tenting screws used in the screw tent-pole technique. Both kinds of screws produced positive results.

Titanium mesh alone or in combination with biomaterials was used to perform alveolar crest regeneration. Deshpande et al. (12) presented a successful case of vertical and horizontal ridge augmentation in the anterior maxilla using autograft, xenograft, and titanium mesh with simultaneous placement of implants. Alagl et al. (13) described a patient who presented with a localized, combined, horizontal, and vertical ridge defect in the anterior maxilla. The patient was treated using titanium mesh and alloplast material mixed with a nano-bone graft to treat the localized ridge deformity

for future implant installation. Ribeiro Filho et al. (14) studied the use of titanium mesh and recombinant human bone morphogenetic protein 2 (rhBMP-2) for the repair of major bone defects in the alveolar bone. They found that the combination of rhBMP-2 and titanium mesh provided effective augmentation of the atrophic anterior maxilla prior to implant placement.

In our case report, we used a GBR technique: heterologous bone associated with a resorbable membrane fixed with mini screws for bone augmentation. We used a resorbable membrane in this case report since it has several advantages. Resorbable membranes are typically made from materials like collagen or synthetic polymers, which are well-tolerated by the body. Since resorbable membranes break down over time, there's no need for a second surgery to remove them. Resorbable membranes minimize the risk of infection associated with long-term membrane exposure. They promote guided tissue regeneration by maintaining space for bone growth while gradually resorbing. Clinicians find them easier to manipulate during surgery due to their flexibility.

On the other hand, the disadvantages of resorbable membranes must be considered. The rate of resorption can vary among patients, affecting the duration of membrane function. Resorbable membranes may lack the structural integrity of non-resorbable ones, potentially leading to membrane collapse. If resorption occurs too quickly, it can expose the graft site prematurely. Resorbable membranes tend to be more expensive than non-resorbable alternatives. Some patients may experience mild inflammation during resorption.

CONCLUSIONS

GBR involves the use of barrier membranes to guide the growth of new bone at sites where bone volume is deficient. It is commonly applied in implant dentistry to enhance bone quality and quantity for successful implant placement. GBR aims to create a favorable environment for bone regeneration by preventing soft tissue infiltration into the defect site and allowing osteogenic cells to populate the area. GBR may be successful if some guidelines are followed: proper case selection, adequate primary stability of the membrane, good wound closure, and patient compliance during the healing phase. GBR is a valuable technique in implant dentistry, allowing predictable bone regeneration and successful implant outcomes.

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Case Report

DILATED ODONTOMA: A REPORT OF TWO CASES

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ABSTRACT

Dilated odontoma is an extremely rare developmental anomaly that represents the extreme and most severe type III dens invaginatus. In this work, we present two cases of dilated odontoma of the maxilla that were detected randomly by radiography. In the first case, the dental element presented difficulties in eruption. In the second case, the tooth appeared microdontic.

KEYWORDS: *dilated odontoma, Dens in dente, dens invaginatus*

INTRODUCTION

Dilated odontoma (DO) is a developmental tooth anomaly that results from the infolding of the enamel organ into the dental papilla before the calcification of the dental tissues. DO currently does not feature in the most recent classifications of odontogenic tumors as an independent entity (1). The term dilated odontoma describes the most severe variant of Dens in dente, or Dens invaginatus (Type III), characterized by crown and/or root dilation of the affected tooth (2).

The most commonly used classification is that proposed by Oehlers (2), who described *Dens in dente* according to invagination degree in three forms:
Type I: which is a minor form, the enamel-lined invagination is contained within the crown of the tooth, not rising above the cemento-enamel junction;
Type II: the enamel-lined invagination extends apically beyond the cemento-enamel junction but remains within the root;
Type III: the enamel-lined invagination advances apically beyond the cementoenamel junction and riddles apically to create an apical or periodontal foramen. In this type, also called dilated odontoma, the tooth has an oval or circular shape with a radiolucent interior. It shows a single structure, usually with a main soft tissue mass (3).

The prevalence of DO ranges from 0.25% to 7.74%, even though it occurs in deciduous and permanent dentitions (4). It often involves the permanent maxillary lateral incisors, the maxillary central incisors, premolars, canines, and rarely the posterior teeth. It is infrequent in the mandible, mainly in the molars (5). Its etiology is controversial and remains unclear. Several authors have proposed various theories, are abnormal pressure from surrounding tissue, apical proliferation of ameloblast or local growth retardation, invagination of the crown before calcification, and genetic factors (5, 6)

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In this work, we present two cases of dilated odontoma in the superior maxillary detected by chance on the radiograph in the unerupted tooth.

CASE REPORT I

An 8-year-old female patient expressed pain in the right maxillary region. She reported a history of painful symptoms and frequent abscesses in this area. Her medical history was uneventful, and no hereditary factor was found. The panoramic radiograph revealed the presence of type III *Dens invaginatus* with a single root associated with the agenesis of the lateral incisor (Fig. 1).

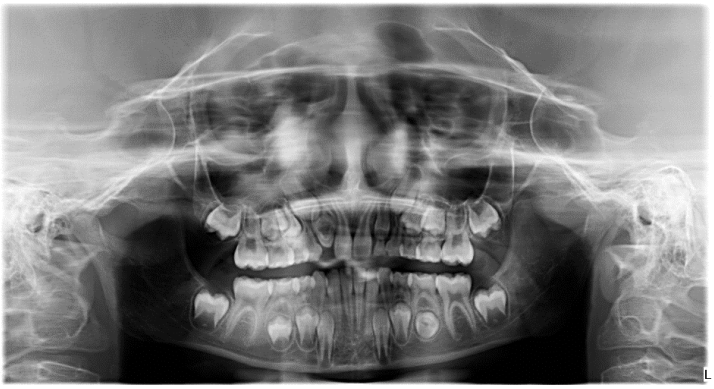

Fig. 1. *Case report I: representative OPT images of maxillary lateral incisor with dilated odontoma.*

Computerized 3D imaging provides images of sufficient quality to evaluate the morphology of an invagination in situ fully. The tooth was extracted after antibiotic therapy (Fig. 2), and primary closure was achieved easily.

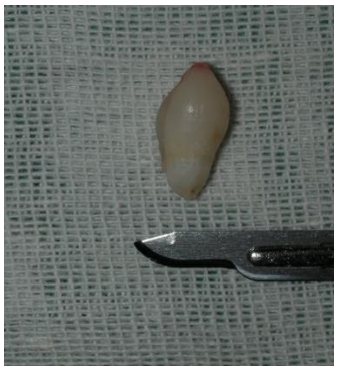

Fig. 2. *Case report I: lateral view of the extirpated dilated odontoma.*

CASE REPORT II

A 10-year-old female patient reported a history of recurrent abscesses and pain in the right maxillary region. The patient was in good general health, and no family members had dental abnormalities. Extra-oral examination revealed no significant results. Intra-oral examination showed dental anomalies of the maxillary right lateral incisor (Fig. 3).

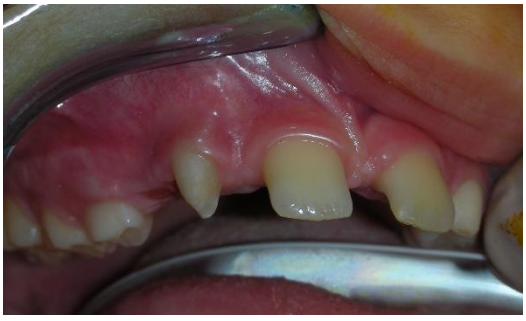

Fig. 3. *Case report II: clinical photograph of erupted dilated odontoma.*

The OPT radiograph showed a random finding of agenesis of 12 and dilated odontoma concerning the unerupted maxillary right lateral incisor (Fig. 4).

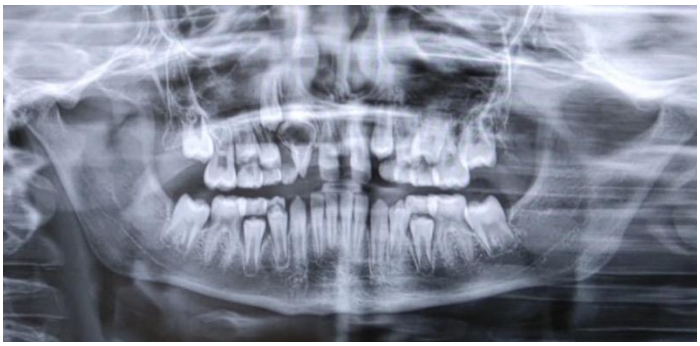

Fig. 4. C*ase report II: representative OPT images of maxillary lateral incisor with dilated odontoma.*

After local anesthesia, a mucoperiosteal flap was raised, and the cortical bone was removed, exposing the dental anomalies. It was easily shelled out, and the surgical flap was repositioned and sutured. Healing was uneventful.

DISCUSSION

Dilated odontoma morphologically shows a completely inverted structure of hard tissue due to the severe invagination of the enamel organ into the developing dental papilla, presenting radiographically as a shell-like structure with an outer radiopacity and a central core of radiolucency (1). The lesion is spherical mainly in appearance; hence the term "dilated". Dilated odontoma represents the extreme and most severe type III *Dens invaginatus* (6-8), whereas odontoma is a common benign odontogenic tumor containing all the various component tissues of teeth. They seemed to result from the budding of extra odontogenic epithelial cells from the dental lamina. This clump of cells creates a mass of tooth tissue that may be deposited in a weird configuration and consists of normal enamel, dentin, cementum, and pulp. The lesion is a complex odontoma when tooth components are well recognized and tooth-like structures are formed. Complex odontomas exhibit an amorphous conglomeration of enamel and dentin, and the most ordinary site is the posterior mandible, showing a well-defined radiopacity encircled by a radiolucent rim. Compound odontomas exhibit multiple rudimentary tooth-like structures and are more typical in the anterior maxilla. Both are frequently associated with an unerupted tooth (8-10).

Based on what has been said so far, considering dilated odontoma a form of odontoma is incorrect. The current WHO classification of odontogenic tumors does not establish dilated odontoma as a specific entity in the general spectrum of odontogenic tumors and within the context of the odontomas. It might be helpful and less misleading to change the terminology of dilated odontoma, as suggested by several authors (6).

Different authors have suggested various theories to explain the development of dilated odontoma. Even though the role of a pathogenic noxa (traumatic, viral, mechanical) on the morpho-differentiation phase of dental development is now established, the exact etiology and pathogenesis are still unclear (4-6). Genetic factors have also been evaluated (11). Dens invaginatus, like many other dental abnormalities (12, 13), are present in various syndromes (5). The most frequent are Williams, Nance-Horan, and Ekman-Westborg-Julin syndrome, based on genetic disorders (14-17).

In conclusion, the present work describes two cases of maxillary incisor teeth, severe type III *Dens invaginatus* or dilated odontoma incidentally detected in an 8-year-old female and a 10-year-old male. Even though the etiology and pathogenesis of this lesion remain to be defined, several studies try to investigate the mechanical properties and the histomorpho-structure of *Dens invaginatus* by using microradiography, micro-hardness tester, light microscopy, CLSM (18-20), and a morphometric software analysis (4, 21, 22) to achieve more pieces of information about this entity. Further investigation is needed to understand its origin fully.

