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PhD THESIS IN ORAL SCIENCES

"Alveolar Bone Augmentation prior to Dental Implant Placement: Volume Analysis & Applications of Pre-augmentation Soft Tissue Expansion (STE) and Regeneration with 3D Printed Scaffolds"

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DEDICATION

To my beloved family,

Mom & Dad

Lama & Gheid

For always believing in me

For the endless love, trust and support

"Believe in yourself and all that you are. Know that there is something inside you that is greater than any obstacle"

~ Christian D. Larson

TABLE OF CONTENTS

PREVIEW

Research Rationale

Bone regeneration procedures are frequently required prior to dental implant placement, to obtain an adequate amount of alveolar bone and thus increase the success of implant therapy outcome.

However, these procedures require deep periosteal incisions and/or vertical releasing incisions for flap advancement to facilitate primary closure as severe alveolar bone resorption is accompanied by lack of soft tissues amount *(figures 1A, 1B).*

Flap advancement procedures might compromise vascularization to the surgical site and increase patient morbidity **(Mormann & Ciancio 1977; Jivraj & Chee, 2006; Esposito et al, 2007).** Moreover, wound dehiscence and bone graft exposure have been frequently reported with these regenerative techniques, compromising the final therapy outcomes **(Jensen & Terheyden, 2009).** Therefore, these techniques are often limited to highly skilled surgeons, especially in the posterior mandible, as the rehabilitation of posterior mandible is very challenging to clinicians in modern dental practice **(Laino et al, 2014).**

In attempt to help most of clinicians perform ridge augmentation procedures, increase the success rate of regenerative outcomes and decrease patient morbidity, we propose a new protocol of ridge augmentation consisting of two steps: pre-augmentation soft tissue expansion to enhance the quality and quantity of soft tissues, followed by alveolar bone regeneration with 3D printed scaffolds.

Regarding soft tissue expansion, a comprehensive literature review was conducted along with a case series of this new approach. Regarding alveolar bone regeneration with 3D printed scaffolds, a comprehensive literature review was conducted along with a

retrospective study, to analyze the anatomy and corresponding virtual grafts of the posterior mandible and understand whether a custom-made or a pre-fabricated scaffold is needed for this purpose.

Therefore, this dissertation represents a preliminary protocol for a future new approach in alveolar ridge augmentation procedures, taking into consideration the volumetric analysis of expanded soft tissues and virtual grafts in the posterior mandible.

This type of analysis might be helpful in the future development of a personalized regenerative approach by utilizing the suitable soft tissue expander and its corresponding scaffold volume for each individual patient.

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Figure (1A): Severe bone resorption in the posterior mandible, accompanied by limited amount of soft tissues

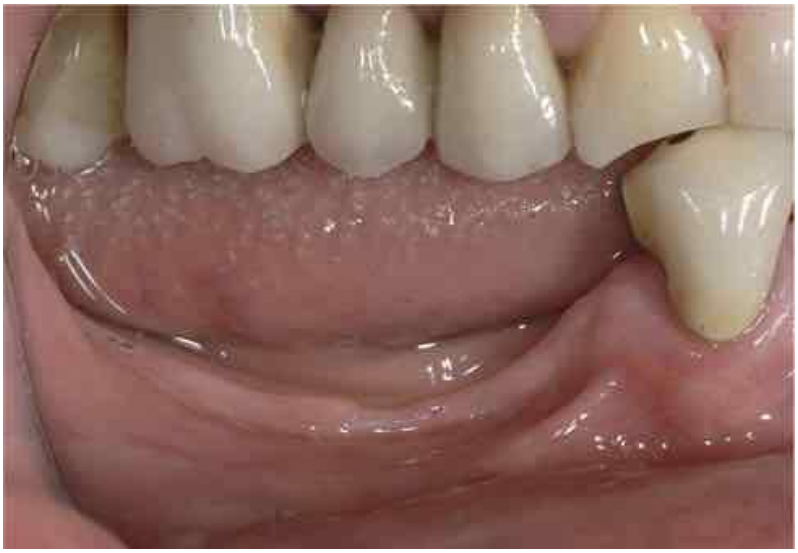
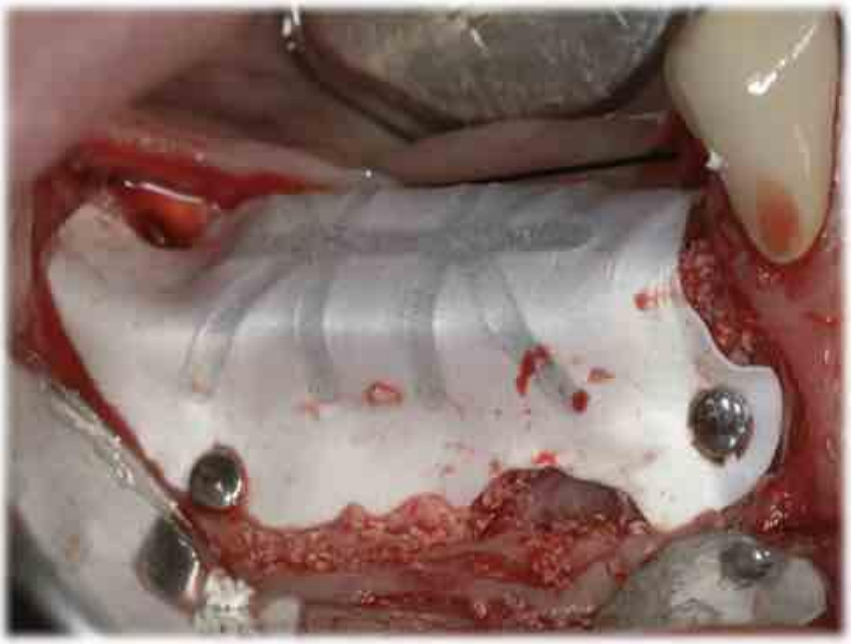


Figure (1B): Traditional ridge augmentation procedure requiring vertical and deep periosteal incisions for flap advancement to reach primary closure



"Courtesy of Rasperini, G. University of Milan, Department of Biomedical, Surgical and Dental Sciences, Foundation IRCCS Ca' Granda Polyclinic, Milan, Italy"

CHAPTER 1

Pre-augmentation Soft Tissue Expansion: An Overview

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1.1. ABSTRACT

OBJECTIVES: The aim of this review was to explore the development of soft tissue expanders, their different types and their potential applications prior to bone augmentation and implant placement.

MATERIALS & METHODS: A review of pertinent literature was performed using PubMed to comprehend the dynamics of soft tissue expanders and determine the current position of their pre-augmentation applications.

RESULTS: There is promising, albeit preliminary information regarding the benefits of pre-augmentation soft tissue expansion (STE). Findings cannot be generalized due to relatively small sample size of the scarce clinical studies available in literature.

CONCLUSIONS: Further clinical trials with larger sample sizes and long-term follow-up are needed before soft tissue expanders can be confidently applied in everyday clinical practice.

KEYWORDS: guided bone regeneration, soft tissue expanders, soft tissue expansion, soft tissue management

1.2. INTRODUCTION

Periodontal disease is one of the most common diseases of the oral cavity and it is prevalent in about half of the American adults **(Eke et al, 2012).** Left untreated periodontal disease can progress and cause destruction of the attachment apparatus and loss of the supporting alveolar bone, eventually ending in tooth loss. In fact, periodontal disease is listed as the major cause of tooth loss in adults **(Jenkins et al, 1988).** Yet, dental caries also contributes to high incidence of edentulism **(Copeland et al, 2004).**

If there is no early replacement of lost teeth, bone resorption will start and progress, causing significant changes of both, the horizontal and vertical dimension of the alveolar ridge **(Liu & Kerns, 2014).** Most of these alterations occur within the first three months after extraction; **Schropp & co-workers (2003)** reported a loss of 50% of crestal width within these early stages. Current literature confirms that tooth extraction without replacement may result in a reduction of 40% of bone height and 60% of bone width within the course of 2-3 years after tooth loss **(Ashman, 2000).**

In cases of severe bone resorption, vertical and/or horizontal bone augmentation procedures are mandatory prior to placement of implants to achieve adequate dimensions of the alveolar ridge which in turn, is necessary to accomplish successful outcomes of implant therapy, especially in anterior maxilla which is an area of high aesthetic priority.

Different techniques have been described for bone grafting: Bone block and/or guided bone regeneration (GBR) are used for horizontal bone augmentation, with a good predictability and satisfactory final outcomes **(McAllister & Haghighat, 2007).** Vertical bone augmentations are technique-sensitive as well but even more challenging. Several surgical techniques can be applied, such as: vertical GBR, onlay grafting, inlay grafting and distraction osteogenesis **(Rocchietta et al, 2008; Esposito et al, 2009).** Moreover, vertical bone augmentations are associated with high complication rates mainly soft tissue

dehiscence, which are the primary cause for bone graft exposures **(Lundgren et al, 2008).** As a negative consequence, such complication can lead to eventual partial or complete loss of the bone augmentation material. Wound dehiscence with subsequent bone graft exposure may occur in up to 20% of vertical bone augmentations **(Jensen & Terheyden 2009; Kaner & Friedmann 2011).** Similarly, **Proussaefs & Lozada (2005)** reported about 25% of bone graft exposures in patients who got vertical bone augmentation with autogenous bone blocks. This complication rate was even higher (50%) in a work published by **Roccuzzo & co-workers (2007).** In general, high incidence of bone graft exposures has been documented in the literature **(Verhoeven et al, 1997; Chiapasco et al, 2004).** Due to the aforementioned high complication rate, the use of short dental implants was suggested as an alternative to grafting procedures in atrophic areas **(Esposito et al 2011).** This might be an acceptable option in the posterior area. However, in the zone of aesthetic priority, severe bone resorption has to be compensated by augmentation procedures to achieve satisfactory aesthetic results.

Since high complication rate have been observed with different vertical bone augmentation techniques, it can be extrapolated that such complications might not be associated with the applied augmentation technique per se, but rather with the execution and precision of the surgical procedure, mainly with management and manipulation of the soft tissues..

To ensure a successful final outcome of any surgical procedure, a tension-free (passive) primary closure of the soft tissues is important to preserve the vascularization of the tissues **(Cordaro et al, 2002)** and to reduce the risk for subsequent post-surgical infections **(Wang & Boyapati 2006).**

Since soft tissues follow the underlying bony contour **(Sonick & Hwang, 2007),** severe alveolar bone resorption in either the maxilla or the mandible are usually accompanied by a limited amount of soft tissues, which impairs a tension-free primary closure of the soft

tissues. This might be even more compromising when large amount of bone grafting materials are to be used (e.g. bone block grafts) in vertical and/or horizontal bone augmentation procedures.

As a consequence in an attempt to achieve complete and tension-free primary soft tissue closure over the grafted area, flap advancement is usually performed by mobilizing the muco-periosteal flap by deep periosteal releasing incisions. This approach has been recommended following major bone grafting procedures to achieve the aforementioned goals **(Greenstein et al, 2009)**. Vertical releasing incisions negatively affect the perfusion of the muco-periosteal flap **(Mormann & Ciancio 1977; Jivraj & Chee, 2006; Esposito et al, 2007)** and since preservation of sufficient blood flow is essential for the nutrition of the soft tissues, a decrease in flap vascularization increases the risk of soft tissue dehiscence **(Nakayama et al, 1982).** Moreover, periosteal releasing incisions compromise the integrity of the periosteum overlying the bone graft, which results in: diminished blood supply to the bone graft **(Abrahamsson et al, 2010)**, less new bone formation and poor bone remodeling activity **(Zhang et al, 2008).** The periosteum is a fundamental source of osteoblasts and their precursor cells **(Allen et al, 2004)** and hence, presence of vital periosteal progenitor cells on the surface of bone grafts accelerates bone healing **(Xie et al, 2007).**

Moreover, flap advancement may result in a reduction of the vestibule and a coronal displacement of the muco-gingival junction, which may compromise the final aesthetic result and impair cleansing around prostheses on implants **(Jung et al, 2014).**

When extensive flap advancements are required, even if flap passivity has been achieved with releasing incisions, the risk for wound dehiscence may increase with negative consequences for the underlying bone graft **(Lundgren et al, 2008; Burkhardt & Lang, 2010).** Moreover, incomplete soft tissue coverage results in a limited contact with area between bone and flap which in turn is necessary for re-vascularization of the bone graft

(Moghadam, 2009) and which is important in the prevention of an accelerated resorption of the bone **(Zerbo et al, 2003).** It has been documented that flap tensions results in wound dehiscence, irrespective of flap thickness **(Burkhardt & Lang, 2010).** In a clinical study on implant patients, wound dehiscence occurred in 40%-100% of sites exposed to high flap tensions **(Burkhardt & Lang, 2010).**

In general, flap mobilization seems to increase the risk for soft tissue dehiscence, and, as a consequence, to compromise the survival of the underlying bone graft. Attempts to minimize the risk of post-surgical soft tissue dehiscence have been made by utilizing extra-oral approaches in bone augmentation. Placement of bone grafts through extra-oral incision was encouraged to avoid the risk of intra-oral incision breakdown **(Bell et al, 2002).** This approach was also adopted to expose the facial blood vessels for anastomoses with free fibula flaps (segment of bone with vascularized pedicle), to compensate for the poor vascularization of soft tissues associated with atrophy **(Rohner et al, 2002; De Santis et al, 2004; Chiapasco et al, 2011).** However, these methods are invasive as they involve patient hospitalization and an increase in morbidity rate.

Notably, a considerable amount of bone graft volume resorbs during the postoperative healing phase and as a part of the remodeling process regardless of other factors **(Cordaro et al, 2002; McAllister & Haghighat, 2007).** A compromised mucosal vascularization and lack of tissue integrity will accelerate bone resorption, beyond the commonly seen remodeling activity **(Lundgern et al, 2008; Rothamel et al, 2009)**. Hence to prevent such unfavorable results, improvement of the quantity and quality of soft tissues overlying bone grafts must be taken into consideration **(Kaner & Friedmann, 2011)**.

An increase of soft tissue volume overlying bone is well-documented in distraction osteogenesis, which induces an expansion of both, bone and covering soft tissues **(Rocchietta et al, 2008; Esposito et al, 2009).** However, bone distraction is a complex and technically

demanding procedure. It requires a special device that must be serious complications **(Uckan et al, 2002).**

Alternatively, less-invasive methods to create a surplus of soft tissues and therefore, reduce the risk for mucosal dehiscence have been investigated. To these belong: periosteal distraction **(Schmidt et al, 2002; Kessler et al, 2007; Sencimen et al, 2007; Oda et al, 2009; Tudor et al, 2010)** and tissue engineered periosteum **(Schönmeyr et al, 2009; Warnke et al, 2009)**. In spite of the promising results, these methods are still experimental and have been tested only in animal experiments. Further investigations are obligatory to validate the eligibility for their clinical applications.

1.2.1. Soft Tissue Expansion

Soft-tissue expanders have been introduced in implant surgery, as pre-augmentation devices, to avoid the complications associated with bone grafting procedures **(Kaner & Friedmann, 2011; Mertens et al, 2015).** The concept of soft tissue expansion is based on the biological properties of various soft tissues, such as skin or mucous membranes, to react to applied mechanical forces by true tissue growth (cell proliferation) **(Neumann et al, 1957).** This phenomenon can be observed in abdominal skin during pregnancy, obesity, or muscle growth or as a result of traditional habits like lip and neck expansion as part of African traditions **(Johnson et al, 1993).** Soft tissue expanders have the capability to enlarge soft tissue volumes without altering its thicknesses and to generate tissues with appropriate color match and texture similar to that of the original tissues **(Fang et al, 2013)**. One of the clinical indications of such technique is the preoperative expansion of the oral mucosa when large bone augmentations are planned. An over amount of soft tissues might reduce the need for periosteal incisions and guarantee a passive flap closure covering the bone graft. Further, intra-oral applications of soft tissue expanders include the repair of lip and/or palate clefts.

The use of soft tissue expanders became popular in the field of plastic surgery since 1976 (**Uijlenbroek et al, 2011)** The applications are well established for many indications, ranging from correction of skin burn after burn wounds, scars, alopecia, congenital nevi to post-mastectomy breast re-construction **(Berge et al, 2001; Ronert et al, 2004; Obdeijn et al, 2009; Chummun et al, 2010)**. Recently, the "concept" of soft tissue expansion has been introduced in orthopedics. In a clinical report, the successful application of an "external" soft tissue expander to achieve skin closure in open fractures was described **(Formby et al, 2013).**

1.2.1.1. History & Types of Soft Tissue Expanders

Neumann first developed soft tissue expanders in **1957,** by applying a subcutaneous rubber balloon to expand skin tissues in order to repair an ear defect. Nonetheless, it was not until the early 80's when the real interest in soft-tissue expanders re-surfaced, particularly in breast reconstruction **(Radovan, 1982)** and treatment of burns **(Argenta et al, 1983).** The expanders used in these early stages were made of silicone rubber, with an external valve penetrating the skin for manual inflation by serial injections, as illustrated in *figure (1).*

The amount of soft tissue gain with conventional expanders has been reported to be dependent on the type of expanded tissues and the shape of the expanders **(Brobmann & Huber, 1985; van Rappard et al, 1988).** It was observed that tissue gain was more pronounced with rectangular and crescent forms compared with round-based expanders **(Johnson et al, 1993).**

Despite the positive results with conventional expanders, they have several disadvantages; such as repetitive inflations, which may increase the treatment time up to several months The intermittent modality of external inflations creates pressure peaks with a reduction of the tissue vascularity **(Pietila, 1990)** which in turn may cause an expander perforation through the soft tissues **(Wiese, 1993).** A lack of perfusion caused by pressure peaks reduces

the local oxygen partial pressure of the soft tissues and therefore increases the risk for expansion failures **(Berge et al, 2001).** Additionally, serial injections increase the costs of treatment, and morbidity of the patients as well as the risks for adverse effects by repeated punctures. Despite these drawbacks, conventional soft tissue expanders are still used in plastic surgical procedures. Due to the above-mentioned shortcomings, the use of conventional expanders is limited in craniofacial defects **(van Damme et al, 1992).**

To overcome the disadvantages of conventional soft tissue expanders, **Austad & Rose (1982)** developed a self-inflating osmotic soft tissue expander, without an external port and no need for repetitive inflations. The new type of expander was based on a semi-permeable silicone membrane, which contained hypertonic sodium chloride solution. The osmotic gradient allowed a continuous inflow of body fluids into the expander. As a consequence, the expander volume increased with concomitant soft tissue growth. As a negative effect, leaks occurred from the shell of the expander to the surrounding tissues resulting in tissue necrosis. These complications made the device inappropriate for clinical application. To overcome this major drawback, **Wiese (1993)** developed a novel self-inflating osmotically active soft tissue expander made of hydrogel. It consists of both: a polymer network (cross-linked hydrogel of co-polymers based on methyl-methacrylate and N-vinyl-pyrrolidone) **(Wiese, 1993; Wiese et al, 1999; Wiese et al, 2001)** and a variable aqueous component **(Refojo, 1975).** Since 1999, this hydrogel expander has been designed and manufactured under the name of Osmed® (Ilmenau, Germany) which is the first commercially available self-inflatable osmotic expander and has been FDA-approved since 2001.

The biomaterials used are the same like in contact lenses and offer a high biocompatibility without eliciting any toxic effects, adverse immune reactions, infections or any other systemic manifestations and most importantly they do not provoke any localized inflammatory reactions in the soft tissues **(Wiese et al, 2001)**. Incorporation of methacrylate, in general,

produces ionic hydrogels due to the presence of carboxyl moieties, which results in a greater osmotic potential and subsequent amplification of the swelling capability in comparison to non-ionic hydrogels **(Wiese et al, 2001).** Inclusion of "methyl" methacrylate, specifically, in osmotic hydrogel expanders results in an increased swelling ratio **(Wiese, 1993; Wiese et al, 1999; Wiese et al, 2001)** when compared to "hydroxyethyl" methacrylate **(Downes et al, 1992).**

The presence of cross-links renders the polymer network insoluble in aqueous media **(Bell et al, 1996),** thus the expander has the ability to swell and retain large volumes produced by swelling and not dissolve in the aqueous media. In an effort to test different biomaterials, **Varga & colleagues (2009)** developed a hydrogel osmotic soft tissue expander made of acrylamide (AAm), acrylic acid (AAc) or N-isopropylacrylamide (NIPAAm). Although NIPAAm hydrogels were proven to be the most appropriate biologically and mechanically for applications in plastic and reconstructive surgeries, these expanders were only tested *in vivo* and remain to be validated in clinical trials.

Since osmotic expanders abolish the need for serial injections, they inflate continuously by osmotic gradients without the need for additional interventions. A constant expansion compared to an intermittent inflation results in the formation of new cells, tissue growth **(van Rappard et al, 1988),** and a greater amount of final tissue gain **(Wee et al, 1992; Bennett & Hirt, 1993; Bascom & Wax, 2002).**

Absence of an external filling port minimizes the bulkiness of the expansion device **(Swan et al, 2012),** which facilitates the positioning of the expanders. With a starting volume of just 10% of the final volume, osmotic expanders are initially smaller in size than conventional expanders **(Ronert et al, 2004).** As a consequence, osmotic expanders require smaller incision for insertion **(Chummun et al, 2010),** which reduces surgical trauma *(figures 2A, B).* Miniaturized osmotic expanders have been successfully used in clinical ophthalmology

(Schnittkowski et al, 2003) and opened new indications in pediatric surgery **(Obdeijn et al, 2009).**

There are two generations of Osmed® hydrogel soft tissue expanders. The first-generation lacks a silicone envelope surrounding the surface of the hydrogel, which results in extremely rapid expansion, in the early stages after insertion with consequent complications **(Rees et al, 2008)**. Importantly, rapid inflation does not result in an actual increase in soft tissue volume since tissues need time to adapt **(Uijlenbroek et al, 2011).** Early studies showed that tissues might return to their pre-expansion status in the case of rapid expansion **(Johnson et al, 1993).**

To avoid such undesirable outcomes, a second-generation of osmotic hydrogel soft tissue expanders, coated with silicone, have been introduced in 2001 **(Ronert et al, 2004).** Both generations are displayed in *figure (3).*

The perforations in the "impermeable" silicon shell allow the influx of surrounding fluids. The number of perforations controls the inflow rate, which in turn limits the speed of expansion **(Kaner & Friedmann, 2011).** Compared to the first generation, a less steep swelling curve of the second-generation expanders represents a continuous expander growth with less pressure peaks **(Ronert et al, 2004; Anwander et al, 2007).** In other words, inclusion of silicone coating adjusts the expansion speed overall, which gives more time for the newly formed tissues to adapt, more time for wound healing and results in greater amount of expanded tissue **(Wee et al, 1992),** and effective soft tissue generation **(Wiese, 1993; Wiese et al, 2001)**. Comparisons between osmotic expanders and conventional expanders are summarized in *table (1).*

In the following, we will refer to Osmed® expanders as they are the most widely applied commercially available expansion devices with sufficient evidence-based data.

1.2.1.2. Shapes, Dimensions and Expansion Time & Speed of Osmotic Expanders

Osmotic soft tissue expanders are available in different shapes with diverse "prior to insertion/post-insertion" dimensions to match their area of application. For example, round shape is mainly used in breast reconstruction while rectangular shape is recommended for defect coverage after excision of large skin tumors and burns **(Ronert et al, 2004).** For intra-oral uses, the manufacturer recommends hemispheric and cylindrical shapes. "Pre-insertion/post-insertion" dimensions are accurately defined for each expander, which simplifies surgical planning.

Once inserted, osmotic expanders have the capability to expand ten times of their original volume **(Chummun et al, 2010)**, within the time of approximately 6-8 weeks post-insertion **(Obdeijn et al, 2009).** Results from studies concluded that expanders could reach 6 times their original volume, after two weeks after insertion **(Abrahamsson et al, 2010).**

This duration primarily depends on the anatomical location, size of the defect **(Ronert et al, 2004)** and on the dimensions of the expander **(Mertens et al, 2015).** Expanders with bigger pre-insertion/post-insertion dimensions require more time to achieve their final size. Accordingly, the duration of expansion may vary from 10 days to 8 weeks **(Ronert et al, 2004).** Furthermore, the expansion speed, which dictates the time frame to complete swelling, depends on the shape of the expander.

Round expanders for breast reconstructions are left for approximately 4-6 months, as they are replaced later on by permanent implants.

Since osmotic expanders have different shapes and dimensions each model apparently has its own swelling curve. It is impossible to modify the swelling characteristics of a certain type of expander after insertion **(Uijlenbroek et al, 2011)** and therefore, the appropriate expander model should be chosen prior to surgical placement.

1.2.2. Intra-oral Applications of Soft Tissue Expanders

In craniofacial surgery, the application of expanders was first described by **Argenta & VanderKolk (1987).** The use of soft tissue expanders prior to bone augmentation of the severely atrophic mandibular ridge has been encouraged by early reports **(Lew et al, 1988; Wittkampf, 1989; Schwartz & Relle, 1990; Bahat & Handelsman, 1991, Lew et al, 1991, Zeiter et al, 1998).** However, conventional expanders in the shape of silicone balloons were applied in all these cases and they included few patients without long-term follow-up, in terms of stability or relapse of expanded soft tissues and outcomes of hard tissue procedures following expansion. Recently, osmotic hydrogel soft tissue expanders of the second generation have been investigated in intra-oral applications.

Uijlenbroek & co-workers (2011) tested osmotic soft tissue expanders in an animal study. To validate the effectiveness and efficiency of soft tissue expanders in various intra-oral applications, the researchers placed the expanders in the palatal mucosa of goats. As the palatal mucosa is very firm in these animals, the researchers hypothesized that a similar expansion would be successful in the oral cavity of humans. Expanders were implanted for 40 days, using either a “tunnel” approach or a “flap” approach. After swelling the expanders had created a surplus of soft tissues with an excellent shape and no signs of inflammation. Histological analysis revealed no signs of bone resorption, despite the pressure exerted on bone, which is equal to the amount of pressure needed to expand the soft tissues. Regarding the expander insertion techniques, no difference was observed between the tunnel and the flap approach. With the tunnel technique, fixation of the expander was more challenging compared to the flap approach due to restricted view and limited freedom of handling the expander. Based on the manufacturer’s guidelines for intra-oral use of the expanders, insertion is recommended with the “tunnel” technique. This approach prevents a complete flap reflection for expander placement.

In an *in vivo* experiment, **Abrahamsson & colleagues (2009)** placed sub-periosteal osmotic soft tissue expanders in mandibles of rabbits. In each rabbit, two sites were assigned: test site in contact with the base of the expander, and control site which was the flat end of the expander, fixed by a mini-screw and has no expanding capacity. Two weeks post-expansion, clinical inspection showed no signs of soft tissue dehiscence or infections and histological examination revealed periosteal expansion without any signs of inflammatory reactions or bone resorption. In fact, new bone formation at the edges of the expanded periosteum was evident, while there were no signs of bone formation in the control area.

In a following experiment, the authors applied the same animal model, and protocol in order to evaluate the outcomes of post-expansion bone augmentation by GBR (with particulate onlay bone graft and covered either by titanium mesh or bioresorbable mesh) **(Abrahamsson et al, 2010)**. Three months post-augmentation, it was evident that tissue expanders were able to create a sub-periosteal space and new bone formation was allowed underneath the mesh and at the edges of the expanded periosteum. The effect of soft tissue expansion on the outcomes of GBR with two different grafting materials was evaluated in another animal study **(Abrahamsson et al, 2011).** In agreement with previous findings, soft tissue expanders were able to create a surplus of soft tissues including periosteum, which facilitated mucosal coverage of the bone graft without occurrence of soft tissue dehiscence. New bone formation was found under the titanium mesh regardless of the type of bone graft. Nonetheless, it must be noted that a lack of soft tissue dehiscence or related complications in this report may be attributed to the adopted extra-oral surgical approach. The authors chose such an approach as the access via the oral cavity in rabbits was restricted **(Abrahamsson et al, 2010).**

There are just few clinical data available which describe the mucosal expansion prior to bone augmentation; two case series **(Kaner & Friedmann, 2011; Mertens et al, 2015)** and one randomized controlled clinical trial **(Abrahamsson et al, 2012)** could be found in literature.

In a randomized controlled clinical trial, **Abrahamsson et al (2012)** applied sub-periosteal soft tissue expanders in ten patients requiring bone augmentation prior to implant placement. Two weeks post-insertion, the expanders have been removed and GBR was carried out with either a particulate onlay graft protected by titanium mesh and a collagen membrane (test group), or a cortical bone block graft, harvested from the ramus, without any previous soft-tissue expansion (control group). The authors chose GBR as the bone grafting method in the test group for two reasons: 1) Predictable results have been reported with regard to bone fill **(Degidi et al, 2003),** and 2) promising results after mucosal expansion have been described in previous animal experiments **(Abrahamsson et al, 2010; Abrahamsson et al, 2011)**.

In the test group two patients showed minor perforations of the soft tissues due to expander placement close to incision line. In these two cases, however, soft tissue expansion was sufficient to completely cover the bone graft with the mucosal flap, without any complications. In the control group, periosteal incisions were required to allow flap advancement and achieve full coverage of the bone grafts.

Changes in soft tissue profile of the attached gingiva were evaluated at baseline and 6 months after augmentation in both groups and additionally at post-expansion in the experimental group, by using an objective 3D metering device. This device is based on digital light stripe projection, which deflects whenever the surface alters in topography. During the procedure, a clinical picture is also taken. Deflection data are registered through a sensor and stored in a computer with appropriate software. Data is evaluated and displayed as a color-coded picture of the topography **(Wälivaara et al, 2007).**

The software matched calibrated pictures for each patient in both groups at different time points. A line was drawn on the matched pictures of at the level of the attached gingiva and over the bone-augmented area. The lines opened up in a diagram, which resulted in two or three curves depending on the number of the measuring occasions. These curves demonstrated soft

tissue profile at the specific area where the lines were drawn. Alterations in soft tissue profile overtime were determined by measuring the height difference between the curves. The mean soft tissue profile gain at the attached gingiva level was 2.9 ± 1.1 mm when compared to baseline, while it decreased to 2.3 ± 2.1 mm at the time of implant placement, when compared with the starting point. The control group showed a soft profile change of 1.5 ± 1.4 mm at the time of fixture installation. Even if the test group showed increased gingival dimensions after surgeries, the differences were not statistically significant. The authors did not measure the total volume change in soft tissues, as they only wanted to determine overall stability of created soft tissues by evaluating soft tissue profile changes overtime. Although soft tissue profile became less prominent after healing of bone graft when compared to pre-augmentation soft tissue profile, this result was statistically insignificant.

Six months post-operatively, the test group showed a minimal resorption of bone graft in the vertical dimension of just 27% and a tendency for resorption in the horizontal aspect (14%). Corresponding to earlier findings in the literature **(Chiapasco et al, 2006; McAllister & Haghighat, 2007)**, vertical bone resorption was more pronounced than lateral one.

On the other hand, the control group showed a statistically significant bone resorption in both, the vertical (42%) and horizontal (28%) dimension. Overall, bone resorption in the experimental group was less pronounced than that in the control group. However, the difference just reached statistical significance when smokers have been excluded from the calculation. Smokers have been included in the study as they might be candidates for such an approach in everyday clinical practice.

The favorable outcome with expansion could be attributed to the direct contact of the bone graft with periosteal progenitor cells. One might speculate that a reduced bone graft resorption in the test group is based on different augmentation modalities in test and control group.

Despite the satisfactory results reported with soft tissue expansion in animal and human clinical trials, the authors recommended further refinements of the soft tissue expansion technique particularly in smoking patients. Such refinements may mainly focus on the risk reduction of complications such as soft tissue perforation **(Nyström et al, 2009; Lindfors et al, 2010).**

Similarly, positive outcomes of pre-augmentation soft tissue expansion were reported by **Kaner & Friedmann (2011)** in a case series. In contrast to the previous study, the osmotic expanders have been placed in submucosal pouches. The rationale for the altered location was to prevent replacement of periosteum with collagen-rich connective tissues lacking osteoblasts and precursor cells, which can have negative effects on the healing of subsequent bone graft.

