UNIVERSITÀ DEGLI STUDI DI MILANO

CORSO DI DOTTORATO IN SCIENZE ODONTOSTOMATOLOGICHE
XXXIII CICLO

DIPARTIMENTO DI SCIENZE BIOMEDICHE, CHIRURGICHE E ODONTOIATRICHE

TESI DI DOTTORATO DI RICERCA

"THE REGENERATION OF EDENTULOUS ATROPHIC RIDGES: PROS, CONS, AND EFFECTIVENESS OF THREE DIFFERENT SURGICAL OPTIONS"

MED/28

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A.A.2019-2020

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1 - PRELIMINARY CONSIDERATIONS

During the three-year doctoral period, the candidate developed three research projects which, although different, are all related to one of the most controversial topics of advanced implant dentistry which include procedures to make it possible the use of dental implants also in case of severely atrophic edentulous ridges. During the last decade, a variety of surgical procedures have been proposed, each of them with specific indications, contraindications, advantages and disadvantages. Despite the publication of numerous systematic reviews and meta-analyses focused on this fascinating topic, much controversy is still present between clinicians and researches.
The aims of the PhD candidate have been to focus on three main areas related to the correction of severely atrophic edentulous ridges in order to: 1) update the present knowledge on this particular field thank to a systematic analysis of the available literature; and 2) develop three different research projects specifically dedicated to regeneration/reconstruction procedures.

More in detail, one line of research was focused on the evaluation of medium to long-term results of dental implants placed in severely atrophic jaws reconstructed with autogenous calvarial bone blocks covered with bovine bone mineral and collagen membranes. Seventy-two patients and 330 implants were involved in this retrospective study with a mean follow-up after implant loading of 8 years, ranging from 3 to 19 years.

The second project, similar in structure to the previous one, was designed to evaluate retrospectively the medium and long-term results (with a mean follow-up of 10 years) of implants placed in atrophic edentulous ridges grafted with autogenous mandibular blocks covered with bovine bone mineral and collagen membranes. Seventy-five patients, 82 sites, and 182 implants were involved in this study.

The third line of research allowed the candidate to design a prospective study which involved edentulous and atrophic patients treated with guided bone regeneration using CAD-CAM customized titanium meshes. The effectiveness of digitally customized titanium meshes in association with autologous bone particles and bovine bone mineral for the regeneration of atrophic edentulous sites, and the survival rate of implants placed in the reconstructed areas were evaluated. A histomorphometric analysis of mandibular regenerated bone were also performed.
Forty-one patients, 53 sites, and 106 implants were involved in this study and the follow-up of implants before and after loading ranged from 2 to 23 months (mean: 11 months), and from 1 to 15 months (mean: 6 months), respectively.
This latter research is of particular interest, as the literature supporting this particular technique is scarce, and available data are difficult to be compared, because they have been collected in a non- systematic way and mainly retrospectively.

In all these studies, a dedicated questionnaire, adapted from the OHIP-14 survey, was performed to evaluate patients' satisfaction (patient reported outcome measurements – PROMs) as regards the treatment received.

It was therefore possible to elaborate a discussion of the results on two different levels: the first associated to the specific regenerative / reconstructive technique; the second allowed a comparison among the different treatment solutions.

Thanks to the variety and wideness of parameters analyzed, data collected from these three studies may be very useful for clinicians, researchers, and for the development of future research on this field.

2 - THE SCENARIOS AND CLINICAL IMPLICATIONS

Bone defects of the jaws may generate a series of problems, such as a disharmonic facial contour, and impaired functions, including speech and swallowing. Moreover, the loss of teeth and the supporting alveolar jaw bone can lead to significant impairment of mastication.

After dental extractions, a wound healing cascade takes place in the fresh extraction socket, involving both hard (alveolar bone) and soft tissues (periodontal ligament, gingiva). The sequence of these biological reparative processes results into a variable horizontal and vertical alveolar ridge resorption, both on the buccal and palatal/lingual side. Even if resorption occurs mainly in the first three months after tooth loss (Schropp et al. 2003), this phenomenon appears to be progressive and may result in aesthetic and functional challenges during the replacement of missing teeth, in particular when an implant-supported prosthesis is requested.

In the last decades, experimental and clinical studies have showed that osseointegration is highly predictable, and dental implants represent a reliable means for restoring dental function in partially and completely edentulous patients. (Schropp, et al., 2003; Becktor et al. 2004, Araujo, & Lindhe, 2005; Pietrokovski, et al., 2007; Esposito et al. 2008; Chiapasco, et al., 2009; Jung et al. 2012; Pjetursson et al. 2012; van Velzen et al. 2015; Hammerle, & Tarnow, 2018).

Although surgical and prosthetic procedures are well consolidated, treatment planning in oral implantology has undergone a huge evolution. Implants were originally placed using the concept of "surgically and anatomically driven implant placement" in fully edentulous patients: implant placement was primarily determined by the location of available bone, and the main goal was to allow adequate bone anchorage to provide a functionally efficient prosthetic rehabilitation. Following these principles, oral surgeons and prosthodontists if on one side were able to restore mastication, on the other one they were not always able to achieve an ideal aesthetic result.

As oral implants have also been used for the rehabilitation of partially edentulous patients, esthetic outcomes have become more important because implant-supported partial prostheses have to integrate with the adjacent natural dentition, both from a functional and an aesthetic point of view. A good aesthetic result can be achieved only if implants are placed in a precisely planned position determined by the prosthetic needs. Therefore, the concept of "**restoration-driven implant placement**" has been introduced to optimize both function and esthetics (Garber, & Belser, 1995).

Consequently, adequate hard and soft tissue volumes as well as correct intermaxillary relationships are key prerequisites to obtain a good clinical outcome. However, long-standing edentulism and periodontal disease, trauma sequelae, ablation for tumors or osteoradionecrosis, and congenital malformations, may determine hard and soft tissue deficiencies of such an entity to hamper not only the aesthetic outcome but also the possibility of placing implants.

Therefore, in case of: a) insufficient bone volume to host even short (< / = 6 mm) and/or narrow (< 3mm) implants in a prosthetically driven position; b) proximity of anatomical structures, such as the inferior alveolar nerve, the maxillary sinus, and the nasal floor; c) unfavorable vertical and/or horizontal intermaxillary relationships, the bone volume and/or the surrounding soft tissues (keratinized mucosa) must be augmented (Chiapasco et al. 2006, 2009; Chiapasco, & Casentini, 2018).

In order to recreate more favorable conditions, different bone reconstructive/regenerative techniques have been proposed, including:

- distraction osteogenesis (Chin & Toth 1996; Chiapasco et al. 2004; Jensen et al. 2011);
- bone splitting (Bruschi, et al., 2017; Scipioni, et al., 2008);
- guided bone regeneration (Lang et al. 1994; Chiapasco et al. 1999; Simion et al. 2001; Buser et al.

2002; Hammerle et al. 2002; Urban et al. 2013);

- maxillary sinus floor elevation (Pjetursson et al. 2008, 2009);
- onlay grafting with allogeneic (Waasdorp & Reynolds, 2010; Krasny, et al., 2015) or autogenous bone blocks (Von Arx et al. 2006; Chiapasco et al. 2006-2009; Esposito et al. 2008, 2009; Sbordone et al. 2009; Nkenke & Neukam 2014; Smolka 2014; Schwartz-Arad et al. 2016).

As the rehabilitation with implant-supported prostheses of partially or totally edentulous patients is often complex from a prosthetic and surgical viewpoint, and a multidisciplinary approach is often recommended, another key aspect is represented by an accurate treatment plan, which must include different, consecutive steps.

Starting from the aforementioned notion of "Prosthetically Guided Implant Placement", Chiapasco & Casentini (2018) codified the concept of "**Prosthetically Guided Regeneration – PGR**" to create rational diagnostic and treatment protocols for complex implant cases presenting with bone defects.

The keynote aspects of this classification is that the final and ideal prosthetic plan is the reference point to which all plannings of regenerative procedures must be aimed to: briefly, the prosthesis is the leading aspects which will pilot all the remaining treatments.