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Case Report

INTENTIONAL REPLANTATION

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ABSTRACT

Intentional replantation, also defined as therapeutic replantation, consists of the extraction of a compromised tooth element, extra-oral endodontic treatment, and immediate repositioning of the tooth in its extraction socket after evaluation of the root surface. The predictability of this technique is influenced by root anatomical factors, non-traumatic extraction techniques, rapid root filling, and non-traumatic extra-oral manipulation of the periodontal ligament, which allow this approach to be considered practicable and scientifically valid in selected cases and patients. Multiple studies have recently demonstrated success rates of intentional repetition of 88% and 95%, giving this technique greater predictability. The aim of this article is to examine the steps of this procedure by presenting two clinical cases.

KEYWORDS: *intentional replantation, extraction, compromised tooth, endodontic treatment*

INTRODUCTION

Considered for years as a 'last resort' when faced with an otherwise compromised tooth and the only alternative to dental avulsion, in recent years, thanks also to the appearance of numerous case series, intentional replantation has been re-evaluated and is considered a technique to be adopted in exceptional circumstances (1)

The indications for this technique may be of a strictly dental nature, such as the impossibility of carrying out a correct orthograde root canal therapy, a reprocessing, or an apicoectomy, in the presence, however, of good knowledge and mastery of extractive and endodontic techniques (2-4). It can also be useful in the presence of carious root lesions, which are difficult to approach in a single session (monophasic).

Compared to the past, the continuous evolution of the procedure has led to changes in tooth extraction and apex preparation techniques, the handling of the tooth during the surgical phase, and the materials used for root-end filling.

Intentional replantation involves several surgical and endodontic steps that must be carried out with precision to achieve a predictable result (2, 5).

During the extraction procedures, the preservation and, therefore, non-damage of the periodontal ligament is the basis of the success of the therapy, which requires a non-traumatic avulsion for both the element and the alveolus and the shortest possible extra-oral element permanence time to avoid ankylosis and external root resorption considered negative prognostic factors (1, 6, 7, 8).

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There are elements that must be evaluated for a tooth to be chosen for the clinical procedure of deliberate reimplantation. A fundamental factor related to the element to be re-implanted is the time the tooth is in the extraoral environment because success decreases significantly after 60 minutes. The tooth element to be replanted must be intact, without root fractures, and the alveolus must also be free of comminuted fractures that would lead to probable failure of the replantation.

Complex root anatomy, pre-existing grade III tooth mobility, and the presence of furcation lesions are considered factors that negatively influence the prognosis.

The advantages of this approach are connected with the restoration of a tooth element that would otherwise have been extracted, with possible functional and aesthetic benefits, with considerable facility and speed on the practitioner's part in performing the retrograde treatment in a more conservative and less traumatic manner for the surrounding hard and soft tissues.

Reviews in the literature report a success rate for intentional reimplantation ranging from 70 to 96% of cases (1, 4-7, 9).

CASE REPORTS

In this paper, we describe two cases of therapeutic replantation. Although there is no universally accepted clinical protocol for intentional replantation in the literature, the surgical-endodontic technique we used involves various steps (1, 10).

The first case concerns a 27-year-old male patient with good general health and excellent oral hygiene. Tooth element 2.8 presented painful symptoms attributable to a carious lesion penetrating the pulp (Fig. 1).

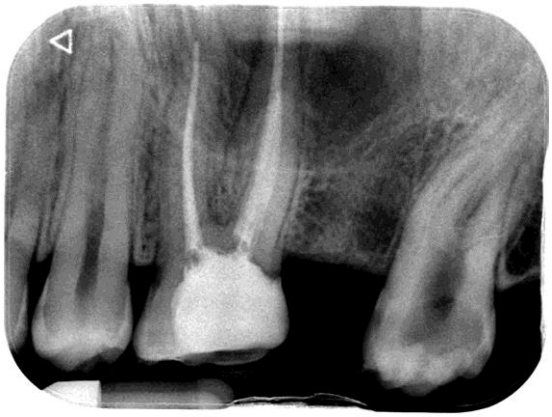


Fig. 1. *Lesion penetrating the pulp.*

It was therefore decided to choose intentional replantation and simultaneous reconstruction of the element in an extraoral environment, and after appropriate radiological evaluation, the following protocol was followed:

- preoperative rinses with 0.2% chlorhexidine for 1 minute to reduce the bacterial load in the oral cavity;
- plexic anesthesia with a vasoconstrictor (Septanest, articaine hydrochloride, 1:200,000) avoiding intra-ligamentous anesthesia to avoid possible periodontal ligament damage;
- atraumatic extraction of element 2.8, only with surgical forceps, again to preserve the periodontal ligament;
- After tooth extraction, root inspection is performed with the aid of a dental operating microscope, 40x Labomed, to check for fractures, additional canals or foramen, isthmuses, or apical deltas.

The tooth element is held between the forceps branches during all procedures. A turbine was used to access the chamber, which was cooled with sterile saline. Orthograde access to the root canals was performed at first by manual probing and later with rotating NiTi instruments (Reciproc, Dentsply). Root canal obturation was done using System B (Sybronendo) and Bio dentine (Septodont).

Subsequently, apicoectomy with a high-speed handpiece, approx. 3 mm retrograde root canal was prepared using ultrasound and retrograde obturation with bioceramic cement (BioRoot, Septodont) (Fig. 2). The tooth element was repositioned in the alveolus, which was protected by sterile gauze during the extra-oral procedures. It was stabilized by a 3-0 silk X-shaped suture to seek a better fit of the gingiva with the tooth (Fig. 3). The sutures were removed after 14 days, and the radiographic and intraoral control examination to assess stability was carried out at 3 months (Fig. 4a, b).

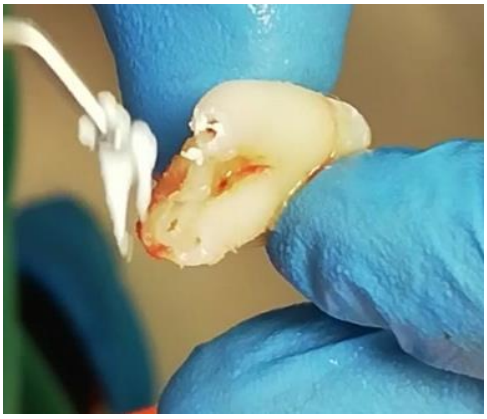


Fig. 2. *Root canal preparation.*

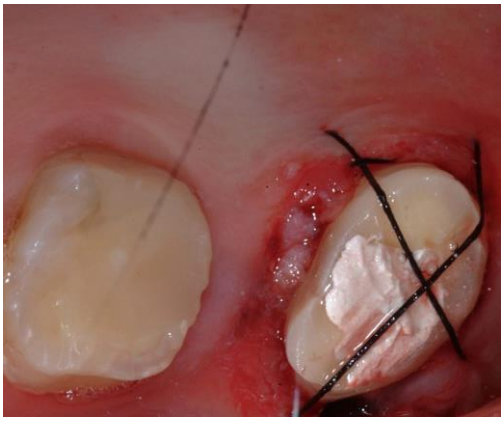
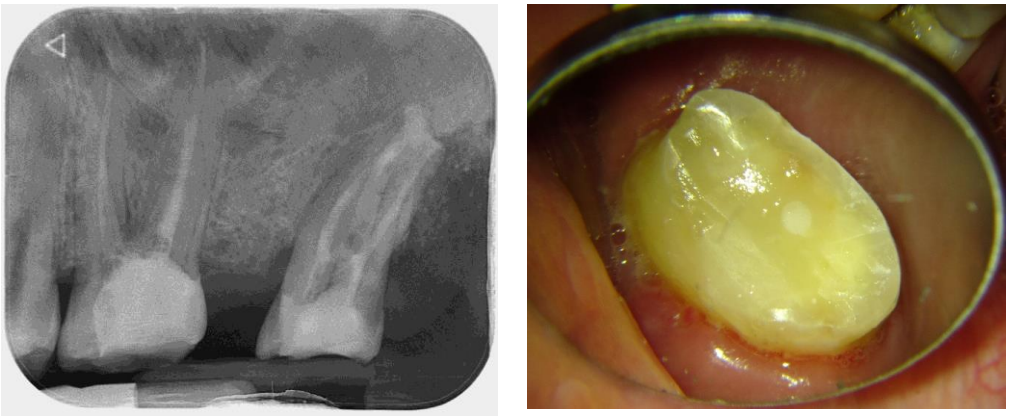


Fig. 3. *Tooth element stabilized by a 3-0 silk X-shaped suture.*



a. b.

Fig. 4. ***a****): Radiographic and* ***b****): intraoral control examination to assess stability at 3 months.*

The second replantation case was performed on an endodontically treated tooth element with secondary root caries apically at the bone margin of tooth 4.7 (Fig. 5).

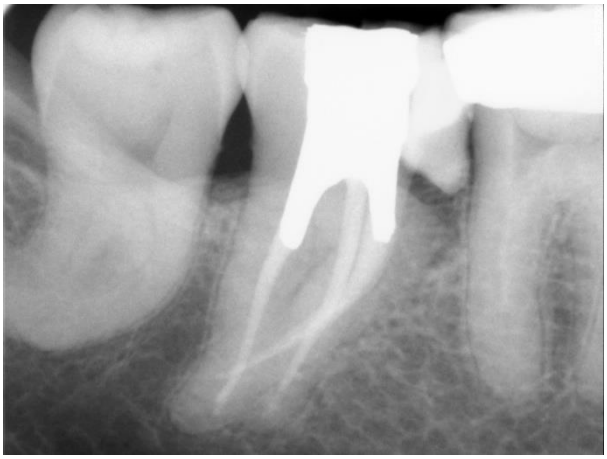


Fig. 5. *Secondary root caries at the bone margin.*

The therapeutic approach of the intentional replantation involved only apicectomy with retrograde filling in the extraoral phase.

The protocol performed included the same steps as in the previous case, with the exception of endodontic orthograde therapy, which had already been performed (Fig. 6). In the intraoperative endoral X-ray, we can observe how the root apices were apicectomised by at least 3 mm and note the bioceramic cement used for the retrograde filling. Subsequently, the patient was regularly followed up and examined. According to the protocol, we performed endoral control X-rays after 6 months to observe how the tooth is stabilized in its seat (Fig. 7).