Out of twelve patients enrolled in the study, two experienced soft tissue perforations by the expanders and had to be retrieved prior to final expansion. Perforation occurred due to infection four weeks post-insertion in one patient, while the choice of an oversized expander was the cause in the other one; a fact that emphasizes the selection of an appropriate size of the expander. Perforated sites were allowed to heal for 6 weeks, and then retreated with smaller expanders.

After 60 days in situ, all the expanders reached their final volume and vertical bone augmentation was carried out either with onlay grafting (autogenous bone block harvested from the ileum in three patients) or GBR (ramus graft covered with Bio-Oss and a collagen membrane, in nine patients). During removal of the expander, a surrounding capsule of soft tissue could be observed which did not show signs of inflammatory infiltration upon histological analysis. The expanded tissues showed good quality, and the space created by the expanders allowed a tension-free primary closure. Despite the occurrence of a minor exposure of bone graft in one patient following vertical augmentation in the posterior maxilla, spontaneous healing occurred following local debridement without any further complications.

In the present study, pre-augmentation soft tissue expansion decreased the incidence of post-

operative graft exposure to 4% in comparison to previous studies of vertical bone augmentation without prior soft-tissue expansion of 23% (**Verhoeven et al, 1997),** 27.3 % **(Chiapasco et al, 2004),** 25% **(Proussaefs & Lozada, 2005),** 33.3% & 50 % **(Roccuzzo et al, 2007).** After 4-6 months of bone graft healing and just before implant placement, cone beam computed tomography (CBCT) analysis were performed and a revealed high vertical bone gain of 7.5 ± 2.4 mm, in comparison to findings from a recent systematic review, in which mean vertical bone gain was reported to be 4.8 mm with various augmentation methods **(Jensen & Terheyden, 2009).** It must be noted that, the amount of bone resorption was not measured in this case series. Bone biopsies were histologically analyzed with micro-computed tomography (micro-CT), revealing appropriate bone volume density (BV/TV) with distinct trabecular structure. Implants have been placed after bone augmentation, with uneventful healing.

In an alternative case series, sub-periosteal osmotic expanders were applied by tunnel approach in eight patients with severe atrophy in the maxilla or mandible before bone augmentation **(Mertens et al, 2015)**. Quality and quantity of newly created soft tissues were evaluated together with post-operative soft-tissue related complications such as: perforation, infection, dehiscence, necrosis and pain. Expansion time varied between 20, 40 and 90 days depending on the size of the defect and dimensions of the expander. Upon insertion, patients reported a slight pressure in the area, but without any symptoms of pain. The only soft-tissue related complication was mucosal perforation accompanied with eventual pre-mature loss of the expander. Such complication occurred in two patients; one had a history of a previous trauma in the area of implantation, and the other had experienced a cleft surgery, and did not follow the postsurgical instructions of abstaining of wearing the prosthesis. Both patients showed signs of mucosal scars prior to insertion of the expander. This suggests that case selection for soft tissue expansion is essential in order to avoid complications and that presence of scars at the surgical site might be a contraindication for soft tissue expansion. This hypothesis could not be

confirmed in previous animal studies **(van Damme et al, 1994; van Damme et al, 1997),** and soft tissue expansion was independent of the presence of scarred tissue. On the contrary, skin expansion decreased the limiting effect of scar tissues on restriction of mid-facial growth **(Edington et al, 1998)**, implying the option of expanding compromised soft tissues. Although presence of scars might not be an "absolute" contraindication for expansion, it must be taken into consideration that compromised soft tissues usually have a lower expanding capacity compared to normal ones **(Fang et al, 2013).**

In contrast to the previously mentioned case series **(Kaner & Friedmann, 2011),** re-treatment with soft tissue expanders was not carried out. All other patients experienced uneventful healing free from any complications. Final expansion of the vestibular mucosa was achieved, and all the expanders reached their final size, limiting the amount of gain of keratinized mucosa. It has to be mentioned that the quality of the expanded tissues was lining and not masticatory mucosa. The authors explained that this might be related to the applied expansion technique, as all the expanders were placed in the vestibule and thus were only surrounded by alveolar mucosa.

After removal of the expanders, two recipient sites showed sign of resorption of the underlying bone. This observation didn't have any adverse consequences and the corresponding areas have been successfully augmented later on.

Vertical and/or horizontal bone augmentations were performed either with autogenous or synthetic block grafts. No periosteal releasing incisions were needed to achieve primary soft tissue closure over the bone graft, except in the two patients who experienced soft tissue perforation and pre-mature removal of the expanders. Post-operative healing of the grafts was uneventful. At the time of implant placement, all bone grafts were successfully healed and soft tissue quantity was sufficient to passively close the mucosal flaps. With the current expansion techniques, careful evaluation of the amount of keratinized and non-keratinized soft tissues is

required in order to accordingly plan soft tissue expansion prior to bone or implant surgery. . It has been reported that a lack of attached gingiva and presence of mobile soft tissues might impair the fixation of the expander **(Park et al, 2013).** On final clinical follow-up, no complications were reported and none of the placed implants were lost.

To summarize: Based on these three clinical studies, it can be concluded that soft tissue expansion prior to bone augmentation may reduce the risk of mucosal dehiscence with subsequent bone graft exposure. Additional randomized controlled clinical trials; with an adequate sample size and long-term follow up are needed to confirm these findings. Summary of these clinical studies are found in *table 2.*

In two published case reports by **Park & colleagues (2013)**, sub-periosteal hydrogel osmotic expanders were used prior to vertical bone augmentation in severely resorbed mandibular ridges. They were left in situ for either three or six weeks. At the time of bone grafting, tension-free and complete closure of the augmented bone with overlying soft tissues was achieved. After healing, the grafted bone was hard and intact clinically, and implants could be placed without any complications. Similar positive outcomes were documented with pre-augmentation soft tissue expansion, in a patient with significant bone resorption in the posterior area of the mandible **(von See et al, 2010 a).**

Another indication where soft tissue expanders have been applied is the repair of lip and/or palate clefts. *In vivo* studies demonstrated variable outcomes with the applications of soft tissue expanders. In a rabbit cleft lip model, an over amount of soft tissue was generated by expansion of the labial surface area, resulting in reduced postoperative lip pressure and improved mid-facial growth **(Edington et al, 1998).** Conversely, in a cleft lip and palate model in cats **(van Damme et al, 1997)** even if soft-tissue expansion of the palatal muco-periosteum was feasible, retardation of transversal growth was reported as iatrogenic side effects from active expansion.

Moreover, in a previous cat model by the same researchers active expansion resulted in palatal bone resorption **(van Damme et al, 1994).**

In a clinical study, **Kobus (2007)** used hydrogel soft tissue expanders as an adjunctive in two-stage repair of cleft palate in children over the period of fifteen months. The clinician intended to limit their palatal scarring and therefore, preserve maxillary growth. Out of the nineteen children enrolled in the study, seven ended up with fistulae despite the adjunctive application of soft tissue expanders. The high rate of fistulae was explained by the lack of silicon coating around the surface of osmotic expanders (first-generation) which resulted in a super-quick expansion with concomitant wound dehiscence. **Swan & colleagues (2008)** criticized the fast expansion technique in children and stated that the available soft-tissue expanders tend to expand equally in all directions (i.e. isotropic), which is not feasible in a confined area like the palate. They recommended that directionally dependent expansion must be developed to allow for swelling in transverse direction only.

A novel anisotropic self-inflating hydrogel tissue expander was recommended that could improve future clinical applications of soft tissue expansion in cleft palate defects, eyelid and nasal tip reconstruction.

This novel expander, based on methyl methacrylate and vinyl-pyrrolidone designed to display anisotropy, showed a capacity for considerable expansion and a controlled modifiable expansion rate **(Swan et al, 2011).** Anisotropy was induced through compression of hydrogel copolymer at elevated temperatures (annealing) and expansion rate was controlled by incorporation of a semi-permeable silicone membrane, *in vitro.* Efficacy of this novel expander was later tested *in vivo*, by sub-periosteal implantation in hard palates of pigs **(Swan et al, 2012)**. Uncoated and silicone-coated expanders were compared 6-weeks post-expansion. Similar to all published findings in literature, uncoated devices resulted in rapid expansion causing muco-periosteal ulceration, while coated ones displayed a more controlled expansion.

Coated expanders showed a significant increase in soft tissue volume without any evidence of acute inflammation. Formation of soft tissue capsule was present around these expanders and expander-mediated erosion of palatal bone could be observed. Despite the promising results, clinical research is needed to investigate the outcomes of anisotropic expansion in specific intra-oral applications.

1.2.3. Technical Guidelines for Insertion of Soft Tissue Expanders in Pre-augmentation Applications

The placement of soft tissue expanders in the oral mucosa is technique- sensitive and caution must be taken especially in tunnel techniques. Their use in a moist environment might influence the operation time, as soft-tissue expanders start to swell once in contact with the fluids. Thus, placement of soft tissue expanders requires high technical skills of the surgeon. Screw-fixation is mandatory to avoid migration when subjected to chewing or expanding forces. To facilitate screw-fixation, osmotic expanders are fabricated with a flat end on one side, as shown in *figure (4).* This flat area has no expanding capacity. However, care must be taken that even if screw-fixed, the expander can still migrate if placed close to the incision line or mucosal perforation may occur as negative side effects **(Manders et al, 1984; Radovan, 1984; Wieslander et al, 1991; Abrahamsson et al, 2012)** Usually, expanders are removed after successful mucosal expansion and immediately prior to bone augmentation.

Tissue expansion can be done repeatedly in the same area **(Kaner & Friedmann, 2011)** and increasing forms of expanders can be indicated in large defects **(Mertens et al, 2015)**. These findings are confirmed by studies dealing with soft tissue expansion in plastic surgery **(Roposch et al, 1999; Huo et al, 2009; Liu et al, 2011).**

1.2.4. Effect of Expansile Pressure and Location of the Expander on the Underlying Bone

During the soft tissue expansion process, the underlying bone surface serves as a counter-bearing area for the expansile stress exerted by expanders **(Stuehmer et al, 2009).**

These pressures could evoke bone reactive changes, such as bone resorption.

There are conflicting findings in the literature about the reciprocal effects between soft tissue expanders and bone; while some studies reported about bone resorption **(Hemmer et al, 1987; Antonyshyn et al, 1988 a; Fudem & Orgel, 1988; Tominaga et al, 1993; van Damme et al, 1994; El-Saadi & Nasr, 2008, Mertens et al, 2015)**, or decreased bone density **(Stuehmer et al, 2009),** others did not observe any signs of bone loss (**Uijlenbroek et al, 2011)** or even documented new bone formation **(Abrahamsson et al, 2009; Abrahamsson et al, 2010; Abrahamsson et al, 2011).**

Despite these contradictions, bone resorption and deformation have been well documented with conventional expanders, used in children and adults **(Hemmer et al, 1987; Fudem & Orgel, 1988; Paletta et al, 1989; Penoff, 1990; Schmelzeisen et al, 1999).** Expansion-mediated bone deformity might be a minor clinical finding **(Sinow et al, 1991).** Nevertheless, in some cases the bone deformations are not completely resolved after expander removal and deformities will remain **(von See, 2010 b).** The causative underlying effect might be the pressure peaks associated with conventional expanders. It is well documented that osteoclastic activity increases in areas subjected to higher pressure **(Tominaga et al, 1993),** especially when a certain threshold level has been exceeded **(Sato et al, 1998).**

Early studies suggested that decreased bone thickness and erosion are usually evident on the bone surface below the expander **(Johnson et al, 1993),** while increased bone thickness, volume and bone deposition are apparent at the periphery of the expanders most of the time, as a part of hyper-compensation mechanism **(Johnson et al, 1993).** Hyper-compensation occurs in the form of increased bone apposition and microcirculation **(Svindland et al, 1995),** following

hypo-perfusion of the underlying bone when the periosteum is elevated **(Kowalski et al, 1996)** for expander placement.

To decrease the risks for bone resorption, applied forces must be distributed over a large surface area, like it happens with second-generation expanders. Nonetheless, bone resorption can occur with osmotic expander when placed sub-periosteally, even without pressure peaks. Sub-periosteal placement of hydrogel expanders may impair microcirculation of the bone (**Rucker et al, 2005),** which in turn causes bone resorption **(Hemmer et al, 1987)** due to limited nutrition via the periosteum **(Chanavaz, 1995).**

Bone resorption with sub-periosteal expanders was confirmed in a rat model **(Stuehmer et al, 2009);** a significant decrease in bone density and thickness in the area underneath the expander was observed 21-days post-expansion. This was attributed to the position of the expander directly on bone.

Similarly, **Mertens & colleagues (2015)** observed bone resorption with sub-periosteal expanders in two patients but without any negative effects on the final outcomes. Interestingly, although the authors attributed these findings to pressure on bone, one of the patients had been fitted with two expanders but showed bone resorption under one expander only. The authors didn't interpret this finding.

In contrast, **Abrahamsson & co-workers** suggested that placing the expanders in a sub-periosteal location induced slow expansion of the periosteum which resulted in new bone formation at the periphery of the expanders **(Abrahamsson et al, 2009; Abrahamsson et al, 2010; Abrahamsson et al, 2011)** without any signs of bone resorption underneath these devices. They assumed that the slow expansion of the periosteum activated osteogenic cells, which enhanced bone formation. In fact, different studies confirmed that lifting the periosteum slowly could result in new bone formation, as it has been described for periosteal distraction **(Schmidt et al, 2002; Kessler et al, 2007; Sencimen et al, 2007).** In an attempt to prevent

direct contact between expanders and bone, **Kaner & Friedmann (2011)** implanted the expanders in submucosal pouches, which resulted in positive outcomes, without any signs of bone resorption.

Further findings from the literature suggest that bone resorption is in fact related to the "amount" of pressure forces exerted on the bone surface when the expanders are placed sub-periosteally in direct contact with bone and not on its position per se. Permanent pressure on bone, surpassing a certain threshold can result in bone necrosis **(Carlsson, 2004).** The maximum force exerted by expanders is reported to be 32.4 kPa **(Wiese, 1993),** and it has been demonstrated that persistent compressive pressure of a threshold surpassing 6.86 kPa leads to significant bone resorption due to reduced perfusion **(Sato et al, 1998)** in rats. However, critical pressure force is expected to be much higher in humans **(Mertens et al, 2015)**. This might explain why resorption with sub-periosteal osmotic expanders was reported in rats **(Stuehmer et al, 2009),** but not in humans **(Abrahamsson et al, 2012).** In a rabbit model, there were no signs of bone resorption due to exerted pressure on bone **(Abrahamsson et al, 2009),** which proposes that critical pressure also differs between different animals.

Distribution of pressure over a large area could minimize the probability of surpassing a certain threshold and thus reduces the risks for bone resorption. **von See & co-workers (2010 b)** used a calvarial rat model to investigate whether simultaneous insertion of mechanical devices along with the expanders could result in a better distribution of the load on bone and over a larger surface area. Four groups were compared: expanders alone, expander with underneath titanium plate, expander with underneath titanium mesh and control group. Twenty-one days post-expansion, micro-CT images and histological analysis revealed significant decrease in hydroxyapatite density and marked lacunae beneath the osmotic hydrogel expanders when they were applied without underlying titanium mesh or plate, while such decrease in bone density was reduced when titanium mesh was placed, and totally prevented with titanium plate.

Moreover, bone thickness was decreased with expanders solely or expanders implanted on titanium mesh, but not with expanders placed on titanium plates. A compensatory increase in bone thickness at the peripheries could be observed in all the test groups but not in the control group. Although, titanium mesh and titanium plate acted as pressure distributors, titanium plate disseminated forces more effectively because expanders directly placed on bone or titanium mesh, induced connective tissue lacunae in the bone underneath the expander. As a consequence, morphologic changes can only be prevented with the utilization of titanium plates, as bone resorption cannot be avoided with titanium mesh underneath the expander.

In conclusion, bone resorption has been very well documented with conventional expanders, but inconsistent with osmotic expanders. With sub-periosteal expanders, bone resorption has been reported *in vivo* in rats, due to low-pressure threshold in small animals, while there were conflicting findings in studies on humans. Further clinical investigations are mandatory to determine the pressure threshold of expanders in humans that if surpassed, might cause bone resorption. Evaluation of the bone surface reaction to applied soft tissue expansion is also needed to confirm the preliminary findings from the present clinical studies.

1.2.5. Effect of Connective Tissue Capsule Surrounding the Expanders on Bone Augmentation

Connective tissue capsule formation is a common finding around the expanders when they have been retrieved **(Pasyk et al, 1984).** Data from early literature suggests that dense fibrous capsule can develop around the tissue expanders and completely surround them within few days after insertion **(Austad et al, 1982; Pasyk et al, 1982; Argenta et al, 1985; Pasyk et al, 1988).** On the other hand, the capsule thickness rapidly thins out after expander removal **(Johnson et al, 1993).** These findings could not be confirmed by recent studies, which showed that soft tissue capsule does not form unless the expander is left in location for more than two weeks **(Kaner & Friedmann, 2011; abrahamsson et al, 2012; Mertens et al, 2015).**

In the previously mentioned study, **Kaner & Friedmann (2011)** placed soft tissue expanders in a submucosal pouch without elevation of the periosteum, to avoid replacement of periosteum with fibrous connective tissue. Soft tissue capsule may negatively affect the healing of bone following augmentation. Periosteum is a fundamental source for osteoblasts and their precursor cells **(Allen et al, 2004).**

Encapsulation of sub-periosteal expanders can be avoided if they are left in situ for just a short period of time; **Abrahamsson et al (2012)** removed sub-periosteal expanders 14 days post-insertion without any signs of fibrous tissue encapsulation. This had positive effects on bone augmentation, documented in their rabbit models; there was an evident direct contact between progenitor cells populating the periosteum and the bone graft in histological analysis **(Abrahamsson et al, 2010; Abrahamsson et al, 2011).** Although encapsulation wasn't reported, it was evident in earlier rabbit models, in which all expanders were covered by collagen-rich capsule within two weeks after insertion **(Abrahamsson et al, 2009).**

Encapsulation was evident when sub-periosteal expanders were retrieved after 20, 40 or 90 days **(Mertens et al, 2015)**. Fibrous tissue encapsulation seems to be inevitable if the expanders are left in place for a long time, regardless their location; it was obvious with submucosal soft tissue expanders that were left in location for 6o days **(Kaner & Friedmann, 2011).**

Based on these findings, it was suggested that caution must be taken not to leave sub-periosteal expanders for a long time; otherwise fibrous connective tissue replacement of the periosteum should be expected **(Abrahamsson et al, 2012)**.

Mertens & colleagues (2015) did not report any negative outcomes following bone augmentation and implant placement, despite the development of a soft tissue capsule around the sub-periosteal expanders. A recent *in vivo* study revealed that, although sub-periosteal implantation of expanders resulted in complete ischemia of the periosteum and was replaced by

fibrous connective tissue within 14 days, these tissues had significantly higher density of micro-vessels than a healthy periosteum which didn't have any negative effects on vascularization to the bone **(von See et al, 2010 c).** Such findings might justify bone augmentation immediately following the completion of soft tissue expansion, even if there is complete replacement of the periosteum with a fibrous tissue capsule. Earlier studies included recommendations for a delayed bone grafting procedure after soft tissue expansion **(LaTrenta et al, 1988)**.

More clinical trials are needed to evaluate the effect of connective tissue capsule formation on subsequent bone augmentation.

1.2.6. Effect of Soft Tissue Expanders on Microcirculation & Soft Tissue Vascularization

Integrity of vascularization is important to ensure successful outcomes of the surgical procedures. Different studies have been conducted to evaluate the effect of soft tissue expansion on vascularization of the soft tissues.

In an *in vivo* study on beagle dogs, **Kaner et al (2014)** evaluated the effect of submucosal soft tissue expansion on mucosal microcirculation. Following surgical interventions, there is a hyperemic response of the periosteal and supra-periosteal blood vessels during the first three days post-operatively **(Caffesse et al, 1981; Nobuto et al, 2005).** Although microcirculation was reduced after local anesthesia, there was a reduction in post-operative hyperemic response during the first three days post-surgery. This fact may be attributed to minimal surgical trauma, as preparation of a submucosal pouch only requires a minimally invasive surgical approach without any need for elevation of the periosteum. The study conductors concluded that microcirculation is minimally and momentarily disturbed by insertion of expanders, which explains the positive outcomes with submucosal expanders, in previous investigations **(Kaner & Friedmann, 2011).**

In another animal model on beagle dogs, the authors evaluated microcirculation in vertical bone augmentation following soft tissue expansion **(Kaner et al, 2015)**. Augmentation surgery impaired microcirculation in control group, but didn't cause further decrease in sites treated with expanders, beyond that of local anesthesia. Two weeks post-augmentation, microcirculation was significantly lower for the control group, compared to test group, and although no signs of wound dehiscence were reported in the test group (with expanders), eight wound dehiscence were evident in the control group (without expanders). Based on that study it seemed that soft tissue expansion might lower the impairment of microcirculation caused by vertical ridge augmentation, and reduce the incidence of soft tissue dehiscence.

Even if expander losses were low in a case series of patients, 30% of the expanders were lost in this animal study. The authors attributed this finding to possible continuous uncontrolled mastication on the surgical sites, despite the proper surgical execution.

In another *in vivo* study on rats, **von See & colleagues (2010 d)** reported a higher density of micro-vessels in the soft tissue surrounding the augmentation material when pre-augmentation soft-tissue expansion was utilized, in comparison to grafted area without a prior soft tissue expansion**.** This was in agreement with early studies, which confirmed an increase in the vascularity of the expanded tissues. Histological findings revealed that rapid angiogenesis is evident with increased number of blood vessels at the junction between connective tissue capsule and host tissues, which contributes to an actual increase in the vascularity of the expanded soft tissues **(Johnson et al, 1993).** Subsequent bone augmentation did not have any influence on functional micro-vessel density caused by soft tissue expansion. Complete osseointegration of the bone graft was possible when the mucosal perfusion around the augmentation area was not compromised **(von See et al, 2010 d).** High vessel density of soft tissues seems to play a role in blood supply to the underlying bone **(Chanavaz, 1995).**

Absence of periosteal perfusion was observed in bone augmentation without previous soft tissue expansion caused by surgical dissection of the periosteum **(Kowalski et al, 1996).** Additionally, the periosteum will be subjected to tensile forces during the surgical procedure that further impairs the patency of the vessels **(von See et al, 2010 d).**

It can be concluded, that regardless of the location of the expanders, tissue expansion tends to increase vascularization of the soft tissues, and also reduces adverse effects on micro-circulation following bone augmentation.

Summary of relevant *in vivo* studies are presented in *table 3.*

1.2.7. Long-term Outcomes of Applications of Osmotic Hydrogel Soft Tissue Expanders

Since the application of soft tissue expanders for intraoral mucosal expansion is relatively new, no long-term results are available. Clinical guidelines may be extrapolated from studies that describe the use of osmotic expanders in plastic surgery.

Chummun and co-workers (2010) published their five-years experience with soft-tissue expanders through retrospective data collection. Ten patients have been treated with soft-tissue expanders either for alopecia, scars or burn contracture. Six patients had an uneventful post-operative healing and the required amount of soft tissues was obtained without any complications. The other four patients developed different degrees of wound infection. Based on the high complication rate, the authors suggested that identifying a suitable anatomical location and proper case-selection seem to be mandatory in order to avoid any complications. Similarly, **Obdeijn et al (2009)** reported a high complication rate in a three-years clinical experience. From nine patients treated, complications of infections, ischemia of the skin and expander migration were reported in five patients. In two cases, complications were attributed to previous irradiation in the area where the expander was inserted. As a matter of fact, previous irradiation and infected areas are contraindications for implantation of tissue expanders **(Ronert et al, 2004).** However, if using soft-tissue expanders are indicated,

radiation therapy should only start after the completion of the expansion phase **(Ronert et al, 2004)**.

Obdeijn & co-workers (2009) expanded scalp skin in five patients, and confirmed by previous data, they found high complication rate when expanders are applied in the head and neck areas **(Antonyshyn et al, 1988 b).** Nonetheless, data from a 15-years retrospective study revealed that a high complication rate with head and neck soft tissue expansion could be noticed but the severity of complications was of minor importance **(Belghith et al, 2012).**

The authors recommended that indications for soft tissue expansion must be considered carefully, not to change the advantages of tissue expansion into a disadvantage by increasing complications **(Obdeijn et al, 2009).**

A more positive experience with soft tissue expanders was published by **Ronert & colleagues (2004)** in which they used expanders in 58 patients for different extra-oral indications, mainly in breast reconstructions, over the period of four years. They reported a success rate of 81.5% in expanders without a silicone envelope, while it was up to 91% with silicone-coated expanders. The authors considered the final outcome as successful when there was adequate soft tissue gain with good final aesthetic appearance.

Regarding soft tissue expansion in pediatric patients high complication rates, most commonly infection, have been reported **(Pisarski et al, 1988; De Agustin et al, 1993; Iconomou et al, 1993; Gibstein et al, 1997; Neale et al, 1998; Hurvitz et al, 2005).** Complication rates in children undergoing soft tissue expansion have been reported to be high, ranging between 20% and 40% **(Friedman et al, 1996).**

According to published data, the source of infections could be distant from the location of the expander **(Mason et al, 1999),** such as endogenous sources like pharyngitis or otitis media, that suggest the relocation of the etiological bacteria from the infection site to the expander through dissemination by hematogenous or lymphatic pathways. Despite using conventional soft tissue

expanders, which already increase the risk of infections, **Adler & co-workers (2009)** concluded that infection did not hamper further expansion or successful reconstruction, in concordance with other reports **(Radovan, 1984; Antonyshyn et al, 1988 a).**

Although the role of antibiotics to prevent bacterial seeding on the expander from remote infected sites still needs to be evaluated, it might be preferable to prescribe antibiotics in individuals with high risk of developing infections. This might be valid as well for the application of intra-oral soft tissue expanders. Additionally, we suggest that expander placement adjacent to teeth must be prepared by a careful periodontal screening and treatment in order to avoid wound infections.

To avoid expander infection, **Wacke & co-workers (2011)** studied hydrogel osmotic expanders as a drug delivery system for antibiotics, *in vitro.* Expanders were incubated with either tobramycin or ofloxacin, in a setting that simulates the orbit of a newborn. Results showed that antibiotic release from the expander to the surrounding environment was sufficient and in effective concentrations that can be useful in preventing post-implantation infections in future clinical applications, and also eliminating the adverse effects associated with administration of systemic antibiotics. Such expanders should also be tested for intra-oral applications, as they might decrease infection complication rates.

For intra-oral applications, soft tissue expansion should be avoided in irradiated and actively infected sites.

1.2.8. Recommendation & Future Directions

Applications of soft tissue expanders prior to bone augmentation and placement of endosseous implants are still in a preliminary phase. Despite the initial promising results from the presented clinical studies, further clinical investigations are mandatory to work out clinical guidelines and protocols to define indications and contraindications for pre-augmentation applications. The

effect of soft tissue expansion on the bone needs to be determined, as well as the effect of the location of the expander and the amount of applied pressure.

The relationship between soft tissue expansion and tissue biotype (thick vs. thin) has not been addressed yet in literature. Thus, the effect of tissue biotype on the final outcomes of pre-augmentation soft tissue expansion needs to be investigated. Moreover, the relationship between the rate of expansion and tissue biotype and the determination of a suitable expander insertion technique for each biotype should be evaluated as well.

1.3. CONCLUSIONS

An ideal expander requires the following characteristics, as described by **Mazzoli & colleagues (2004):** 1) it should be easy to manipulate and place especially in sites with small access, 2) it should expand gradually and controllably over a short period of time, yet tolerable on long term, without inducing pressure spikes resulting in complications, such as: infections and extrusion of expanders. These requirements are met with the osmotic expanders, mainly second generation. Based on the results presented in this review, there is promising, albeit preliminary information regarding the benefits of pre-augmentation soft tissue expansion and a use of soft tissue expanders in everyday clinical practice cannot be recommended at the time being. The previous findings cannot be generalized due to relatively small sample size. Further clinical trials with a larger sample sizes and long-term follow-up are needed before implementing soft tissue expanders into everyday clinical practice.