In the PGR protocol different degrees of atrophy were divided in 5 classes and each of them was associated to specific surgical regeneration procedures including both soft and hard tissues.

More in detail, the first step of the PGR concept is to evaluate the feasibility of an implant -supported restoration from a prosthetic point of view. Plaster casts can be used to provide a diagnostic wax-up that should simulate, with wax of different colors, the ideal ridge profile and the anatomy of teeth to be replaced. The wax-up allows:

a) the recognition of intra- and inter-maxillary discrepancies or asymmetries between the jaws;

b) caliper measurement of the wax thickness simulating the vertical/horizontal augmentation that may be necessary to achieve the ideal morphology.

Once the prosthetic plan has been finalized and a ridge defect has been identified, the next step will be a precise evaluation of bone defect morphology in relation to the prosthetic needs. In other words, this will allow to understand if the bone volume is adequate to place implants in a prosthetically driven way (in order to achieve what has been planned with the wax-up) or if grafting procedures are needed. The wax-up will be duplicated to produce a diagnostic template with radiopaque tooth/teeth or radiopaque reference point/points in the center of each crown, reproducing the ideal tooth/teeth position. Then, a computerized tomography will be performed wearing the diagnostic template in the mouth and this will make it possible to obtain the following information:

- residual bone dimensions;
- localization of important anatomic structures;
- soft tissue thickness;
- the relationship between the residual hard and soft tissues and the planned restoration.

If the existing bone volume does not allow implant placement, depending on the existing defect, it will also be possible to choose the most appropriate augmentation procedure and the ideal diameter and length of the planned implants.

After this prosthetically driven diagnostic protocol, it is possible to classify a clinical case in five classes (class 0–5):

- **class 0**: post-extractive socket;
- **class 1**: healed socket with minimal reduction of bone volume. The bone volume is sufficient to place an implant fully embedded in the residual bone with no prosthetic compromises but soft tissues thinning or non-ideal support may determine the presence of a "cosmetic defect". In these cases no hard tissue correction is needed but a soft tissue augmentation with an autogenous connective tissues or volume stable matrices are recommended to improve the final aesthetic result;
- **class 2**: healed socket with a moderate horizontal deficit. Implant(s) can be placed in the correct prosthetically driven position but a simultaneous hard tissue augmentation procedure is indicated because of unavoidable creation of dehiscences or fenestrations;
- **class 3**: healed socket with a significant horizontal alveolar bone deficit. In this case, local anatomy not only doesn't allow implant placement in in appropriate position but primary stability is difficult or impossible to achieve as implant will present the majority of its surface outside of the bone. Therefore, a preliminary regeneration/reconstruction of the defect is necessary and, after an adequate healing period (ranging from 4–9 months, depending on the technique selected and the grafting material used), implant can be placed in a second-stage surgical procedure;
- **class 4**: healed socket with a combined horizontal and vertical defect. As in class 3, a regenerative/reconstructive procedures are mandatory and a delayed implant placement is recommended.

The three projects will be focused on the treatment of class 3 and 4.

As already anticipated, augmentation of atrophic ridges can be obtained with a variety of surgical procedures, including distraction osteogenesis, bone splitting, guided bone regeneration, maxillary sinus floor elevation, onlay grafting with allogeneic or autogenous bone blocks.

Project 1 and 2 are focused on the clinical and radiographic outcome of reconstruction of class 3 and 4 with autogenous bone blocks taken from the mandibular ramus or the calvarium, respectively. The reconstruction with autogenous onlay bone grafts still represents a versatile and very well documented procedure (Chiapasco et al. 2006-2009; Aloy-Prósper et al. 2015; Aghaloo et al. 2016) which allows the correction of the majority of defects in different clinical scenarios, with no limitations regarding the defect extent.
Among the different donor sites, the mandibular ramus and the calvarium have demonstrated reliable results because of their rich cortical component and the reduced resorption rate (Chiapasco, et al., 2018-2020).
The mandibular ramus is one of the most frequently used and favourable results have been reported in terms of bone volume stability and implant survival rates over time, ranging from 60% to 100% for machined surface implants (mean: 81.6%) and from 90% to 100% for rough surface implants (mean: 94.2%) (Clementini, et al., 2011; Chiapasco, et al., 2014 - 2020).
However, limits are represented by the quantity of available bone which is frequently insufficient in case of extended class 3 and, in particular, class 4 defects.
In case of large defects, where intra-oral donor sites may be not sufficient in terms of bone quantity, calvarial bone offers an excellent donor site (Donovan et al. 1994; Tessier et al. 2005; Iizuka et al. 2004; Carinci et al. 2005; Smolka et al. 2006; Chiapasco et al. 2007, 2013; Gleizal & Beziat 2007; Gutta & Waite 2009; Quiles et al. 2015; Restoy-Lozano et al. 2015; Mertens et al. 2013, 2017). Favorable results have been reported in terms of bone volume stability and implant survival rates over time, ranging from 60% to 100% for machined surface implants (mean: 81.6%) and from 90% to 100% for rough surface implants (mean: 94.2%) (Clementini et al. 2011; Chiapasco, et al., 2014 - 2018).

Project 3 will be focused on the clinical and radiographic outcomes of guided bone regeneration of the same classes by means of customized Titanium meshes in association with a mixture of particulated autogenous bone and bovine bone mineral in granules.

GBR procedures are also well documented and, according to the extent and/or type of defect (class 3 and 4), can be performed using autogenous bone particles or non-autogenous bone substitutes in association with resorbable (class 3) or non-resorbable membranes, including titanium meshes (class 3 and 4).
As regards Titanium meshes, these have been successfully used for years augmentation (Roccuzzo, et al., 2007; Her, et al., 2012; Miyamoto, et al., 2012; Briguglio, et al., 2019).
However, some drawbacks and complications have been reported: the available meshes are generally rectangular foils of different thickness which must be trimmed and bended according to the defect to be reconstructed, requiring clinical skill and experience. This procedure can be time consuming and the final shape not ideal; furthermore, angles and edges created during modelling, if irregular and sharp, may expose soft tissues to mechanical trauma, which may lead to perforation of the flap and exposure of the mesh.
As an alternative to traditional titanium meshes and to provide a solution to the over-mentioned disadvantages, a new generation of titanium meshes have been recently proposed. These latter, in order to be precisely adapted to the bone defects, are created with a CAD/CAM technology This makes it possible to produce a precise three-dimensional scaffold by means of laser sintering (Sumida, et al., 2015; Sagheb, et al., 2017; Seiler, et al., 2018).

3 - AIM OF THE THREE YEAR RESEARCH PROJECTS

The aim of the first project, a retrospective longitudinal cohort study, is to present the medium to long-term outcome of bone reconstructions with calvarium grafts by evaluating: a) complication rate of the reconstructive procedure; b) bone resorption before implant placement; c) peri-implant bone resorption; d) implant-related complications; e) implant survival rate; and, f) patient's satisfaction inquired with a dedicated questionnaire.

The aim of the second project, a retrospective longitudinal cohort study, is to present the medium- to long-term clinical and radiographic results of implant-supported prostheses in patients reconstructed with autogenous bone blocks taken from the mandibular ramus, covered with bovine bone mineral particles and a collagen membrane. The following outcomes were recorded: a) complication rate of the reconstructive procedure; b) bone resorption before implant placement; c); peri-implant bone resorption; d) implant-related complications; e) implant survival and success rates and f) PROMs inquired with a dedicated questionnaire.

The aims of the third project, a prospective, single-arm clinical study, are to evaluate: a) the effectiveness of digitally customized titanium meshes in association with autologous bone particles and BBM and covered with collagen membranes for the regeneration of atrophic edentulous sites; b) the survival rate of implants placed in the regenerated areas and, c) PROMs inquired with a dedicated questionnaire.