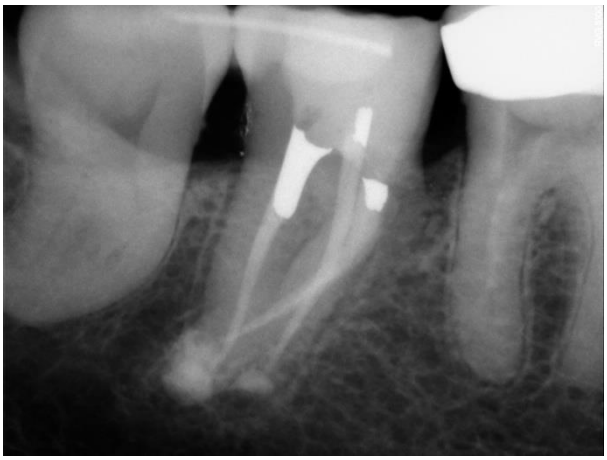


Fig. 6. *Intraoperative endoral X-ray.*

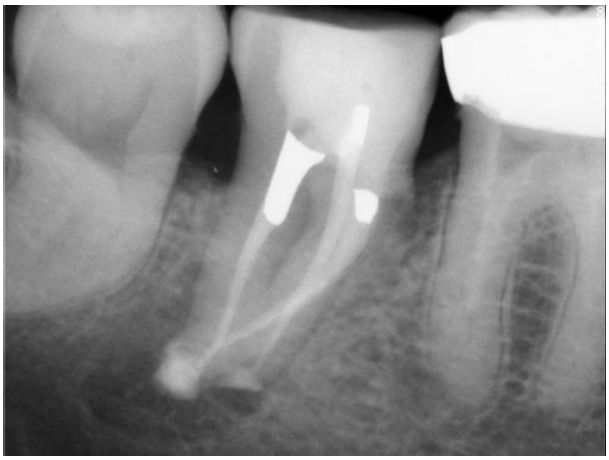


Fig. 7. *Endoral control X-rays after 6 months.*

DISCUSSION

The improved operative efficiency of this technique, as demonstrated in recent literature, has resulted in the fact that in the presence of difficult conservative restorations and anatomical limitation or difficult surgical access that complicates or contraindicates the execution of apicectomy, intentional replantation is considered an alternative method in the resolution of post-treatment endodontic pathology or to more complex techniques of functional prosthetic restoration of dental elements with a simplified and monophasic approach, in selected cases.

Atraumatic extraction techniques, periodontal ligament preservation, and a correct and rapid root canal filling technique are the basis of clinical success. This approach, long considered only for hopeless teeth, is getting an overdue but increasingly consistent recognition in the literature and should be considered as a possible alternative to avulsion and subsequent implant rehabilitation in terms of long-term success and cost-effectiveness (2, 11-15).

CONCLUSIONS

This technique has a rapid evolution in the operative phases and materials used. The presence of two specialists, an oral surgeon and an endodontist, is recommended to reduce the extra-oral retention time of the element and thus increase the success rates of reimplantation. At the same time, an extraction expertise (that preserves the root anatomy and periodontal complex) is essential.

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Experimental Study

GENE EXPRESSION OF STEM CELLS TREATED WITH MARINE-DERIVED POROUS CARBONATED ALGA IN VITRO

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ABSTRACT

Marine-derived porous carbonated red algae (MDPCRA) is a bone substitute for bone regeneration in dentistry. It is manufactured through a controlled process, resulting in a porous scaffold with interconnected pores and a high surface area conducive to cell attachment, proliferation, and tissue ingrowth. MDPCRA exhibits excellent biocompatibility, osteoconductivity, and resorbability, making it an ideal substrate for bone regeneration and remodeling. Dental follicle stem cells (DFSCs) are a population of mesenchymal stem cells found within the dental follicle surrounding developing teeth. DFSCs exhibit self-renewal capacity and multipotent differentiation potential, allowing them to differentiate into various cell types, including osteoblasts, cementoblasts, adipocytes, and periodontal ligament fibroblasts. To verify how MDPCRA stimulates bone regeneration, we treated dental DFSCs with MDPCRA to obtain information regarding the expression of genes related to osteoblast differentiation. In DFSCs, after 24 h of treatment, MMP15 was up-regulated. After 4 days of treatment, MMP15 still increased, and IL6 and SP7 were also upregulated. In conclusion, MDPCRA can stimulate several genes in DFSCs involved in osteoblast differentiation.

KEYWORDS: *marine-derived porous carbonated red alga, dental follicle, stem cells, bone regeneration, gene expression*

INTRODUCTION

Among various bone substitutes for bone regeneration in dentistry is a marine-derived porous carbonated red alga (MDPCRA). It is manufactured through a controlled process, resulting in a porous scaffold with interconnected pores and a high surface area conducive to cell attachment, proliferation, and tissue ingrowth (1, 2). MDPCRA exhibits excellent biocompatibility, osteoconductivity, and resorbability, making it an ideal substrate for bone regeneration and remodeling. It has been used in various oral surgical procedures for bone augmentation. It is a bone graft substitute, providing structural support and promoting new bone formation (3, 4). Unlike autografts, MDPCRA eliminates the need for additional donor-site surgery. Its porous structure and interconnected pore network facilitate vascularization and tissue integration, promoting faster healing and improved clinical outcomes. Moreover, it resorbs gradually over time, allowing for replacement by host bone tissue (5, 6).

Dental follicle stem cells (DFSCs) are a population of mesenchymal stem cells found within the dental follicle surrounding developing teeth. DFSCs exhibit self-renewal capacity and multipotent differentiation potential, allowing

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them to differentiate into various cell types, including osteoblasts, cementoblasts, adipocytes, and periodontal ligament fibroblasts. DFSCs can be isolated from the dental follicle tissue of impacted third molars or extracted teeth undergoing orthodontic treatment. Various isolation techniques, such as enzymatic digestion and explant culture, have obtained a heterogeneous population of DFSCs from dental follicle tissues (7, 8). DFSCs are promising for various therapeutic applications in regenerative dentistry and oral maxillofacial surgery. They have been investigated for their potential to regenerate periodontal tissues, including the cementum, periodontal ligament, and alveolar bone, in cases of periodontal disease, dental trauma, and tooth loss. Additionally, DFSCs have shown potential for use in dental implantology, root canal therapy, and craniofacial bone defects (9, 10). Their immunomodulatory properties render them attractive candidates for treating inflammatory and autoimmune oral diseases.

To verify how MDPCRA stimulates bone regeneration, we treated dental DFSCs with MDPCRA to get information regarding gene expression related to osteoblast differentiation.

MATERIALS AND METHODS

Dental Follicle Stem Cells (DFSCs) Isolation

A dental follicle was collected during third molar extraction and digested for 1 h at 37°C in a solution containing 1 mg/ml collagenase type I and 1 mg/ml dispase, dissolved in phosphate-buffered saline (PBS) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, and 500 µg/ml clarithromycin. The solution was then filtered using 70 µm Falcon strainers (Sigma Aldrich, St Louis, Mo, U.S.A.) to separate mesenchymal stem cells from fibroblasts. Stem cells were cultivated in α-MEM culture medium (Sigma Aldrich, St Louis, Mo, U.S.A.) supplemented with 20% Fetal Bovine Serum (FBS), 100 µM 2P-ascorbic acid, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Sigma Aldrich, St Louis, Mo, U.S.A.) The flasks were incubated at 37°C and 5% CO_2, and the medium was changed twice weekly (6).

DFSCs were characterized by immunofluorescence for the cytoskeletal component vimentin, positive mesenchymal stem cell markers CD90 and CD73, and the negative marker CD34, as described in Sollazzo et al. (11).

Cell treatment

DFSCs were seeded at a concentration of 1.0×10^5 cells/ml Algipore (Dentspay Italia SRL, Roma, Italy) at the concentration of 10 mg/ml in 9 cm^2 (3 ml) wells containing DMEM supplemented with 10% serum and antibiotics.

Another set of wells containing untreated cells was used as a control. The treatment was carried out at two time points: 24 h and 4 days. The cells were maintained in a humidified atmosphere containing 5% CO_2 at 37°C. At the end of the treatment period, the cells were lysed and processed for total RNA extraction.

RNA isolation, reverse transcription, and quantitative Real-time PCR

According to the manufacturer's instructions, total RNA was isolated from the cells using RNeasy Mini Kit (Qiagen, Hilden, Germany). The pure RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

cDNA synthesis was performed starting from 500 ng of total RNA using the PrimeScript RT Master Mix (Takara Bio Inc., Kusatsu, Japan). The reaction mixture was incubated at 37 °C for 15 min and inactivated by heating at 70°C for 10 s.

cDNA was amplified by real-time quantitative PCR using an ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). All PCR reactions were performed in a 20 µL volume. Each reaction contained 10 µl of 2x qPCRBIO SYGreen Mix Lo-ROX (PCR Biosystems, Ltd., London, UK), 400 nM of each primer, and cDNA.

Custom primers belonging to the “extracellular matrix, adhesion molecule” pathway, “osteoblast differentiation,” and “inflammation” pathway were purchased from Sigma Aldrich. The selected genes grouped by functional pathways are as follows: osteoblast differentiation [SPP1 (Osteopontin), SPARC (Osteonectin), RUNX2 (Runt-related transcription factor 2), ALP (Alkaline phosphatase), BGLAP (Osteocalcin), FOSL1 (FOS-like antigen 1), SP7 (Osterix), ENG (Endoglin)], extracellular matrix, adhesion molecule [COL1A1 (Collagen type I alpha1), COL4A1 (Collagen type IV alpha 1), MMP14 (Matrix Metallopeptidase 12), MMP15 (Matrix Metallopeptidase 15)], inflammation [IL6 (Interleukin 6), IL6R (Interleukin 6 Receptor)] and RPL13 (Ribosomal protein L13) as reference gene. All experiments were performed using non-template controls to exclude reagent contamination. PCR was performed using two analytical replicates. The amplification profile was initiated by incubation for 10 min at 95 °C, followed by a two-step amplification for 15 s at 95°C and 60 s at 60°C for 40 cycles. In the final step, melt curve dissociation analysis was performed.

Statistical analysis

The gene expression levels were normalized to the expression of the reference gene (RPL13) and expressed as fold-changes relative to the expression in untreated cells. Quantification was performed using the delta-delta Ct method (12).

RESULTS

DFCSs were phenotypically characterized using immunofluorescence. Fig. 1a shows a cytoskeletal filament stained with vimentin. The cell surfaces were positive for mesenchymal stem cell markers CD90 (Fig. 1b) and CD73 (Fig. 1c) and negative for markers of hematopoietic origin CD34 (Fig. 1d).

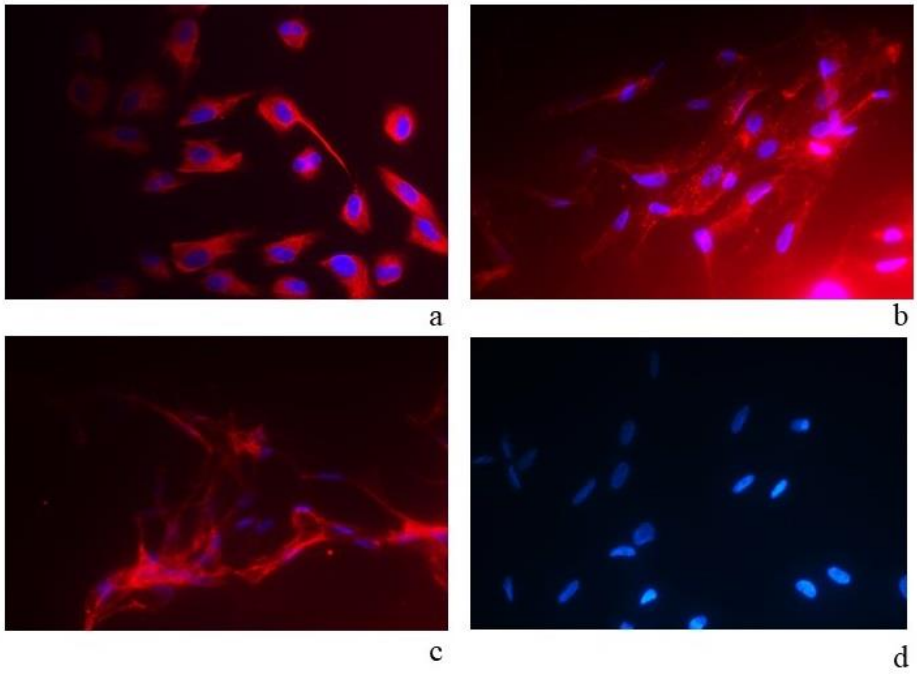

Fig. 1. *DFCSs by indirect immunofluorescence (Rhodamine). Immunofluorescence staining of vimentin (a), mesenchymal stem cell marker CD90 (b), CD73 (c), and hematopoietic markers CD34 (d). Nuclei were stained with DAPI. Original magnification x40.*

MDPCRA treatment of DFSCs was analyzed by quantitative Real-Time PCR after 24 h and 4 days of treatment, and the expression levels of osteoblast-related genes, extracellular matrix, and inflammation pathways were measured. Table I reports the significant fold-change obtained after 24 h and 4 days for DFCSs (bold).