1.4. ACKNOWLEDGEMENTS

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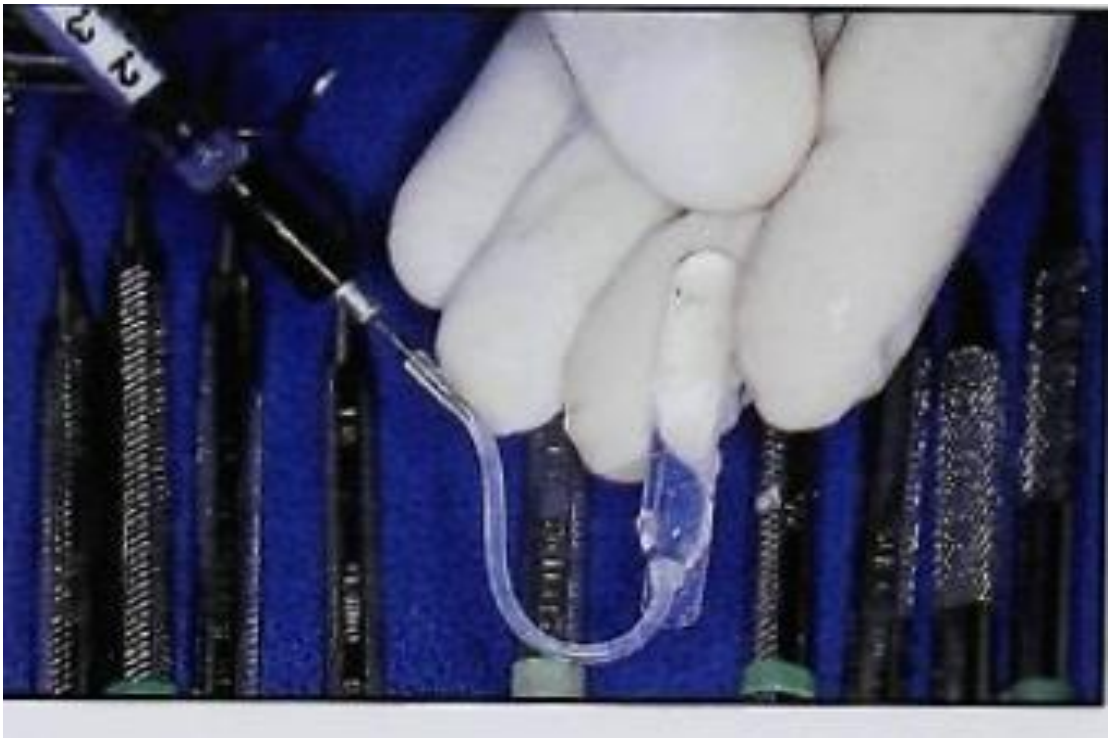
Figure (1): Conventional expander with an external port for serial injections and manual inflation



"Courtesy of Zeiter et al, 1998"

Figure (2A): Small incision is created for the insertion of osmotic soft tissue expander

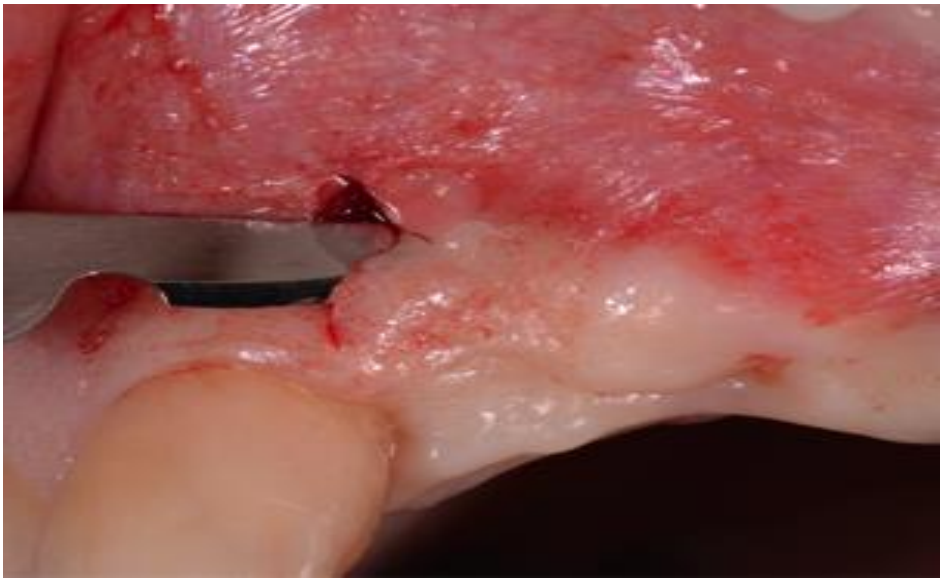
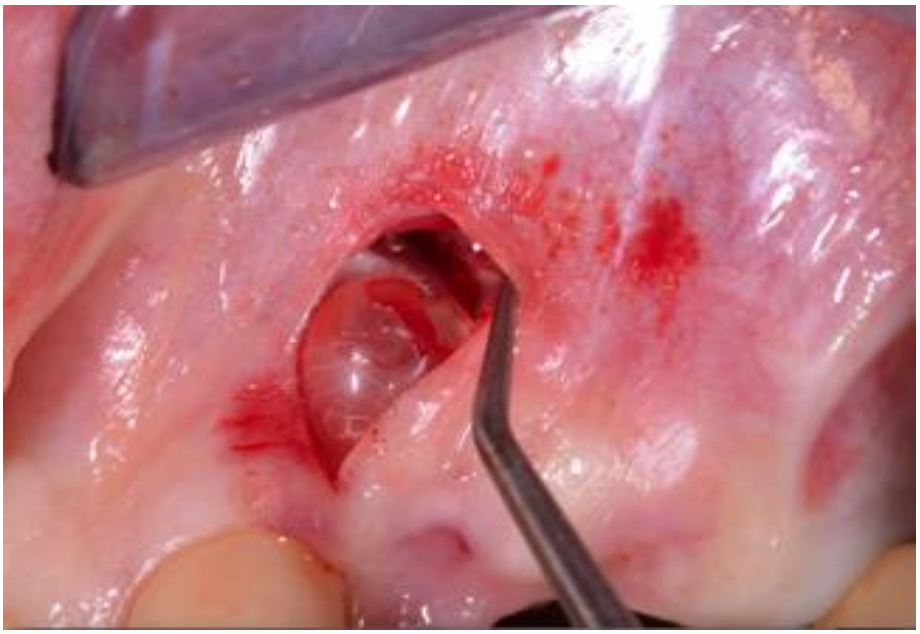
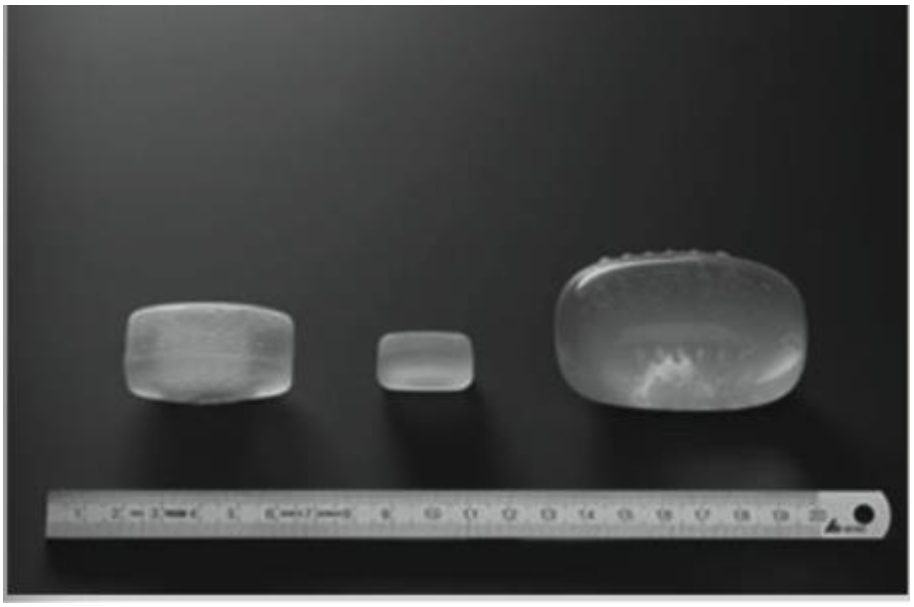


Figure (2B): Osmotic expander inserted through small incision



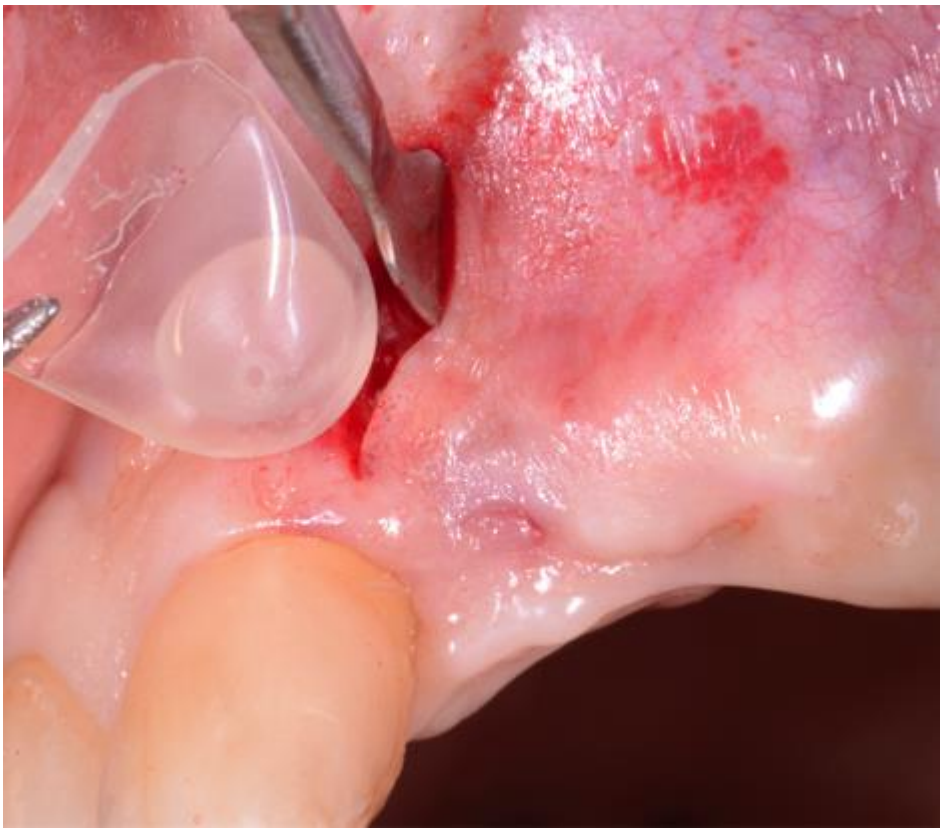
"Courtesy of Rasperini, G. University of Milan, Department of Biomedical, Surgical and Dental Sciences, Foundation IRCCS Ca' Granda Polyclinic, Milan, Italy"

Figure (3): Rectangle osmotic hydrogel expander: from left to right: un-swollen, without silicon shell, swollen



"*Courtesy of Osmed® GmbH (Illmenau, Germany)"*

Figure (4): Osmotic expander with a flat-end to facilitate screw-fixation and prevent migration of the expander. Flat ends have no expansion capability



"Courtesy of Rasperini, G. University of Milan, Department of Biomedical, Surgical and Dental Sciences, Foundation IRCCS Ca' Granda Polyclinic, Milan, Italy"

Table 1. **Comparison between conventional and osmotic soft tissue expanders**

Characteristic	Osmotic expanders	Conventional expanders
First development of "concept"	Austad & Rose (1982)	Neumann (1957)
Presence of an external portal	No	Yes
Size	Small size, needing small incisions for insertion	Bigger than osmotic expanders
Material	Hydrogel cross-linked co-polymers Lack of silicone coating in first generation Presence of silicone coating in second generation	Elastic silicone rubber
Mechanism of expansion	Spontaneously by osmotic forces from surrounding fluids	Manual inflation by serial injections through external portals, by either the clinician, patients, parents or guardians
Use in paediatric patients	Encouraged	Discouraged, due to injection-related pain
Time for expansion	Weeks or months	Weeks or months
Mode of expansion	Continuous Rapid with first generation Gradual and slow in second generation	Intermittent
Development of pressure peaks	Yes (first generation) No (second generation)	Yes
Complications	Minimal rate of complications (mainly with second generation) Chances for relapse with first generation	Infections, pain, ischaemia and perforation
Amount of soft tissue gain	Excellent amount due to constant & gradual pressure (second generation) Lack of actual tissue gain with first generation occasionally	Depends on the expanded host soft tissue and the shape of the expander
Cost	Lower cost	Higher cost because of the need of serial injections
Area of current applications	Craniofacial, intra-oral, ophthalmologic applications and plastic surgery	Limited uses in plastic surgery

"Courtesy of Asa'ad et al, 2016"

Table 2. **Summary of clinical studies on pre-augmentation soft tissue expansion**

Author & year	Type of study & no. of subjects	Treatment groups	Type of expander	Shape of expander & final volume or dimensions	Location of expander	Time needed for full expansion	Soft tissue complications during expansion	Soft tissue quality post-expansion
Kaner & Friedmann (2011)	Case Series (12)	Soft tissue expansion followed by bone augmentation in all patients	Second-generation hydrogel osmotic expanders	Hemisphere with 0.35 ml final volume or Round-ended cylinders with 0.24, 0.7, 1.3 or 2.1 ml final volume	Submucosal (in maxilla or mandible)	60 days	Soft tissue perforation in two patients, due to infection or oversized expander	Excellent quality with enough created space
Abrahamsson et al. (2012)	RCT (20)	Test Group (10): expansion followed by bone augmentation Control Group (10): bone Augmentation only	Second-generation hydrogel osmotic expanders	Shape: N/A Final Dimensions: 5.6 × 11 × 6 mm	Sub-periosteal (in maxilla or mandible)	14 days	Soft tissue perforation and projection of expander through incision line in two patients	Surplus amount of soft tissue
Mertens et al. (2015)	Case Series (8)	Soft tissue expansion followed by bone augmentation in all patients	Second-generation hydrogel osmotic expanders	Hemisphere or cylinder with 0.24, 0.35, 0.7, 1.3 and 2.1 ml final volume	Sub-periosteal (in maxilla or mandible)	20 or 40 or 90 days (depending on the size of the defect)	Soft tissue perforation in two patients with previous history of trauma or cleft surgery	Excellent soft tissue quality and quantity but no increase in keratinized gingiva

RCT, randomised clinical trial; NA, not announced.

"Courtesy of Asa'ad et al, 2016"

Signs of bone resorption post-expansion	Bone-grafting technique	Need for periosteal releasing incisions/flap advancement	Adverse outcomes post-augmentation	Analysis & investigation	Further findings	Other comments
No signs of bone resorption	Vertical bone augmentation with either autogenous block graft or GBR	Tension-free closure was achieved in all patients	Very low incidence of graft exposure (4%).	(i) Clinical (ii) Radiological (CBCT) (iii) Histological (iv) Micro-CT analysis of bone core biopsy	(i) Mean vertical bone gain highly increased (7.5 ± 2.4 mm) (ii) Encapsulation of all expanders on retrieval without any signs of inflammatory infiltrate on histological analysis (iii) Good bone volume density on micro-CT (0.1614 ± 0.0582)	(i) The authors did not measure amount of bone resorption post-augmentation. (ii) Patients who had perforations were retreated again with expanders after time for healing was allowed.
NA	Test Group: Horizontal and vertical bone augmentation with GBR Control Group: Horizontal and Vertical augmentation with autogenous block graft	Only in the control group	Test Group: statistically significant bone loss in vertical dimension only (27%) ($P = 0.04$) Control Group: statistically significant bone loss vertically (42%) and horizontally (23.5%) ($P = 0.01$, $P = 0.024$, respectively)	(i) Clinical (ii) Objective 3D metering device	(i) Test group showed less vertical and horizontal resorption of the bone graft in comparison with the control group, and this was statistically significant with the exclusion of smokers. (vertical, $P = 0.12$, horizontal, $P = 0.049$). (ii) Soft tissue profile was less prominent after healing of bone grafts, but this was statistically insignificant.	(i) Authors excluded smokers from the final analysis. (ii) Authors attributed better results in control group either to soft tissue expansion or to different grafting techniques between groups. (iii) Authors did not measure the final gained soft tissue volume.
Two recipient sites showed signs of bone resorption, due to pressure from expansion, without further consequences	Vertical and/or horizontal augmentation with either autogenous or synthetic block grafts	Only in the two patients who pre-maturely lost the expanders	None. All grafts were successful at the time of dental implant placement	Clinical	(i) Encapsulation of all expanders on retrieval (ii) Expansion time depended on the defect size and expander dimensions	(i) Patients reported slight pressure when the expanders were placed, without pain. (ii) Patients who had perforation were not retreated with expanders. They were only treated with bone augmentation. (iii) Expanders improved quantity and quality of soft tissues but did not alter the type of the original soft tissue subjected to expansion

"Courtesy of Asa'ad et al, 2016"

Table 3. **Summary of *in vivo* studies on pre-augmentation soft tissue expansion**

Author & year	Animal model & no. of subjects	Treatment groups	Type of expander	Shape of expander & final volume or dimensions	Location of expander	Time needed for full expansion	Analysis & investigations	Important findings
Abrahamsson et al. (2009)	Rabbits (8)	In each rabbit, two sites were involved: Test site: soft tissue expander Control site: the flat end of the expander, which has no capability for expansion.	Second-generation hydrogel osmotic self-inflatable expanders	Shape: NA Final Dimensions: 5.6 × 11 × 6 mm	Sub-periosteal (mandible, extra-oral approach)	14 days	(i) Clinical (ii) Histological	(i) No signs of soft tissue dehiscence or infections (probably due to extra-oral approach). (ii) No inflammatory infiltration. (iii) Connective tissue encapsulation. (iv) New bone formation at the edge of expanders. (v) Surplus amount of soft tissue was created. (vi) No signs of bone resorption as due to pressure.
Stuehmer et al. (2009)	Rats (18)	First group: soft tissue expander in direct contact with bone Second group: soft tissue expander separated from bone by polydioxanone (PDS) foil Third group: control	Second-generation hydrogel osmotic self-inflatable expanders	Hemisphere, 0.7 ml	Sub-periosteal (Calvaria)	21 days	(i) Histological (ii) Radiological (micro-CT) (iii) Immunohistochemistry	(i) No signs of inflammatory reactions. (ii) Significant decrease in hydroxyapatite density in first group. (iii) Statistically significant decrease in bone thickness beneath expanders in first and second groups ($P < 0.05$). (iv) Bone thickness at the peripheries did not change in all groups.
Abrahamsson et al. (2010)	Rabbits (13)	Soft tissue expander followed by GBR (onlay bone graft + either titanium mesh or bioresorbable mesh) in all rabbits	Second-generation hydrogel osmotic self-inflatable expanders	Shape: NA Expanders increased 6 times their original volume (initial dimensions were 2.5 × 7.5 × 3 mm).	Sub-periosteal (mandible, extra-oral approach)	14 days	(i) Clinical (ii) Histological	(i) Soft tissue dehiscence was recorded in two of the sites with bioresorbable meshes. (ii) Surplus amount of soft tissue was created. (iii) Mean bone fill was 65% under the titanium mesh and 85% under the bioresorbable mesh ($P < 0.05$). (iv) New bone formation was in direct contact with both types of mesh.
von See et al. (2010b)	Rats (28)	First group: soft tissue expander only Second group: soft tissue expander placed on titanium mesh Third group: soft tissue expander placed on titanium plate Fourth group: control group	Second-generation hydrogel osmotic self-inflatable expanders	Hemisphere, 0.7 ml	Sub-periosteal (Calvaria)	21 days	(i) Radiological (Micro-CT) (ii) Histological	(i) Hydroxyapatite density was significantly decreased beneath the expander when no pressure distributor was used, or with underneath titanium mesh. (ii) Compensatory increase in hydroxyapatite density at the periphery when no pressure distributor was used, or with titanium mesh only. (iii) No change in hydroxyapatite density with titanium plates. (iv) Marked destruction underneath all the expanders. (v) Increased bone thickness at the periphery underneath all expanders (compensatory). (vi) Bone beneath titanium plate was comparable to control histologically
von See et al. (2010d)	Rats (16)	Test group: soft tissue expansion followed by bone augmentation with autogenous bone graft Control group: bone augmentation with autogenous bone graft only	Second-generation hydrogel osmotic self-inflatable expanders	Hemisphere, 0.7 ml	Sub-periosteal (clavaria)	21 days	(i) Histological (ii) Intravital microscopy (microcirculation monitoring)	(i) In control group: bone graft did not become completely integrated with the underlying bone. (ii) In test group: complete osseointegration of bone graft, vascular connection between bone graft and underlying bone, numerous osteoblasts and active osteocytes. (iii) Functional micro-vessel density soft tissue overlying bone graft was significantly higher in test group ($P < 0.05$).

"Courtesy of Asa'ad et al, 2016"

Table 3. *(continued)*

Author & year	Animal model & no. of subjects	Treatment groups	Type of expander	Shape of expander & final volume or dimensions	Location of expander	Time needed for full expansion	Analysis & investigations	Important findings
Abrahamsson et al. (2011)	Rabbits (11)	Soft tissue expansion followed by GBR (autogenous particulate bone or Bio-Oss covered by titanium mesh overlied by collagen membrane)	Second-generation hydrogel osmotic self-inflatable expanders	Shape: NA Final dimensions: 5.6 × 11 × 6 mm	Sub-periosteal (mandible, extra-oral approach)	14 days	(i) Clinical (ii) Histological (iii) Scanning electron microscopy (SEM)	(i) No signs of dehiscence infections, or perforation. (ii) No signs of inflammatory response. (iii) New bone formation under mesh. (iv) Mean bone fill was 58.1 ± 18% with autogenous bone and 56.9 ± with Bio-Oss. (v) Surplus amount of soft tissue was created. (vi) Generated bone was denser with Bio-Oss.
Kaner et al. (2014)	Dogs (10)	Soft tissue expansion only	Second-generation hydrogel osmotic self-inflatable expanders	Cylinder, 0.7 ml	Submucosal (mandible)	42 days	Laser Doppler flowmetry (LDF)	(i) Local anaesthesia caused significant decrease of blood flow at baseline ($P < 0.05$). (ii) No additional significant decrease of blood flow upon completion of surgery. (iii) Blood flow showed significant increase 1 and 3 days post-surgery when compared to measurement post-anaesthesia ($P < 0.05$). (iv) Submucosal implantation of soft tissue expanders resulted in momentary disturbance of micro-circulation.
Kaner et al. (2015)	Dogs (10)	Test Sites: soft tissue expansion followed by vertical augmentation with GBR (onlay autogenous graft + granular biphasic calcium phosphate + resorbable membrane) Control sites: bone augmentation as in test sites but without prior expansion	Second-generation hydrogel osmotic self-inflatable expanders	Cylinder, 0.7 ml	Submucosal (mandible)	35 days	(i) Laser Doppler flowmetry (LDF) (ii) Receiver operating characteristic (ROC) curves	(i) Primary closure was achieved in test groups while there was a need for releasing incisions in control group. (ii) Surplus amount of soft tissue was created in test group. (iii) In control group, 8 dehiscences were found. (iv) Post-augmentation, test sites showed significantly better perfusion than control sites without preceding soft tissue expansion ($P = 0.012$). (v) Three days post-surgery, perfusion was still significantly decreased in control sites ($P = 0.005$), while microcirculation in test sites had returned to pre-surgical levels. (vi) Micro-circulation levels post-augmentation significantly predicted subsequent would dehiscence ($P = 0.006$)

NA, not announced.

"Courtesy of Asa'ad et al, 2016"

CHAPTER 2

Pre-augmentation Soft Tissue Expansion (STE): Case Series & Volumetric Analysis

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2.1. ABSTRACT

OBJECTIVES: To investigate the clinical outcomes, complications and volume gain of pre-augmentation soft tissue expansion (STE).

MATERIALS & METHODS: Tissue expanders were implanted in seven patients requiring vertical and/or horizontal bone augmentation. Guided bone regeneration (GBR) was carried out either after 20, 40 or 60 days of STE. Vertical and horizontal bone gains were analyzed with cone beam computed tomography (CBCT) scans. Optic scanning and superimposition of cast models fabricated from pre- and post- augmentation alginate impressions were used for volumetric analysis.

RESULTS: Seven sites in seven patients were treated with STE. Perforation occurred in two sites; early failure due to the tightness of the prepared pouch, and late failure due to minor cracks of the silicon shell covering the expander as a result of handling of the expander body with a dental tweezer. Post-expansion, primary wound closure was easily achieved at augmentation without any subsequent graft expositions. Six months post-augmentation, CBCT analysis revealed high vertical bone gain (mean = 7.3 ± 1.2 mm). Mean of horizontal bone gain was 5.5 ± 2.9 mm.

Volumetric analysis of three successful expansion cases revealed a mean volume increase of 483.8 ± 251.7 mm^3. Soft tissue volume increase corresponded only to the 0.24 ml cylinder expander (volume increase = 259.4 mm^3), while this increase was almost half of the final expander volume for the 0.7 and 1.3 ml cylinder expanders (436.1 mm^3 & 755.9 mm^3 respectively).

Volumetric analysis of the late expansion failure reflected soft tissue shrinkage, which might suggest that STE affects tissues by tension and does not cause real volume gain. All dental implants were osseointegrated in the patients that underwent subsequent dental implant therapy.

CONCLUSIONS: High vertical bone gain and minimal post-surgical complications were associated with bone augmentation procedures preceded by soft tissue expansion (STE).

KEYWORDS: guided bone regeneration, soft tissue expanders, soft tissue expansion, soft tissue management

2.2. INTRODUCTION

Placement of endosseous implants has revolutionized modern dentistry, with a constantly increasing number of patients seeking replacement of lost teeth with this modality of treatment.

Since the overall success of dental implant therapy depends on the presence of adequate bone volume at implant sites **(Javed et al, 2013),** sufficient vertical and horizontal amounts of alveolar ridge prior to dental implant placement are essential especially in the anterior maxilla, which is a highly demanding aesthetic region.

Bone augmentation can be carried out using different techniques: bone blocks and/or guided bone regeneration (GBR) are applied for horizontal bone augmentation **(McAllister & Haghighat, 2007).** Vertical bone augmentation employs more challenging and technique-sensitive methods: vertical GBR, onlay grafting, inlay grafting and distraction osteogenesis **(Rocchietta et al, 2008; Esposito et al, 2009)** and is frequently associated with high complication rates such as soft tissue dehiscence and subsequent exposure of bone grafts into the oral cavity **(Jensen & Terheyden, 2009).**

Consequently, soft tissue expanders have been introduced in implant therapy, as pre-augmentation devices, to avoid the complications associated with bone-grafting procedures **(Kaner & Friedmann, 2011; Abrahamsson et al, 2012; Mertens et al, 2015; Asa'ad et al, 2016).** The currently used soft tissue expanders made of hydrogel, which is the same material used to fabricate contact lenses, are designed and manufactured since 1999 under the name of Osmed® (Ilmenau, Germany), which is the first commercially available self-inflatable osmotic expander and has been FDA- approved since 2001.

Up to date, there is scarce clinical evidence describing soft tissue expansion (STE) prior to bone augmentation procedures: only two case series **(Kaner & Friedmann 2011; Mertens et**

al, 2015) and one randomized controlled clinical trial **(Abrahamsson et al, 2012)** are available in literature. These studies have evaluated the outcomes of bone regeneration, but neither has provided clear technical guidelines on the intra-oral clinical utilization of these devices nor volumetric analysis of soft tissues. Only post-expansion changes in the profile of the attached gingiva was evaluated in one randomized controlled clinical trial **(Abrahamsson et al, 2012).** The authors did not measure the total volume change of soft tissues, as they only aimed to determine the overall stability of the expanded soft tissues by evaluating their profile changes over-time.

Based on these observations, we conducted a clinical study on pre-augmentation soft tissue expansion, utilizing Osmed® expanders, to gain insight on the safety and effectiveness of this approach. We also performed volumetric analysis by optic scanning, to evaluate the changes in soft tissue volume post-expansion.

2.3. MATERIALS & METHODS

This clinical study was conducted in the period between May 2016 and September 2017.

2.3.1. Study Participants & Inclusion Criteria

From the pool of patients attending the Dental Clinic of the Ospedale Maggiore Policlinico, University of Milan- Italy, seven participants requiring alveolar bone augmentation and dental implant placement were included in this clinical investigation. All patients were enrolled into the study after explaining its objectives and obtaining their verbal and written informed consent.

Study participants fit the following inclusion criteria:

1. Patients in need for bone augmentation procedures in vertical and/or horizontal dimensions, either in the maxilla or mandible, prior to dental implant placement
2. The edentulous area of interest had insufficient amount of soft tissues
3. In partially edentulous areas, neighboring teeth should have no clinical signs of caries, periapical infections or periodontal inflammation. If active periodontal disease was present, the periodontal condition had to be stabilized first
4. Patients without any reported systemic diseases (ASA-1 or ASA-2 according to the classification of the American Society of Anaesthesiologists)
5. Non-smokers or ex-smokers who have quit smoking since at least one or more years prior to enrollment in the study

The exclusion criteria were the following:

1. Self-declaration of pregnancy

2. Patients on medications that would adversely affect the outcomes of implant therapy and bone regeneration procedures (e.g. bisphosphonates, anti-resorptive drugs)

2.3.2. Implantation of Soft Tissue Expanders

Based on the extension and location of the edentulous area, intra-oral cupola expanders (final volume, 0.35 ml) or cylinder expanders (final volumes, 0.24 ml, 0.7 ml & 1.3 ml) were applied (Osmed®, Ilmenau, Germany). Expanders were left in situ for 20, 40 or 60 days, depending on the final volume of the utilized expander. The appropriate expander was selected using a specific surgical template corresponding to the initial and final volumes of the expander *(figure 1A).*

Expanders were inserted using the same surgical technique previously described in literature **(Mertens et al, 2015).** Briefly, expanders were inserted in a sub-periosteal pouch prepared under local anesthesia and controlled with the specific surgical template *(figure 1B)* to ensure the device is easily fit without tension into the prepared pouch. The expander was handled carefully by holding its flat end with a tweezer *(figure 2A).* To prevent any dislocation or potential migration, expanders were fixed with a bone fixation screw *(figure 2B),* at the flat end, which does not have an expansion capability. In all cases, the surgical site was closed utilizing a mattress suture. No antibiotics were prescribed, and sutures were removed 10 days after expander insertion. Any complications, such as expander expulsion were documented throughout the expansion period.

2.3.3. Expander Removal & Bone Augmentation

When the expansion phase was successfully completed *(Case 1: figures 3A, B),* expander removal and bone augmentation were scheduled at the same appointment. Depending on the dimension of alveolar bone resorption, vertical and/or horizontal bone augmentation was performed.

Under local anesthesia, a mid-crestal incision was performed and a full mucoperiosteal flap was released, avoiding any releasing incisions and the expander and its fixing screw were removed. Bone surface was carefully examined for any signs of potential resorption due to pressure from the expander. *(Case 1: figures 4A, B, C. Case 2: figures 5A, B, C).*

In all cases, bone augmentation was performed using particulate autogenous bone harvested with bone scraper from the surgical site, mixed with xenograft (Bio-Oss, Geistlich, Wolhusen, Switzerland). In case of vertical bone augmentation, the graft was covered with titanium reinforced PFTE membrane (Cytoplast, Osteogeneics Biomedical Inc, Lubbock, Tex., USA) *(Case 1: figure 6A, B),* while collagen membrane was used (Bio-Gide, Geistlich, Wolhusen, Switzerland), in the case of horizontal bone augmentation *(Case 2: figure 7A, B).* Tension-free primary closure was achieved in all cases without utilizing deep periosteal and/or vertical releasing incisions *(Case 1: figure 6C, Case 2: figure 7C).*

For all patients, administration of antibiotics started one hour before the augmentation surgery (amoxicillin/calvulanic acid, 2g) and continued for 7 days every 12 hours. Chlorhexidine mouthwash (0.2%) was recommended for daily use (3 times/day for 14 days). Ketoprofene (50 mg) was prescribed as an analgesic. Patients were followed-up weekly and sutures were removed two weeks after surgery. Any complications such as soft tissue dehiscence, membrane exposure and bone graft expulsion were documented throughout the bone healing period.

2.3.4. Dental Implant Placement

In all patients, implants (MegaGen Implant, Gyeongbuk, South Korea) were placed 6-9 months following bone augmentation *(Case 2: figure 8A).* All implants were submerged and sutures were removed 7-14 days later.

2.3.5. Radiographs

Panoramic radiographs *(Case 2: figure 8B)* or intra-oral radiographs were taken soon after dental implant placement was completed. However, cone beam computed tomography (CBCT) scans were taken for all patients, before placement of soft tissue expanders and 4-6 months following bone augmentation procedures *(Case 1: figures 9A, B).*

Vertical and horizontal bone gains were calculated on CBCT, as previously described **(Kaner & Friedmann, 2011).** Briefly, subtraction of bone height or width "before augmentation" from bone height or width "before placement of dental implants" was performed using measurement tool of the CBCT software at landmark sites.

2.3.6. Volumetric Analysis by Optic Scanning

Volumetric analysis was performed using previously described methods **(Schneider et al, 2011; Thalmair et al, 2013)** with some modifications.

Briefly, alginate impressions were taken for each patient, one immediately before expander insertion and one soon after expander removal and simultaneous bone augmentation, i.e. when the expansion phase was successfully completed. Two master casts for each patient were made utilizing pre-expansion and post-expansion impressions. To estimate the volumetric changes, an optic scanner and computer-aided design (CAD) software were used.

The cast models were optically scanned with a 3D camera (Cerec 3D, Sirona Dental Systems GmbH, Bensheim, Germany) and digitalized creating STL files (Standard Tessellation Language). The STL files of pre- and post-expansion models were imported into CAD software (Geomagic Studio 2013, Raindrop Geomagic, NC, USA) *(Case 2: figures 10A, B)* and were superimposed by using the buccal surface of adjacent teeth as a reference point *(Case 2: figure 10C),* using the best-fit algorithm. Afterwards, volume change in the expansion area was calculated using another CAD software (Catia V5, Rand Worldwide Inc, Maryland, USA). The expanded tissues were highlighted with the software, allowing for

volume change calculation *(Case 2: figure 11A).* The expanded area was then extracted into STL format, allowing for superimposition of this area over pre-expansion STL file for further confirmation *(Case 2: figures 11B, C).* Volume analysis was done by the same calibrated examiner (F.A).

2.4. RESULTS

Seven patients (5 females, 2 males, mean age= 50.4 ± 9.6 years, age range= 39 – 60 years) were included in this clinical investigation. Expanders were placed at seven surgical sites. One patient dropped out after soft tissue expansion has failed, therefore, only six patients completed the study.

During the expansion period, healing was uneventful in 5 patients, while the expansion procedure failed in two patients due to perforation of the expanders through the mucosa.