4 - MATERIALS & METHODS

4.1 – RECONSTRUCTIVE PROCEDURE WITH CALVARIUM – PROJECT 1

- ***Study design***

From 1998 to 2014, 72 patients (18 male patients, 54 female patients) with ages ranging from 16 to 72 years (mean: 48 years), presenting with partial or total edentulism of the jaws in association with relevant bone defects and requesting an implant-supported prosthetic restoration, were enrolled in this study.
Patients presented edentulous areas in the mandible and/or maxilla with: a) the absence of sufficient bone volume to host even short (</= 6 mm) and/or narrow (< 3mm) implants; and b) unfavorable vertical and/or horizontal intermaxillary relationships which might compromise the final prosthetic outcome because of the impossibility of placing implants in a prosthetically driven position.
Exclusion criteria for the surgical procedure were the following: a) severe kidney and/or liver disease; b) congenital or acquired immunodeficiency; c) ongoing antiblastic chemotherapy at the time of first examination; d) sequelae of radiotherapy in the head and neck area; e) oral mucosa diseases, such as lichen planus; f) plaque index ≥ 2 (Silness & Löe 1964) and gingival index ≥ 2 (Löe 1967); g) non-compliant patients; h) tobacco abuse (>10 cigarettes per day) or alcohol abuse; i) non compensated diabetes; j) ongoing bisphosphonate therapy with oral or parenteral administration; k) active periodontal disease at the time of first examination (in these cases, patients underwent etiologic therapy, motivation in personal oral hygiene, and were re-evaluated for surgical treatment).
The exclusion criteria for enrolment in this retrospective study were: a) patients inability to attend an updated follow-up visit with clinical and radiographic data collection; b) patients inability to compile a questionnaire on patients related outcome measures; c) loss of clinical data concerning the surgical and prosthetic treatments. Reasons for exclusion were recorded during data collection.
Documentation for all clinical cases included: a) intra-oral photographs of the initial clinical situation; b) panoramic radiograph and a complete series of peri-apical radiographs for partially edentulous patients; c) impressions and plaster casts with diagnostic wax-up for the fabrication of diagnostic/surgical templates with radio-opaque markers; d) pre-operative computed tomography scans performed with diagnostic templates in place to evaluate residual bone volumes and the correlation of the residual crest with the ideal prosthetic crown position.

All the selected patients were reconstructed with autogenous onlay bone grafts harvested from the parietal calvarium (table 1). After a waiting period ranging from 5 to 9 months to allow the integration of the grafts, 330 implants were placed (201 implants in the upper jaw and 129 in the mandible) and all of them were let to heal in a submerged fashion. Three to 9 months later, implants were exposed and the prosthetic phases started.
In 16 patients (97 implants), because of a significant coronal displacement of the buccal flaps at the end of the reconstructive procedure to allow primary wound healing, a loss of vestibule depth and the absence of keratinized tissue on the crestal aspect of the reconstructed areas was present. Thus, at the time of implant placement, a vestibuloplasty in association with free gingiva grafts taken from the palate was performed, to restore adequate vestibulum depth and to achieve an adequate width of the keratinized mucosa around implants.
All patients were restored with a provisional implant-supported prosthesis that was substituted with the final one 3 to 12 months later.

This observational study was performed in accordance with the principles stated in the Declaration of Helsinki and the Good Clinical Practice Guidelines, and was approved by the Ethics Committee of the University of Milan, Italy (Registration No. 2016/ST/244; Protocol of Final Approval No. 7347/2017). An informed consent, providing all alternative treatments available (including advantages and disadvantages of each of them) as well as a detailed description of the reconstructive procedure with calvarium, including potential side effects and complications, was signed prior to treatment by all patients. Moreover, patients were thoroughly informed about: a) alternative donor sites for the reconstructive procedures, including iliac crest and mandibular ramus (when this latter may offer a sufficient quantity of bone); and b) post-operative morbidity related to each donor site.

All patients were informed that their data would be used for statistical analysis and publication.

Table 1 - Patients' demographic and clinical data related to bone reconstructions

No	Sex	Age	Smoker	Site of defect	Missing teeth	Date of Grafting	Type of reconstruction	Donor site	Pericranium	Sinus lift	Complication before impl placement	Date of screw removal and impl placement	Pre-implant bone resorption (mean)
1	F	30	no	Mand (p)	31-36	Feb-98	vert + hor	calvarium	no	no	Dehiscence	Nov-98	1mm
2	F	34	no	Mand (p)	34-36	Apr-98	Vert + hor	calvarium	no	no		Dec-98	0mm
3	F	56	no	Max (t)	18-28	Jan-99	Vert + hor	calv + il. Chips	no	bilateral		Sep-99	0 mm
4	F	23	no	Max (p)	12-11-21-22	Oct-01	Vert + hor	calvarium	no	no		Apr-02	1mm
5	M	25	no	max (p)	12-11-21-22-23-24-25-26-27-28	Jan-03	vert + hor	calvarium	no	no		Jun-03	0mm
6	M	20	no	Max (p)	12-11-21-22-23	Feb-03	Vert + hor	calvarium	no	no		Jul-03	0 mm
7	F	32	no	Max (p)	11-21-22-23	May-03	Vert + hor	calvarium	yes	no		Oct-03	0.5 mm
8	F	50	no	Max (p)	22-28	Feb-04	Vert + hor	calvarium	No	Left side	Dehiscence	Oct-04	0 mm
9	M	63	no	Max (p)	23-28	Feb-04	Vert + hor	calvarium	No	Left side		Sep-04	0 mm
10	F	47	no	Max (t)	18-28	Mar-04	Vert + hor	calvarium	yes	bilateral		Sep-04	0 mm
11	M	59	no	Max (p)	18-13; 24-28	Jun-04	Vert + hor	calvarium	No	bilateral		Dec-04	0 mm
12	M	41	no	Max (p)	21-24	Oct-04	Vert	calvarium	No	no		Apr-05	0.5 mm
13	F	44	yes	Mand (t)	38-48	Dec-04	Vert	calvarium	No	no		Jun-05	0 mm
14	F	41	no	Max (p)	14-24	Jan-05	Vert + hor	calvarium	No	no		Jul-05	0 mm
15	F	64	no	Mand (t)	38-48	Jan-05	Vert + hor	calvarium	yes	no		Jun-05	0 mm
16	F	60	no	Mand (p)	48-45; 35-38	Mar-05	Vert	calvarium	No	no		Sep-05	0 mm
17	F	16	no	Mand (p)	42-34	Apr-05	Vert + hor	calvarium	yes	no		Nov-05	0 mm
18	F	57	no	Max (p)	15-28	Apr-05	Vert + hor	calvarium	No	Left side		Oct-05	0 mm
19	M	20	no	Max (p)	11-21-22	Jan-06	Vert + hor	calvarium	No	no		Jul-06	0 mm
20	F	61	no	Max (t) Mand (p)	18-28; 36-34; 46-44	Jan-06	Vert + hor	calvarium	yes	bilateral		Jul-06	0 mm
21	M	30	yes	Max (p)	13-25	Feb-06	vert + hor	calvarium	No	no		Sep-06	0mm
22	M	56	no	Max (p) Mand (p)	18-28; 13-16; 24-28; 41-48	Jun-06	Vert + hor	calvarium	No	bilateral		Nov-06	0 mm
23	F	67	no	Max (t)	18-28	Sep-06	Vert + hor	calvarium	No	bilateral		Feb-07	0 mm
24	M	27	no	Max (p)	13-21	Jan-07	Vert + hor	calvarium	No	no		Jul-07	0 mm
25	F	60	no	Mand (p)	34-38	Jan-07	Vert + hor	calvarium	No	no		Jul-07	0.5 mm
26	M	61	yes	Max (t)	18-28	Jun-07	Vert + hor	calv + il. Chips	No	bilateral	Dehiscence	Oct-07	0.5 mm
27	F	39	no	Mand (t)	38-48	Apr-08	Vert	calvarium	yes	no		Sep-08	0 mm
28	F	72	no	Mand (t)	38-48	May-08	Vert + hor	calvarium	yes	no		Oct-08	0 mm
29	F	54	yes	Max (t)	18-28	May-08	Vert + hor	calvarium	yes	bilateral	Dehiscence	Oct-08	0.5 mm