Significantly up-regulated genes showed ≥ 2-fold change in expression (P value ≤ 0.05) while significantly down-regulated genes showed ≤ 0.5-fold change in expression (P value ≤ 0.05). In DFSCs, after 24 h of treatment, MMP15 was up-regulated (Table I). After 4 days of treatment, MMP15 was still increased and IL6 and SP7 were up-regulated.

Table I. *Gene expression in DFCSs after 24h and 4 days of treatment. Numbers express the fold changes of the de-regulated genes in treated cells vs. untreated cells. ND – not determined. In bold significant gene expression level.*

	24 h	**4 gg**
SPP1	nd	nd
SPARC	0.3	0.3
RUNX2	0.4	0.5
ALP	0.3	0
BGLAP	nd	nd
FOSL1	0.6	1.3
SP7	0.8	**2.6**
ENG	0.6	1
COL1A1	0.3	0.1
COL4A1	0.6	0.5
MMP14	**1.5**	1.3
MMP15	**2**	**2.4**
IL6	1.6	**2.1**
IL6 R	1.2	1.2

DISCUSSION

Matrix Metalloproteinase 15 (MMP 15), also known as membrane-type matrix metalloproteinase 2 (MT2-MMP), is a member of the matrix metalloproteinase (MMP) family of enzymes that play crucial roles in the degradation and remodeling of the extracellular matrix (ECM). MMP XV is unique among MMPs because of its localization to the cell membrane and its ability to activate other MMPs and process various ECM components (13-16).

MMP15 is synthesized as an inactive zymogen that undergoes post-translational modifications to become enzymatically active. It consists of several domains, including a signal peptide, prodomain, catalytic domain containing the zinc-binding motif essential for proteolytic activity, hemopexin-like domain, and transmembrane domain that anchors it to the cell membrane. The prodomain acts as an autoinhibitory module that prevents premature enzyme activation. The expression and activity of MMP15 are tightly regulated at multiple levels. Transcriptionally, MMP15 expression is induced by various stimuli, including growth factors, cytokines, and mechanical stress, through the activation of specific transcription factors, such as AP-1 and NF-κB. Post-translational modifications, including glycosylation and proteolytic cleavage, regulate MMP15 activity and localization. Moreover, tissue inhibitors of metalloproteinases (TIMPs) and other endogenous inhibitors modulate MMP15 activity, maintaining a balance between ECM degradation and synthesis (17, 18).

MMP15 is primarily localized in the cell membrane, where it functions in pericellular proteolysis and ECM remodeling. One of MMP15's unique functions is its ability to activate other MMPs such as MMP2 and MMP13, by cleaving their prodomains, thereby amplifying ECM degradation. MMP15 also cleaves various ECM components, including collagen type I, fibronectin, and laminin, thereby facilitating cell migration, invasion, and angiogenesis. Beyond its role in ECM remodeling, MMP15 participates in various physiological processes including embryonic development, tissue repair, and immune responses. MMP15 contributes to morphogenetic processes such as branching morphogenesis in the lung and mammary glands during development. In wound healing and tissue repair, MMP15 facilitates the clearance of damaged ECM and promotes the migration of fibroblasts and endothelial cells to the site of injury (19, 20).

Interleukin 6 (IL6) is a multifunctional cytokine that plays a pivotal role in immune regulation, inflammation, and tissue homeostasis. Beyond its classical role in the immune system, IL 6 has emerged as a critical regulator of bone metabolism, influencing both the bone formation and resorption processes.

IL6 exerts its effects through the IL6 receptor (IL6R) and signal-transducing component glycoprotein 130 (gp130), which form a complex upon IL6 binding. This IL6/IL6R/gp130 complex activates intracellular signaling pathways, including the Janus kinase/signal transducer and activator of transcription (JAK/STAT), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K)/Akt pathways. These pathways regulate gene expression, cell proliferation, differentiation, and survival (21-23).

IL6 plays a dual role in regulating osteoblast functions. Under physiological conditions, low concentrations of IL6 stimulate osteoblast proliferation and differentiation and promote bone formation. IL6 enhances the expression of osteogenic genes such as runt-related transcription factor 2 (RUNX2) and osteocalcin, facilitating osteoblast maturation and mineralization. Additionally, IL6 promotes osteoblast survival by activating anti-apoptotic pathways. IL6 also influences osteoclast function, albeit indirectly, through its effects on other cell types. IL6 stimulates RANKL production by osteoblasts and stromal cells, thereby promoting osteoclast differentiation and activity. Additionally, IL6 enhances the macrophage colony-stimulating factor (M-CSF) expression, which is essential for osteoclast precursor proliferation and survival (23).

IL6 plays a complex role in bone metabolism and influences both the bone formation and resorption processes. Under physiological conditions, IL6 stimulates osteoblast activity and promotes bone formation, whereas dysregulated IL6 signaling in pathological conditions leads to bone loss and an increased risk of fractures.

Understanding the molecular mechanisms underlying IL6's effects on bone cells and its involvement in bone-related disorders is essential for developing targeted therapies to preserve bone health and to treat osteoporosis, rheumatoid arthritis, and other bone-related diseases. Ongoing research into IL6 biology and its therapeutic potential holds promise for improving treatment outcomes and addressing unmet medical needs in skeletal disorders (23).

CONCLUSIONS

In conclusion, MDPCRA is a bone graft substitute with various applications. Its biocompatibility, osteoconductivity, and resorbability make it an attractive option for promoting bone regeneration and tissue remodeling in a wide range of clinical scenarios. Here, we demonstrated that MDPCRA can stimulate several genes of DFSCs involved in osteoblast differentiation. However, we know that more experiments are needed to establish the global effect of MDPCRA on bone formation.

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Evaluation Study

HISTOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF HUMAN GINGIVAL FIBROBLASTS ON DIFFERENT TITANIUM SURFACES

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ABSTRACT

The aim of the research is to evaluate the response of cells such as fibroblasts to titanium surfaces treated differently. The reason why this type of cells was chosen is related to their role in the bone healing process and in the abutment adhesion, forming a barrier that protects the underlying bone. Human gingival fibroblasts cells (HGF) were cultured for 18 and 72 hours on machined titanium grade 5 (Ti6Al4V) interstitial elements (ELI) titanium disks, coated disks with titanium nitride (TiN) and sandblasted disks with hydroxyapatite (HA) (OsseoGRIP). Cell morphology was analyzed by scanning electron microscopy (SEM), while focal adhesion kinase (FAK) protein was analyzed by confocal laser scanning microscopy (CLSM). From the results of this study, it is clear that SEM and CLSM showed great HGF cell adhesion and filopodium-like extensions on the isotropic nanorough surface (OsseoGRIP), especially after 72 hours. FAK protein was localized along cellular extensions on the OsseoGRIP disks. Within the limits of the study, we could observe that the micro-geometric differences of the various surfaces analyzed lead to a difference in cell growth in qualitative and quantitative terms. In fact, the less rough TiN surfaces are those that show less fibroblast growth. This consideration may be important in implant systems that require a long transmucosal canal where it would be desirable, in order to compose a coronal seal important for implant and bone, to achieve good growth and adhesion of the connective part. For this is useful the application of the machined and OsseoGRIP type surfaces which, being rougher, allow better seal. OsseoGRIP surfaces, thanks to their 0.5 µm surface nano-roughness, allow both to obtain a good seal from soft tissues and to control bacterial adhesion.

KEYWORDS: *OsseoGRIP, bone healing process, adhesion, titanium, endosseous implants*

INTRODUCTION

Titanium is a metal that, in its pure state, is shiny, white, and quite ductile. It has a high melting point (1668°C) and a rather low modulus of elasticity (similar to that of bone), which makes it particularly flexible and able to absorb the

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masticatory loads and then transmit them to the bone without developing tension, fundamental property to use it in dental implants together with its excellent biocompatibility.

The titanium used for the realization of endosseous implants is defined as "commercially pure" (cpTi) and has been classified by ASTM (American Society for Testing and Materials) in grades 1 to 4, depending on the oxygen content (increasing from 0, 18 to 0.40) and iron (increasing from 0.20 to 0.50). This increase corresponds to an improvement in the mechanical characteristics (1).

For the realization of dental implants, it is also possible to use titanium alloys such as Ti6Al4V (titanium grade 5) composed of 90% titanium, 6% of aluminum, 4% of vanadium, 0.25% of iron and 0.2% of oxygen (2). There is also a version of this alloy with low levels of interstitial elements (ELI), which is an excellent choice in those circumstances where a combination of high strength, lightness, toughness and corrosion resistance is required.

The titanium implant surfaces can be subjected to additive treatments (coating with hydroxyapatite or calcium phosphate, titanium plasma-spray, and ion deposition) or subtractive type (electropolishing, mechanical polishing, sandblasting, acid attack, and oxidation), which create irregularities in the surfaces (3).

Surfaces with a clear orientation of irregularities are defined as anisotropic; those that do not have an easily identifiable orientation are instead called isotropic.

Subtractive techniques

Turning/cutting/smoothing: the surfaces treated this way are defined as "machined". The cutting device used is a rotating carborundum disk, which creates a jagged and irregular design on the titanium surfaces with a low finish degree. Instead, the turning is made with a stainless-steel instrument, through which a highly anisotropic surface is obtained with microscale irregularities. To have a more delicate finish degree, the surfaces can be exposed to a smoothing process by using grit-papers or diamond clothes made of abrasive particles such as corundum (SiC) of various sizes or with alumina powder (4).

Sandblasting

Sandblasting consists of the realization of surface irregularities through the collision with microscopic particles of variable size (2). Sandblasting can be made with titanium dioxide particles (5), alumina, hydroxyapatite, or rutile, and the surface roughness is directly proportional to the size of the used particles. Surfaces treated with larger particles have a greater roughness and, therefore a greater contact area (4).

Regarding the sandblasting with hydroxyapatite (HA) particles (OsseoGRIP surfaces), a comparison was made with the machined surfaces. The impact of HA particles causes plastic deformation and induces an increase in the surface available for osseointegration. The surface does not have a clear orientation and contrarily to the machined ones, in which peaks and valleys are evident, appears homogeneous. Based on these characteristics, a 67% osseo-implant was considered after 12 weeks, greater than 62% of the machined surfaces (6).