In one of these two sites, the expander was expelled due to crack formation of the silicon shell as a result of handling the body of the expander with a dental tweezer *(figures 12A, B, C).* It must be noted that the patient was wearing a removable partial denture during the expansion period despite being advised not to do so. Therefore, taking into consideration the patient's needs, the base of the denture was relieved to accommodate soft tissue expansion in the area. Nevertheless, it seems that wearing a denture, even if relieved, might have contributed to crack propagation in the silicon shell, eventually creating a perforation within the shell and subsequently causing expulsion of the expander at a very late stage of STE.

In the other failed site, a cupola expander was inserted in a very tight mucosal pouch due to the anatomical location of the expansion site, which was the first molar. In this case, insertion of the expander in the classical horizontal direction was not possible due to the necessity to fix the expander at the flat end close to the mental nerve, so the prepared pouch was a bit tight to avoid any nerve injury. The expander was expelled within the first week of insertion.

Neither sites were retreated with expanders; one patient dropped out of the study, and the other patient underwent bone augmentation two months after failed expansion.

In six patients, three sites underwent vertical augmentation and five sites were regenerated horizontally; two of these sites were combined with vertical augmentation. Tension-free primary wound closure was easily achieved in all cases, without the need for periosteal deep

incisions and/or vertical releasing incisions. It must be noted that deep periosteal releasing incisions were needed to advance the flap over the bone grafting material in the case of failed expansion.

Following bone augmentation procedures, wound healing was uneventful, without any reported soft tissue dehiscence, graft expulsion and/or membrane exposure.

Six months post-augmentation, CBCT analysis revealed that the mean vertical bone gain was 7.5 ± 1.3 mm, while horizontal bone gain for successfully expanded cases was 5.5 ± 2.9 mm. For the early failed expansion case, horizontal bone gain was 2mm *(table 1).* Due to the long follow-up of the study, only three patients received dental implants in the augmented areas (one patient received one dental implant and two patients received two implants each). Diameter of placed implants ranged between 3.5 and 4 mm, while length range was 7- 10 mm. All five implants were successfully osseointegrated and scheduled for prosthetic rehabilitation. In those patients, no further soft tissues management was needed, even in terms of soft tissue augmentation.

- **Volumetric Analysis Results**

Volumetric analysis was not possible for two patients with successful STE due to severe gag reflex while taking alginate impressions. Regarding the failed cases, volumetric analysis was only done for the case in which late expansion failed occurred, taking into consideration that the post-expansion alginate impression for this case was taken two weeks after failed expander removal.

Results of volumetric analysis are shown in *table 2.* For the three successful expansion cases, the soft tissue volume increase was 259.4 mm^2 for the 0.24 ml cylinder expander, 436.1 mm^2 for the 0.7 ml cylinder expander and 755.9 mm^2 for the 1.3 ml cylinder expander (mean volume increase of the three cases = 483.8 ± 251.7 mm^3). Soft tissue increase corresponded

only to the 0.24 ml cylinder expander while this increase was almost half of the final expander volume for the 0.7 and 1.3 ml cylinder expanders.

Regarding the late-stage failed expansion case, volumetric analysis at two weeks post-expander removal revealed very minimal amount of surplus tissues (54.4 mm^3).

2.5. DISCUSSION

In current literature, there are limited available clinical data that describe pre-augmentation soft tissue expansion; two case series (**Kaner & Friedmann, 2011; Mertens et al, 2015)** and one randomized controlled clinical trial (**Abrahamsson et al, 2012).**

Kaner & Friedmann (2011) were the first to describe the use of osmotic tissue expanders prior to vertical ridge augmentation, reporting a mean vertical bone gain at the time of dental implant placement of 7.5 ± 2.4 mm) in twelve patients. The present case series revealed similar findings, as mean vertical bone gain was 7.5 ± 1.3 mm. These findings might suggest that vertical bone augmentation preceded by STE might result in predictable vertical bone gain. In fact, a recent systematic review reported that mean vertical bone gain was 4.8 mm with classical bone augmentation procedures **(Jensen & Terheyden 2009),** which could highlight the importance of pre-augmentation using STE.

In the present study, mean horizontal bone gain for successfully expanded cases was 5.5 ± 2.9 mm, which is somewhat comparable to other findings in literature regarding bone gain following horizontal bone augmentation without preceding STE **(Elnayef et al, 2015).**

Surplus amount of soft tissues by STE allows for a passive primary closure of the flap minimizing post-surgical complications that would compromise bone fill, such as membrane and/or bone graft exposure. Interestingly, neither of these complications occurred in the current case series and a very low incidence of graft exposure was reported by **Kaner & Friedmann** (4%). When compared to other studies in literature, higher incidence of bone graft exposition was reported with vertical bone augmentation without preceding soft tissue expansion; 23% **(Verhoeven et al, 1997),** 27.3 % **(Chiapasco et al, 2004),** 25% **(Proussaefs & Lozada, 2005),** 33.3% & 50 % **(Roccuzzo et al, 2007).**

Despite the similar findings between the present study and the previously published case series **(Kaner & Friedmann, 2011),** it must be noted that the present study had a very small

sample size and did not exclusively investigate vertical bone augmentation. Furthermore, the method of expander insertion differed between both studies; we placed the expanders sub-periosteally as we hypothesized it might be easier and less demanding surgically, while the submucosal approach has been advocated by **Kaner & Friedmann (2011)** in an attempt to reduce the risk of bone resorption due to pressure exerted on bone surface by the expander. Nonetheless, signs of bone resorption after expander removal were evident on the bone surface at one site in the present study and at two sites in a different clinical study in which sub-periosteal implantation of expanders was also employed **(Mertens et al, 2015).**

In a randomized controlled clinical trial, no signs of bone resorption were reported with the sub-periosteal approach which could be due to the much shorter duration of the expansion phase; expansion period of two weeks was chosen by the authors without following the manufacturer's guidelines, in order to avoid the formation of connective tissue capsule around the expander, which might replace the periosteum **(Abrahamsson et al, 2012).**

Complications related to osmotic tissue expanders reported in the literature have been attributed to different causes; infections, wearing a removable denture, expanding scarred tissues and perforations either due to utilization of an excessively large expander or due to expander placement too close to the incision line **(Kaner & Friedmann, 2011; Abrahamsson et al, 2012; Mertens et al, 2015).** In the present clinical investigation, one expander failed because it was placed in a tight pouch due to anatomic considerations, and the other expander perforated the tissues at a very late stage into the expansion. Expander perforation into soft tissues at a very advanced stage of expansion has not been previously reported in literature. Therefore, we have looked carefully into the causes that might have contributed to this adverse event at a very late stage of expansion. Clinical photos taken during the surgical procedure revealed that the expander body, and not its flat end, was handled by a sharp instrument (tweezer). This might have led to the formation of a minor

crack on the shell that propagated during the expansion phase, until the hydrogel body perforated through the crack all the way into the soft tissues. Moreover, it must be noted that the patient in this case was wearing a removal partial denture despite being advised not to do so. Although the denture base was relieved to accommodate the expansion of tissues, it seems that the removable denture might have contributed to crack propagation in the silicon shell during the active expansion phase, leading to silicon shell perforation and subsequent expulsion of the expander.

Up to date, only one clinical study evaluated soft tissue changes during STE. In their randomized controlled clinical trial, **Abrahamsson et al (2012)** measured soft tissue stability of the attached gingiva at baseline and 6 months after augmentation in control and test groups and additionally at post-expansion in the test group, by using an objective 3D metering device. The mean soft tissue profile gain at the attached gingiva level was 2.9 ± 1.1 mm when compared to baseline, while it decreased to 2.3 ± 2.1 mm at the time of implant placement, when compared with the baseline. The control group showed a soft profile change of 1.5 ± 1.4 mm at the time of fixture installation. Even if the test group showed increased gingival dimensions after surgeries, the differences were not statistically significant. The authors did not measure the total volume change in soft tis- sues, as they only wanted to determine overall stability of created soft tissues by evaluating soft tissue profile changes over time. Although soft tissue profile became less prominent after healing of bone graft when compared to pre-augmentation soft tissue profile, this result was statistically insignificant.

In attempt to evaluate the total volume change, we have done volumetric analysis using previously described methods **(Schneider et al, 2011; Thalmair et al, 2013)** with some modifications. For the three successful expansion cases, soft tissue volume increase corresponded only to the 0.24 ml (240 mm^3) cylinder expander (volume increase = 259.4

mm^3), while this increase was almost half of the final expander volume for the 0.7 ml (700 mm^3) and 1.3 ml (1300 mm^3) cylinder expanders (volume increase = 436.1 mm^3 & 755.9 mm^3 respectively). These findings suggest that it is difficult to reach a complete volume increase with bigger final volume expanders, probably due to higher pressure distribution to the underlying bone surface. However, this hypothesis needs to be confirmed in future studies, also comparing the volume increase results between different expander insertion approaches, i.e. sub-periosteal versus submucosal insertion techniques.

Regarding the volumetric analysis of the case in which expansion failure happened at a late stage, and since histological analysis was not possible for this study, we thought that volumetric analysis of this particular case might give an insight if the expansion process is just merely tension on soft tissues, or might represent a real volume gain. Very minimal volume of surplus tissues was observed, two weeks after expander removal. The amount of expanded tissues was higher at the time of expander removal than two weeks after, as seen through clinical photos, even though no quantification was made of such volume reduction. Therefore, and based on volumetric analysis, tissue shrinkage has occurred two weeks after expander removal, which could reflect that the expansion process creates only tension on the tissues without any actual volume gain.

To summarize, STE might be a useful pre-augmentation approach specifically for vertical bone augmentation, as it results in high vertical bone gain with minimal post-surgical complications. The ideal clinical scenario for this specific application would be the need of vertical bone augmentation in the posterior mandible with limited amount of present soft tissues, consisting only of alveolar mucosa.

Findings of the present study must be interpreted with caution as it has some limitations; study sample was too small due to difficulty in patient recruitment from the Dental Clinic. Volume analysis does not provide information on the actual volume changes in the tissues,

as the post-expansion impressions were taken while expanders were still in situ. However, the volumetric analysis still gives an insight on the overall soft tissue volume changes; STE probably results only in tissue tension rather than real volume gain and the distribution of expansion pressure to the underlying bone surface, with sub-periosteal large expanders, might prevent full soft tissue volume increase corresponding to the final expander volume.

These preliminary findings of the need to be confirmed in future clinical studies with a large sample size, comparing different expander insertion approaches as well.

2.6. CONCLUSIONS

Despite the limitations of this study, it can be concluded that soft tissue expansion might be a beneficial pre-augmentation approach, especially in the vertical dimension. The ideal area for this specific application would be the posterior mandible with the presence of alveolar mucosa. Further clinical studies with larger sample size are needed to confirm these preliminary findings.

2.7. ACKNOWLEDGEMENTS

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mandible: a longitudinal study. *The International Journal of Oral & Maxillofacial Surgery* **26:** 351–357.

Figure (1A): Specific surgical templates used to choose the appropriate soft tissue expander. Each template has two ends that reflect the initial and final expander volumes

Courtesy of: Osmed® GmbH (Ilmenau, Germany)

Figure (1B): Sub-periosteal pouch prepared under local anesthesia and controlled with the surgical template

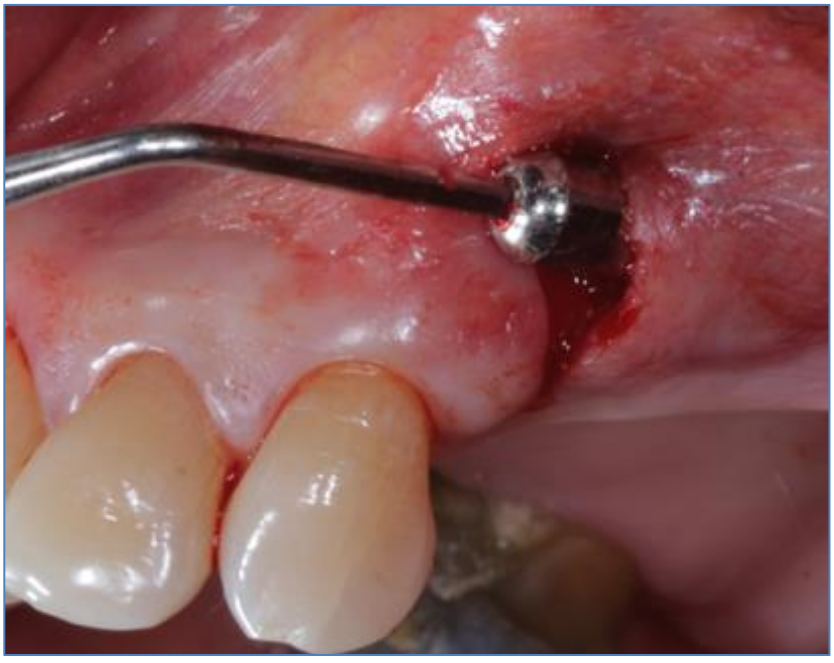


Figure (2A): During cupola expander insertion, the expander is handled carefully by holding its flat end with a tweezer

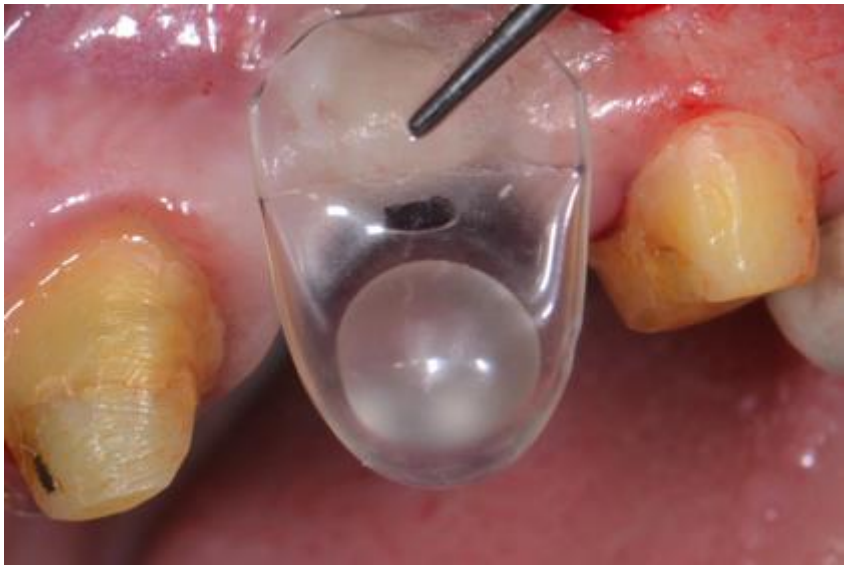


Figure (2B): Insertion of bone fixation screw at the flat end to prevent potential expander migration

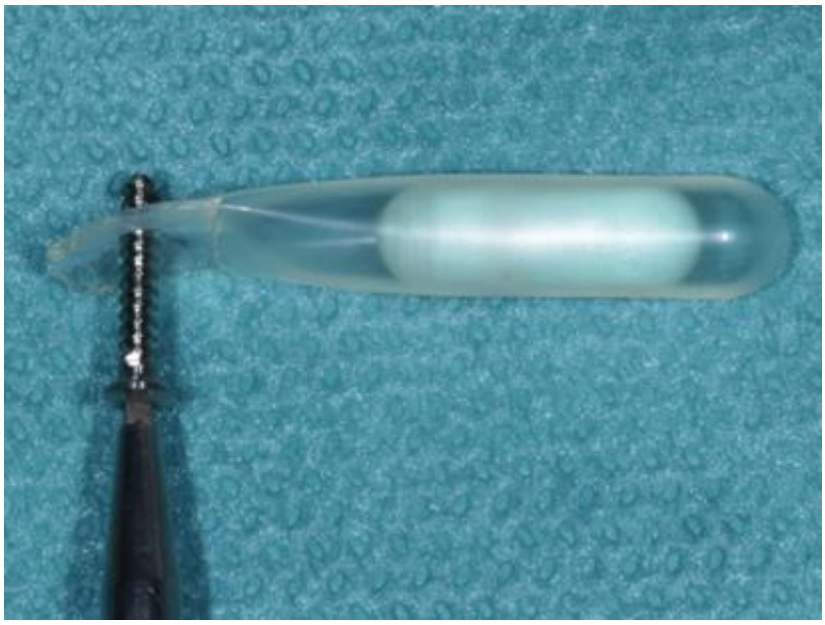


Figure (3A): Poor quantity and quality of soft tissues prior to STE

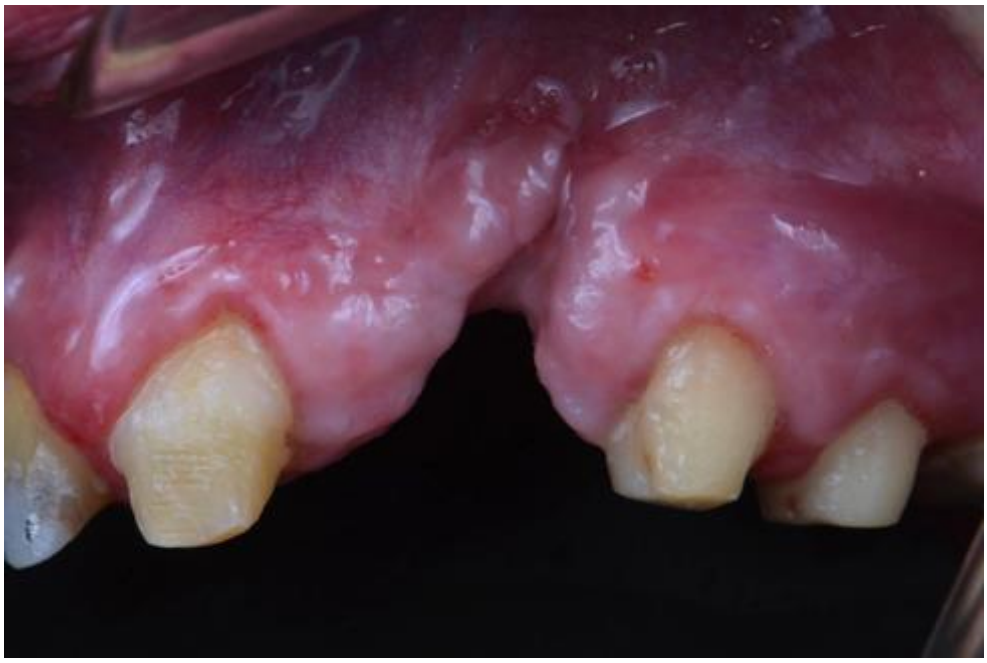


Figure (3B): Expansion phase successfully completed after 40 days of insertion

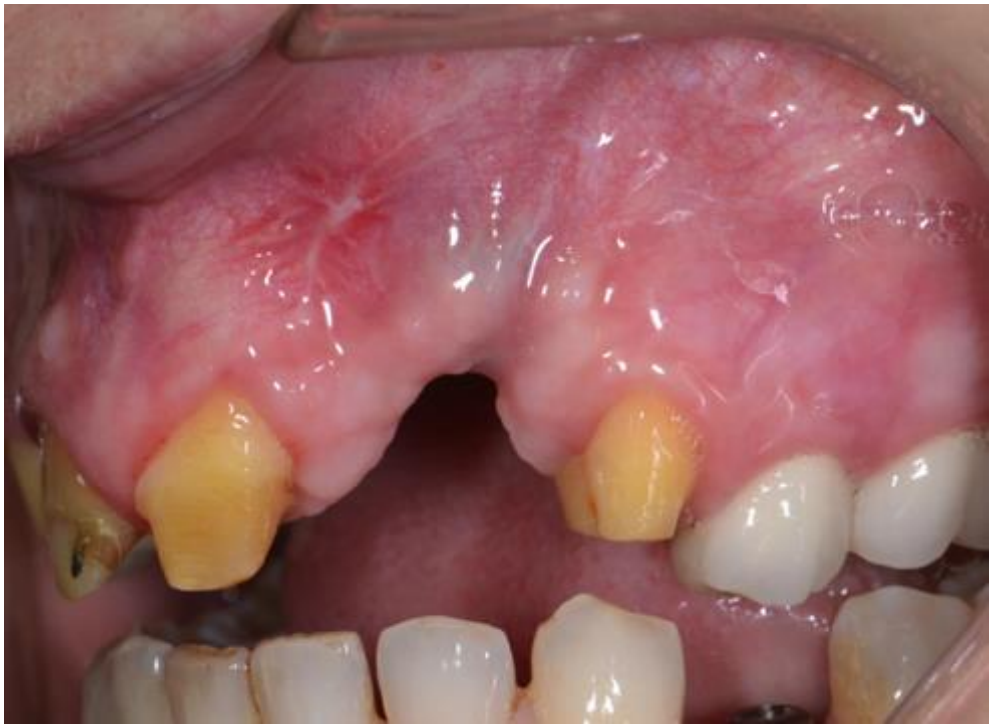


Figure (4A): Full thickness flap was elevated to expose the expander and its fixation screw for removal

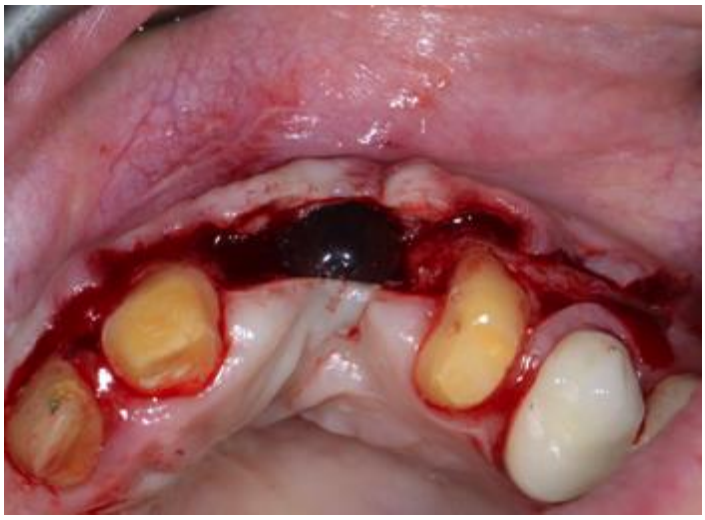


Figure (4B): Expander was removed and the surface of the bone was carefully examined, also to determine the amount of defect

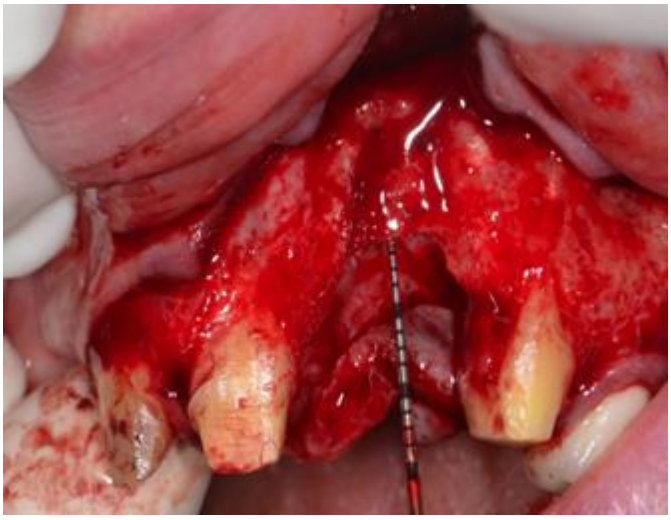


Figure (4C): Cupola expander of 0.35 ml final volume was successfully removed. Fluid inside the expander is evident

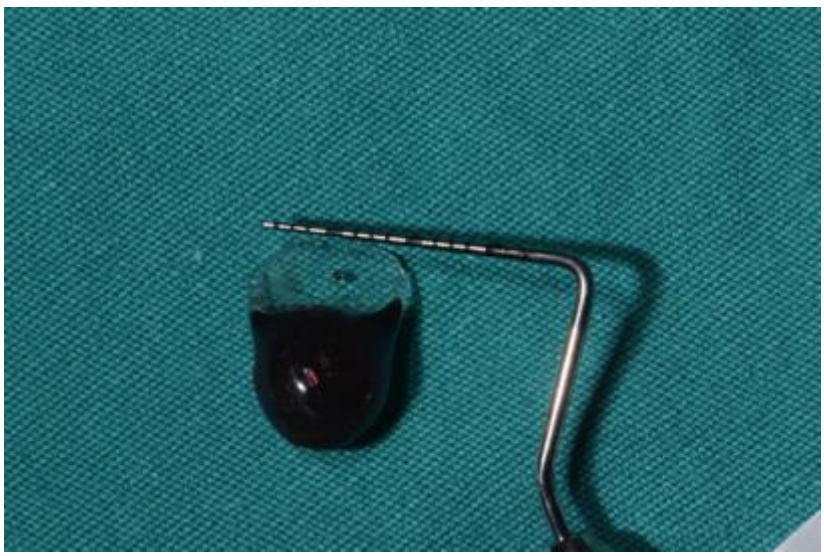


Figure (5A): Full thickness flap was elevated to expose the expander and its fixation screw for removal

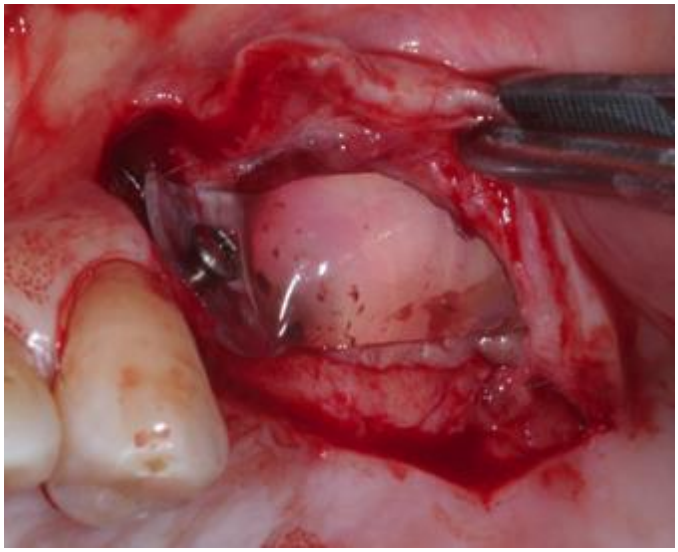


Figure (5B): Expander was removed, signs of bone resorption due to expansion pressure is evident

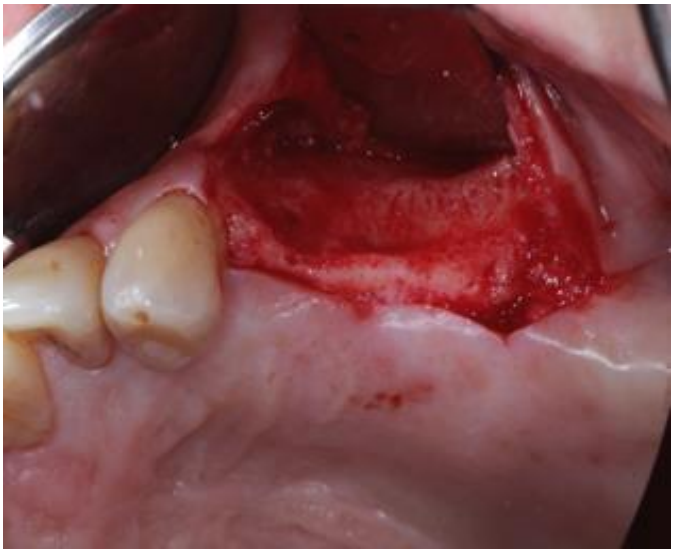


Figure (5C): Cylinder expander of 0.7ml final volume was successfully removed. Fluid inside the expander is evident

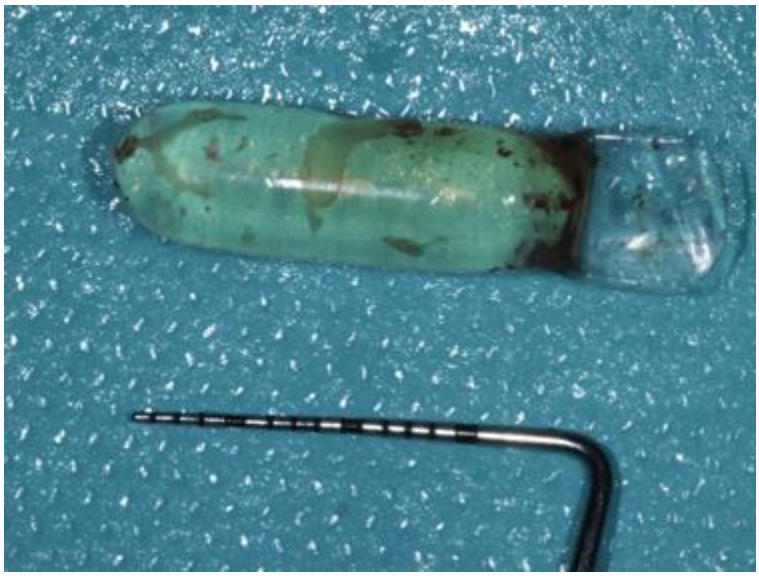


Figure (6A): Vertical & horizontal augmentation were done utilizing xenograft with autogenous bone

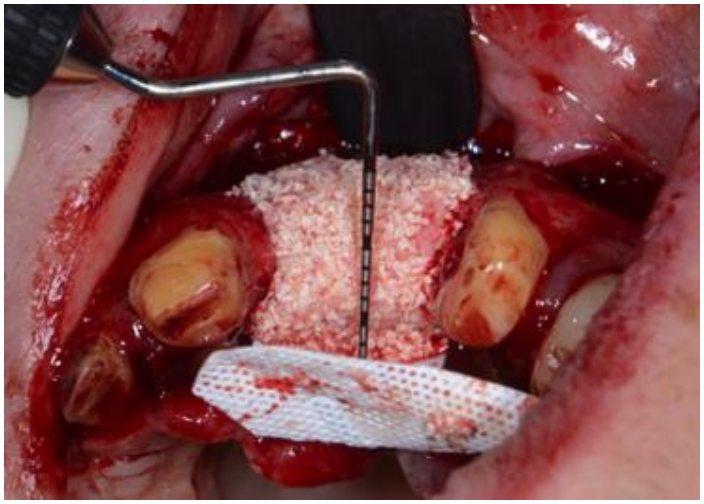


Figure (6B): Bone graft was covered with titanium reinforced PTFE non-absorbable membrane

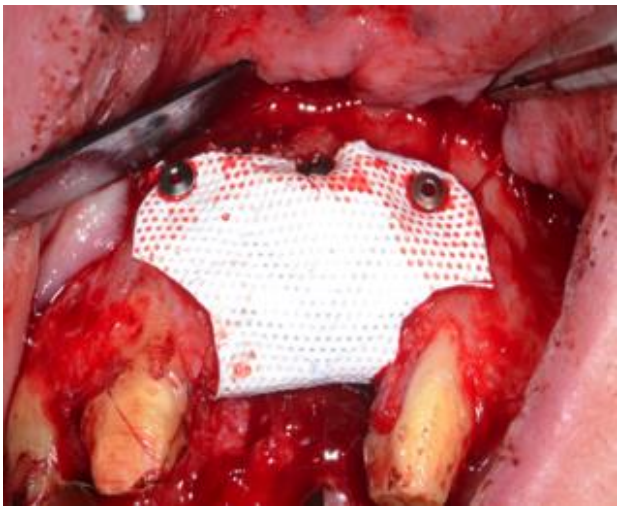


Figure (6C): Primary, passive wound closure was achieved without vertical or periosteal releasing incisions