30	F	63	no	Mand (p)	48-45	Jun-08	Vert	calvarium	yes	no		Nov-08	0 mm
31	F	59	no	Max (p) Mand (t)	18-13; 23-27; 38-48	Jun-08	Vert + hor	calvarium	yes	bilateral		Nov-08	0 mm
32	F	41	yes	Max (t)	18-28	Oct-08	Vert + hor	calvarium	yes	bilateral		May-09	0 mm
33	F	58	no	Max (t)	18-28	Jan-09	Vert + hor	calv + il. Chips	yes	bilateral		Jun-09	0 mm
34	F	59	no	Mand (p)	33-38	Feb-09	Vert + hor	calvarium	yes	no		Sep-09	0 mm
35	F	61	no	Max (p) Mand (t)	18-14; 24-28; 38-48	Apr-09	Vert + hor	calvarium	yes	bilateral	Dehiscence	Nov-09	0 mm 0.5 mm 0 mm
36	M	58	no	Mand (p)	44-46	Apr-09	Vert + hor	calvarium	no	no		Oct-09	0mm
37	F	69	no	Max (p)	18--12	May-09	Vert + hor	calvarium	yes	right side		Dec-09	0 mm
38	F	50	no	Mand (p)	46; 36	Jun-09	Vert + hor	calvarium	yes	no		Dec-09	0.5 mm
39	F	58	no	Mand (p)	48-43	Jul-09	vert+hor	calvarium	no	no		Jan-10	0mm
40	F	68	no	Mand (p)	48-43; 35-38	Jul-09	Vert + hor	calvarium	no	no		Dec-09	0 mm
41	F	66	no	Mand (p)	48-44; 34-38	Sep-09	Vert	calvarium	yes	no		Jan-10	0 mm
42	F	36	no	Max (p)	11-21-22	Oct-09	Vert + hor	calvarium	no	no		Feb-10	0 mm
43	M	60	no	Mand (p)	34-38	Dec-09	Vert + hor	calvarium	no	no		Jul-10	0mm
44	F	60	no	Mand (p)	34-38; 45-48	Jan-10	Vert + hor	calv	yes	no		Jun-10	0 mm
45	F	45	yes	Max (p)	16-18;26-28	Feb-10	Vert + hor	calv + il. Chips	yes	bilateral		Sep-10	0 mm
46	F	20	no	Max (p)	18-12; 22; 24-25	Mar-10	Vert + hor	calvarium	yes	no		Sep-10	0 mm
47	F	58	no	Max (p)	25-28	Apr-10	Vert + hor	calvarium	no	no		Nov-10	1mm
48	F	51	no	Max (t)	18-28	May-10	Vert + hor	calvarium	yes	no		Nov-10	0 mm
49	F	53	no	Mand (p)	35-38	May-10	Vert + hor	calvarium	yes	no		Nov-10	0 mm
50	F	42	no	Max (t)	18-28	Jun-10	Vert + hor	calv + il. Chips	yes	bilateral		Oct-10	0.5 mm
51	F	26	no	Mand (p)	48-44; 35-38	Sep-10	Vert + hor	calvarium	yes	no		Mar-11	0 mm
52	F	60	no	Mand (p)	31-38	Sep-10	Vert + hor	calvarium	no	no		Apr-11	0mm
53	F	47	no	Max (t) Mand (t)	18-28; 38-48	Oct-10	Vert + hor	calvarium	yes	bilateral		Jul-11	0 mm
54	F	51	yes	Max (p) Mand (p)	18-24; 34-38	Oct-10	Vert + hor	calvarium	yes	no		Apr-11	0 mm
55	M	19	no	Max (p) Mand (p)	15-14; 21-22;24;42-31	Oct-10	Vert + hor	calvarium	yes	no		Mar-11	0 mm
56	F	51	no	Mand (p)	46-45; 35-38	Jan-11	Vert + hor	calvarium	yes	no		Jun-11	0 mm
57	M	45	yes	Mand (p)	35-38	Feb-11	Vert + hor	calvarium	yes	no		Sep-11	0mm
58	F	55	no	Mand (p)	41-48	Mar-11	vert + hor	calvarium	yes	no		Oct-11	0 mm
59	M	45	no	Mand (p)	33-38	Apr-11	vert + hor	calvarium	yes	no		Sep-11	0.5mm
60	M	55	no	Max (p)	22-28	May-11	Vert + hor	calvarium	no	Left side	Dehiscence	Nov-11	0.5mm
61	F	33	no	Max (p)	12-11-21-22-23	Sep-11	vert + hor	calv + il. Chips	yes	no		Feb-12	0mm

62	F	42	no	Mand (t)	38-48	Feb-12	Vert + hor	calvarium	no	no		Sep-12	0.5mm
63	F	60	no	Mand (p)	34-38	Jun-12	Vert + hor	calvarium	yes	no		Dec-12	0mm
64	F	53	no	Max (p)	13-18	Jul-12	Vert + hor	calvarium	no	no		Feb-13	0.5 mm
65	M	40	no	Max (p)	22-28	Sep-12	Vert + hor	calvarium	no	no		Mar-13	0mm
66	F	66	no	Max (t)	18-28	Sep-12	Hor	calv + il. Chips	yes	right side		Jun-13	0 mm
67	F	18	no	Mand (p)	48-45	Jan-13	vert+hor	calvarium	no	no		Jun-13	0 mm
68	F	48	no	Mand (p)	48-43	Mar-13	vert + hor	calvarium	yes	no		Sep-13	0mm
69	M	50	yes	Max (p)	12-11-21-22	Apr-13	Vert + hor	calvarium	no	no		Oct-13	0mm
70	F	61	yes	Mand (p)	46-44; 34-38	Jun-13	Vert + hor	calvarium	yes	no		Feb-14	0.5 mm
71	F	56	no	Max (t)	18-28	Jun-13	Vert + hor	calvarium	yes	bilateral		Nov-13	0 mm
72	F	56	no	Mand (p)	48-44	Sep-13	Vert + hor	calvarium	no	no		Apr-14	0mm

Legend: mand, mandible; max, maxilla; (p), partially edentulous; (t), totally edentulous; vert, vertical; hor, horizontal; calvarium* = calvarium + iliac spongiosa chips.

- ***Clinical Protocol***
    - *Pre-operative patient preparation*

All patients were prepared to surgery according to the following protocol: a) professional oral hygiene 2 weeks prior to surgery (when residual dentition was present); b) local antisepsis with 0.2% chlorhexidine mouthwashes starting 3 days before surgery, and; c) antibiotic prophylaxis consisting of intravenous infusion of ceftriaxone (2 grams) at the time of general anesthesia induction (first surgical session) or oral administration of amoxicillin and clavulanate (2 grams) one hour before surgery at the time of implant placement.

- *Reconstructive phase*

The first surgical step consisted of the reconstruction of the atrophic sites with autogenous calvarial onlay bone grafts. All patients were treated under general anesthesia with naso-tracheal intubation and controlled hypotension to reduce bleeding during surgery. Surgical access to the donor site was obtained through a para-sagittal or hemi-coronal incision in the parietal area. The outer cortex was exposed and one or more bone segments (according to surgical needs), including only the outer cortex, were harvested by means of piezo-electric instruments or fine fissure burs and curved chisels. The bone segments were immediately soaked into sterile saline.