Etching

The implant is immersed in acid substances that remove a small amount of metallic material, thus creating a surface micro-roughness. The most commonly used acids are HCL, H_2SO_4, and HF, increasing the available surface and transforming anisotropy into isotropy (7). These are the only acids capable of reacting with titanium surface oxide, which normally has a very low chemical reactivity. What occurs at the level of the implant surface are redox reactions, which are responsible for the metal dissolution (8). The dual acid-etching technique involves the immersion of titanium implants for some minutes in a mixture of concentrated HCl and H2SO4 heated above 100°C. This method increases osteoconductive by attaching osteogenic cells and fibrin, resulting in direct bone formation (9).

Sandblasted and acid-etched surface (SLA)

The combination of these two treatments is perhaps the most used. This technique involves first a sandblasting phase with 250-500 μm particles that create macrostructures and then a second phase in which micro-irregularities are created using acids (13). Through sandblasting, an optimal roughness is guaranteed for a mechanical fixation. Then, the etching smooths the sharp peaks and may add a high-frequency component on the implant surface with potential importance for protein adhesion, which is important for the early bone-healing process (10).

Oxidation

The titanium itself has a surface oxide film. Still, the oxidized implants are made by applying a thicker layer of oxide obtained by heat treatment or by placing the implant in a galvanic cell with an electrolyte [H_2SO_4, H_3PO_4,

CH_3COOH, NaOH, $Ca(OH)_2$]. Current (80 V) is then passed through the galvanic cell, and the oxide will grow from a thickness of 5 nm to 1 mm or more (10, 11). In addition to increasing the thickness of the TiO2 film, there is an increase in roughness and surface area (12). More precisely, a dissolution of the oxide layer along the convective current lines and its thickening in other areas occurs. Dissolution creates micro and nano-pores on the implant surface (13).

Additive techniques

Titanium Plasma-Spray (TPS): this technique requires a titanium powder to be introduced into a high-temperature plasma torch. The particles then collide with the implant surface on which they condense and fuse to form a film about 30 μm thick. The resulting TPS coating will have a roughness of about 7μm, increasing the implant's surface area (14).

Hydroxyapatite coating

This technique involves the addition of HA on the implant surface to increase the osteoconduction. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is considered a bioactive material, as it allows the realization of calcium phosphate-rich layers on the surface (15) and is also capable of absorbing large amounts of fibronectin and vitronektin, which favor the adhesion of osteoblasts and therefore osseointegration (16). The release of calcium phosphate in the peri-implant region determines the precipitation of biological apatite on the implant surface, which acts as a matrix for the growth and adhesion of osteogenic cells. There are several methods for coating the titanium surface. Still, the one used in clinical practice is the plasma-spray technique, which involves a mixture of amorphous calcium phosphate with calcium phosphate crystals and hydroxyapatite (2). The procedure is analogous to coating with TPS and involves the deposition of a film with a thickness varying from a few micrometers to a few millimeters.

Titanium nitride coating

Titanium alloys are known for their high strength, low density, and ability to resist corrosion. Despite this, they are not very resistant to abrasion, which can dissolve the surface oxide layer. Titanium nitride (TiN) is instead known for its particular hardness and mechanical strength, with a low dissolution of titanium ions, and this type of coating allows to solve the problem of poor resistance to abrasion of the titanium alloy. These conclusions were observed in the study by Tamura et al. (12) after analyzing a nitride titanium surface. Regarding the abrasion resistance, the surface treated with nitride showed much better characteristics; for this reason, the use of this surface is recommended in those circumstances where both a good biocompatibility and a good resistance to abrasion is required, as in the case of 'abutment (17).
Surface topography can be divided into macro, micro, and nano roughness (13, 14):

- Macro roughness: this feature can range from millimeters to microns and, if appropriate, can directly improve the initial implant stability and long-term fixation by mechanical interlocking the rough surface irregularities and the bone (13). However, a major risk with high surface roughness may be an increase in peri-implantitis (14).
- Micro roughness: it ranges from 1-10 microns and determines superior growth and interlocking of bone at the implant interface (13).
- Nano roughness: it is widely used in implant dentistry and ranges from 1-100 nm. The roughness is believe to promote absorption of proteins and adhesion of osteoblasts, improving osteointegration (13). Nanoscale topography can be obtained by various methods, such as sandblasting, ionization and etching.

The aim of the research is to evaluate the response of cells such as fibroblasts to titanium surfaces treated differently. This type of cells was chosen due to their role in the bone healing process and in the abutment adhesion.

MATERIALS AND METHODS

Sample preparation

The study was carried out using:

- machined Ti6Al4V ELI titanium disks;
- coated disks with TiN;
- sandblasted disks with HA (OsseoGRIP).

All the samples had a diameter of 15 mm and a thickness of 1.5 mm and were previously sterilized in an autoclave at 160 ° for 2 hours. Acrylic resin discs (Sintodent and Jet Lang) of the same size as the test samples, also sterilized in an autoclave, were used as the control group.

Cells and cell culture

The study used human gingival fibroblasts HGF obtained from ATCC (ATCC® PCS-201- 018 ™). Cells were seeded at a density of 1.5 x 10^5 cells/cm^2 in tissue culture plastic dishes containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics as penicillin and streptomycin in a fully humidified atmosphere consisting of 95% air, 5% CO_2 at 37°C. For SEM the cells were seeded on the titanium disks (1.5 x 10^4 cells/disk) and incubated for 18 or 72 hours.

To analyze the expression of FAK protein was used a confocal laser scanning microscopy (CLSM). The cells were seeded on the titanium disks with a density of 1.5 x 10^5 cells/disk as well as on the tissue culture plastic wells, which were used as control. The cells were incubated for 18 or 72 hours.

SEM analysis of cell morphology

HGF cell morphology was analyzed by SEM (Zeiss EVO, Germany) after a culture of 18 or 72 hours. The samples were prepared as follows: all the disks with cultured cells were washed twice in PBS, phosphate-buffered saline, to remove nonattached cells and then twice with 0.1 M SCB, sodium cacodylate buffer, pH 7.4, at 37°C for 5 min. The cells on the disks were fixed for 3 hours at 4°C according to Karnovsky's method (4% paraformaldehyde, 3% glutaraldehyde, 0.1 M SCB, pH 7.4). After fixation, the disks with HGF were washed twice in PBS and then dehydrated using a graded ethanol series. The procedure ended with critical point drying and ion-sputter coating.

CLSM analysis of immunofluorescent staining

The expression of FAK protein and actin was analyzed by immunofluorescent staining with CLSM (confocal laser scanning microscopy) after HGF cell culture for 18 or 72 hours. The samples were prepared as follows:

All the disks with cultured cells were washed twice in PBS to remove nonattached cells. Then the cells were fixed in 4% paraformaldehyde in PBS for 10 mint 37°C and permeabilized with 0.1 Triton X-100 in PBS for 5 min at 37°C. The cells were labeled with rabbit anti-FAK antibody for 1h at 37°C and then overnight at 4°C. After washing the cell layer with 0.2% Triton X-100 in PBS, anti-rabbit IgG were added for 30 min at 37°C. Finally, the cellular actin was stained using rhodamine-phalloidin.

RESULTS

The samples were analyzed by SEM after 18 and 72 from the positioning of the HGFs. Following we can appreciate the images of the various samples (Fig. 1-4).

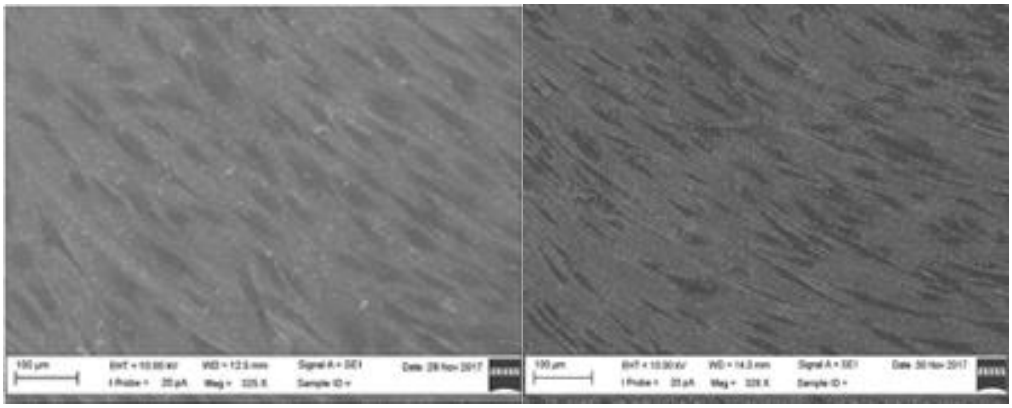

Fig. 1. *Ti6AL4V ELI alloy coated with TiN after 18 hours of fibroblast growth (left) and after 72 hours (right).*

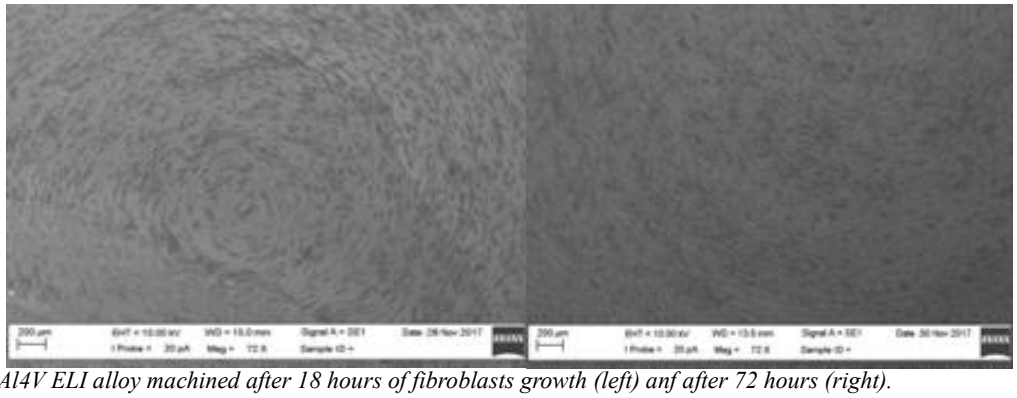

Fig 2. *Ti6Al4V ELI alloy machined after 18 hours of fibroblasts growth (left) anf after 72 hours (right).*

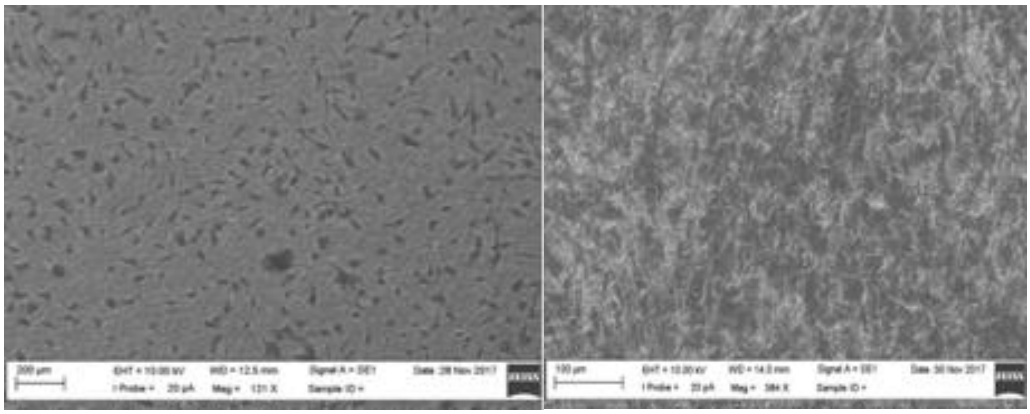

Fig. 3. *Ti6Al4V ELI alloy coated with HA after 18 hours of fibroblasts growth (left) and after 72 hours (right).*