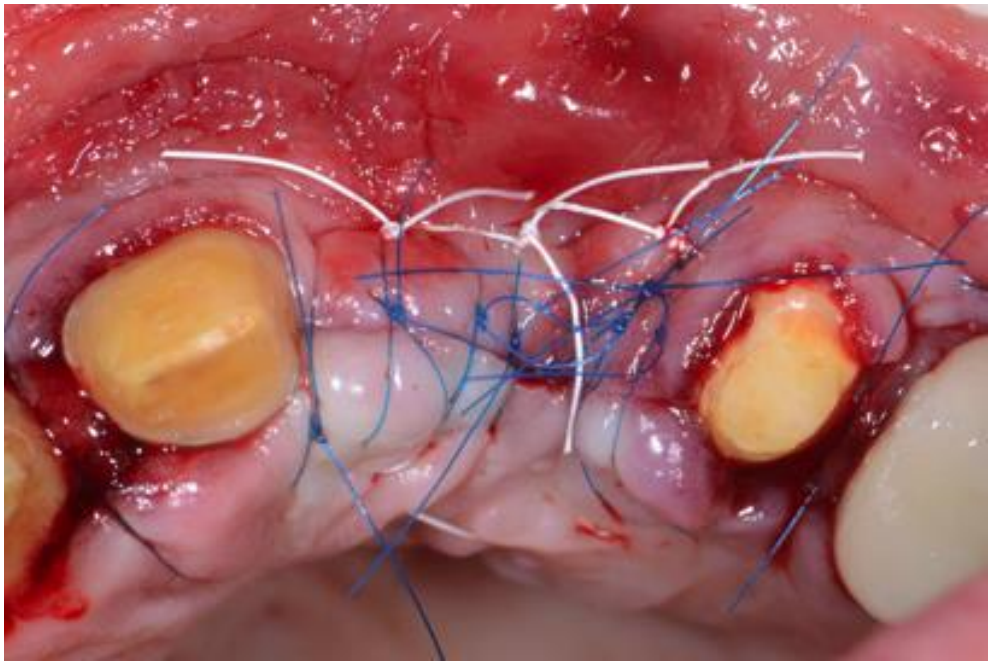


Figure (7A): Horizontal augmentation was done utilizing xenograft with autogenous bone

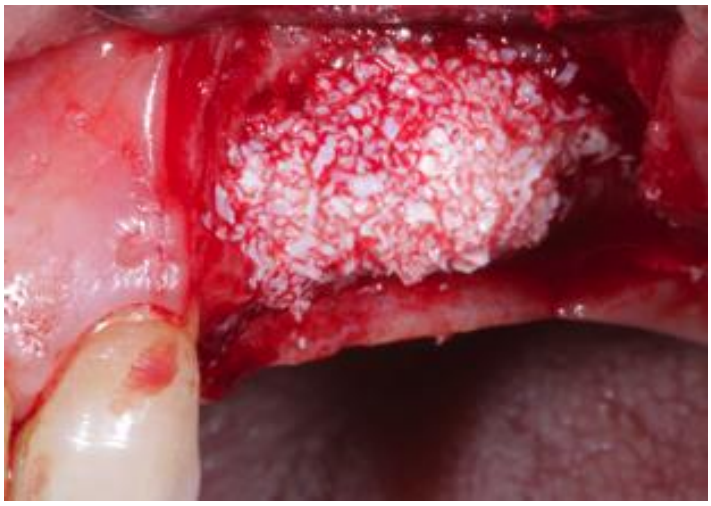


Figure (7B): Bone graft was covered with collagen absorbable membrane

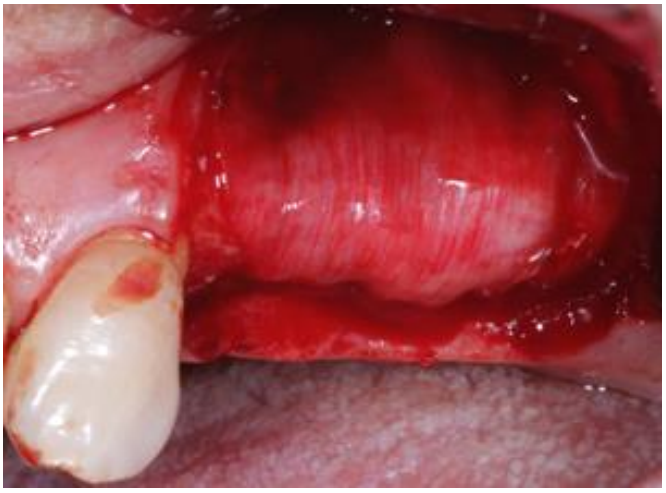


Figure (7C): Primary, passive wound closure was achieved without deep periosteal releasing incisions

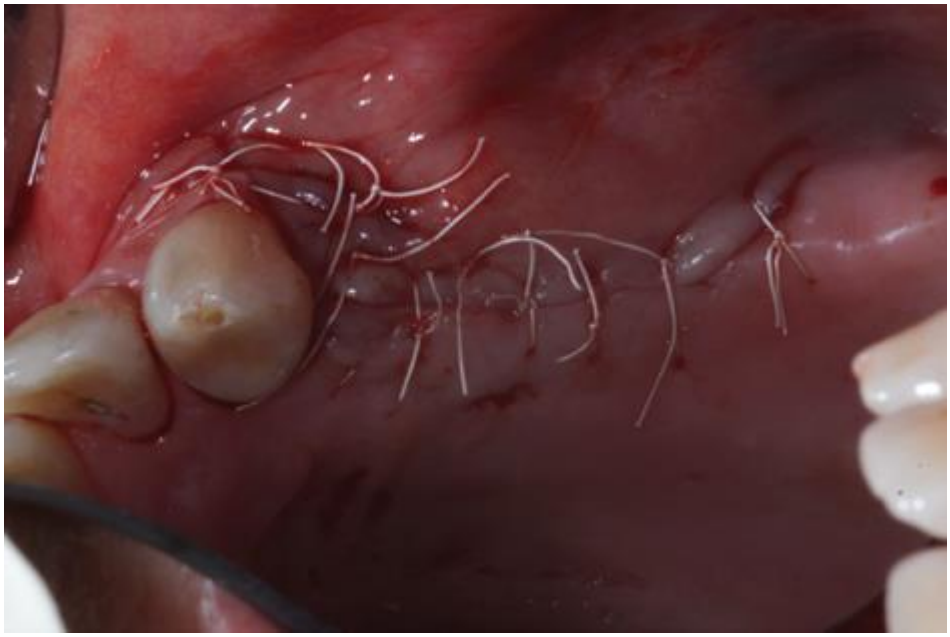


Figure (8A): Dental implant placement at the area that was expanded and augmented

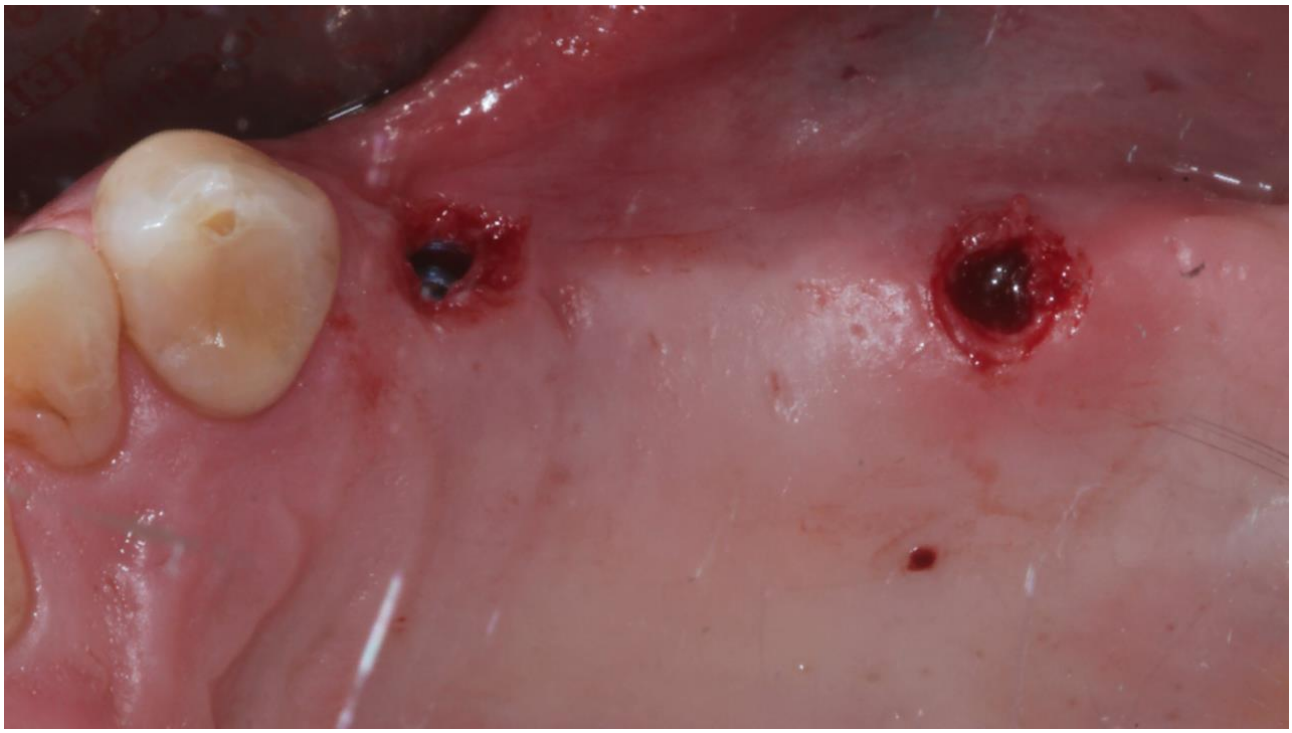


Figure (8B): Section from panoramic radiograph representing the same site following dental implant placement

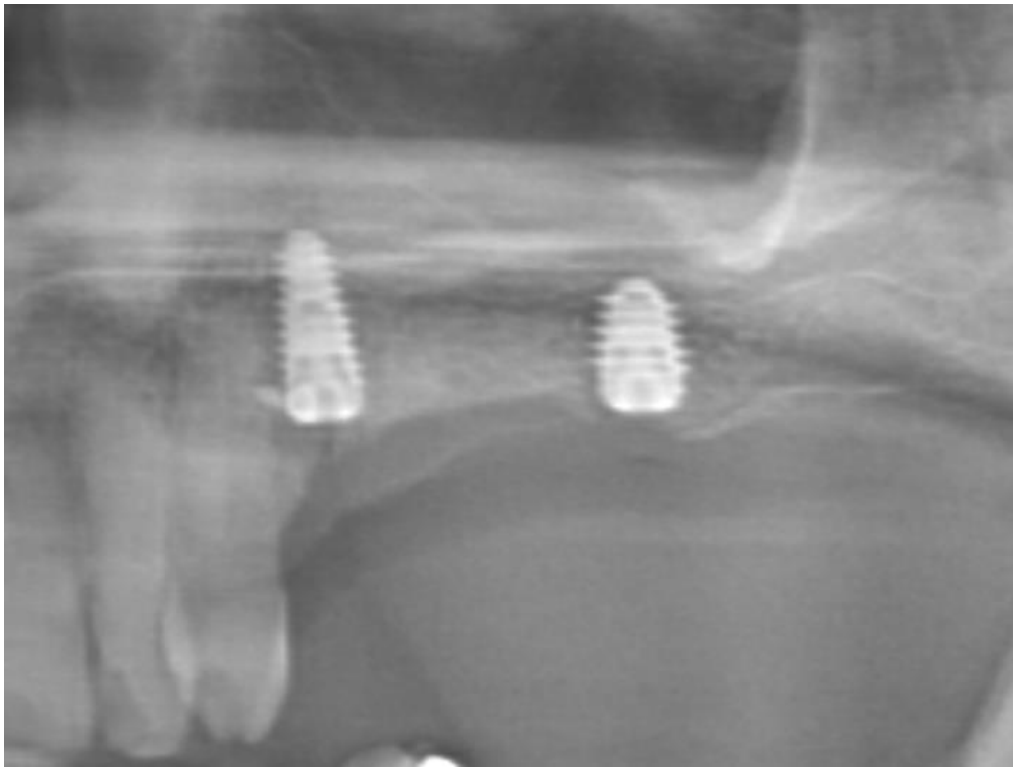


Figure (9A): CBCT image at baseline prior to STE

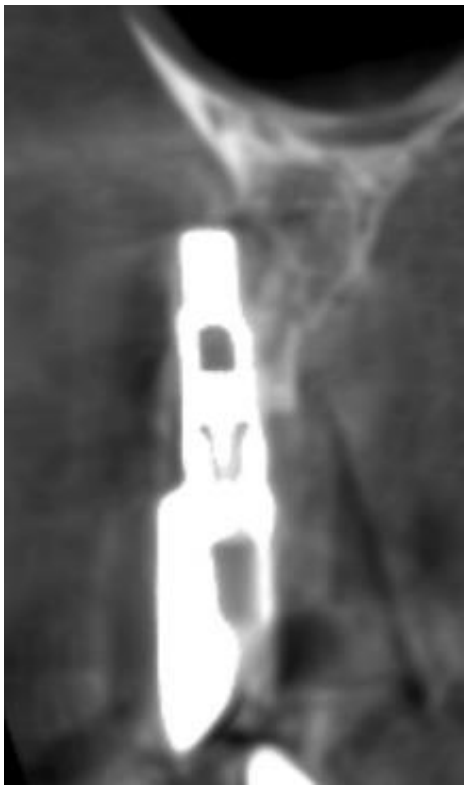


Figure (9B): CBCT image at 6 months post-augmentation

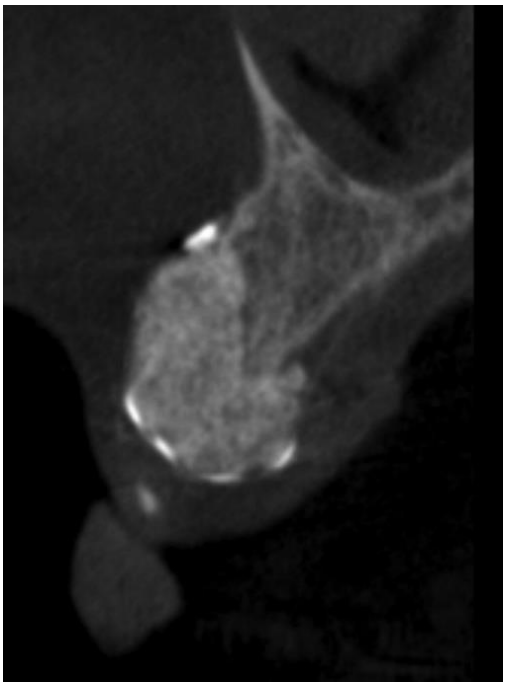
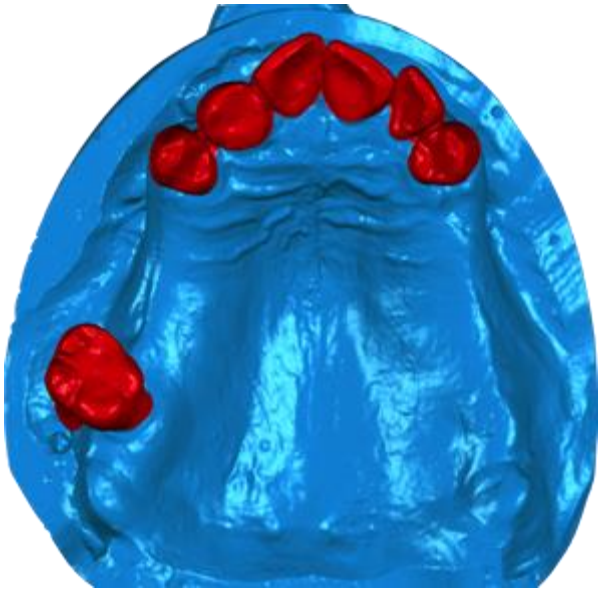
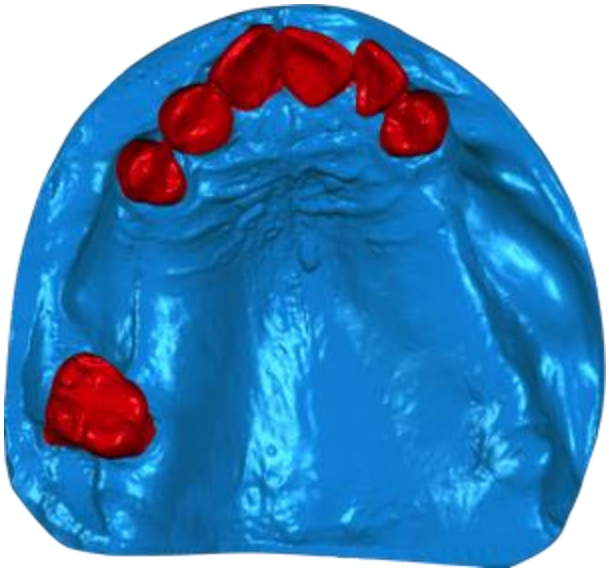


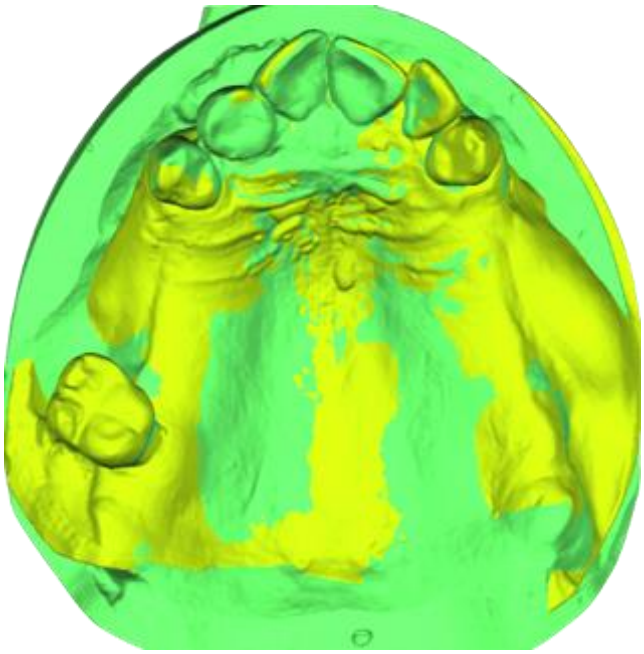
Figure (10): Volumetric analysis in the upper left maxilla. (A): Optically scanned pre-expansion model. (B): Optically scanned post-expansion model. (C): Superimposed pre- & post- expansion models



A

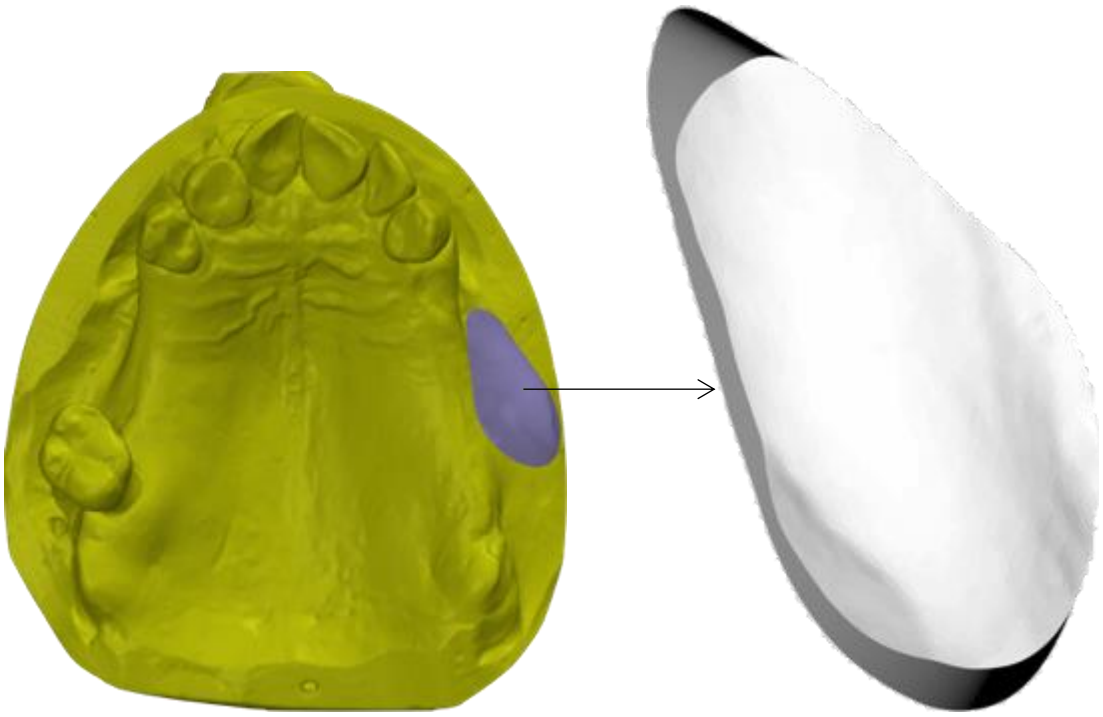


B



C

Figure (11): Calculation of volumetric changes. (A): Expanded tissues are highlighted with CAD software, and volume change is calculated. (B): The expanded area in STL format (coronal view), which can be superimposed on pre-expansion STL file for further confirmation of calculation. (C): The expanded area in STL format (lateral view)



A B

C

Figure (12A): The body of the expander was handled with a tweezer during insertion, creating minor cracks

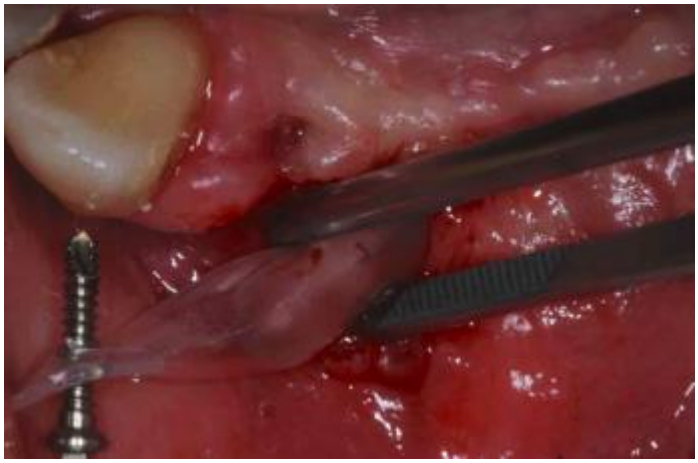


Figure (12B): Late failure of soft tissue expansion as seen by tissue perforation and expulsion of the expander

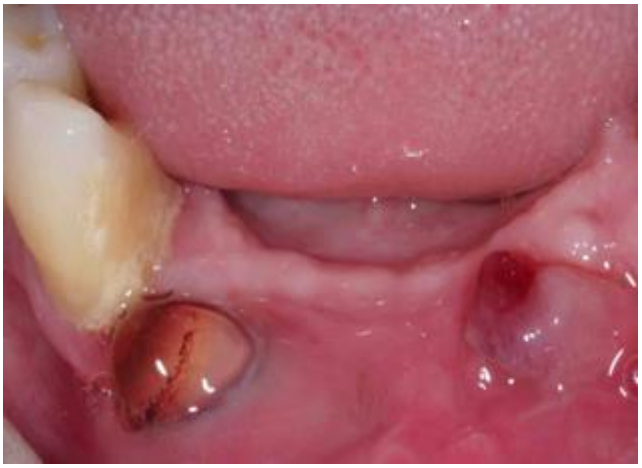


Figure (12C): Perforation of the silicon shell, due to propagation of the crack during expansion

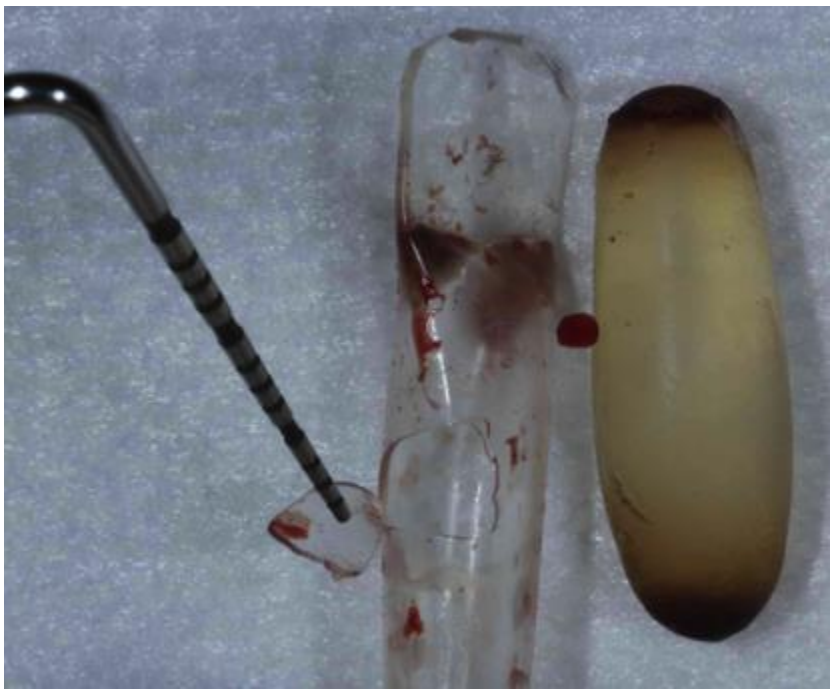


Table (1): Bone fill calculations for the six out of seven participants

Expansion Zone	Final Expander Volume & Shape	Augmentation Dimension	Amount of Bone Fill in Vertical Direction	Amount of Bone Fill in Horizontal Direction
Right Posterior Maxilla	0.24 ml, Cylinder	Horizontal	N/A	3 mm
Left Posterior Maxilla	0.7 ml, Cylinder	Horizontal	N/A	3 mm
Right Posterior Mandible	1.3 ml, Cylinder	Vertical	8 mm	N/A
Left Anterior Maxilla	0.35 ml, Cupola	Vertical & Horizontal	8 mm	8 mm
Right Anterior Maxilla	0.35 ml, Cupola	Vertical & Horizontal	6 mm	8 mm
Right Posterior Mandible**	0.35 ml, Cupola	Horizontal	N/A	2 mm

N/A= Not applicable

** Early expansion failure occurred in this case

Table (2): Volume gain analysis done by superimposing the pre- & post- augmentation optic scans of cast models

Expansion Zone	Initial Expander Volume	Final Expander Volume	Total Expansion Days	Expansion Days as Recommended by the Manufacturer for the Specific Final Volume	Soft Tissue Volume Gain	Success of Expansion
Right Posterior Maxilla	0.045 ml (45 mm^3)	0.24 ml (240 mm^3)	20 days	20 days	259.4 mm^3	Successful
Left Posterior Maxilla	0.15 ml (150 mm^3)	0.7 ml (700 mm^3)	40 days	40 days	436.1 mm^3	Successful
Right Posterior Mandible	0.25 ml (250 mm^3)	1.3 ml (1,300 mm^3)	60 days	60 days	755.9 mm^3	Successful
Anterior Mandible **	0.15 ml (150 mm^3)	0.7 ml (700 mm^3)	38 days	40 days	54.4 mm^3	Late Soft Tissue Expansion Failure

** Post-expansion impression was done two weeks after removal of failed expander

CHAPTER 3

3D Printed Scaffolds & Biomaterials: Review of Alveolar Bone Augmentation & Periodontal Regeneration Applications

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3.1. ABSTRACT

To ensure a successful dental implant therapy, presence of adequate vertical and horizontal alveolar bone is fundamental. However, an insufficient amount of alveolar ridge in both dimensions is often encountered in dental practice due to the consequences of oral diseases and tooth loss.

Although post-extraction socket preservation has been adopted to lessen the need for such invasive approaches, it utilizes bone-grafting materials, which have limitations that could negatively affect the quality of bone formation. To overcome the drawbacks of routinely employed grafting materials, bone-graft substitutes such as 3D-scaffolds have been recently investigated in the dental field.

In this review, we highlight different biomaterials suitable for 3D-scaffold fabrication, with a focus on "3D-printed" ones as bone graft substitutes that might be convenient for various applications related to implant therapy. We also briefly discuss their possible adoption for periodontal regeneration.

Keywords: Bone graft, biomaterials, 3D printed scaffolds, bone tissue engineering, periodontal regeneration

3.2. INTRODUCTION

Placement of endosseous implants has revolutionized modern dentistry, with a constantly increasing number of patients seeking replacement of lost teeth with this modality of treatment.

Since the overall success of dental implant therapy depends on the presence of adequate bone volume at implant sites **(Javed et al, 2013),** sufficient vertical and horizontal amounts of alveolar ridge prior to dental implant placement are essential especially in the anterior maxilla, which is a highly demanding aesthetic region.

Bone augmentation can be carried out using different techniques: bone blocks or guided bone regeneration (GBR) is mainly applied for horizontal grafting **(McAllister & Haghighat, 2007).** Vertical bone augmentation employs more challenging and technique-sensitive methods: vertical GBR, onlay grafting, inlay grafting and distraction osteogenesis **(Rocchietta et al, 20018; Esposito et al, 2009)** and is frequently associated with high complication rates such as soft tissue dehiscence and subsequent exposure of bone grafts into the oral cavity **(Jensen & Terheyden, 2009).**

In an attempt to overcome the obstacles related to vertical bone augmentation, short dental implants have been suggested as an alternative in the atrophic areas **(Esposito et al, 2011).** Despite being an acceptable option in the posterior areas of both jaws, bone grafting is still obligatory in anterior regions with severe bone resorption to achieve final satisfactory aesthetic results.

Bone grafts serve as filling materials with alternating properties of space-maintenance, blood-clot stabilization and scaffolding **(Pellegrini et al, 2013),** by providing a temporary template to support migration of cells from the periphery of the grafted area **(Pagni et al, 2012).** Bone grafting materials are divided into: autografts, allografts, xenografts and alloplasts, each with its own set of advantages and disadvantages **(Oryan et al, 2014).** As a result, researchers are

constantly working on exploring new bone graft substitutes with more predictable regenerative outcomes and minimal complications. To this end, tissue engineering has become more commonly used for oral bone grafting procedures.

The specific field of tissue engineering that mainly focuses on enhancing bone regeneration and repair by creating substitutes to traditional bone grafting materials is referred to as bone tissue engineering (BTE) **(O'Keefe & Mao, 2011)** which started about three decades ago and has been witnessing a tremendous growth ever since **(Amini et al, 2012).** This could be ascribed to the high regenerative potential of bone in comparison to other tissues in the body, thus serving as a paradigm for general principles in tissue engineering **(Fisher & Reddi, 2003).** A classic BTE paradigm includes the following three key components: biomaterials to provide a scaffold for new tissue growth, cells and signaling molecules **(Ikada, 2006; Amini et al, 2012).**

Within this model, scaffolds can either be acellular or cellular upon implantation. In the former, the overall architecture and geometry promote the recruitment of local stem cell and or/osteoprogenitor cells **(Kinoshita & Maeda, 2013),** which could be possible with "smart" cues and attachment motifs within the scaffold architecture. On the other hand, the latter strategy involves implantation of a scaffold combined with stem cell and or/osteoprogenitor cells **(Kinoshita & Maeda, 2013),** that can be incorporated by two methods: (i) cell seeding into a "prefabricated" scaffold, a commonly applied tissue engineering strategy ii) cell encapsulation during scaffold fabrication made of hydrogel polymer matrix **(Nicodemus & Bryant, 2008),** based on the immobilization of cells within a semi-permeable membrane. This technique protects cells from the immune system **(Murua et al, 2008)** and permits uniform cell distribution within the construct **(Bryant & Anseth, 2003).**

In this narrative review, based on orthopedic and dental studies available on Pubmed, MEDLINE and Google Scholar, we focus on the first key component of the tissue-

engineering paradigm for applications in alveolar bone and periodontal tissue regeneration, because scaffolds are considered the key players for successful bone regeneration **(Kinoshita & Maeda, 2013).** Biomolecules and cellular elements of the paradigm for this specific application are discussed elsewhere **(Pilipchuk et al, 2015).**

3.2.1. Properties of 3D Scaffolds for Applications in Alveolar Bone & Periodontal Tissue Regeneration

Although conventional bone grafting materials serve the role of a supporting matrix**,** they have several disadvantages: allografts, xenografts and alloplasts are brittle, poorly processable into porous forms, and unable to generate structures tailored to the specific needs of patients. Likewise, they are unable to maintain the desired generated tissue volume under mechanical forces, hindering their ability to provide a proper template for effective cell interaction **(Pagni et al, 2012).** Although autografts may have the ability to withstand mechanical forces, they are difficult to shape and conform to a bony defect **(Damien & Parsons, 1991),** which is of significant concern in the craniofacial region.