During bone harvesting, care was taken to avoid the sagittal sinus by maintaining a safety distance of at least 1.5 cm from the sagittal suture. Bone scrapers were also used to harvest cortical bone chips from the outer margins of the harvesting area to obtain supplementary bone to fill empty spaces between the calvarial blocks and the recipient sites. Haemostasis in case of bleeding from diploic vessels was obtained with surgical wax. In 37 patients, also segments of pericranium were harvested from the same sites to cover the bone grafts at the end of the reconstructive procedure to reduce the risk of graft exposure in case of wound dehiscence, as demonstrated by some studies (Autelitano et al. 2008; Chiapasco et al. 2013). The surgical access was closed, with subcutaneous resorbable stitches (Polyglactin 4/0 sutures) associated with a cutaneous suture (nylon or silk 3/0 sutures). In 8 patients affected by extreme atrophy, in which the calvarial chips were not sufficient to fill adequately empty spaces between the recipient site and the calvarial blocks, cancellous bone chips were also taken through a small cutaneous incision (1 to 2 cm approximately) with a trephine also from the anterior iliac crest.

The second phase, performed during the same session, consisted of the reconstruction of the atrophic site/s. A midcrestal full-thickness incision with medial and distal releasing incisions was performed to gain access to the defect. Care was taken to remove any remnant of soft tissues from the bone surface to be reconstructed. Bone blocks were then modelled to obtain adaptation to the recipient site and then fixed with titanium micro (1.5mm in diameter) or mini (2mm) screws. All remaining gaps between the bone blocks and the recipient sites were filled with the bone chips taken from the calvarium and/or the iliac crest. A layer of slowly resorbable biomaterial (Bio-Oss®, Geistlich Biomaterials, Switzerland) mixed with autogenous bone chips was placed over the grafts and stabilized with collagen membranes (Bio-Gide®, Geistlich Biomaterials, Switzerland), to eliminate sharp edges, to harmonize the shape of the reconstructed area, and to reduce the risk of bone resorption, as suggested by some authors (Von Arx and Buser 2006). Details of the reconstructions according to type of defects are reported in table 1.

In 22 patients presenting with maxillary atrophy and expanded maxillary sinuses (16 patients presenting with bilateral expanded sinuses and 6 patients with unilateral expansion), the bone reconstruction was associated with a sinus floor elevation with a lateral approach (38 sinuses) (table 1). Sinuses were grafted with a mixture

of particulated calvarial bone and demineralized bovine bone mineral (Bio-Oss®, Geistlich Biomaterials, Switzerland) with a one-to-one ratio, approximately.
After the completion of the reconstructive phase, periosteal releasing incisions were performed in all patients to allow a tension-free, water-tight closure of the flaps. Flap closure was performed with both horizontal mattress and simple interrupted sutures using 4/0 or 5/0 silk or polyglactin sutures.
The following post-surgical instructions were prescribed: a) application of ice packs over the reconstructed surgical site for 6-8 hours after surgery; b) oral antibiotics (amoxicillin and clavulanate - 2 grams per day for 6 days); c) post-surgical non-steroidal analgesic therapy for 5 to 7 days after surgery (ketoprofen and lysin salt 80 mg. – 2/3 per day for 2 to 5 days according to patient post-operative pain was prescribed in the majority of patients; paracetamol 1000mg. with the same dosage was prescribed only in two patients with allergy to ketoprofen); d) cold and liquid diet for the first two days after surgery and a soft diet until suture removal; e) 0.2% chlorhexidine mouthrinses, twice per day for 21 days, combined with normal oral hygiene manoeuvres on the remaining dentition (if present). When a concomitant sinus elevation was performed, patients were instructed not to blow their nose for the first 15 postoperative days and to sneeze with their mouth open to avoid any pressure peak in the upper respiratory tract which might damage the elevated Schneiderian membrane. Patients were also suggested not to fly or to make underwater activities for the following 3 to 4 weeks postoperatively. Sutures were removed 14 to 21 days after surgery.
During the post-operative period, edentulous patients were not allowed to wear removable prostheses that could stress the reconstructed ridges for a minimum of 6 weeks. In the following period, and until implant placement, prostheses relined with soft materials were allowed just for “cosmetic” use, with the prohibition to use them for chewing hard food. Patients presenting with partial edentulism in the anterior maxilla/mandible were provisionally rehabilitated with fixed or removable partial prostheses (e.g.: Maryland bridges or vacuform retainers including resin teeth), provided that they had no contact with the reconstructed areas. Finally, partially edentulous patients in the posterior maxilla/mandible, if not requesting temporary prosthetic restorations, were left to heal without any prostheses.

- *Implant phase*

Four to 9 months after the reconstructive procedure, implants were placed in the reconstructed areas following indications of surgical templates constructed with a prosthetically driven approach. Titanium micro and mini-screws placed at the time of bone grafting where removed only if they might interfere with implant placement. Sixty-three patients were treated under local anesthesia, and 9 under general anesthesia. The choice was made according to the extension of the defect, the number of implants to be placed, the duration of the surgical session, and the patients’ compliance. A total of 330 implants (201 implants in the upper jaw and 129 in the mandible) were placed and all of them were let to heal in a submerged fashion. Patients were discharged with the same post-operative instructions given for the reconstructive phase. Three to 9 months later, implants were exposed and the prosthetic phases started. At the time of implant exposure, 16 patients (97 implants) presenting with reduced or absent keratinized mucosa in the reconstructed areas, were treated with a vestibuloplasty in association with free gingiva grafts taken from the palate, to restore adequate vertical dimensions of the buccal sulcus and to achieve an adequate width of the keratinized mucosa around implants.

- *Prosthetic phase*

All patients received a provisional implant-supported prosthesis for 3 to 9 months to achieve an adequate soft tissue adaptation and conditioning. Finally, definitive prostheses were delivered. Details concerning the type of prosthesis are reported in table 2.

Table 2 - Patients' demographic and clinical data related to implant placement and prosthetic restorations

No	Sex	Age	Site of defect	Date of implant placement	No. Of implant	Complication after implant placement	Date implant loading	Type of prosthesis	follow-up	lost to follow-up	vestibuloplasty + FGG
1	F	30	Mand (p)	Nov-98	4		Apr-99	FPP	84	2006	no
2	F	34	Mand (p)	Dec-98	3		May-99	FPP	221		no
3	F	56	Max (t)	Sep-99	9		Jul-00	FCP	207		no
4	F	23	Max (p)	Apr-02	2		Oct-02	FPP	181		no
5	M	25	max (p)	Jun-03	5		Dec-03	2 FPP	132	2014	yes
6	M	20	Max (p)	Jul-03	4		Nov-03	FPP	96	2011	no
7	F	32	Max (p)	Oct-03	4	perimplantitis 21 and 22	Mar-04	FCP	163		yes
8	F	50	Max (p)	Oct-04	5		Mar-05	FPP	151		no
9	M	63	Max (p)	Sep-04	3		Feb-05	FPP	152		no
10	F	47	Max (t)	Sep-04	8		Mar-05	FCP	151		no
11	M	59	Max (p)	Dec-04	7		May-05	FCP	149		yes
12	M	41	Max (p)	Apr-05	3		Sep-05	FPP	145		no
13	F	44	Mand (t)	Jun-05	3		Oct-05	FCP	120	2015	yes
14	F	41	Max (p)	Jul-05	5		Oct-05	FPP	144		yes
15	F	64	Mand (t)	Jun-05	3		Oct-05	IRO	144		no
16	F	60	Mand (p)	Sep-05	4		Dec-05	2 FPP	142		no
17	F	16	Mand (p)	Nov-05	4		Mar-06	FPP	139		yes
18	F	57	Max (p)	Oct-05	5		Jan-06	FPP	141		no
19	M	20	Max (p)	Jul-06	2		Nov-06	FPP	132		no
20	F	61	Max (t) Mand (p)	Jul-06	12		Nov-06	FCP (max); 2 FPP (mand)	132		yes
21	M	30	Max (p)	Sep-06	5		Jan-07	FPP	44	2010	yes
22	M	56	Max (p) Mand (p)	Nov-06	10		Mar-07	3 FPP	127		no
23	F	67	Max (t)	Feb-07	6		Jun-07	FCP	124		yes
24	M	27	Max (p)	Jul-07	3		Nov-07	FPP	120		yes
25	F	60	Mand (p)	Jul-07	3		Oct-07	FPP	121		no