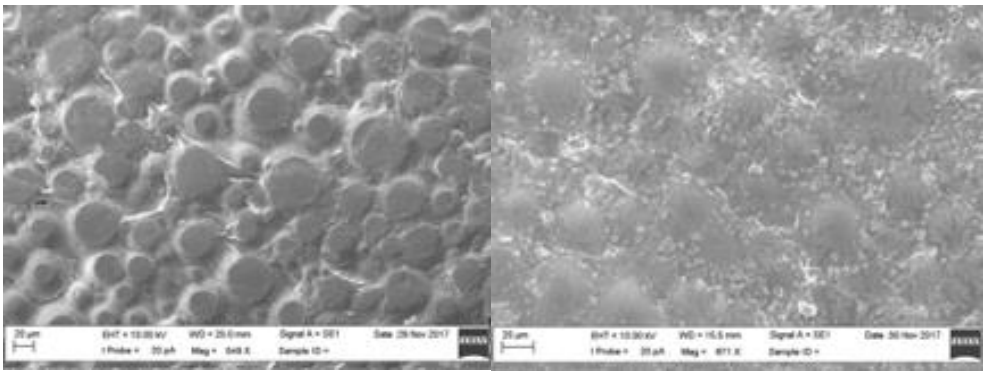

Fig. 4. *Resin Jet Lang after cell placement. The presence of an isolate fibroblast is highlighted.*

DISCUSSION

After an implant is inserted, a biological bone healing process is triggered. At the level of the bone, in the first few hours, a clot is formed that contains growth factors that influence the mesenchymal cells and intensify the activity of inflammatory cells. Over the next four days, the clot is gradually reabsorbed and replaced by granulation tissue containing blood vessels, leukocytes, macrophages, and mesenchymal-like fibroblast cells. Macrophages will take care of engulfing damaged tissue and cells and releasing growth factors and cytokines that further promote migration and differentiation of mesenchymal cells. The latter provides for the deposition of a new extracellular matrix, and together with an intense

angiogenesis mediated by endothelial cells, a temporary connective tissue is generated. Subsequently, the osteoprogenitor cells migrate and differentiate into osteoblasts. The formation of osteoid tissue is observed, followed by hydroxyapatite deposition around the 7th day with the consequent formation of immature bone or braided fibers bone (14th day) and then of mature lamellar bone (3 months) after a remodeling phase (4, 5).

Fibroblasts are the most numerous cellular elements within the connective tissue (about 65% of the total cell population), responsible for the synthesis of glycoproteins and proteoglycans of the amorphous matrix as well as the tropocollagen, the main fibrous component of the extracellular matrix (15). When an implant is introduced into the maxilla, it must interface correctly with 3 types of cells: osteoblasts, epithelial cells, and fibroblasts. The latter two adhere to the transmucosal component of the implant, forming a barrier that protects the underlying bone (13). The attachment of connective tissue to the implant blocks the apical migration of the junctional epithelium, preventing the loss of crestal bone (16). The topographic and physico-chemical properties of the implant surface affect hard and soft peri-implant tissues. As explained above, different treatments are used to increase the roughness of the implant and favor osseointegration. On the contrary, the transmucosal portion of the implant itself is generally made of smooth-turned titanium to minimize bacterial colonization (14). Regarding the type of surfaces that favor the attachment of fibroblasts, it has been observed that the fibroblasts grown on surfaces with micro-topography treated with double acid attack showed a greater production of type I collagen and fibronectin compared to those grown on the machined or smooth surfaces (17). According to the study by Miao et al. (13), after evaluating a sample with a polished surface, one with micro-roughness, and one with nano-sparse, the surfaces with nano-roughness are the ones that most stimulate the adhesion of epithelial cells and fibroblasts, increasing the percentage of survival of the implant. On this surface, the cells express more vinculin, a protein that achieves focal adhesion. Greater vinculin expression is associated with greater adhesion strength.

Guida et al. (14) also observed that a greater surface area and better tissue-titanium contact are obtained on nanostructured surfaces. This type of surface can be obtained through anodization and is characterized by irregularities with a uniform and isotropic distribution over the entire surface.

It has been suggested that a certain roughness is necessary to obtain an excellent seal of the soft tissues and to counteract the migration of the epithelium, always bearing in mind that the same roughness favors bacterial adhesion and plaque formation and, therefore, it must not exceed the value (Ra) of 0.2 μm. The nanostructured surfaces obtained with anodic oxidation, which have such a low roughness to fall into the "smooth" category according to Alberktsson and Wennerberg (6), on the one hand, favor the formation of a soft tissue seal and, on the other appear to be little contaminated by the oral bacteria (14).

From the results of this study, in agreement with the literature, it is clear that with regard to machined surfaces, fibroblasts orientate themselves along the anisotropic irregularities of the surface, resulting in lower adhesion and reduced type I collagen production compared to other surfaces.

The OsseoGRIP surface is characterized by a greater nanorugosity than the TiN (0.5 μm) and does not present a clear orientation of the irregularities, thus falling into the category of isotropic surfaces. This surface, unlike the machined one in which peaks and valleys are evident, appears homogeneous, favoring a clearly greater cell adhesion as well as adequate plaque control thanks to its moderate roughness.

The surface coated with TiN is characterized by the presence of a nitride layer with a thickness of 2 μm, composed of TiN and Ti_2N, and an irregular roughness with a size of 0.32 μm, which determines a modest cell growth.

In general, surfaces with nano-roughness are those that most stimulate the adhesion of fibroblasts, as on this surface the cells express a greater amount of vinculin, a protein that achieves focal adhesion. Greater vinculin expression is associated with greater adhesion strength.

CONCLUSIONS

Within the limits of the study, we could observe that the micro-geometric differences of the various surfaces analyzed lead to a difference in cell growth in qualitative and quantitative terms. In fact, the less rough TiN surfaces are those that show less fibroblast growth. This consideration may be important in implant systems that require a long transmucosal canal where it would be desirable, in order to compose a coronal seal important for implant and bone, to achieve good growth and adhesion of the connective part. For this is useful the application of the machined and OsseoGRIP type surfaces which, being rougher, allow better seal. Those OsseoGRIP in particular, thanks to their 0.5 μm surface nano-roughness, allow both to obtain a good seal from soft tissues and to control bacterial adhesion.

With regard to osseointegration, OsseoGRIP appears to be clearly advisable due to its ability to grow precursors of bone cells while maintaining a moderate surface roughness that allows to counter bacterial colonization. During the process of osseointegration in the first few hours we observe the formation of a clot containing growth factors which

influence the mesenchymal cells and intensify the activity of inflammatory cells. Over the next four days the clot is gradually reabsorbed and replaced by granulation tissue, containing blood vessels, leukocytes, macrophages and mesenchymal-like fibroblast cells. Macrophages will take care of phagocytizing the damaged tissue and cells, as well as releasing growth factors and cytokines that further promote migration and differentiation of mesenchymal cells. The latter provide for the deposition of a new extracellular matrix and together with an intense angiogenesis mediated by endothelial cells, a temporary connective tissue originates. Subsequently the osteoprogenitor cells migrate and differentiate into osteoblasts and the formation of osteoid tissue is observed, followed by hydroxyapatite deposition around the 7th day with consequent formation of immature bone (14th day) and then of mature lamellar bone (3 months) after a remodeling phase. The surface of the OssGRIP is the one that most stimulates this process.

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Observational Study

LIP AUGMENTATIONS WITH AGAROSE DERMAL FILLER: A 10-YEAR EXPERIENCE

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ABSTRACT

The process of facial aging is generally derived from intrinsic and extrinsic processes. The most significant changes occur in the dermis, where the amount of glycosaminoglycans and proteoglycans decreases. In general, aging is an atrophy process. The lips are the foundation on which the remainder of the perioral region is centered. The aging process of the lips begins with a proliferative phase from birth to pubescence; full, youthful-appearing lips are a direct effect of glandular and muscular hypertrophy. After puberty, a gradual atrophy of these structures occurs. The aging process includes the skin and supporting structures such as the teeth, muscles, and bone. Lip rejuvenation for the treatment of perioral rhytids is a procedure commonly requested by patients who are typically older than 50 years of age, smokers, or former smokers. The present study analyzed the use of the agarose gel for perioral rejuvenation. Newer advances in the use of fillers include agarose gel. A general increase in the indications for the use of soft tissue augmentation techniques was reported. Agarose gel has proven to be a reliable and predictable tool for perioral rejuvenation.

KEYWORDS: *filler, rejuvenation, perioral tissues, agarose, gel*

INTRODUCTION

The appearance of the mouth, teeth, and surrounding tissues provides insight into a number of factors such as age, social status, hygiene, and physical well-being and, in general, can even affect and influence one's perception and attitude (1). These structures are the most exposed parts when speaking and contribute to the first general impression, playing an important role in every occasion and conversation (2).

Teeth can hugely impact the aesthetic appeal of a smile, and the techniques used to improve their appearance, such as veneers, ceramic crowns, and the less invasive composite restorations, are fundamental in case of extensive tooth loss, diastema's closures, malpositions, and in the treatment of imperfect odontogenesis and severe dyschromia. It is also important to note that the aesthetic and functional results should be achieved using minimally invasive restorative techniques, preserving structures that contribute to defining and supporting the smile.

Color plays a pivotal role in the aesthetics of the lower third of the face; the idea of the white and perfectly shaped teeth spread during the Roman civilization. Patrician women were used to whiten their teeth by rubbing them with cloths soaked in urea-related compounds. Teeth tend to become darker over time, and white teeth have always been

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associated with a youthful smile. These changes are followed by the modification of lips and surrounding soft tissues derived from physiological aging. Teeth shape and related pathologies can also have a fundamental impact on the smile's appearance. In addition, the consequences of an improper vertical dimension, overjet, and overbite can cause irreversible changes to the muscles and skin of the perioral region. It can be deduced that rejuvenation or remodeling of the perioral region for the dentist represents a complementary intervention in addition to dental treatment. Various techniques such as peeling, connective tissue massage, biostimulation, and electroporation with collagen precursors and fillers are employed for this aim (3).

Fillers are widely used in dentistry as a complementary intervention to dental treatment, especially in complex prosthetic rehabilitation. They are divided into resorbable and non-resorbable. Absorbable fillers tend to be degraded by the action of certain enzymes, which reduce their tissue-supporting effect within 3-8 months. Permanent fillers guarantee a long-lasting effect and have a higher percentage of complications (temporary or permanent) that limit their clinical use (4).

Fillers made their appearance in the 1960s in the form of silicone oil. Today, increasingly innovative and technologically advanced materials ensure the integrity and complete biocompatibility of such products.