BTE has opened new doors for regeneration through the introduction of scaffolds, which possess three-dimensional (3D) architectures that closely mimic native extra-cellular matrix (ECM). Such arrangements eventually enhance cell adhesion, proliferation, differentiation and overall tissue regeneration **(Seunarine et al, 2006).** As a matter of fact, scaffold properties are influenced by the used biomaterials and must be specific for the application while in harmony with the native environment to ensure that the defect area is replaced with a healthy, functional tissue matching the original one and without reparative scar formation **(Castillo-Dalí et al, 2015).**

In general, scaffolds must exhibit an adequate degree of hydrophilicity **(Li & Chang, 2004; Goddard & Hotchkiss, 2007),** roughness **(Hoffmann et al, 2014)** and specific surface topography; a topographic landscape on micro- and sub-micrometer scales must be developed

to replicate the natural process of bone regeneration **(Cheng & Kisaalita, 2010).** Nano-topography increases the overall surface area, surface to volume ratio and surface roughness **(Park, J. et al, 2007),** that enhance the adhesion between osteoblasts and the underlying scaffold surfaces **(Webster & Smith, 2005).** As for micro-scale features, they facilitate cell penetration, vascularization, diffusion of nutrients **(Hollister et al, 2002)** and offer better spatial organization for cell growth and ECM production **(Woodard et al, 2007).** Development of a multi-scale scaffold has been also emphasized in periodontal tissue regeneration **(Park, C.H. et al, 2010).**

Other important design features are overall porosity, pore size and interconnectivity. As human cancellous bone demonstrates a total porosity between 30% and 90% **(Karageorgiou & Kaplan, 2005),** any construct enclosing voids within this range is considered suitable for bone regeneration. However, extremely high porosity can jeopardize the overall mechanical stability of a scaffold by reducing its overall compressive strength **(Bose et al, 2012).** For alveolar bone regeneration applications, an overall porosity of 70% was applied in pre-clinical and clinical studies **(Vaquette et al, 2012; Costa et al, 2014; Goh et al, 2015).** Regarding pore diameter, a range between 150 μm and 500 μm facilitates vascularization and penetration of new tissues **(Muschler et al, 2004)** without compromising the scaffold's mechanical strength **(Amini et al, 2012)** or cell penetration into inner surface areas **(Dhandayuthapani et al, 2011).** These consequential events are also dictated by the presence of an interconnected pore network, which is essential for cell growth into the interior of the scaffold to prevent core necrosis **(Chang & Wang, 2011).**

For successful bone regeneration, the template should demonstrate mechanical strength close to native tissues to support target cells, surrounding and newly formed tissues until full regeneration is achieved, mainly in load-bearing areas **(O'Brien, 2011; Mitsak et al, 2011).** In order to maintain this process, degradation rate of a scaffold should be in concordance with

the remodeling processes of the target tissue **(Hutmacher, 2000).** For dento-alveolar reconstruction, degradation within 5-6 months is considered appropriate **(Yeo et al, 2008).**

In addition, as implanted scaffolds should be biocompatible and bioactive, the utilized biomaterials should not elicit any inflammatory or cytotoxic reactions **(Chen et al, 2012)** and must evoke a specific biological response at the interface of the material, which results in the formation of a bond between the tissues and the material **(Hench et al, 1971).**

Although the previously presented features constitute the basics in scaffold designing for bone regeneration, it must be noted that the design and balance between biomaterials and scaffolds is a complex and interdisciplinary matter. Furthermore, this aspect can become more complex when alveolar bone regeneration is attempted along with cementum and periodontal ligament tissues. In this scenario, spatial organization is necessary by utilizing a multiphasic scaffold, which encloses variable architectural and chemical composition to closely capture the structural organization of native tissue and/or its cellular and biochemical composition **(Ivanovski et al, 2014).** Therefore, "compartmentalization" is essential for controlling the spatiotemporal events resulting in effective periodontal complex regeneration **(Ivanovski et al, 2014),** which could prevent tooth ankylosis**.** This can be achieved by ensuring a compartmentalized formation of bone and functionally- oriented periodontal ligament fibers (PDL) that are integrated over time **(Ivanovski et al, 2014).** *Figure (1)* illustrates a multiphasic scaffold with channel-like *"fiber-guiding architectures"* of the PDL compartment displaying a thickness of 0.250 mm to mimic the width of adult periodontal ligament space **(Park, C.H. et al, 2012).**

3.2.2. Applied Biomaterials in 3D Scaffolds Fabrication for Tissue Regeneration

As biomaterials strongly influence the overall properties of a scaffold, it is important to comprehend their individual characteristics to allow for appropriate selection in specific applications and taking into consideration that biomaterials differ in their cellular affinity

(Khang, 2015), which influences adhesion, proliferation and the overall regeneration outcome. As cell adhesion is mediated via integrins, such differences between biomaterials can be further explored. Below, we present biomaterials that can be applied in the regeneration of alveolar bone, periodontal tissues regeneration and are compatible with new scaffold fabrication techniques.

3.2.2.1. Biodegradable Natural Polymers

Natural polymers, which include proteins and polysaccharides, are the first biomaterials to be recruited in different clinical applications because of their high biocompatibility, good cell recognition, enhanced cellular interactions in the surrounding environment **(Nair & Laurencin, 2007),** and hydrophilicity **(El-Sherbiny & Yacoub, 2013).** Due to these properties, they have been thoroughly investigated as hydrogels in the earliest work of cell encapsulation in tissue engineering, demonstrating successful results **(Mikos et al, 1994; Cao et al, 1998; Sims et al, 1998; Perka et al, 2000; Lee & Mooney, 2001).**

Collagen is one of the most widely expressed proteins in the human body, providing strength and structural stability to many tissues from skin to bone **(Pastorino et al, 2014).** Being the major organic component of the ECM in native bone makes collagen an attractive biomaterial for BTE applications **(Aravamudhan et al, 2013).** It is well documented that collagen matrices promote cell adhesion, proliferation and osteogenic differentiation of bone marrow stromal cells, *in vitro* **(Pastorino et al, 2014).** Similarly, the denatured form of collagen termed *gelatin* **(Côté et al, 2004)** enhances osteoblast adhesion, migration and mineralization as it contains several biological and functional groups that promote such activities **(Meyer & Wiesmann, 2006).**

Regarding polysaccharides, *Chitosan* is a popular biomaterial in bone tissue engineering due to its appealing characteristics; it displays antibacterial and anti-fungal activities, rapid blood clot formation and analgesic properties **(Aranaz et al, 2009),** all of which render chitosan

useful in wound healing acceleration that would minimize the risk of scaffold contamination and post-operative infections, thus preventing eventual exposure and failure of the scaffold.

For the same applications, *alginate* is another commonly investigated polysaccharide. It is highly processable into different scaffold types, which encourages its employment in BTE, regenerative medicine **(Sun & Tan, 2013)** and has been the most studied biomaterial for encapsulation of living cells **(Murua et al, 2008).** Interestingly, alginate and chitosan do not exist within the human body, but they display structural similarities to glycosaminoglycans (GAGs) found in the ECM of human tissues such as bone **(Holzapfel et al, 2013),** making them attractive candidates for applications in tissue regeneration.

Despite their good biological properties, the previously mentioned natural polymers lack *bioactivity* **(Raucci et al, 2012),** which is the key factor in promoting hard tissue formation. They also share weak mechanical characteristics and somewhat rapid degradation rate **(Florczyk et al, 2011; Sun & Tan, 2013; Cao et al, 2014)** through enzymatic reaction **(Lenz, 1993).**

To overcome such undesired properties, scaffolds based on natural polymers are usually combined with bioactive materials (e.g. bioceramics) or mechanically strong ones (e.g. synthetic polymers or metals), depending on the area of application (e.g. load-bearing or not). Interestingly, although bioceramics are mechanically weak as well, they tend to increase the overall compressive strength of natural polymer based-scaffolds **(Kane et al, 2015).**

3.2.2.2. Biodegradable Synthetic Polymers

Biodegradable synthetic polymers have generated interest in BTE because of their relatively low-cost and ability to be produced in large quantities with long-shelf life in comparison to their natural counterparts **(Dhandayuthapani et al, 2011).** The most investigated biomaterials of this group are *aliphatic polyesters* which include: polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA), and their copolymer polylactic-co-glycolic acid (PLGA).

Polycaprolactone (PCL) is the most popular aliphatic polyester in medical applications; it has been used in medical devices for the last 30 years **(Goh et al, 2015)** and has been investigated in craniofacial repair **(Gough et al, 2003).** PCL is an excellent candidate for BTE applications due to its biocompatibility **(Pitt, 1990),** suitability for various scaffold fabrication techniques **(Williams et al, 2005),** remarkably slow degradation rate and mechanical stability **(Mitsak et al, 2011).** It is suggested that the latter two traits might allow for better maintenance of generated bone volume and its contour over time. However, PCL is hydrophobic in nature **(Zhu et al, 2002)**, which is also responsible for the inferior cell affinity and poor cellular responses and interactions to the surface **(Lim et al, 2015).** Similarly, *polylactic acid* (PLA) and *poly(lactic-co-glycolic acid) (PLGA)* are as hydrophobic while *polyglycolic acid (PGA)* is hydrophilic, keeping in mind that these polymers still have higher rates of degradation in comparison to PCL **(Chen & Thouas, 2015).** But in general, aliphatic polyesters display a slow degradation rate in correlation to natural polymers and bioceramics **(Yildirimer** & **Seifalian, 2014).** Synthetic polymers degrade by hydrolysis **(Lenz, 1993)**, which can be in the form of bulk degradation or surface erosion **(Göpferich, 1996; von Burkersroda, 2002).** Most of the available polyesters degrade by the former mechanism **(Davison et al, 2015)** characterized by hydrolysis within the interior part of the biomaterial, resulting in an empty shell formation, while the size is maintained for a considerable amount of time **(Li, 1999).** This feature is considered appealing for scaffold utilization as a bone graft substitute and less suitable for drug-delivery purposes. Still, aliphatic polyesters release acidic byproducts upon degradation, which can result in tissue necrosis and subsequent scaffold failure with chronic exposure **(Amini et al, 2012).** Therefore, they are usually combined with bioceramics that enhance the bioactivity of a construct and tend to neutralize the acidic byproducts by elevating the overall pH value for the scaffold **(Tamjid et al, 2013)** to maintain tissue health. Counteracting acidic byproducts and overall-pH buffering can also be achieved when

polyesters are combined with metals **(Brown et al, 2015).** Despite the acidic by-products and the lack of *bioactivity*, aliphatic polyesters are moldable for fabrication into the required shapes and have good mechanical properties **(Sabir et al, 2009; Gong et al, 2015).**

3.2.2.3. Bioceramics

Bioceramics are inorganic biomaterials constituting different categories, among which are calcium phosphate bioceramics and bioactive glass with very well documented applications as bone-fillers in dental applications **(Sarkar & Banerjee, 2010).** Calcium phosphate bioceramics enclose hydroxyapatite (HAp), tricalcium phosphate (α-TCP and β-TCP) and biphasic calcium phosphate (BCP), all of which can also be in the form of injectable cements (pastes) that are moldable, easy to handle and harden when left in situ. Moldable calcium phosphate materials allow for intimate adaptation to complex defects, which is difficult to accomplish with conventional bone grafting materials **(Thein-Han & Xu, 2011).**

Bioceramics are attracting more attention in bone reconstruction due to their unlimited availability, bioactivity, excellent biocompatibility, hydrophilicity, similarity to native bone inorganic components**,** osteoconductivity **(Woodard et al, 2007)** and reported potential osteoinductivity **(LeGeros, 2002),** which is the ability to induce ectopic bone formation by instructing the surrounding *in vivo* environment to do so **(Blokhuis & Arts, 2011).** This potential activity can be attributed either to the surface of bioceramics which absorbs and exhibits osteoinductive factors, or due to gradual release of calcium and phosphate ions into the surrounding environment, subsequently stimulating the differentiation of osteoprogenitor cells into osteoblasts. Both theories are yet to be confirmed **(Barradas et al, 2011).** The importance of incorporating calcium phosphates in 3D scaffolds for alveolar bone regeneration has already been demonstrated in literature **(Costa et al, 2014).**

The most investigated calcium phosphate ceramic in BTE is *Hydroxyapatite (HAp)* because it shares the same chemical composition of native bone minerals, which positively influences

adhesion and proliferation of osteoblasts **(Huang et al, 2004).** Despite this important feature, HAp takes a long time to degrade when in its "crystalline form" *in vivo*, causing the remaining particles to impede complete bone formation and increase the risk for infection and exposure in oral and maxillofacial regions **(Szpalski et al, 2010).** Consequently, applications of crystalline HAp are being eventually substituted by amorphous hydroxyapatite, which has a faster degradation rate **(Zhao et al, 2011).** Modification of HAp degradation rate can also be achieved by its combination with other biomaterials of faster kinetics, such as natural polymers **(Johnson et al, 1996).**

The second most widely studied calcium phosphate ceramic is *β-tricalcium phosphate (β-TCP),* because of its ability to form a strong bone-calcium phosphate bond **(LeGeros, 2002)** and its degradation rate which is faster than HAp **(Oryan et al, 2014).** Interestingly, when tricalcium phosphate is combined with HAp, a mixture termed biphasic calcium phosphate (BCP) is produced **(Nery et al, 1990).** In comparison to other calcium phosphate ceramics, BCP has significant advantages in the terms of controlled bioactivity, stability while promoting bone ingrowth especially in large bone defects **(Lobo et al, 2010)** and controllable degradation rate **(Ramay & Zhang, 2004)** as BCP has a higher degradation rate than HAp, yet, slower than that of β-TCP **(Petrovic et al, 2012).**

Another biomaterial that belongs to bioceramics and is investigated in BTE is *bioactive glass (BG),* which is a silicon oxide with substituted calcium **(Pilipchuk et al, 2015).** When exposed to body fluids, a layer of calcium phosphate forms on the surface of bioactive glass, which chemically binds to bone **(Hench, 2006).** The specific type of bioglass used as a synthetic graft in intraoral applications (termed 45S5 Bioglass®) **(Pilipchuk et al, 2015)** has a very slow degradation rate because it converts to a HAp-like material in the physiologic environment **(Huang et al, 2006a; 2006b).** Typically, bioceramics degrade via multiple mechanisms: physio-chemical dissolution accompanied by possible phase transformation,

multinucleated cell-mediated degradation and mechanical fragmentation due to loss of structural integrity by the former two mechanisms **(Davison et al, 2015).**

Although bioceramics have inviting qualities, they are extremely brittle, difficult to shape into desired structures because of their stiffness and low flexibility and moldability **(Kim, H.W. et al, 2005).** They have weak mechanical strength **(Hench, 1991)** and fracture toughness **(Tevlin et al, 2014),** which limit their applications to non-load bearing areas. However, their combination with mechanically strong biomaterials, such as synthetic polyesters or metals tends to eliminate brittleness, difficulty in shaping and weak mechanical strength **(Zhang & Wu, 2013; Długoń et al, 2014).**

3.2.2.4. Metals

Metallic biomaterials are extensively applied in dental and orthopedic fields to support the replacement of lost bone structures because of their excellent mechanical properties **(Staiger et al, 2006; Alvarez & Nakajima, 2009);** they display high strength, toughness and hardness, in comparison to polymers and ceramics, making them suitable for applications in load-bearing areas **(Hallab et al, 2004).** It is reported that metals enhance the mechanical properties of a scaffold by decreasing the pore size **(Kim, J.H. et al, 2014).**

Within this group of biomaterials, titanium and titanium-alloys are encouraged in bone regeneration due to their high biocompatibility, appropriate mechanical properties and elasticity **(Wu et al, 2008).** Different studies reported that titanium-based 3D scaffolds display good hydrophilicity, which enhances mineral deposition and encourages cell attachment and proliferation *in vitro* **(Wu et al, 2008)** and new bone formation without any signs of inflammation or necrosis *in vivo* **(Haugen et al, 2013).**

Nonetheless, lack of biodegradability of titanium and titanium alloys is a major disadvantage and might discourage their applications in bone regeneration due to the need of a second

surgery for removal, which can compromise patient satisfaction and increase health care costs **(Staiger et al, 2006).**

In the past decade, magnesium and magnesium alloys have been thoroughly researched and found to be extremely appealing materials for orthopedic applications (**Staiger et al, 2006)** with great potential in BTE; they have mechanical properties close to native bone and are completely biodegradable **(Staiger et al, 2006)** which eliminates the need for a second surgery to retrieve the scaffold. Although Magnesium & Magnesium alloys degrade by corrosion **(Persaud-Sharma & McGoron, 2012),** their by-products are biocompatible and don't elicit adverse reactions that could negatively affect surrounding tissues **(Heublein et al, 2003).**

Magnesium and its alloys are osteoconductive, play a role in cell attachment **(Staiger et al, 2006)** and tend to increase the expression of osteogenic markers *in vitro* **(Yoshizawa et al, 2014).** Although pure magnesium has a rapid rate of degradation *in vivo* **(Gray & Luan, 2002),** this can be controlled through the utilization of magnesium alloys **(Zhang, E.L. et al, 2009),** or by coating pure magnesium with titanium **(Zhang, E.L. et al, 2005)** or ceramics **(Geng et al, 2009).** Similar to natural and synthetic polymers, metals lack *bioactivity*.

In regards to all the previously described biomaterials, each has remarkable characteristics and individual limitations. Henceforth, it is very common to combine two or more different biomaterials to produce a "*synergistic effect*" in the overall resulting properties **(Erol et al, 2012)** and improve the mechanical, biological and degradation kinetics of a scaffold **(Cascone et al, 2001).** Additionally, bone tissue is made of organic and inorganic components **(Manjubala et al, 2008),** thereby making it more difficult for one biomaterial to simulate the complex bone tissue environment and possess the required characteristics of the target tissue **(Castillo-Dalí et al, 2015).** These scaffolds are referred to as "composite" or "hybrid" and whenever three biomaterials are incorporated, the term "ternary" can be used. Composite

scaffolds used for BTE applications are divided into: "*polymer/ceramic", "ceramic/metal"* and *"polymer/metal".* The former type is the most popular among composites and has been thoroughly studied by researchers in the orthopedic field for the last five years **(Polo-Corrales et al, 2014),** although literature confirms that various composite scaffolds support attachment, proliferation, and differentiation of osteoblasts while maintaining the final shape of newly formed bone **(Polo-Corrales et al, 2014).**

Composites, whether ternary or not, consist of a major component (matrix) and minor components (filler); the material which constitutes more than 50% of the blend is considered the major element, while the material/materials that are less than 50% represent the minor component **(Thuaksuban et al, 2011).**

3.2.3. Advances in 3D Scaffold Fabrication Techniques

Different techniques are employed in the fabrication of 3D scaffolds, with the conventional methods including: particle leaching, gas foaming, freeze drying, phase separation, fiber meshes/fiber bonding, melt molding and solution casting **(Kinoshita & Maeda, 2013).** However, with these techniques, heterogeneities in pore size, porosity, interconnectivity and architecture are unavoidable, which can complicate drawing conclusions from experiments that assess the effect of scaffold properties on newly formed tissues **(Thimm et al, 2011).** Moreover, these techniques might not applicable for the fabrication of a custom-made scaffold with finely tuned architectures that replicates the complexity of native tissues and precisely conforms to the shape of a certain defect.

With the development of solid-freeform fabrication (SFF) techniques, also known as rapid prototyping (RP), it became possible to create scaffolds with precise external shape, internal morphology and "reproducible" three-dimensional architecture, despite their complexity **(Moroni et al, 2006).**

These technologies represent additive manufacturing as they build complex structures layer by layer by "3D printing", with one of the following techniques: inkjet printing, laser assisted printing (e.g. Selective Laser Sintering "SLS" & Stereolithography "SLA") and extrusion printing (e.g. Fused deposition modeling "FDM"**) (Obregon et al, 2015).** Each printing method is compatible with specific biomaterials and differs in resolution. For example, laser-assisted methods enable printing diverse biomaterials with wide range viscosities **(Koch et al, 2010),** overcoming the limitations of inkjet printing in which low-viscosity inks are needed to prevent clogging of the nozzle of the printing machine that would eventually compromise printing quality, while extrusion printing is limited to thermoplastic biomaterials such as PCL **(Chia & Wu, 2015; Obregon et al, 2015).** In regards to bio-printing, inkjet, laser-assisted and extrusion-based techniques are utilized in printing of living cells and constructs **(Obregon et al, 2015).** In consequence, these technologies can be further explored in cell encapsulation and cell-based therapies, especially that they allow for a controlled positioning of cells with precision, which could mimic the tissue interface and the surrounding microenvironment. However, these applications are generally limited to hydrogel scaffolds **(Yeong et al, 2004),** made of natural or synthetic polymers **(Chia & Wu, 2015).** Different methods of 3D printing are demonstrated in *figure (2)* **(Obregon et al, 2015).**

These new techniques utilize computer-aided design (CAD) and computer-assisted manufacturing (CAM) technologies to 3D print a desired structure based on a CAD file that has already defined the exact dimensions of a scaffold **(Yeong et al, 2004).** This approach can be applicable in fabricating constructs that conform to a specific anatomical shape; in a typical clinical case scenario, CAD models are produced based on images from computed tomography (CT) scans of a patient-specific bone defect to develop a "custom-made" bone graft substitute which could be helpful in regenerating defects with complex geometry **(Ma, 2008)** as illustrated in *figure (3)* **(Park, C.H. et al, 2014 a).** Image-based 3D printed scaffold

following this scheme displayed promising results in pre-clinical investigations in periodontal regeneration with the need of further assessment for future employment in clinical practice **(Park et al, 2012; Park et al, 2014 a).** In literature, few studies have focused on the concept of custom made scaffolds for alveolar bone regeneration, by using subtractive technology (milling of a commercially available block, dictated by CAD/CAM technologies), which might not be as sophisticated due to the lack of layer by layer addition **(Figliuzzi et al, 2013, Mangano, A. et al, 2014, Mangano, F.G. et al, 2015).**

Although RP techniques are capable of producing constructs with satisfying mechanical strength by precisely controlling overall geometrical design and porosity, these characteristics can still be limited by the machine's resolution and material repertoire, as mentioned earlier. Due to the lack of sufficient resolution to fabricate nano- and sub-micrometer structures, a combination of RP techniques with different fabrication methods such as electrospinning **(Nandakumar et al, 2013)** has been proposed to allow for the construction of efficient biomimetic constructs.

3.2.4. Applications of 3D-Printed and/or Compartmentalized Scaffolds in Alveolar Bone & Periodontal Regeneration

With the increased need for "optimal" tissue regeneration, 3D "printed" scaffolds have been recently investigated in different periodontal applications: guided bone regeneration (GBR), guided tissue regeneration (GTR), vertical bone augmentation, sinus augmentation and socket preservation, showing variable outcomes of success.

PCL has been the most utilized biomaterial in these applications, probably because of its well-documented positive outcomes in hard tissue regeneration in the field of orthopedics **(Polo-Corrales et al, 2014).**

Regarding periodontal tissue regeneration, a novel anatomically shaped human-molar & rat-incisor 3D printed PCL/HAp scaffold showed promising results in terms of inducing

regeneration by "cell homing" instead of cell delivery in a rat model **(Kim, K. et al, 2010).** In another rat model **(Park, C.H. et al, 2012; Park, C.H. et al, 2014 a)** the concept of "compartmentalization" was applied to achieve regeneration of cementum, alveolar bone and periodontal ligament, by utilizing a custom-made 3D-printed PCL scaffold, which enclosed an alveolar bone interface and a PDL-interface with fiber-guiding architectures. The biphasic construct allowed for regeneration of obliquely oriented periodontal fibers, cementum-like tissue, alveolar bone and allowed for greater control of tissue infiltration when compared to random porous scaffolds. Similarly, multiphasic periodontal tissue regeneration was achieved with a 3D printed PCL/HAp triphasic scaffold that allowed for spatiotemporal delivery of multiple proteins, *in vivo* **(Lee, C.H. et al, 2014).**

Recently, a biphasic PCL scaffold utilizing two scaffold fabrication techniques and cell sheet technology was investigated in the regeneration of the alveolar bone and periodontal tissues **(Vaquette et al, 2012).** In fact, cell sheet technology was tested as a part of the scaffold to provide biomechanical support during wound healing process, which was lacking in a material-free approach of cell sheet technology in periodontal regeneration **(Akizuki et al, 2005).** The scaffold enclosed two compartments manufactured by two different techniques and of different biomaterials: the bone compartment was constructed from β-TCP/PCL by fused deposition modeling (FDM), then thermally incorporated with an electrospun PCL membrane enclosing cell sheets, representing the PDL compartment. After being tested in a subcutaneous rat model, results demonstrated successful regeneration of cementum, alveolar bone and periodontal ligament. Early bone markers confirmed that FDM bone compartment promoted early bone formation. However, there was no functional orientation of the PDL fibers, as no specific cell oriented architecture was contained in the design. To address this finding, the researchers developed a second generation of the same scaffold **(Costa et al, 2014)** but with certain modifications of the PDL compartment, by including superimposed

concentrically oriented rings in the membrane, fabricated by melt electro-spinning to allow for some level of tissue organization. This compartment was also more porous to improve cell interactions and vascularization. The bone interface was modified to enhance alveolar bone regeneration by coating the β-TCP/PCL construct with calcium phosphate (CaP). By employing the same animal model, results revealed higher bone formation with improved PDL fiber orientation and vessel ingrowth.

Despite the promising results *in vivo*, 3D PCL-based scaffolds showed less promising outcomes in clinical studies.

A *"pre-fabricated"* 3D PCL scaffold printed by FDM was tested for socket preservation in a randomized clinical trial **(Goh et al, 2015).** Although the scaffold maintained the ridge height better after 6-months, this finding can be expected because no filler was used in the control group. The efficacy of PCL-based scaffolds as space fillers in socket preservation should be interpreted with caution, because comparison with other socket preservation techniques is still lacking. Most importantly, the scaffolds showed minimal signs of degradation 6-months following intervention and fibrous invasion was reported in one patient due to manual shaping for friction fit within the extraction socket. One might conclude that *"custom-made"* 3D printed PCL-scaffolds based on medical imaging could show more favorable results by allowing a precise adaptation to the bony defect. However, adverse outcomes were reported when a custom-made image-based 3D fiber-guided PCL/HAp scaffold printed by SLS was applied in GTR in a recent case report, as shown in *figure (4)* **(Rasperini et al, 2015).** After thirteen months of scaffold implantation, soft tissue dehiscence was reported with histological and molecular weight analysis revealing that almost 76% of the scaffold mass remained with minimal bone repair. This result can be interpreted by the very slow degradation profile of PCL in addition to its inferior cell affinity and weak osteoconductive activity. This final outcome might have also been compromised due to the low resolution of the 3D printing

technology. Interestingly, one might attribute the end result to the acidic by-products upon degradation, as well. Nonetheless, this matter is debatable, as some data in literature have revealed that metabolic pathways easily remove PCL byproducts and thus PCL doesn't produce a local acidic environment as other aliphatic polyesters **(Sinha et al, 2004; Woodruff & Hutmacher, 2010).**

The slow degradation of PCL has been considered appealing in hard tissue regeneration **(Mitsak et al, 2011),** but this might be valid for orthopedic applications only, because there are key differences in behavior between the long bones and alveolar bone with bone remodeling being slower in the former in comparison to the latter **(Dixon et al, 1997).** Although it is very well documented that bioceramics tend to control the degradation rate of polyesters **(Díaz et al, 2014),** the percentage of HAp that was combined with PCL in this case report (4%) might not have been sufficient to accelerate the degradation profile. As a matter of fact, accelerated degradation of PCL was achievable with much a higher percentage of HAp in an *in vitro* investigation **(Díaz et al, 2014).**

Interestingly, this fiber-guiding scaffold model in GTR was successful in pre-clinical studies on rats **(Park, C.H. et al, 2012; Park, C.H. et al, 2014 a).** The discrepancy in results could be due to the differences between rats and humans in terms of healing window, anatomic structures and host responses **(Rios & Giannobile, 2011)**.

Another biomaterial that has been widely tested as part of 3D scaffolds for periodontal applications is bioceramics, mainly in sinus and bone augmentation procedures. In a sheep animal model, a pre-fabricated 3D printed scaffold, made of biphasic ceramic (α-TCP +HAp) was compared to bovine bone (Bio-Oss) and particulate β-TCP for vertical bone augmentation **(Carrel et al, 2016).** The scaffold eliminated the need for membranes and provided better mechanical support to the newly formed tissues, which can be explained by the fact that when α-TCP comes into contact with body fluids, it converts to HAp which has a very slow

degradation rate. Similarly, a 3D-printed BCP scaffold "(30%)/HA, (60%)/ β-TCP, (10%) α-TCP" showed favorable results as a bone graft substitute for sinus augmentation *in vivo* in terms of abundant deposition of newly formed bone tissue within the biomaterial pores, which could be promising in future clinical applications **(Mangano, C. et al, 2015).**

Specific conclusions can be extrapolated from the previous studies about the use of certain biomaterials in scaffolding for various periodontal applications. For example, the use of PCL as the only biomaterial in a scaffold could be discouraged mainly due to its slow degradation rate which can lead to wound dehiscence and subsequent failure of tissue regeneration and also due to its inferior cell affinity **(Lim et al, 2015)**. If combined with bioceramics, an increase in the weight percentage of the bioceramic should be utilized to accelerate the degradation profile. Likewise, increased porosity of the bulk scaffold construct can assist with more rapid tissue ingrowth that can further drive the degradation process. Other aliphatic polyesters might be discouraged as well due to their acidic byproducts unless counteracted by the combination of bioceramics or metals. In a recent *in vitro/in vivo* investigation, magnesium/PLGA scaffold was applied in socket preservation, in which magnesium was able to counteract the acidic degradation of PLGA, thus decreasing the risk for tissue inflammation and eventually enhancing osteogenesis **(Brown et al, 2015).** Still, it should be kept in mind that the ideal percentage of biomaterials to eliminate the risk of adverse effects may be difficult to determine for clinical uses.

Regarding GTR, where contact with bacteria and exposure are more likely to occur, natural polymers could be the best choice for this specific application, such as chitosan which has antibacterial properties that could decrease the chance of bacterial contamination and subsequent exposure. Gelatin can also be recommended in this application, and it has already been investigated *in vitro* as the biomaterial of a "periodontally-inspired" scaffold, created by directional freeze casting **(Park C.H. et al, 2014 b).** Despite having a relatively low

compressive resistance, gelatin displayed attractive biological properties because intrinsic cell interactions with the scaffold surface are still possible with the presence of adhesive RGD motifs, making cell affinity and growth more significant **(Hersel et al, 2003).** To overcome the mechanical weakness of gelatin, it was proposed to incorporate this platform into the previously described synthetic polymer-based, fiber-guiding 3D scaffold system **(Park, C.H. et al, 2012; Park, C.H. et al, 2014 a).**

It should be kept in mind that natural polymers must be combined with mechanically strong materials; in GTR applications, the scaffold serves a dual role: a grafting material and a membrane. Since space maintenance is required for periodontal regeneration, it is essential to utilize a mechanically strong scaffold.

For applications in alveolar bone regeneration, augmentation and socket preservation, scaffolds made of bioceramics can be recommended. Nonetheless, using bioceramics alone can be questionable for clinical applications, because of their weak mechanical properties. To overcome such limitations, bioceramics can be combined with mechanically strong biomaterials as mentioned earlier.