26	M	61	Max (t)	Oct-07	6		Apr-08	FCP	114		no
27	F	39	Mand (t)	Sep-08	4		Feb-09	FCP	104		no
28	F	72	Mand (t)	Oct-08	5		Feb-09	FCP	104		no
29	F	54	Max (t)	Oct-08	6		Jan-09	FCP	83	deceaded 2017	yes
30	F	63	Mand (p)	Nov-08	1		Apr-09	FPP	102		no
31	F	59	Max (p) Mand (t)	Nov-08	10	perimplantitis 13-25; implant removal	Mar-09	FCP (mand); FPP (max)	103		no
32	F	41	Max (t)	May-09	8	perimplantitis 14-15-24-25	Sep-09	FCP	97		no
33	F	58	Max (t)	Jun-09	8	perimplantitis 27-25-24-23-14-15	Nov-09	FCP	96		no
34	F	59	Mand (p)	Sep-09	3		Feb-10	FPP	92		no
35	F	61	Max (p) Mand (t)	Nov-09	10		Feb-10	FCP (mand), 2 FPP (max)	92		no
36	M	58	Mand (p)	Oct-09	2		Feb-10	FPP	10	2010	no
37	F	69	Max (p)	Dec-09	3	perimplantitis 13	Mar-10	FPP	91		no
38	F	50	Mand (p)	Dec-09	2		Mar-10	FPP	91		no
39	F	58	Mand (p)	Jan-10	3		Jun-10	FPP	88		no
40	F	68	Mand (p)	Dec-09	4		Mar-10	FPP	91		no
41	F	66	Mand (p)	Jan-10	5		May-10	2 FPP	89		no
42	F	36	Max (p)	Feb-10	2		Jun-10	FPP	24	2012	no
43	M	60	Mand (p)	Jul-10	3		Nov-10	FPP	24	2012	no
44	F	60	Mand (p)	Jun-10	4	implant removal 36	Nov-10	2 FPP	84		no
45	F	45	Max (p)	Sep-10	3		Jan-11	2 FPP	81		no
46	F	20	Max (p)	Sep-10	6		Feb-11	2 FPP	80		yes
47	F	58	Max (p)	Nov-10	2		Mar-11	FPP	48	2015	no
48	F	51	Max (t)	Nov-10	6		Mar-11	FPP	79		yes
49	F	53	Mand (p)	Nov-10	2		Apr-11	FPP	78		no
50	F	42	Max (t)	Oct-10	6	perimplantitis 15-12-17	Feb-11	FCP	80		no
51	F	26	Mand (p)	Mar-11	4		Jul-11	2 FPP	76		no
52	F	60	Mand (p)	Apr-11	4		Jul-11	IRO	48	2015	no

53	F	47	Max (t) Mand (t)	Jul-11	13		Jan-12	2 FCP	70		yes
54	F	51	Max (p) Mand (p)	Apr-11	9		Oct-11	2 FPP	72		yes
55	M	19	Max (p) Mand (p)	Mar-11	5		Sep-11	4 FPP	73		no
56	F	51	Mand (p)	Jun-11	5		Oct-11	2 FPP	72		no
57	M	45	Mand (p)	Sep-11	2		Feb-12	FPP	68		no
58	F	55	Mand (p)	Oct-11	4		Feb-12	FPP	68		no
59	M	45	Mand (p)	Sep-11	3		Dec-11	FPP	70		no
60	M	55	Max (p)	Nov-11	3	implant removal 26	Mar-12	FPP	67		no
61	F	33	Max (p)	Feb-12	3		Jul-12	FPP	64		no
62	F	42	Mand (t)	Sep-12	4		Jan-13	IRO	57		no
63	F	60	Mand (p)	Dec-12	3		Mar-13	FPP	55		no
64	F	53	Max (p)	Feb-13	3		Jun-13	FPP	52		no
65	M	40	Max (p)	Mar-13	4		Jul-13	FPP	51		no
66	F	66	Max (t)	Jun-13	5		Sep-13	FCP	49		no
67	F	18	Mand (p)	Jun-13	2		Nov-13	FPP	47		no
68	F	48	Mand (p)	Sep-13	3		Feb-14	FPP	44		yes
69	M	50	Max (p)	Oct-13	2		Feb-14	FPP	44		no
70	F	61	Mand (p)	Feb-14	4	perimplantitis 36	Jul-14	2 FPP	40		no
71	F	56	Max (t)	Nov-13	6		Feb-14	FCP	44		no
72	F	56	Mand (p)	Apr-14	3		Jul-14	FPP	40		no

Legend: mand, mandible; max, maxilla; (p), partially edentulous; (t), totally edentulous; FPP, fixed partial prosthesis; FCP, fixed complete prosthesis; ISO, implant-supported overdenture

- ***Analysis***

Patients were followed-up from a minimum of 3 to a maximum of 19 years (mean: 8.1 years) after the start of prosthetic loading.
The following outcomes were recorded: a) complication rate of the reconstructive procedure; b) bone resorption before implant placement; c) implant survival rate and implant-related complications; d) peri-implant bone resorption; and, e) patient's satisfaction inquired with a dedicated questionnaire.

- *Complication rate of the reconstructive procedure*

After the reconstructive phase and until implant placement, clinical controls were carried out at suture removal, one month after surgery, and two weeks before implant placement. Complications regarding the donor and the recipient sites were recorded on the clinical chart of each patient.

- *Bone resorption before implant placement*

A panoramic radiograph was taken immediately after the reconstructive procedure and 2 weeks before implant placement. Graft resorption was evaluated manually at the time of screw removal and implant placement with a periodontal probe by measuring the distance between the head of each screw and the grafted bone. For each screw, the arithmetic mean between the values measured on the mesial and distal aspects of the screw head was calculated to obtain a single resorption value. Measurements were rounded to the nearest half millimeter.

- *Implant survival rate and implant-related complications*

Criteria used to determine implant survival were the following: a) absence of persistent pain or dysesthesia; b) absence of mobility; c) absence of continuous peri-implant radiolucency.
Failed implants were considered those presenting: a) continuous peri-implant radiolucency; b) mobility or c) fractures; d) those causing persistent paresthesia/dysesthesia or e) chronic pain; f) presence of peri-implant infection not responding to medical/surgical treatment.
On the contrary, peri-implantitis not associated with mobility but responding to medical/surgical treatment was considered as an implant-related complication.

- *Peri-implant bone resorption*

Peri-apical radiographs were taken immediately after implant placement, at provisional prosthetic load, at prosthetic finalization, and annually thereafter.
Marginal peri-implant bone resorption was evaluated using a dedicated image processing software (ImageJ® 1.38v U. S. National Institutes of Health, Bethesda, Maryland, USA) after acquisition of digital radiographs or digitalization of conventional films with a Nikon camera (Nikon Corp., Tokyo, Japan). The distance between the implant shoulder and the most coronal point of bone-to-implant contact, mesial and distal to each implant was measured. Dimensional distortion was corrected by setting in the software the known length and diameter of each implant. The radiographs taken immediately after implant placement were considered the baseline value.
All measurements were rounded to the nearest half millimeter. A mean bone resorption value was then calculated for each implant and for each patient.

- *Patient's satisfaction*

At the latest recall, patients were interviewed to evaluate their satisfaction concerning the treatment received. A dedicated questionnaire, adapted from the OHIP-14 survey (Lee et al. 2015), was provided to specifically assess their satisfaction regarding the received procedures. Ten questions were used to evaluate: 1) postoperative pain at the grafted site during the first 2 weeks after surgery; 2) postoperative pain at the donor site during the first 2 weeks after surgery; 3) discomfort due to the inability to use provisional prostheses for 6 weeks after surgery; 4) discomfort during the healing period, following the second postoperative week; 5) discomfort related to the dietary limitations prescribed during the healing phases; 6) frequency of meal interruption because of discomfort following the second postoperative week; 7) discomfort/limitations perceived at work, following the second postoperative week; 8) pain/discomfort perceived after the delivery of the final prosthesis; 9) time after which all forms of pain and discomfort had disappeared; 10) whether the patient, knowing exactly what to be expected post-operatively, would undergo the same reconstructive procedure again. Three Likert scales were adopted for questions 1 to 9, to quantify pain/discomfort intensity (questions 1 to 7: 1 = none, 2 = minimal, 3 = mild, 4 = moderate, 5 = high), frequency of dietary limitations (question 8: 1 = never, 2 = hardly ever, 3 = occasionally, 4 = fairly often, 5 = very often), and how long after the reconstruction discomfort was no more perceived (question 9: 1 = less than 3 months; 2 = less than 6 months, 3 = less than 9 months, 4 = less than a year, 5 = more than a year). Question 10, being dichotomous, was evaluated with the following score: 1= yes; 5 = no.