Soft tissue augmentation techniques began in 1893 when Neuber (5) used autologous fat, which did not guarantee adequate stability. In 1977, Knapp (6) introduced the purification of bovine collagen, and from this point on, it started to be used as filler. It was approved for this specific use by the Food and Drug Administration (FDA) in 1982, and it was, for many years, one of the few resorbable fillers available. Porcine-origin fillers represent another alternative with no risk for BSE transmission and no requirement for intradermal skin testing before use (7).

The introduction of hyaluronic acid has led to a considerable increase in dermatologists, dentists, and plastic surgeons using this type of filler. They started to be used to correct imperfections related to pathologies such as facial asymmetries and lipoatrophy in AIDS patients and even for the rejuvenation of perioral tissues (8). The latest filler proposed by scientific research is the agarose gel, created to enhance durability and eliminate the side effects observed with other fillers (9). Agarose is a saccharide polymer composed of repeated units of 3,6-anhydro-L-galactose and D-galactose. Due to its physical properties, agarose in water forms a hard gel with a 3D plastic mesh that is slowly resorbed and easily extruded through a 30-32 G needle. It is reabsorbed from the site of application by macrophages. After enzymatic destruction by galactosidase, an enzyme belonging to the glycosidase family, it is degraded through the pentose phosphate pathway at the level of the macrophages, platelets, and reticuloendothelial system (RES).

Due to its ability to retain numerous molecules and fluids, agarose gel can increase the volume of soft tissues. In addition, it has a longer duration than hyaluronic acid and collagen since there is no corresponding enzyme to reabsorb it. Its degradation only occurs after being processed by macrophages and then subjected to the action of galactosidases. This mechanism, therefore, guarantees longer-lasting results. Although the desired effect tends to decrease from the 4th month, the duration of agarose gel is 8/11 months. Its injectable, painless (isotonic and isosmotic), colorless, and transparent nature makes it an ideal material for soft tissue correction.

This article aims to share the experience gained over 12 years in using filler to create a pleasant smile in harmonious relation with the perioral tissues.

MATERIALS AND METHODS

Two hundred seventy patients needed volumetric lip augmentation (Fig.1). Agarose gels with concentrations of 1.5 and 2.5 from the companies Ghimas (Easy-Filler, Casalecchio di Reno-Bologna, Italy) and SIFARMA S.p.A (Easy-agarose, Milan, Italy) was used. Special 'total emptying' syringes allowed the use of the entire gel during the treatment.

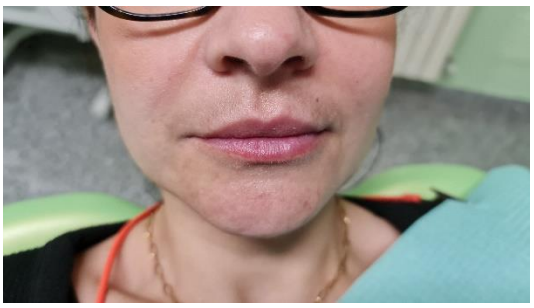

Fig. 1. *Before treatment.*

Patients treated with oral anticoagulants and suffering from severe systemic diseases, such as dialysis patients, were excluded. After the dental treatment, the patients were proposed to eliminate or diminish the skin perioral manifestations induced by chrono/photoaging and worsened by the dental pathologies.

The target area was disinfected and well-illuminated; any residual make-up was carefully removed. In some cases, especially in asymmetries, the area was highlighted with a dermographic pencil before starting the treatment to maintain the references. The needle was inserted into the mid-deep dermis, with the bevel pointing outward beneath the target zone. The correction was performed using the linear technique, releasing the product as the needle was withdrawn. A slow injection was carried out to achieve a better placement of the product in the desired sites, reducing trauma.

In some cases, the treated area was subjected to a gentle massage to promote the homogenous distribution of the material and to correct any excess. In addition, a further infiltration of agarose was performed after 1 or 2 weeks to correct slight asymmetries derived from an uneven distribution of the material (Fig. 2). All patients were advised about the need for periodic follow-ups.

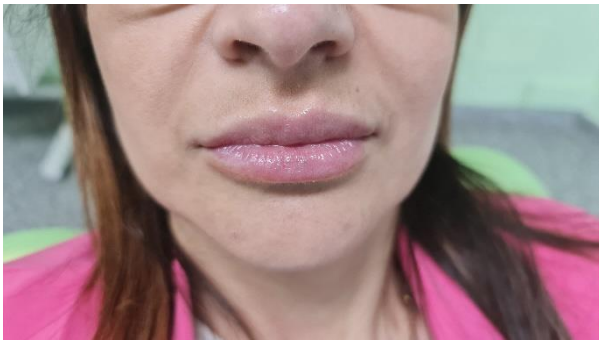

Fig. 2. *After treatment with agarose gel, a marked improvement was noted despite an initial situation difficult to resolve with fillers alone.*

The infiltrations were performed with a 30 G needle into the papillary dermis, preferably with a microponfi or 'nappage' technique and bleaching if necessary. The insertion angle was about 10° (as much as possible parallel to the skin surface), and the amount of material injected depended on the depth of the wrinkle. The infiltration was stopped as soon as the wrinkle was lifted by the agarose (100% correction). 0.6 ml was often sufficient to treat the entire perioral region. After 5 months, the lip's volume was diminished, but the result was still satisfactory. Adjustments were performed with a 0.5 ml syringe.

RESULTS

Two weeks after the first treatment, patients showed a significant improvement in lip volume (Fig. 2). The results remained unchanged for about 4 months after the first infiltration. The effects slowly but gradually wore off from this moment, so the treatment had to be repeated at 6 months (Fig. 3).

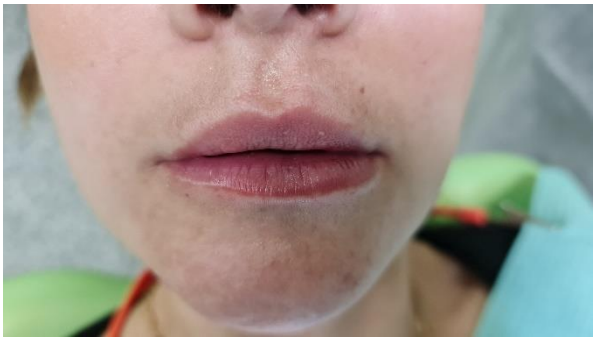

Fig. 3. *After 6 months a reduction in lip volume was observed, a situation requiring further adjustment with agarose filler.*

Dryness, roughness, and the "cigarette-paper" appearance of the epidermis considerably regressed. Tissue elasticity and hydration were significantly increased over the entire treated area. Furthermore, teeth appearances had a more harmonious relationship with the perioral soft tissues, and the results were defined as excellent by both the clinician and the patient. There were no important side effects, and erythema, edema, and ecchymosis related to the needle trauma were transient and regressed within a few days. None of the complications associated with using other fillers, such as nodular reactions, over-infections, abscesses, ulcerations, or migrations, were reported.

DISCUSSION

In the present study, agarose gel allowed attenuation or even erasing the main signs of aging by improving the harmony of the oral and extraoral tissues. As it affects the skin and the entire musculature of the lower third of the face, the perioral region aging is a fundamental issue for the dentist. Drooping labial commissures, undefined mandibular rim, and anterior and downward sliding of the muscle mass of the zygomatic region (with a subsequent drooping buccal corners and accentuation of the nasolabial groove) are the main effects of the aging process. Various conservative/minimally invasive and safe techniques, such as agarose gel, have been adopted to prevent or treat these effects.

Wrinkles are probably the most obvious 'photodamage' blemish and the least tolerated, as they are most often associated, in the collective imagination, with the aging phenomenon. The pathogenesis of wrinkles depends on the area of the skin where they occur. The tension of the mimic muscles plays a fundamental role in preventing folds in the overlying skin of the forehead, glabellar, periocular, neck, and décolleté areas. The correction of skin damaged by photo-exposure alterations or by dental disorders, which can affect the perioral zone, requires numerous methods and substances capable of attenuating or eliminating their effects. Generally, using filler alone is not enough to resolve wrinkles. In such cases, peeling, electroporation, ionophoresis, and botulinum toxin type A have proven to be effective adjunctive therapies (10). The documented numerous complications associated with the use of permanent fillers, such as silicone oil and then acrylamide (11), have led many dentists to not use these valuable tools.

Currently, the recent introduction of cross-linked hyaluronic acid and agarose gel has led to an increased interest among dentists and patients. The ideal filler should be biocompatible, non-toxic, non-allergenic, sterile, stable at the injection site without migrations to the adjacent areas, and easy to handle without side effects. All these characteristics are fully satisfied by the agarose gel. The biocompatibility of agarose is well known. The gel is well tolerated by cells and tissues and, in contrast to other vehicles suspended in solvents, it does not induce immune system responses (12).

In the present study, unwanted complications (e.g., nodule formation, foreign-body granuloma, or areas of necrosis), described often with the use of other fillers (13), were not observed. It is also worth mentioning that agarose gel is currently highly used as a biocompatible vehicle in numerous different fields of application in medicine and is employed as the main substrate in various biocompatibility tests, such as in cytotoxicity (14), genotoxicity (15), mutagenesis (16), sensitization (17) and subcutaneous implantation tests (18).

The extensive literature on the biocompatibility of agarose also highlights its safety in the different research fields, from 3D tissue growth (19) to its clinical use as a substrate for controlled drug delivery systems (20). Agarose gel fillers comprise a variable percentage of agarose (1%, 1.5%, or 2.5%), with the remainder comprising injectable water and sodium chloride. These formulations are essential for maintaining biocompatibility and preventing immune system stimulation or allergic reactions. The infiltration into the dermis enables partial replenishment of the hyaluronic acid physiologically lost and restoring the skin tone (21, 22). The product is injected into the dermis to provide a viscoelastic supplement to the extracellular matrix of the connective tissue, thereby increasing the tissue volume. Perioral soft tissue augmentation techniques represent additional therapeutic tools available to the dentist. The lower third of the face can significantly influence the overall aesthetic appearance, and the smile, along with other face regions and the neck, represents the starting point for treatment plans in dentistry.

Harmonization of the face begins with a smile, which may help to increase mental and physical well-being, self-esteem, and the ability to relate (23). An aesthetically pleasing smile increases awareness of oneself and one's abilities; it has a gratifying effect and satisfies the need for security and fulfillment that characterizes the man of the third millennium.

In recent years, the awareness of the social value of the smile has led to a progressive increase in requests for teeth whitening, veneers, and other treatments on perioral tissues that play a key role in defining the smile. Adequate lip volume, the absence of perioral wrinkles, and elastic, hydrated skin are all major elements of a beautiful and harmonious smile. Teeth appearance must also have a correct dimensional relationship with the gingival and perioral soft tissues in a 'golden' or divine proportion. The smile plays a crucial role in defining the emotional state of the individual, but it is also a great tool for communication, both verbal and non-verbal.

For all these reasons, cosmetic dentistry techniques are procedures that are increasingly in demand by patients. Dentists are driven to pay attention to the perioral soft tissues since dental treatment alone can improve the appearance of the smile. Still, an intervention in the perioral tissues allows them to harmonize and recreate the right golden ratio (12). A direct therapeutic intervention limited to the teeth would accentuate the existing discrepancies between the oral and extra-oral tissues. The patient would appear with 'youthful' teeth, while the lips and surrounding tissues would appear dehydrated, drooping, and have a dull, aged appearance.