In non-load-bearing areas, collagen could be the preferred biomaterial in such a combination. Better outcomes are to be expected with the incorporation of collagen because a bioceramic/collagen mix is the closest replicate of native bone ECM composition **(Wahl & Czernuszka, 2006).**

Specifically, the combination of collagen with *hydroxyapatite* is encouraged in bone tissue regeneration **(Wahl & Czernuszka, 2006)** due to the compositional similarities to native tissue and reasonable degradation rates for clinical uses **(Johnson et al, 1996)**

In bone tissue regeneration, care must be taken that this process might take a long time in case of severe ridge resorption, because bone regeneration through scaffolds commences at the peripheries, where contact points between the biomaterial and native bone exists.

Nonetheless, this can be resolved with advances in tissue engineering and further investigations, by creating different points of bone nucleation through engineering it with stem/osteoprogenitor cells **(Mangano, C. et al, 2015).**

To this end, studies on 3D printed scaffolds in the periodontal field have focused on biomaterials, new and/or functional tissue formation and spatial organization mainly when multiple tissue regeneration is attempted. Accordingly, other characteristics still need to be addressed more thoroughly, such as vascularization, analysis of landscape topography and degradation profile and kinetics. Moreover, *"image-based"* 3D printed scaffolds must be investigated in alveolar bone regeneration prior to placement of dental implants, as there are no published studies on this specific use.

3.2.5. Recommendations and Future Directions

BTE is not only based on cellular and molecular events and interactions, but also on the development of biomaterials and scaffolds with prescribed biomechanical properties, representing a fundamental part of the BTE paradigm.

Dental literature on 3D scaffolds and related biomaterials as alternative to bone grafts are still scarce, with extremely limited clinical trials. Validation of the efficacy of scaffolds tested in animal models are obligatory, because the already published results are not representative due to small defects, graft size and also a completely different healing process in small animals. Randomized controlled clinical trials are mandatory, with adequate number of patients and long-term follow-up of implant therapy following scaffold employment in pre-implant augmentation procedures. Thorough evaluation of biological and mechanical properties, as well as degradation profiles of 3D scaffolds in periodontal applications are needed. The effect of 3D scaffolds on "blood clot stabilization" should be assessed, as it is an important prognostic factor in alveolar bone regeneration **(Pellegrini et al, 2013)** and scaffolds should also be tested as a part of a complete tissue regeneration protocol, in combination with new

techniques in soft tissue management which is the key for optimum regenerative outcomes **(Asa'ad et al, 2016).** Due to the existing limitations of scaffold fabrication techniques, investigations of technique combination must be assessed as an acceptable modality for producing scaffolds with clear-cut scales on different levels. As scaffold stabilization represents an important factor in preventing micro-motion and compromised regeneration outcomes, different stabilization techniques should be investigated as well (press-fit graft, fibrin glue); fixation with screws and pins especially in large defects might compromise the scaffold integrity.

3.3. CONCLUSIONS

Scaffolding matrices are an attractive alternative to bone replacement grafts in surgical procedures related to endosseous implant placement, i.e. vertical and/or horizontal bone augmentation, socket preservation and sinus augmentation. Scaffolding matrices can also be used as a membrane and grafting material in periodontal tissue regeneration. A scaffold should be biocompatible, biodegradable, bioactive and made of a hybrid of biomaterials, as the combination of different biomaterials is superior to a pure material, mechanically and biologically. Nonetheless, it still unknown which combination of materials is optimal for alveolar bone regeneration. Much work lies ahead to translate the promising results of pre-clinical studies into clinical reality.

3.4. ACKNOWLEDGEMENTS

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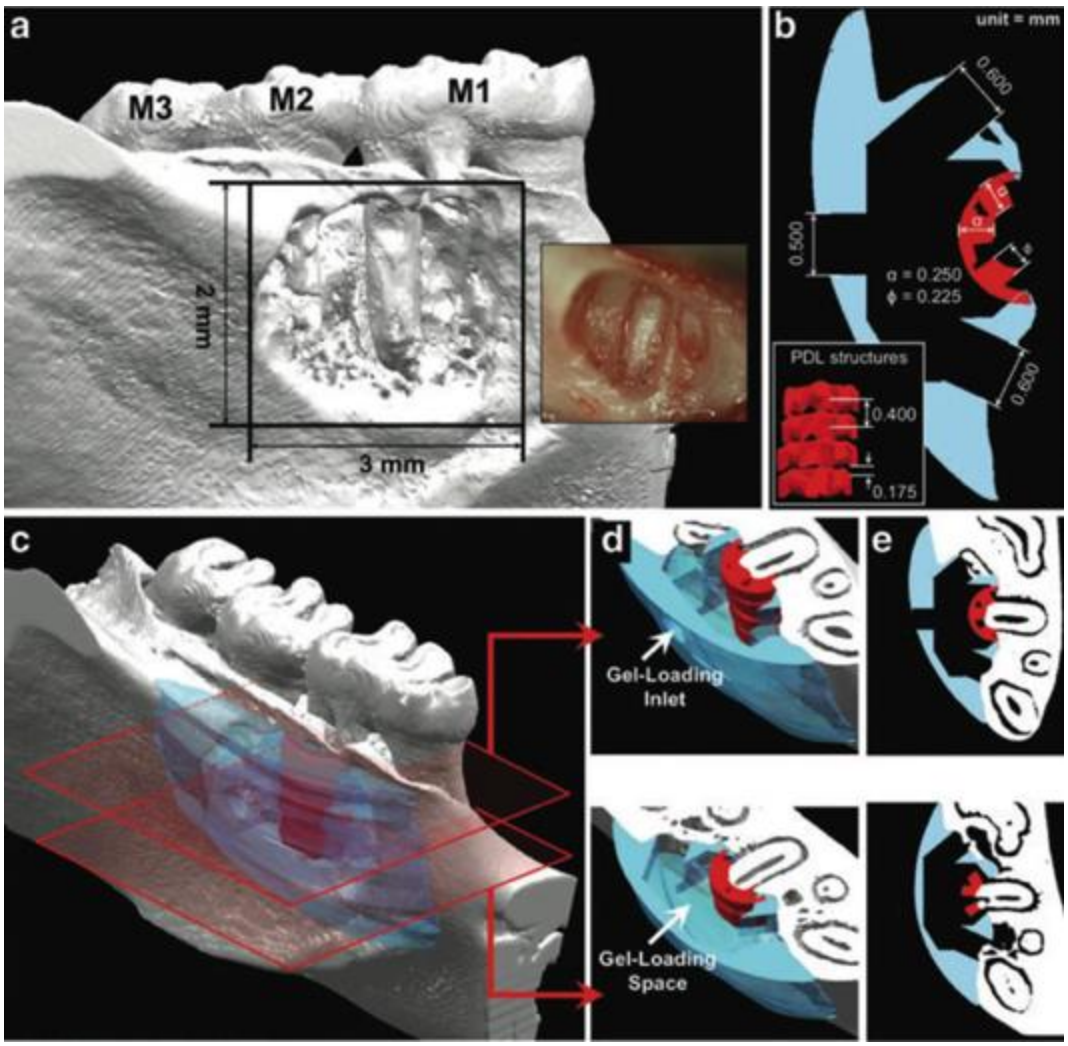
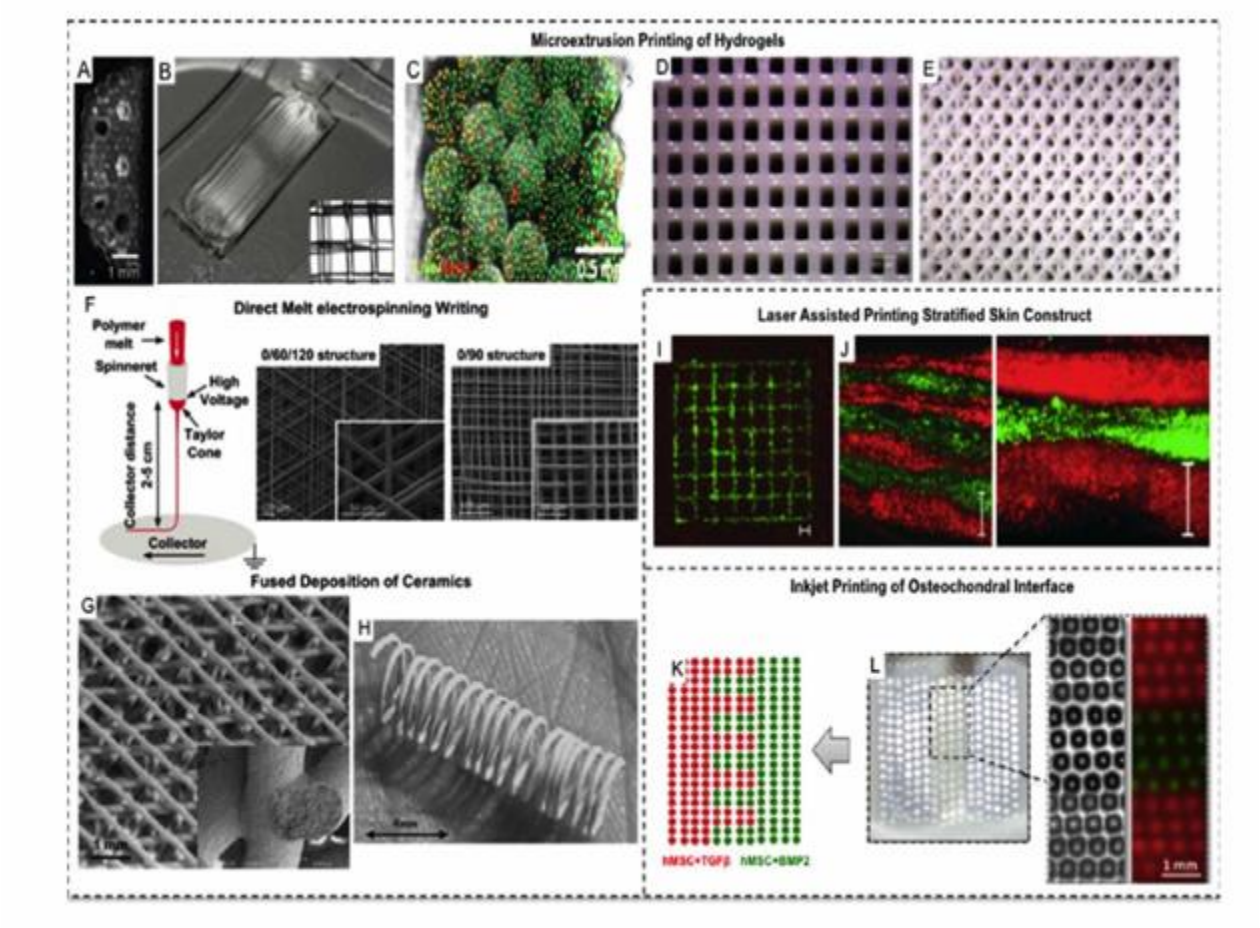
Figure (1): Multiphasic scaffold aimed at multiple tissue regeneration (periodontal ligament, cementum & alveolar bone)



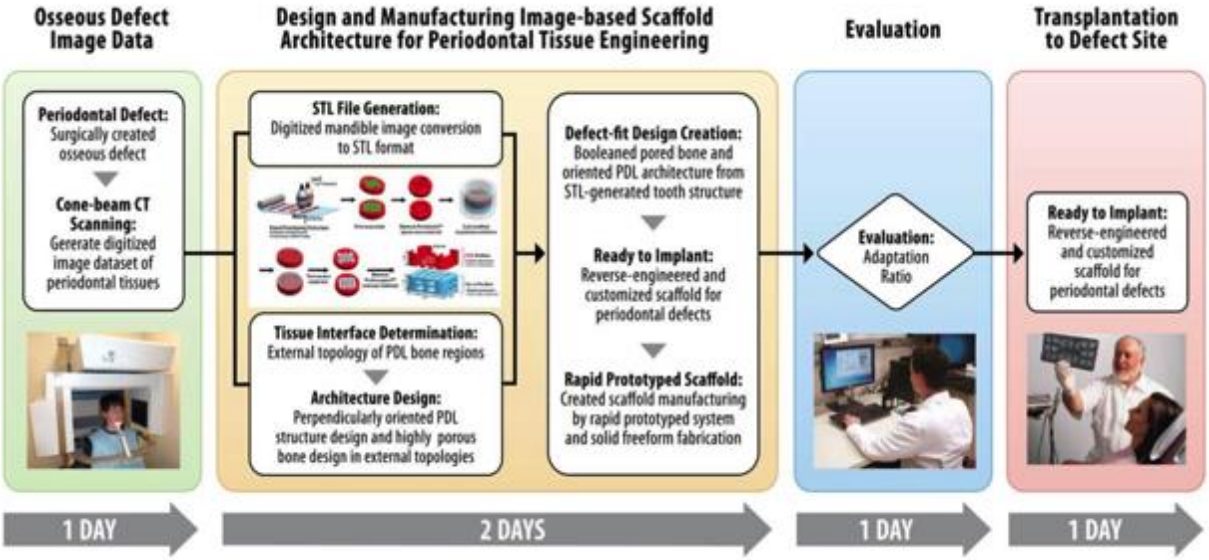
"*Courtesy of Park et al, 2012"*

Figure (2): Different 3D printing methods used to manufacture 3D scaffolds for various applications



"Courtesy of Obregon et al, 2015"

Figure (3): CAD models are produced based on computed tomography (CT) scans of a patient-specific bone defect to develop a custom-made bone graft substitute



"Courtesy of Park et al, 2014 a"

Figure (4): Custom-made 3D printed PCL/HAp scaffold based on images from computed tomography (CT) scans & combined with CAD/CAM technologies for periodontal tissue regeneration

"Courtesy of Rasperini et al, 2015"

CHAPTER 4

Analysis of Alveolar Ridge Atrophy & the Corresponding Digitally Designed Bone Grafts in Posterior Mandibles using Cone Beam Computed Tomography (CBCT) Scans

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4.1. ABSTRACT

OBJECTIVES: To analyze bone resorption patterns in the posterior mandibles and their corresponding digitally designed bone grafts to understand if they come in distinct clusters.

MATERIALS & METHODS: In this retrospective analysis, 120 CBCT scans were analyzed to evaluate the frequency of Cawood & Howell classification, in right and left posterior mandibles. Results were compared between gender and age. The most frequent atrophic class that needs bone augmentation was virtually regenerated in the mandibular segments using specific software. Height, width and length of the obtained grafts were analyzed to conclude if these grafts come in distinct clusters.

RESULTS: Class V was the most frequent atrophic class in comparison to class IV & VI in the left and right posterior mandibles (16%, 20.8% respectively). Severe atrophic stages were more frequent in females ($p= 0.029$ for the left side, $p= 0.007$ for the right side) and in older age groups ($p= 0.008$ for the right side) After virtual regeneration of class V defects, three clusters were evident, differing only in length, based on the number of missing teeth ($p= 0.0001$). Height and width of the virtual grafts were comparable for the three clusters ($p > 0.05$). Mean virtual graft volume was 2,184 mm^3 (four missing teeth), 1,819 mm^3 (three missing teeth) & 1,476 mm^3 (two missing teeth).

CONCLUSIONS: Stage V atrophy was the most frequent resorption pattern in comparison to classes IV & V, in posterior mandibles. Virtual regeneration procedure revealed three clusters of virtual grafts, differing only in the length based on the number of missing teeth. Future studies are recommended to determine the adaptation ratio between virtual and actual grafts to bone surface.

KEYWORDS: mandible, alveolar bone grafting, bone graft, scaffold, cone-beam computed tomography

4.2. INTRODUCTION

To ensure a successful dental implant therapy on the long-term, presence of adequate amount of vertical and horizontal alveolar bone is fundamental **(Javed et al, 2013)**, therefore, alveolar bone augmentation procedures are performed whenever inadequate alveolar bone volume is encountered.

Bone grafting materials utilized for this purpose display alternating properties of space-maintenance, blood-clot stabilization and scaffolding **(Pellegrini et al, 2013),** by serving the role of a temporary matrix to support migration of cells from the periphery of the grafted area **(Pagni et al, 2012).**

Bone grafting materials are divided into: autografts, allografts, xenografts and alloplasts, each having its own set of advantages and disadvantages **(Oryan et al, 2014).** For example, the latter three are brittle, poorly processable into porous forms with incapability to generate patient-tailored structures and are unable to maintain the desired generated tissue volume under mechanical forces **(Pagni et al, 2012).** As for autografts, they are difficult to shape and conform to a bony defect **(Damien & Parsons, 1991),** which is of significant concern in the craniofacial region, despite their ability to withstand mechanical forces.

In consequence, researchers are constantly exploring new bone graft substitutes with more predictable regenerative outcomes. In particular, scaffolds that possess three-dimensional (3D) architectures, which closely mimic native extra-cellular matrix (ECM), have been the subjects of interest. With the recent development of solid-freeform fabrication (SFF) techniques, creation of scaffolds with complex architecture has become achievable **(Moroni et al, 2006).** Such systems utilize computer-aided design (CAD) and computer-assisted manufacturing (CAM) technologies to 3D print a desired structure based on a CAD file that has already determined the scaffold dimensions **(Yeong et al, 2004).** In a typical clinical case scenario, CAD models are produced based on images from computed tomography (CT) scans

of a patient-specific bone defect to develop a custom-made scaffold to regenerate defects with complex geometry **(Ma, 2008),** for review, see **(Asa'ad et al, 2016).**

Nonetheless, creating a "customized" scaffold/bone-graft substitute for every clinical case could be of a very high cost, mainly due to the required armamentarium and set-up. In this regard, providing standardized "pre-fabricated" scaffolds that can be applied in most clinical case scenarios with almost none or minimal modification might be a more cost-effective alternative. As a first step in this direction, frequency analysis of bone resorption patterns and their corresponding digitally designed grafts might be plausible. Such analysis could provide better understanding if the bone resorption patterns come in distinct clusters that allows the production of standardized scaffolds with specific dimensions that can be applied in the majority of clinical cases. This concept was previously investigated by **Metzger & colleagues (2007),** in which the topographical anatomy of the human orbital floor was evaluated for the production of pre-fabricated implants on the base of data obtained from conventional computed tomography (CT).

The aim of the present retrospective study was to analyze bone resorption patterns in the right and left posterior mandible and the corresponding digitally designed bone grafts to evaluate if they come in distinct clusters.

We chose to only evaluate the posterior mandibles because the rehabilitation of this edentulous area with dental implants is very challenging to clinicians in modern dental practice **(Laino et al, 2014).** We also evaluated the severity of Cawood & Howell class in relation to age and gender.

4.3. MATERIALS & METHODS

4.3.1. CBCT Scans & Inclusion Criteria

In this retrospective study, cone beam computed tomography (CBCT) scans from the database of a private dental practice in Como, Italy were selected for frequency analysis of bone resorption patterns and subsequent digital design of the corresponding bone graft.

From the whole database of CBCT scans dated from 2011 until 2016, 245 scans of patients that needed pre-implant bone augmentation in all sextants of the jaws were evaluated. From the 245 scans, 120 scans were selected for the final analysis, as they were of patients that needed bone augmentation procedures in the posterior mandible area. Scan selection was done by the same investigator (F.A) during the period from April- May 2016. As a routine protocol, all patients signed an informed consent agreeing to the use of patients' data for scientific purposes.

The final CBCT scans fit the following inclusion criteria:

1. Scans should be of patients of 35 years of age or greater
2. Scans should be of patients without any reported systemic diseases that affect bone (e.g. osteoporosis), as verified through patient records
3. At least one side of the posterior mandible should be either partially or fully edentulous
4. For the edentulous area to be considered the analysis, it should demonstrate at least two consecutive posterior missing teeth, one of them a molar, as the following: (i) missing first and second molars (ii) missing first, second molars and second premolar, (iii) missing first, second molars & first, second premolars
5. Alveolar bone resorption should be physiological following tooth loss/extraction and not related to trauma or any pathologies, as verified through patient records

The exclusion criteria were the following:

1. Patients who had systemic diseases that would affect the alveolar bone, e.g. osteoporosis
2. Sole presence of edentulous maxillary sextants, as the upper jaw was not the region of interest of this retrospective study

For the final 120 scans, each one included at least one side of the mandible that fit the inclusion criteria for analysis. Afterwards, the contralateral mandibular side was assessed as well; if it fit the inclusion criteria, it was also analyzed, if not, that segment was excluded from the final analysis.

The following "secondary" exclusion criteria were used to eliminate the contralateral segment from the final analysis:

1. Non-consecutive missing posterior teeth
2. One missing posterior tooth
3. Two missing premolars
4. Bounded saddle edentulous ridge
5. The edentulous area was already restored with dental implants

However, it must be noted that in this case, the segment and not the scan was excluded from the overall frequency analysis.

4.3.2. Analysis of Mandibular Bone Resorption Patterns on CBCT Scans

The pattern of the mandibular bone loss was assessed using the classification proposed by **Cawood & Howell classification (1988**), by the same examiner (F.A). This classification system is among the most widely used to categorize edentulous ridges **(Rossetti et al, 2010).** The ridge displays a specific shape during different phases of bone resorption *(figure 1)* that can be clearly identified on CBCT scans, as shown in *(figure 2)* **(Saavedra-Abril et al, 2010).** Cawood & Howell (C&H) classification divides the posterior mandible into six groups as the following:

Class I: dentate

Class II: immediately post extraction

Class III: adequate height and width

Class IV: knife-edge ridge with adequate height and inadequate width

Class V: inadequate height & width

Class VI: depressed ridge form with some loss of basal bone.

In case a mixed classification was present in the analyzed area, the worst classification was registered. Frequency analysis of bone resorption patterns was done by importing DICOM files into OS3D 2.0 software (3DMed, L'Aquila, Italy; www. 3dmed.it). Frequency analysis of bone resorption pattern and the number of missing teeth were compared for age and gender.

4.3.3. Digital Design of the Bone Graft & Virtual Bone Regeneration

Digital design of virtual bone grafts was performed after the frequency analysis was completed. This step was only done for the "most frequent" class of the Cawood & Howell classification in which bone augmentation is required (i.e. the most frequent class among classes IV, V & VI).

By means of an image software (OS3D 2.0, 3DMed, L'Aquila, Italy) the digital data was processed to obtain a 3D image of the bone loss and a virtual graft was designed, simulating a real bone grafting procedure *(figure 3)* as described by **Jacotti and colleagues 2014.** The used software allowed for the determination of length, height and width of each graft. The software also allowed for the verification of intimate adaptation between the virtual graft and bone surface. As a guide for virtual bone regeneration procedure, residual bone height above the mandibular canal was measured, then the "height" of the virtual graft was determined as the height that would allow for a standard height implant to be placed (10 mm) with a 2 mm safety zone above the mandibular canal *(figure 4).* Regarding the "width" of the virtual graft,

it was determined as the width that would accommodate a dental implant of 3.25 mm diameter. As for the virtual graft "length", it was based on the number of the consecutive posterior missing teeth. The 3D planning software allowed for dental implant placement, subsequent virtual bone regeneration and verification of the graft dimension/adaptation *(figure 5).*

4.3.4. Statistical Analysis

The Kruskal-Wallis and Chi-squared tests were used to evaluate the distribution of C&H classes and number of missing teeth in relation to age and gender. Random effects multivariate linear regression models were fitted to evaluate the joint effect of gender, age category and side on the number of missing teeth and to compare length, height and width of virtual grafts **(Rabe-Hesketh et al, 2008).**

Statistical analyses were performed with Stata 13 (StataCorp. 2013)**.** Level of statistical significance was set at $P < 0.05$.

4.4. RESULTS

A total of 120 patients contributed to 240 posterior mandibular segments. A total of 38 mandibular segments were excluded by "secondary exclusion criteria" (14 in the left mandible and 24 in the right mandible). The overall analyzed right and left mandibular segments were 202; 106 in the left mandible and 96 in the right mandible *(figure 6).* Study participants were 47 males and 73 females with an age range between 37 & 92 years old (mean age= 66.2 ± 11.2) *(table 1).*

The study sample was divided into two age groups: < 65 years old and ≥ 65 years old. Most of the study participants were of the second age category (63.3%). For the purpose of this study, we will focus on the results of Cawood & Howell classes that require alveolar bone augmentation (i.e. classes IV, V & VI).

Frequency of Cawood & Howell classes that require bone augmentation in left and right posterior mandibles. Among all participants, the most common atrophic C&H class requiring bone augmentation was class V, in both left (16%) and right (20.8%) mandibles. Class IV occurred in almost 10% of both sides. Class VI was the least frequent C&H class that needed augmentation (3.8% in the left mandible, 5.2% in the right mandible) *(table 2).*

Frequency of Cawood & Howell classes that require bone augmentation in left and right posterior mandibles, in correlation to gender. Females showed higher frequencies of classes IV, V & VI in comparison to males. In fact, 81.8% of class IV, 88.2% of class V, 75% of class VI were found in females in the left mandible, and 70%, 90%, 80% of these classes were found in the right mandible in females, respectively. These results were statistically significant (p value = 0.029 for the left side, p value = 0.007 for the right side) *(table 2).*

Frequency of Cawood & Howell (C&H) classes that require bone augmentation in the left and right posterior mandibles, in correlation to age. Both age categories showed comparable frequencies of classes IV, V & VI in the left mandible (p value > 0.05). However,

patients that were of ≥ 65 years of age had these classes more frequently in the right mandible than in younger patients (12.1% of old patients had class IV, 24.1% had class V & 8.6% had class IV, compared to 7.9% of younger patients having class IV, 15.8% having class V and none having class VI). These results were statistically significant (p value = 0.008) *(table 3).*

Number of consecutive missing posterior teeth in the left and right posterior mandibles, in correlation to gender. In the left mandible, most of the females had either three or four teeth missing (33.8% & 35.4 respectively), while most of the males had three missing teeth (41.5%). These findings were not statistically significant (p value > 0.05). Similar observation and comparative percentages were reported for the number of missing teeth in the right mandible in correlation to gender (p value > 0.05) *(table 4).*

Number of missing teeth in the left and right mandibles, in correlation to age. In the left mandible, almost 37% of study participants who were 65 years or older missed either three or four posterior teeth. On the other hand, younger patients were the least to have four missing teeth, almost by half of that reported in older participants (15%). These results were statistically significant (p = 0.047). Comparable results were also reported for the right side of the mandible (p = 0.006) *(table 5).*

Number of missing teeth in the left and right mandibles in correlation to Cawood & Howell class that requires bone augmentation. Classes IV & V were mostly associated with four missing teeth in the left mandible; 63.6% of class IV and 47.1% of class V cases had four missing teeth, while 50% of class VI was associated with either three or four missing teeth. In the right mandible, 50% of class IV, 50% of class V and 60% of class VI were associated with four missing teeth. These findings were very statistically significant for the right and left mandibles (p= 0.001) *(table 6).*

Results of multivariate linear regression model. Analysis of the joint effect of gender, age category and side on the number of missing teeth, using multivariate linear regression model,

showed that age was an important factor in influencing the number of missing teeth, while gender and side were not as important (p= 0.0001).

As Cawood & Howell class V was the most frequent among classes that require bone augmentation, virtual bone regeneration was performed through digitally designed bone grafts, using specific software. Among the 202 analyzed mandibular segments, 37 segments were of class V Cawood & Howell. Virtual bone regeneration was performed for only 36 segments. One right mandibular segment was excluded from the virtual regenerative procedure due to technical difficulties encountered with the provided CBCT scan of the patient, which did not allow for the procedure to be successfully performed *(figure 7).*

Table (7) shows the height, length & width of the digitally designed grafts of the 36 mandibular segments that had class V atrophic resorption.

Regarding the **"length"** of the virtual graft, the mean was 20 ± 0.6 mm when two teeth were missing (range = 19 - 21 mm). When three teeth were missing, the length mean was 23.9 ± 0.6 mm (range = 23 - 25 mm). In case both premolars and both molars were missing, the length mean was 29.6 ± 0.7 mm (range = 28 - 30 mm). The differences in mean length based on the number of missing teeth was very statistically significant (p= 0.0001).

As for the **"width"** of the virtual graft, it was almost comparable when two (mean = 8.2 ± 0.4), three (8.1 ± 0.3) or four teeth (8.2 ± 0.4) were missing (p > 0.05).

In regards to the **"height"** of the virtual graft, this dimension was also comparable when two (9 ± 0.9), three (9.4 ± 1.2) or four teeth were missing (9 ± 0.8) (p > 0.05).

4.5. DISCUSSION

To the best of our knowledge, this is the first retrospective study to analyze the frequency of Cawood & Howell classes and virtually regenerate the most frequent atrophic class in this classification (i.e. classes that are indicated for bone augmentation procedures). Since there are inconsistent conclusions in literature about the severity of bone resorption in relation to gender, we chose to compare the frequency of Cawood & Howell class between males and females as well. We chose to use this classification **(Cawood & Howell, 1988)** in this retrospective study because it is among the most used to categorize edentulous ridges **(Rossetti et al, 2010)** and the ridge shapes of each class can be easily identified on CBCT scans **(Saavedra-Abril et al, 2010).** Up to date, there is only one study in literature that assessed the frequency of Cawood & Howell atrophic stages. However, this investigation was in a historic nation, and only evaluated the association between age and frequency/severity of atrophy.

Findings of the present study suggest that females show more severe atrophic stage than males. These findings are consistent to what has been previously suggested in literature. **Solar & colleagues (1994)** reported that female gender was an independent risk factor for more severe bone resorption in the mandible. In another study, it was reported that female gender is a risk factor for a greater bone resorption in the posterior mandible **(Kordatzis et al, 2003),** however, this study investigated patients wearing conventional dentures and implant over-dentures. It has been suggested that females have a deeper resorption lacunae, which could explain why they tend to have more severe bone resorption than males **(Devlin et al, 1994).** In another investigation, females showed more severe resorption due to lower bone mineral content of the mandible in young dentate women when compared to young dentate men **(Von Wowern, 1988)** and as known, the less highly mineralized a substrate, the more easily it may be resorbed **(Jones et al, 1995).** These findings were however contradictory to what has been published by

Winter & colleagues (1974), in which males showed more bone loss in the posterior mandible due to the greater biting force.

In the present retrospective analysis, the most frequent Cawood & Howell class was class III in general, but since this study has a specific scope on bone augmentation, we chose to focus on the Cawood & Hawood atrophic classes that represent inadequate alveolar bone volume and thus require bone augmentation procedures, i.e. classes IV, V & VI.

In the present study, class V was the most frequent among the three atrophic classes in all study participants (16% in the left mandible & 20.8% in the right mandible), while class VI was the least frequent. In a retrospective analysis of a historic nation, atrophy stages V & VI were both the most frequent in the older age groups **(Reich et al, 2011).** Since the population of the present retrospective analysis is not historic, the negligible frequency of stage VI frequency can be justified by patients seeking dental treatment at some point before bone resorption progresses to basal bone. As expected, older age group (≥ 65 years) in the current retrospective analysis showed a more severe Cawood & Howell class of resorption in comparison to younger age group (< 65 years). Similar results were reported in historic nation, in which the severity of bone resorption was associated with the individual's age **(Reich et al, 2011).**

As class V was the most frequent among the atrophic classes that require bone augmentation, we performed virtual bone regeneration using specific software to digitally design bone grafts, guided by virtual implant placement, to ensure that the virtual graft dimensions were correct. Based on the analysis of the dimensions of these grafts, three clusters were notable; graft length of 21, 25 and 30 mm based on the number of missing teeth. In these clusters, graft height and width were almost comparable (9 mm and 8 mm respectively). Although we could propose that a pre-fabricated bone graft might be practical to be applied in most case scenarios, this approach might be mostly applicable in females and older age group.