Each patient was asked to fill the questionnaire twice, at the last clinical follow-up and in a subsequent telephone call. Both inquiries were performed by the same investigator. The arithmetical mean of the two answers was recorded per each question. Arbitrarily, the procedure was considered to be successful from a patient's perspective if the questionnaire cumulative score was ≤ 25 and more than 80% of patients would undergo the procedure again.

The same questionnaire has been proposed to patients of project 2 e 3 (table 3).

Table 3 – Questionnaire

		1	2	3	4	5
1	Postoperative pain at grafted site during the first 2 weeks after surgery					
2	Postoperative pain at donor site during the first 2 weeks after surgery					
3	Discomfort due to inability of wearing provisional prostheses for 6 weeks after surgery					
4	Discomfort during the healing period, following the second postoperative week					
5	Discomfort related to dietary limitations prescribed during the healing phases					
6	Frequency of meal interruption because of discomfort following the second postoperative week					
7	Discomfort/limitations perceived at work, following the second postoperative week					
8	Pain/discomfort perceived after the delivery of the final prosthesis					
9	Time after which all forms of pain and discomfort disappeared completely					
10	Whether the patient, knowing exactly what to be expected post-operatively, would undergo the same reconstructive procedure again.	**YES (1) NO (5)**				

- ***Statistical analysis***

Statistical evaluations were conducted with the IBM SPSS Statistics software (Version 24, SPSS Inc., Chicago, IL, USA). Implant survival data were expressed in percentages and analyzed by means of Kaplan–Meier curves to determine the cumulative survival rates. Implants were considered the statistical unit of analysis.

Pearson's chi-square or Fisher's exact test, as appropriate, were used to evaluate: 1) the effect of the use of pericranium on the occurrence of post-reconstruction (pre-implant) complications; 2) the effect of patients' gender, smoking, area of edentulism on the occurrence of adverse events related to implants, considering either the patient, the implant, or the prosthesis as the unit of analysis; 3) the effect of co-interventions (sinus lift, additional bone chips taken from the iliac crest, vestibuloplasty, free gingiva grafts) on the occurrence of post-implant adverse events, considering the patient as the unit of analysis.

Where possible, odds ratio and 95% confidence intervals (CI) were estimated.

A Generalized Estimating Equation (GEE) with the patient as the analysis unit was performed to take into account the clustering of implants into patients, and to evaluate the simultaneous effect of various co-factors and co-interventions on the occurrence of adverse events like implant failure and biological complications (peri-implantitis), and on the marginal bone loss. Implant failures, biological complications, and marginal bone loss were set as dependent variables in three different GEEs. The model was based on the following predictors: a) smoking habits (smoking less than 10 cigarettes a day and non-smoking patients); b) gender; c) age; d) area of implant placement (maxilla or mandible); e) follow-up duration; f) placement of pericranium over the bone reconstruction; g) sinus augmentation; g) combined use of iliac crest bone chips; h) vestibuloplasty with free gingival grafts. The number of implants per patient was considered as the offset variable. The main factors considered for the final analysis were the co-interventions pericranium, sinus augmentation, the use of additional bone chips taken from the ilium, and the patient's factors: gender, smoking and area of implant placement. Significance threshold was set at P=0.05.

Peri-implant bone resorption data were expressed as means (± SD). Descriptive statistical methods were adopted for this outcome. Mean data were calculated utilizing patients as the statistical unit of analysis, because of the vast heterogeneity of clinical scenarios and number of implants placed among the different cases.

4.2 – RECONSTRUCTIVE PROCEDURE WITH MANDIBULAR RAMUS – PROJECT 2

- ***Study design***

This second project was a retrospective longitudinal cohort study evaluating short and medium-to-long term outcomes of patients affected by atrophic edentulous jaws, treated with mandibular ramus block grafts covered by bovine bone mineral and a collagen membrane, and rehabilitated with dental implants.
An initial informed consent was obtained from all patients prior to treatment, providing a detailed description of the procedure, including potential side effects and complications.
In 2017 the authors presented a request to the Ethic Committee of the University of Milan, in order to obtain permission to use all data collected over the years as regards these patients, including charts, radiographs, et cetera. Once obtained, the authors contacted all the previously treated patients (with the exclusion of the unreachable ones) to obtain permission to publish their data in a scientific publication.
A new informed consent, including the purpose of the recall program, the necessity of clinical and radiographic controls, and the patients' authorization to use their data for statistical analysis was signed by all of them.
The study was performed in accordance with the principles stated in the Declaration of Helsinki and the Good Clinical Practice Guidelines, and was approved by the Ethics Committee of the University of Milan, Italy (Registration No. 2016/ST/244; Protocol of Final Approval No. 7347/2017).

- ***Study Population***

From 1997 to 2015, 75 patients (21 males, 54 females) aged between 18 and 78 years (mean: 49 years, STD 14.7), requesting to rehabilitate an edentulous area in the upper and/or lower jaw with an implant-supported dental prosthesis, and presenting with relevant horizontal and/or vertical bone defects in the edentulous area, were recruited for this study. In 7 patients, reconstructive procedures were performed in two sites (for a total of 82 sites).

Patients were enrolled if presented a bone atrophy at the edentulous site whose severity was incompatible with: a) implant placement with simultaneous bone augmentation; b) placement of short (≤6 mm) or narrow (<3 mm) implants; c) sinus floor elevation with no reconstruction of the alveolar bone crest.

Adequacy of the residual bone volume was defined based on the possibility to place dental implants in a prosthetically driven position.

Initial patient screening included: a) evaluation of the residual dentition and periodontal status; b) intra-oral photographs; c) panoramic and peri-apical radiographs; d) preoperative impressions, study models, and manufacturing of a diagnostic wax- up to optimize the final prosthetic restoration. A computed tomography was performed to three-dimensionally assess the residual bone volume in the edentulous spaces.

Exclusion criteria for the surgical procedure were the following: a) severe kidney and/or liver disease; b) congenital or acquired immunodeficiency; c) ongoing antiblastic chemotherapy at the time of first examination; d) sequelae of radiotherapy in the head and neck area; e) oral mucosa diseases, such as lichen planus; f) full mouth plaque score (FMPS) and full mouth bleeding score (FMBS) < 20%; g) non-compliant patients; h) tobacco abuse (>10 cigarettes per day) or alcohol abuse; i) non compensated diabetes; j) active periodontal disease at the time of first examination (in these cases, patients underwent etiologic therapy and motivation in personal oral hygiene and were re-evaluated for surgical treatment). Ongoing

bisphosphonate therapy with oral or parenteral administration was inserted as an exclusion criterion since 2007.

Exclusion criteria for enrolment in this study were the following: a) patients' inability to attend an updated follow-up visit with clinical and radiographic evaluations; b) patients' inability to compile a questionnaire on patient-reported outcome measures; c) loss of clinical data concerning the surgical and prosthetic treatments. Reasons for exclusion were recorded during data collection.

Demographic data and main clinical features (dates of GBR procedure, implant placement, loading, etc) are reported in tables 4 and 5.