CONCLUSIONS

Especially in complex prosthetic rehabilitations, the benefit of a combined approach in perioral tissue rejuvenation is evident. Clinical cases of patients treated with dental and perioral tissue rejuvenation procedures were presented. The results showed that the combination of the techniques mentioned above led to patient-pleasing outcomes.

The treatment of such conditions with agarose gel appeared to be a valid tool, capable of increasing perioral soft tissue volume without undesirable side effects and without requiring frequent infiltrations. By positively modifying the patient's physiognomy without incurring any risk of complications, the agarose gel was reported as an effective therapy for the rejuvenation and the correction of chrono or photo-induced alterations in the perioral region.

The results obtained from using agarose gel are satisfactory and comparable to those obtained from using other gels (24).

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Review

SCLERODERMA SYNDROME AND MUSCLE: A NARRATIVE REVIEW

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ABSTRACT

Skeletal and smooth muscles are both affected by complicated muscle involvement in scleroderma, also known as systemic sclerosis (SSC). The understanding of the cellular and molecular processes underlying the diverse participation of the smooth muscle has significantly increased in recent years. A better knowledge of the clinical features has been made possible by the new techniques for studying smooth muscle cells from the gastrointestinal tract or the vascular wall. In cases of myopathy that are inflammatory in character, it is advised to utilize glucocorticoids, modifying antirheumatic drugs, and calcium channel blockers such as nifedipine and amlodipine. Patients typically do not receive treatment when there is no discernible inflammatory component. The prognosis for SSC is greatly improved by early diagnosis and novel therapeutic options, but it continues to have a severe course and a high chance of premature death. This review summarizes the epidemiology, histopathological factors, and muscle involvement related to SSC and provides insight into ongoing treatment.

KEYWORDS: *scleroderma syndrome, systemic sclerosis, myopathies, non-inflammatory myopathy*

INTRODUCTION

Scleroderma, also known as systemic sclerosis (SSC), is a rare disorder that can cause parts of the skin to become hard and thickened, and it can also cause difficulties with the body's internal blood vessels and organs. The immune system's attack on the connective tissue that lies beneath the skin and that which surrounds internal organs and blood vessels is the root cause of scleroderma. SSC can lead the tissues surrounding joints to become more rigid, limiting the possible range of motion in those joints. In addition, it might cause swelling and pain around the joints that are afflicted. SSC might also present itself with a weakness in the muscles on occasion (1).

If the disease has also affected their muscles, people with scleroderma may experience more severe symptoms, including those involving their heart, lungs, and digestive system. SSC affects the muscles, one of the disease's most critical symptoms; around one-third of all patients report muscle weakness. 15% of these individuals have signs of muscle atrophy, also known as muscle wasting, and 10% have high blood levels of creatine kinase, which is a biomarker of myopathy, also known as muscle sickness. In persons with scleroderma, myopathy has been related to a worse prognosis (likely disease course), but few researchers have studied the associated clinical aspects of this condition (2). The involvement of the muscle in scleroderma is complicated and can affect both smooth muscle and skeletal muscle (3).

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Smooth muscle can be found in the vascular system and the digestive tract. In recent years, there has been a significant increase in understanding of the molecular and cellular mechanisms responsible for the diverse participation of the smooth muscle (4, 5).

A better knowledge of the clinical features has been made possible due to the newly developed techniques for studying smooth muscle cells taken from the vasculature or the gastrointestinal tract. Myositis and non-inflammatory myopathy are two forms of skeletal muscle involvement that are important factors in the debilitation of SSC patients (6). It has been demonstrated that skeletal muscle involvement in SSC can be represented by myositis or non-inflammatory myopathy. Compared to other types of organ involvement, muscle intervention in scleroderma has received comparatively less research attention in terms of its pathophysiology and implications (7). Therefore, this study has been designed to explore the involvement of muscles in scleroderma syndrome.

MATERIALS AND METHODS

We utilize a wide array of search strategies to identify papers that are relevant to their work. When researching and looking for information, we used websites as data collection tools such as Scopus, MEDLINE, PUBMED, and Google Scholar, from 2010-2021. The terms “scleroderma syndrome” and “systemic sclerosis” are examples of key phrases that were employed in the study as well as “muscle”, “myositis”, “myopathy”, “scleroderma syndrome” and “systemic sclerosis”.

RESULTS AND DISCUSSION

Epidemiology

The prevalence of myopathy in scleroderma varies significantly due to the absence of classification criteria that may parse out the variability of muscle disease in scleroderma. This results in a lack of standardization. In earlier research, the presence of muscle disease was determined by the availability of muscle weakness or a mixture of weakness and impaired motor enzymes, aberrant electromyography, or muscle biopsy (8). This definition of muscle disease was based on the assumption that muscle disease could only be diagnosed through biopsy. The lack of diagnostic agreement criteria results in estimates of 5 to 96 percent frequency of SSC-associated muscle participation (9). There is a possibility that SSC is present in as much as 42 percent of myositis patients who also have overlap connective tissue disease (2). Therefore, in addition to obvious cases of overlap myositis, which occur when patients meet assessment criteria for SSC and myositis, there is additional pathogenesis for myopathy and/or weakness in SSC, such as inactivity, malnutrition, or other neurologic diseases. This is in addition to clear cases of overlap myositis.

The diffuse cutaneous SSC subtype, being of African American descent, male, and having a shorter duration of SSC disease are all variables that are considered to be risk factors for SSC-associated myopathy. Muscle histology has contributed significantly to our understanding of myopathy and how it is caused by SSC (10). More recent research has estimated the incidence of muscular weakness in a large scleroderma cohort to be over 25% (11). However, a meta-analysis indicated that the prevalence of proximal muscle weakness was 16%, while the prevalence of myositis was 13% (2). Although the prevalence of myopathy continues to vary depending on the definition used for each study, it is becoming more apparent that SSC patients with concomitant muscle disease have poorer outcomes, including disability and death. This is the case even though the definition of myopathy continues to vary (12).

Histopathologic feature

The basic clinical characteristics are primarily cutaneous symptoms; nevertheless, the involvement of internal organs is what decides the fate of the condition. As the process advances through its many stages, the skin becomes increasingly tauter and thicker. Initial symptoms include swelling of the hands and fingers as well as edema and swelling of the skin more generally. The initial complaint is frequently described as morning stiffness and pain in the joints of the hands. Other symptoms may also be present. The early skin changes of scleroderma might last for several months before the usual skin induration that results from the excessive deposition of collagen and other connective tissue components (13). This induration arises due to an abnormally high level of collagen deposition. Changes in skin pigmentation are one of the most prevalent manifestations of scleroderma. Patients may develop localized hypo- or hyperpigmentation that is frequently follicular rather than the widespread hyperpigmentation that is characteristic of Addison's disease. Skin involvement near the metacarpophalangeal joints is the major criterion for diagnosing systemic sclerosis as the condition in question. The vast majority of these individuals will almost surely be affected by Raynaud's phenomenon. Involvement of the esophagus, most often in the form of esophageal dysmotility, is a hallmark of all types of scleroderma (11).

The muscle histology of patients with weak scleroderma has been the subject of previous research, showing that it is diverse. Histopathologic diagnoses in a single study of 42 scleroderma patients with muscle biopsies ranged from overlapping polymyositis, necrotizing myopathy, dermato-myositis, and fibrosis (14). Other studies have primarily characterized the predominant attributes of patients with weak scleroderma muscle biopsies as necrosis and inflammation (15, 16). This finding supports the conclusion that in the spectrum of overlap myositis, SSC is the most prevalent connective tissue condition associated with idiopathic inflammatory muscular dystrophy, accounting for 42.6% of overlap myositis patients (17).

In scleroderma, fibrosis of the muscles can be a hallmark of the disease's early stages rather than its later stages. In scleroderma muscle apathy, vascular involvement in the muscle histology is more common than in inflammatory myopathies. Scleroderma is a disease that affects the muscles, and a specific histopathologic subtype of the condition called fibrosing myopathy may indicate a more dire prognosis (2).

Muscle involvement

In patients with SSC, a broad vasculopathy is present before the development of tissue fibrosis (18). It is one of the primary pathogenic characteristics that contribute to the development of Raynaud's phenomenon, oral ulcers, pulmonary hypertension, and scleroderma kidney crises (19, 20). When the local vascular injury is not treated correctly, the result is a disorganized accumulation of extracellular matrix (21). The development of profibrotic myofibroblasts is a hallmark of progressive tissue fibrosis (20), precipitated by the disruption of microvascular circulation. The involvement of the esophagus in SSC patients often results in symptoms such as dysphagia and gastroesophageal reflux disease (22). These are two of the most common clinical manifestations of the disease.

Approximately 90 percent of SSC patients may experience some form of esophageal dysfunction (23). Although there may not be any clinical signs in the very early stage, there may be evidence of esophageal involvement on a histological level (24). The development of esophageal dysmotility is significantly influenced by the wasting away of smooth muscle and the formation of fibrosis. Skeletal involvement in SSC might range from general muscle involvement without inflammatory muscle changes to a true inflammatory form of myositis in the affected muscles. There is, however, a “grey area” with individuals who are difficult to categorize as having either one or the other of these conditions. Patients may develop non-progressive, non-inflammatory myopathy due to digestive disturbances, malnutrition, inactivity, or contractures of fibrotic skin. This condition is not progressive and does not cause inflammation. In some instances, it manifests itself as an overlapping condition with inflammatory myopathy in patients who fit the criteria for classification of both disorders (2, 25).

Ongoing therapies

The necessity of inhibiting the autoimmune process as well as inflammation and managing SSC in an organ-specific manner contributes to the complexity of the treatment. Disease-modifying medications and therapies that target specific organs are the mainstays of treatment for SSC, which has a poorly understood etiology. After an accurate assessment of symptoms, the length of the disease, its activity, and any complications, therapeutic recommendations should be made. There is currently no treatment that is specifically indicated for patients suffering from SSC-associated myopathy. Glucocorticosteroids are recommended for use in cases of myopathy that are inflammatory. Patients frequently do not receive treatment if no inflammatory component is detected (2). Treatment for arthritis and muscle pain caused by SSC typically involves either disease-modifying antirheumatic medications or glucocorticoids. Calcium channel blockers, such as nifedipine and amlodipine, are usually prescribed for treating peripheral vasculopathy and digital ulcers. If the patient only shows a moderate reaction, phosphodiesterase type 5 antagonists should be used.

Prostanoids given intravenously are shown to considerably improve the microcirculation and the recovery period of digital ulcers. Even though SSC prognosis can be greatly improved with early detection and the availability of novel therapy options, the disease is nevertheless defined by a harsh course and a high risk of passing away at an early age (2, 26).

CONCLUSIONS

Systemic sclerosis is a connective tissue disease characterized by vascular damage, immune system dysfunction, and muscle and organ fibrosis. SSC treatment requires inhibiting the autoimmune process, inflammation, and organ-specific management. SSC is treated using disease-modifying and organ-specific medicines because its pathophysiology is complex. Symptoms, disease duration, activity, and consequences should inform therapeutic decisions. The prognosis

for SSC is greatly improved by early diagnosis and novel therapeutic options, but it continues to have a severe course and a high chance of premature death.

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