Whether the pre-fabricated scaffold would need a minimum, high or negligible modification at all, is yet to be confirmed in a future study, by calculating and comparing the adaptation ratio of both; virtual and actual grafts. It is has been suggested that chair-side graft shaping and modification highly increase the risk of contamination, subsequent infection that could eventually compromise the outcomes of the bone regeneration procedures **(Jacotti et al, 2014).**

Despite these conclusions, the results of this retrospective study must be interpreted with caution as it has certain limitations. The inclusion criteria of the analyzed CBCT scans were developed for scaffold utilization purposes, thus findings of this study cannot be generalized. The frequency analysis might represent an overestimation of the real situation because the worst Cawood & Howell was registered in case the ridge resorption represented a combination of two different classes. As this is a pilot study, only areas with free end saddle were evaluated, thus excluding bounded saddle areas made of a missing second premolar and missing first molar. Moreover, virtual bone augmentation procedure was guided by virtual placement of dental implants with a standard length (10 mm) and narrow diameter (3.25 mm) and did not take the possibility of utilizing wide-diameter and/or short dental implants in the posterior mandibles into account.

Although we suggested three different volumes of bone grafts based on the number of missing teeth, this aspect represents just one component of the whole clinical paradigm since case management is influenced by various factors that must be taken into consideration; the patient's socio-economic status, application of short dental arch concept, length, diameter, number of dental implants & the utilization of removable prosthesis instead of implant therapy. Therefore, personalized approach is still the best perspective for clinical case management.

In this context, a personalized regenerative approach by utilizing a suitable soft tissue self-inflating expander and its corresponding scaffold volume for each individual patient might seem viable. In fact, the obtained virtual graft volumes in this retrospective study might be

correlated to the available soft tissue expander volumes. For example, the obtained mean virtual graft volume when four posterior mandibular teeth were missing was 2,184.5 mm^3. This corresponds to the 2.1 ml (2,100 mm^3) expander. Therefore, utilizing an expander with this specific volume and its corresponding scaffold volume might seem applicable in a class V atrophic edentulous posterior mandibular area of four missing teeth.

Mean virtual graft volume when three posterior mandibular teeth were missing was 1,819.7 mm^3. The matching soft tissue expander volume in this case might be either 1.3 or 2.1 ml expanders. In case of two missing teeth, the mean virtual graft volume was 1,476 mm^3, which suggests that an expander of 1.3 ml final volume would be the most suitable in this clinical situation. However, future studies focused on soft tissue expanders and their corresponding graft volumes are needed to confirm these findings.

4.6. CONCLUSIONS

Stage V atrophy was the most common among Cawood & Howell classes that require bone augmentation, i.e. inadequate height and inadequate width. Females and older age group showed more severe atrophic stages than males and younger age group. Virtual regeneration of class V defects suggested the possibility of the existence of three clusters of bone grafts, depending on the number of missing teeth. Further studies are needed to evaluate the adaptation ratio between the virtual and actual grafts, to conclude if the grafts need minor or major shaping and modification at chair-side before clinical application.

4.7. ACKNOWLEDGEMENTS

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Figure (1): Cawood & Howell classification of the posterior mandible

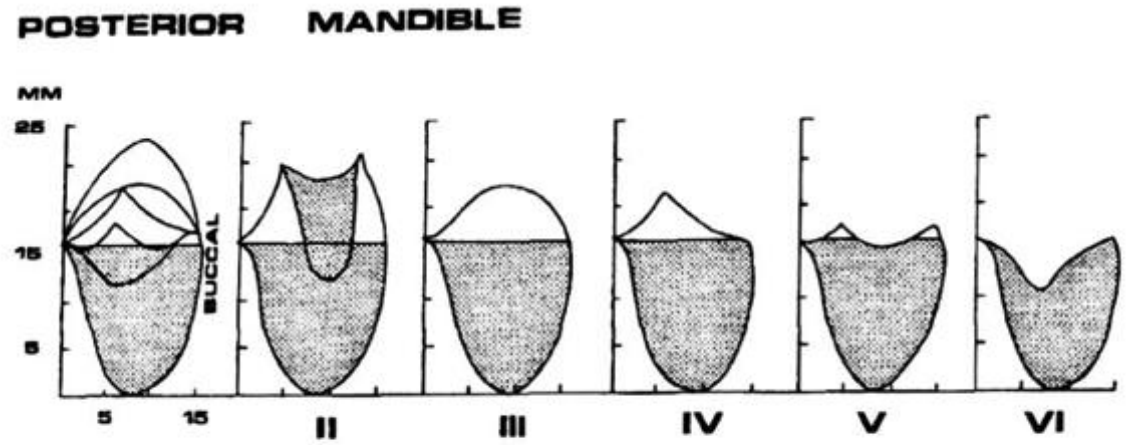
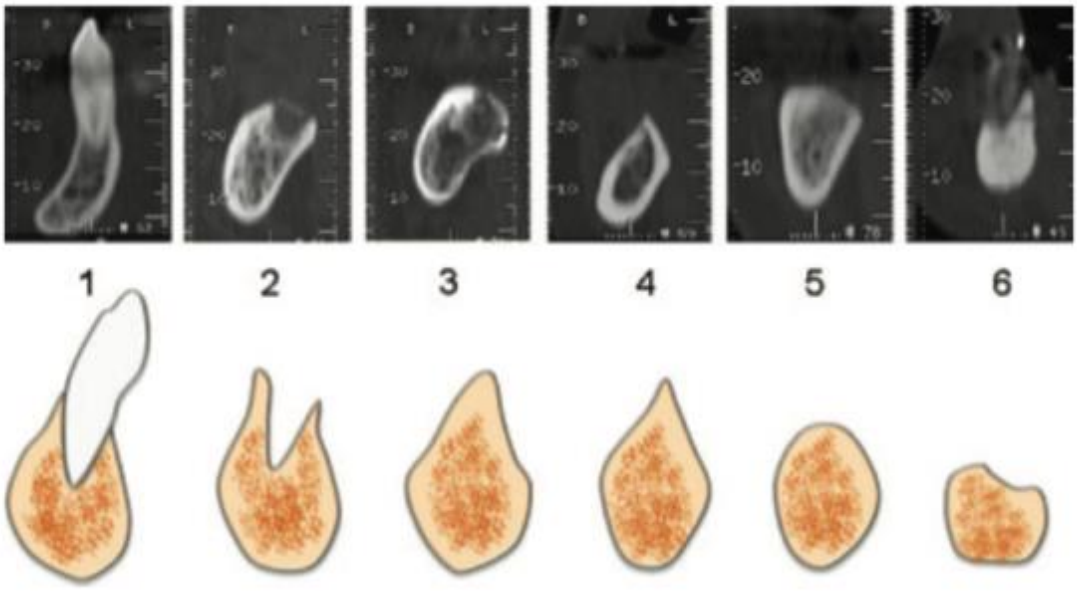


Fig. 5. (A) Classification of anterior mandible (anterior to mental foramina). (B) Classification of posterior mandible (posterior to mental foramina).

"Courtesy of Cawood & Howell, 1988"

Figure (2): Appearance of different classes of Cawood & Howell classification on cone beam computed tomography (CBCT) scans



"Courtesy of Saavedra-Abril et al, 2010"

Figure (3): Creation and positioning of virtual bone grafts, using OS3D 2.0 software

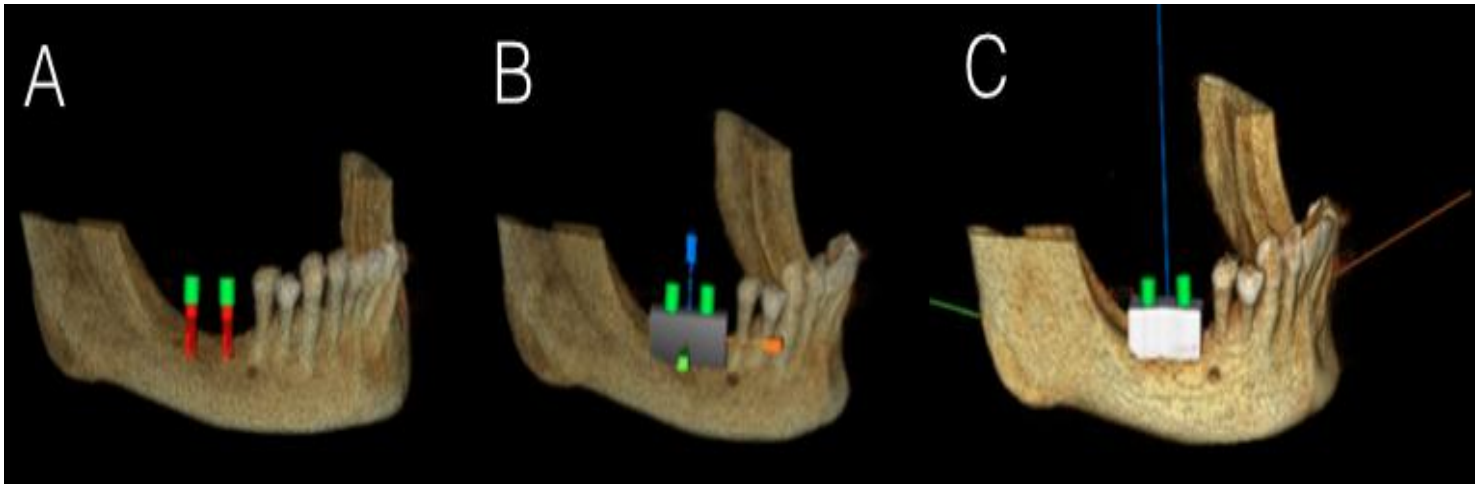


Figure (4): Virtual placement of dental implants using the software as guidance for virtual graft reconstruction

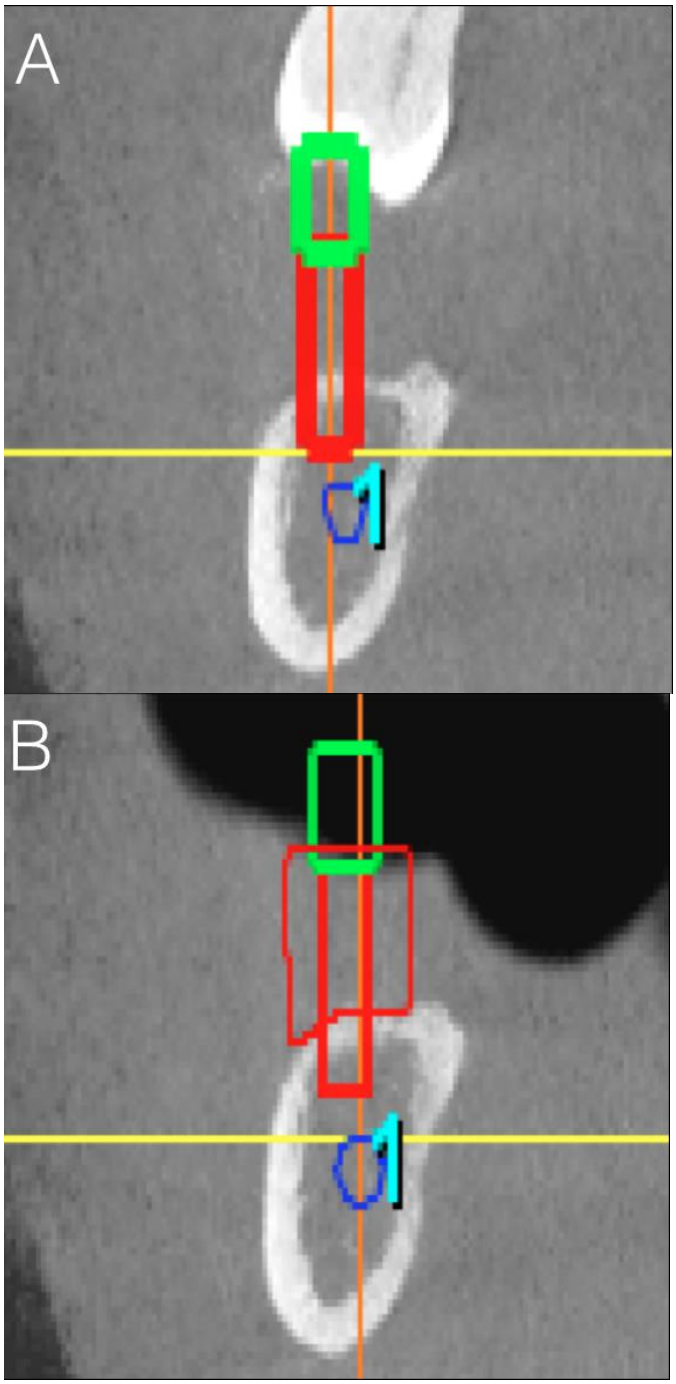


Figure (5): The 3D planning software allowed for dental implant placement, subsequent virtual bone regeneration and verification of the graft dimension/adaptation

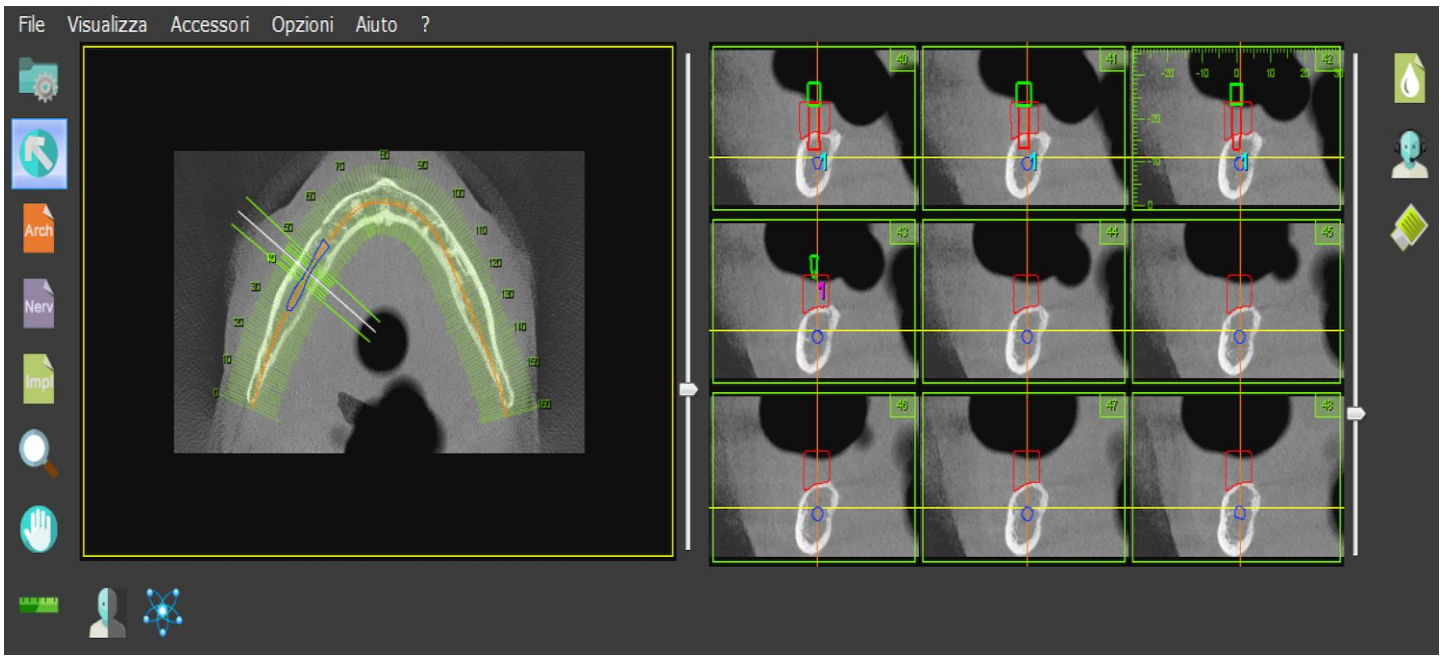


Figure (6): Flowchart of the included CBCT scans

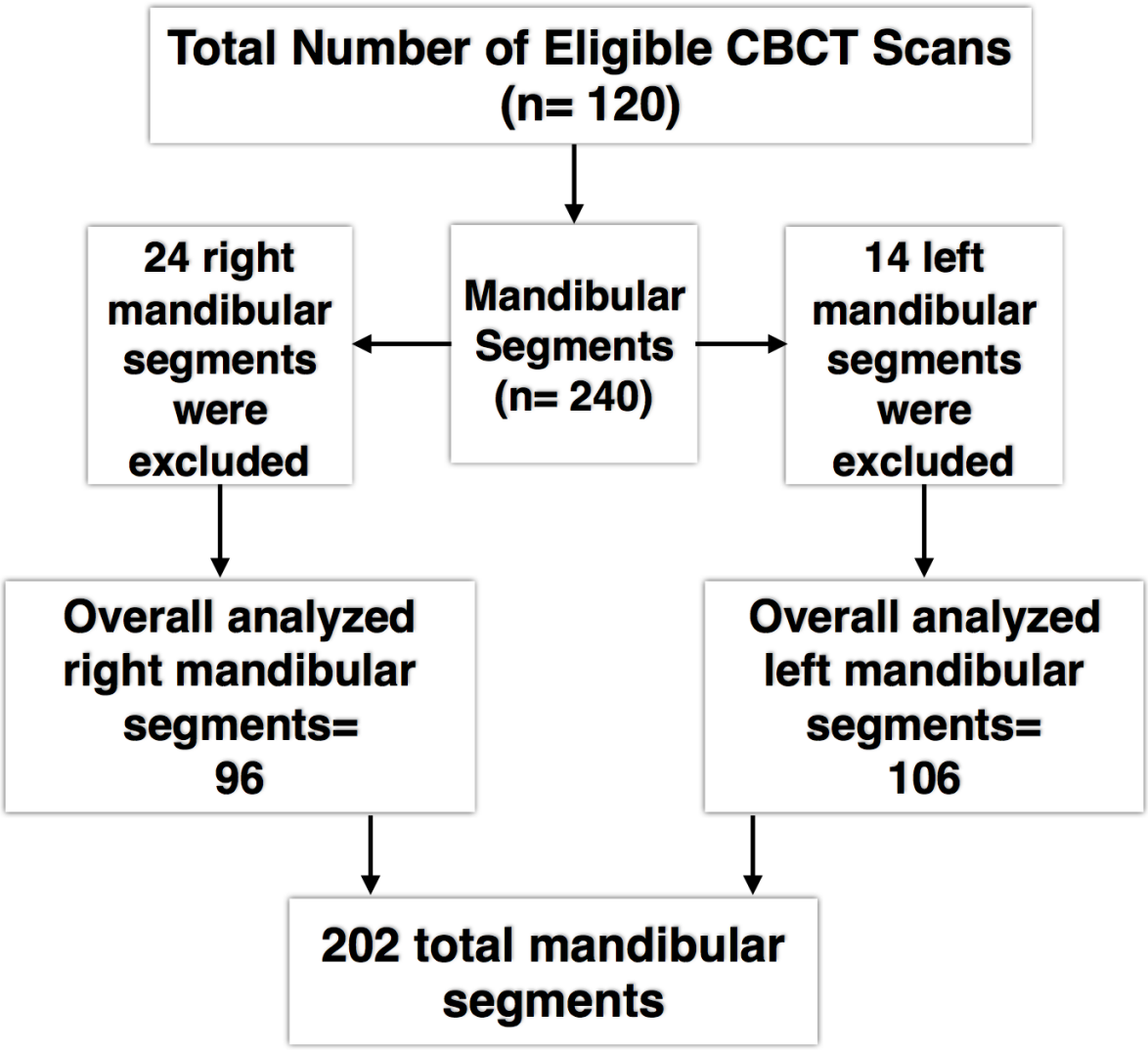


Figure (7): Flowchart of the virtually grafted Cawood & Howell class V mandibular segments

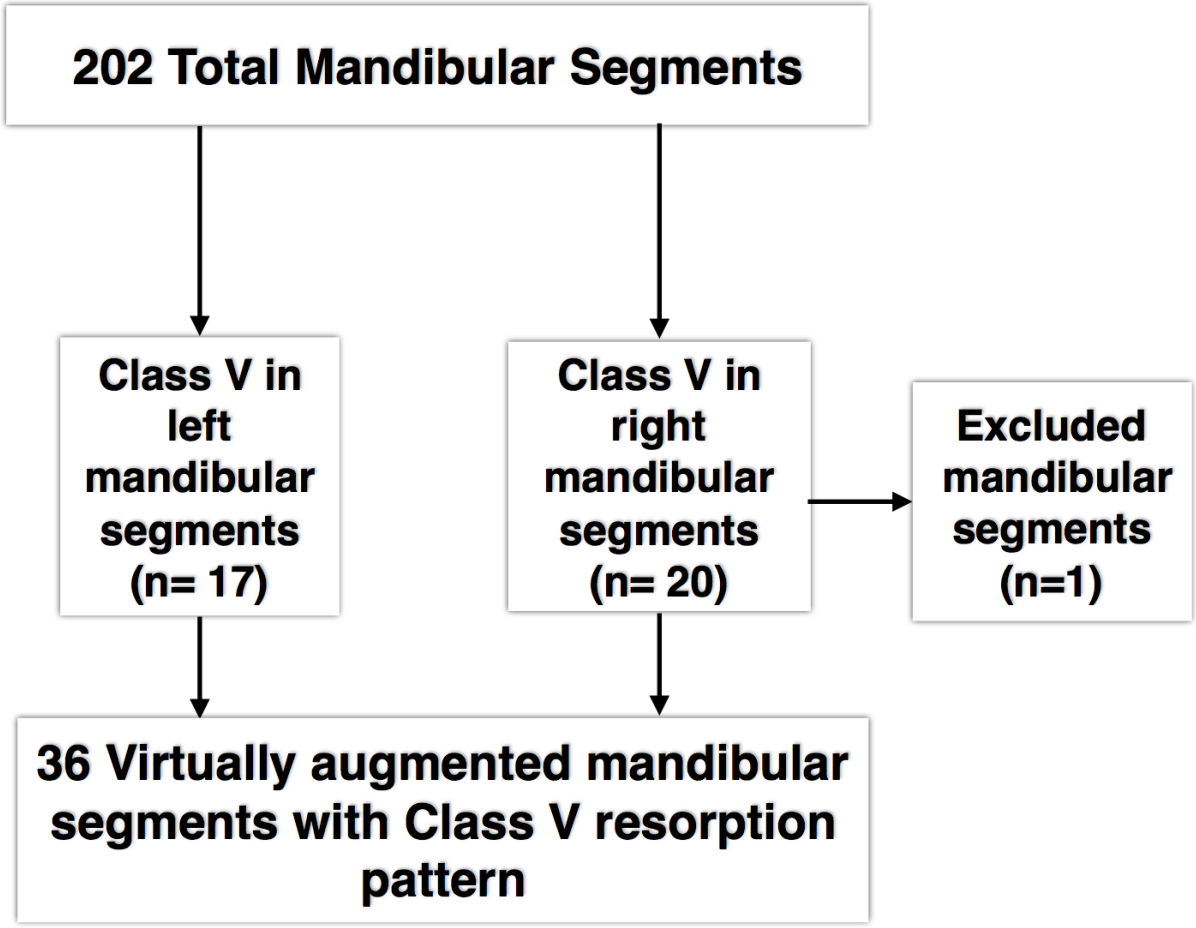


Table (1): Age & gender distribution of the study population

Age Group (Years)	Female n (%)	Male n (%)	Total n (%)
< 65	23 (31.5)	21 (44.7)	**44 (36.7)**
≥ 65	50 (68.5)	26 (55.3)	**76 (63.3)**
Total	**73 (100)**	**47 (100)**	**120 (100)**

Table (2): Frequency of different bone loss patterns in relation to gender, for left and right mandibles

Cawood & Howell (Left Mandible)	Female n (%)	Males n (%)	Total number of mandibular segments n (%)	P-value*
I	7 (10.8)	4 (9.8)	**11 (10.4)**	
II	1 (1.5)	0 (0)	**1 (0.9)**	
III	30 (46.2)	32 (78)	**62 (58.5)**	
IV	9 (13.8)	2 (4.9)	**11 (10.4)**	
V	15 (23.1)	2 (4.9)	**17 (16)**	
VI	3 (4.6)	1 (2.4)	**4 (3.8)**	
Total	**65 (100)**	**41 (100)**	**106 (100)**	**0.029**
Cawood & Howell (Right Mandible)				
I	7 (12.1)	3 (7.9)	**10 (10.4)**	
II	0 (0)	1 (2.6)	**1 (1)**	
III	22 (37.9)	28 (73.7)	**50 (52.1)**	
IV	7 (12.1)	3 (7.9)	**10 (10.4)**	
V	18 (31)	2 (5.3)	**20 (20.8)**	
VI	4 (6.9)	1 (2.6)	**5 (5.2)**	
Total	**58 (100)**	**38 (100)**	**96 (100)**	**0.007**

***** From Chi-squared test

Table (3): Frequency of different loss pattern in relation to age category, for left and right mandibles

Cawood & Howell (Left Mandible)	Age Category (< 65 years) n (%)	Age Category (≥ 65 years) n (%)	Total n (%)	P-value*
I	6 (15)	5 (7.6)	**11 (10.4)**	
II	0 (0)	1 (1.5)	**1 (0.9)**	
III	25 (62.5)	37 (56.1)	**62 (58.5)**	
IV	4 (10)	7 (10.6)	**11 (10.4)**	
V	5 (12.5)	12 (18.2)	**17 (16)**	
VI	0 (0)	4 (6.1)	**4 (3.8)**	
Total	**40 (100)**	**66 (100)**	**106 (100)**	**0.411**
Cawood & Howell (Right Mandible)				
I	9 (23.7)	1 (1.7)	**10 (10.4)**	
II	0 (0)	1 (1.7)	**1 (1)**	
III	20 (52.6)	30 (51.7)	**50 (52.1)**	
IV	3 (7.9)	7 (12.1)	**10 (10.4)**	
V	6 (15.8)	14 (24.1)	**20 (20.8)**	
VI	0 (0)	5 (8.6)	**5 (5.2)**	
Total	**38 (100)**	**58 (100)**	**96 (100)**	**0.008**

***** From Chi-squared test

Table (4): Number of missing teeth in relation to gender, for left and right mandibles

Number of consecutive missing teeth (Left Mandible)	Female n (%)	Males n (%)	Total n (%)	P-value*
0	7 (10.8)	4 (9.6)	**11 (10.4)**	
2	13 (20)	12 (29.3)	**25 (23.6)**	
3	22 (33.8)	17 (41.5)	**39 (36.8)**	
4	23 (35.4)	8 (19.5)	**31 (29.2)**	
Total	**65 (100)**	**41 (100)**	**106 (100)**	**0.32**
Number of consecutive missing teeth (Right Mandible)				
0	7 (12.1)	3 (7.9)	**10 (10.4)**	
2	13 (22.4)	12 (31.6)	**25 (26)**	
3	18 (31)	15 (39.5)	**33 (34.4)**	
4	20 (34.5)	8 (21)	**28 (29.2)**	
Total	**58 (100)**	**38 (100)**	**96 (100)**	**0.39**

* Chi-squared test was used to the number of missing teeth in relation to gender

Table (5): Number of missing teeth in relation to age category, for left and right mandibles

Number of consecutive missing teeth (Left Mandible)	Age Category (< 65 years) n (%)	Age Category (≥ 65 years) n (%)	Total n (%)	P-value*
0	6 (15)	5 (7.6)	**11 (10.4)**	
2	13 (32.5)	12 (18.2)	**25 (23.6)**	
3	15 (37.5)	24 (36.4)	**39 (36.8)**	
4	6 (15)	25 (37.9)	**31 (29.3)**	
Total	**40 (100)**	**66 (100)**	**106 (100)**	**0.047**
Number of consecutive missing teeth (Right Mandible)				
0	9 (23.7)	1 (1.7)	**10 (10.4)**	
2	10 (26.3)	15 (25.9)	**25 (26)**	
3	11 (28.9)	22 (37.9)	**33 (34.4)**	
4	8 (21.1)	20 (34.5)	**28 (29.2)**	
Total	**38 (100)**	**58 (100)**	**96 (100)**	**0.006**

* Chi-squared test was used to evaluate the distribution of number of missing teeth in relation to age and gender

Table (6): Number of consecutive missing teeth in relation to Cawood & Howell classification, for left and right mandibles

Cawood & Howell Left Mandible	No. of missing teeth = 0 n (%)	No. of missing teeth = 2 n (%)	No. of missing teeth = 3 n (%)	No. of missing teeth = 4 n (%)	Total n (%)	P-value*
I	11 (100)	0 (0)	0 (0)	0 (0)	**11 (10.4)**	
II	0 (0)	0 (0)	1 (2.6)	0 (0)	**1 (0.9)**	
III	0 (0)	20 (80)	28 (71.8)	14 (45.2)	**62 (58.5)**	
IV	0 (0)	1 (4)	3 (7.7)	7 (22.6)	**11 (10.4)**	
V	0 (0)	4 (16)	5 (12.8)	8 (25.8)	**17 (16)**	
VI	0 (0)	0 (0)	2 (5.1)	2 (6.5)	**4 (3.8)**	
Total (%)	**11 (100)**	**25 (100)**	**39 (100)**	**31 (100)**	**106 (100)**	**0.001**
Cawood & Howell Right Mandible						
I	10 (100)	0 (0)	0 (0)	0 (0)	**10 (10.4)**	
II	0 (0)	0 (0)	0 (0)	1 (3.6)	**1 (1)**	
III	0 (0)	20 (80)	21 (63.6)	9 (32.1)	**50 (52.1)**	
IV	0 (0)	2 (8)	3 (9.1)	5 (17.9)	**10 (10.4)**	
V	0 (0)	3 (12)	7 (21.2)	10 (35.7)	**20 (20.8)**	
VI	0 (0)	0 (0)	2 (6.1)	3 (10.7)	**5 (5.2)**	
Total (%)	**10 (100)**	**25 (100)**	**33 (100)**	**28 (100)**	**96 (100)**	**0.001**

* The Kruskal-Wallis test was used to evaluate the distribution of number of missing teeth in relation to C&H classification

Table (7): Height, width and length of virtual grafts of Cawood & Howell class V

Virtual Graft Dimension	Two Missing Teeth	Three Missing Teeth	Four Missing Teeth	P- Value*
Length (mean ± SD) (mm)	20 ± 0.6	23.9 ± 0.6	29.6 ± 0.7	**0.0001**
Height (mean ± SD) (mm)	9 ± 0.9	9.4 ± 1.2	9 ± 0.8	**0.49**
Width (mean ± SD) (mm)	8.2 ± 0.4	8.1 ± 0.3	8.2 ± 0.4	**0.71**
Mean Graft Volume (mm^3)	1,476	1,819	2,184	-

***** The Kruskal-Wallis test was used to evaluate the distribution of number of missing teeth in relation to virtual graft dimension

CONCLUSIONS

Recommendations & Future Directions

Based on the results of this dissertation, a new protocol in alveolar bone regeneration prior to dental implant placement can be proposed: pre-augmentation soft tissue expansion (STE) using self-inflating osmotic expanders followed by bone augmentation with the corresponding pre-fabricated scaffolds made of hybrid biomaterials.

This protocol would make alveolar bone regeneration procedures at hand to many clinicians, without limiting this surgical procedure to only highly skilled surgeons.

Although custom-made scaffolds fall within the concept of personalized medicine, the newly suggested protocol with pre-fabricated scaffolds still complies with this modern approach in treating dental patients. For each patient, the most appropriate expander and its volume corresponding pre-fabricated scaffold are chosen after thorough and comprehensive treatment planning. Despite the fact that pre-fabricated scaffolds might receive chair-side modifications, these adjustments would be minimal. The newly suggested protocol might be even more cost-effective, while still falling within the scope of personalized medicine.

To implement this new protocol into the clinical practice, commercially available pre-fabricated scaffolds are still to be developed. Osmotic expanders are commercially available since 1999 and have been FDA approved since 2001. However, scaffold development might be a bit more challenging due to the many components that need to be tested; scaffold design, scaffold properties and the

constituting biomaterials that are the most appropriate for this specific application. Moreover, studies correlating soft tissue expander volumes and the pre-fabricated scaffold volumes will be then needed in order to confirm the applicability of this personalized regenerative protocol. Therefore, much work still lies ahead before translating this protocol to the dental chair.

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