Table 4 - Patients' demographic and clinical data related to bone reconstructions

No	*Sex*	*Age*	*SMOKER*	*Site of defect*	*MISSING TEETH*	*Date of Grafting*	*Type of reconstruction*	*Donor site*	*SL*	*Avarage hor bone gain (mm)*	*Avarage vert bone gain (mm)*	*Complication before implant placement*
1	F	60	N	MAX (p)	14-17	jan-97	hor	ramus	N	3	0	
2	F	53	N	MAND (p)	45-47	oct-00	hor	ramus	N	3	0	
3	F	57	N	MAND (p)	44-47	feb-02	hor	ramus	N	4	0	
4	F	43	N	MAX (p)	14-17	apr-02	hor	ramus	Y	5	0	
5	F	42	N	MAND (p)	34-37	sept-02	vert + hor	ramus	N	4	2	
6	M	50	N	MAX (p)	26-27	nov-02	vert + hor	ramus	Y	2	5	
7	M	50	N	MAND (p)	35-36	jan-03	vert+hor	ramus	N	3	5	
8	F	50		MAND (p)	45-47	feb-03	vert+hor	ramus		3	2	
9	F	40	N	MAND (p)	44-47	feb-03	hor	ramus	N	3	0	
10	F	45	N	MAND (p)	33	mar-03	vert + hor	ramus	N	3	3	
11	F	76	Y	MAX (p)	25-26	jun-03	vert	ramus	Y	0	3	
12	F	43	N	MAX (p)	14-17	mar-04	vert	ramus	Y	0	4	
13	F	41	N	MAX (p)	24-27	apr-04	vert+hor	ramus	Y	4	2	Paresthesia (2 weeks)
14	M	38	N	MAND (p)	43	apr-04	hor	ramus	N	4	0	
					32		hor			4	0	
15	F	65	N	MAX (p)	24-26	may-04	hor	ramus	N	3	0	
16	F	69	N	MAND (p)	36-37	may-04	vert+ hor	ramus	N	2	5	
17	M	64	Y	MAND (p)	45-47	oct-04	vert	ramus	N	0	3	
18	F	51	N	MAND (p)	34-37	nov-04	vert	ramus	N	0	6	
19	F	29	N	MAX (p)	11	nov-04	vert + hor	ramus	N	3	3	
					21					3	3	
20	F	72	N	MAX (p)	25-27	nov-04	vert	ramus	Y	0	5	
21	F	56	N	MAND (p)	45-47	feb-05	vert+hor	ramus	N	3	5	
22	F	40	N	MAX (p)	12--22	mar-05	vert + hor	ramus	N	3	3	
23	M	60	N	MAND (p)	35-37	jun-05	hor	ramus	N	5	0	Paresthesia (2 months)

24	F	47	N	MAND (p)	34-37	sept-05	hor	ramus	N	4	0	
25	F	31	N	MAX (p)	11	sept-05	vert + hor	ramus	N	3	4	
26	F	63	N	MAND (p)	44-47	nov-05	vert + hor	ramus	N	2	4	
27	F	28	N	MAND (p)	34-35	nov-05	hor	ramus	N	4	0	
					44-45		hor	ramus	N	4	0	
28	M	78	N	MAX (p)	26-27	nov-05	vert	ramus	Y	0	4	
29	F	63	N	MAX (p)	15-17	jan-06	vert	ramus	Y	0	4	
30	F	25	N	MAX (p)	11	apr-06	hor + vert	ramus	N	3	3	
					21					3	3	
31	F	22	N	MAX (p)	11- - 22	apr-06	vert + hor	ramus	N	4	2	
32	F	58	N	MAND (p)	35-37	may-06	vert + hor	ramus x 2	N	4	4	
					44-47					2	4	Paresthesia (1 month)
33	M	66	N	MAND (p)	44-47	jun-06	vert	ramus	N	0	4	
34	M	53	Y	MAND (p)	35-37	jun-06	hor	ramus	N	3	0	
35	F	68	N	MAND (p)	44-47	jun-06	vert + hor	ramus	N	3	3	
36	M	35	N	MAX (p)	12- - 22	oct-06	vert+hor (upper) / vert (lower)	ramus	Y	3	3	
				MAND (p)	36-37					0	3	
37	F	43	N	MAX (p)	11	jan-07	vert + hor	ramus	N	5	9	
38	F	68	N	MAX (p)	15-17	apr-07	vert+ hor	ramus	N	1	3	
					26-27					1	3	
39	F	59	Y	MAND (p)	34-36	may-07	vert + hor	ramus	N	4	4	
40	F	42		MAX (p)	25-27	jun-07	vert + hor	ramus	Y	3	5	
41	M	53	Y	MAX (p)	16-17	jun-07	vert + hor	ramus x 2	Y	2	5	dehiscence; curettage solved without problems
					26-27					2	5	
42	F	58	N	MAND (p)	46	sept-07	vert+hor	ramus	N	2	2	
43	M	64	N	MAX (p)	11	oct-07	vert	ramus	N	0	2	

44	F	71	N	MAND (p)	45-47	feb-08	vert+ hor	ramus	N	3	5	
45	M	43	N	MAND (p)	31-36	may-08	vert	ramus	N	0	10	
46	F	52	N	MAND (p)	36-37	may-08	vert+hor	ramus	N	2	5	
47	F	53	N	MAX (p)	24-26	jul-08	vert+hor	ramus	N	2	4	
48	F	68	N	MAND (p)	34-37	jul-08	hor	ramus	N	3	0	
49	M	24	N	MAND (p)	31-41	nov-08	hor	ramus	N	4	0	
50	M	55	N	MAX (p)	24-26	dec-08	vert+hor	ramus	Y	3	5	
51	F	48	N	MAND (p)	45-46	mar-09	vert + hor	ramus	N	5	2	
52	F	48	N	MAND (p)	45-47	may-09	vert + hor	ramus	N	4	4	
53	F	58		MAND (p)	45-47	jun-09	vert + hor	ramus x 2	N	4	5	
					35-37					4	5	
54	M	19	N	MAX (p)	12;11; 21	jun-09	vert + hor	ramus	N	5	5	
55	M	41	N	MAX (P)	15-16	jun-09	vert+hor	ramus	N	3	3	
56	F	48	Y	MAND (p)	35-37	jan-10	vert + hor	ramus	N	5	2	
57	F	18	N	MAND (p)	35	apr-10	hor	ramus	N	3	0	
58	M	59	Y	MAX (p)	26-27	may-10	vert	ramus	Y	0	4	dehiscence; curettage solved without problems
59	F	43	N	MAX (p)	26-27	jun-10	vert	ramus	Y	0	4	
60	F	42	Y	MAX (p)	13-14	jun-10	vert+ hor	ramus	N	3	4	
61	F	30	N	MAND (p)	35	jul-10	vert+hor	ramus	N	3	3	
62	M	25	N	MAX (p)	12	feb-11	hor	ramus	N	5	0	
					22		hor	ramus		5	0	
63	F	24	N	MAND (p)	41	jun-11	vert + hor	ramus	N	8	2	
					35					7	2	
64	F	30	N	MAX (p)	11	apr-12	vert + hor	ramus	N	7	2	
65	F	36	N	MAX (p)	21	jun-12	hor	ramus	N	3	0	
66	M	28	N	MAND (p)	47	apr-13	vert	ramus	N	0	5	

67	F	58	N	MAND (p)	45-47	apr-13	vert+hor	ramus	N	5	10	
68	M	53	N	MAND (p)	36-37	may-13	vert + hor	ramus	N	3	5	
69	F	73	Y	MAND (p)	35-37	nov-13	hor	ramus	N	3	0	
70	F	53	N	MAND (p)	45-46	jan-14	hor	ramus	N	4	0	infection with partial resorption
71	F	43	N	MAND (p)	35-37	feb-14	vert+hor	ramus	N	4	2	
72	F	40	N	MAX (p)	12--22	mar-14	vert+hor	ramus	N	6	3	
					14-15		vert+hor	ramus	N	4	0	
					24-25		vert+hor	ramus	N	4	0	
73	F	54	N	MAND (p)	45-47	jun-14	vert + hor	ramus	N	4	2	
					35-37		vert + hor	ramus	N	4	2	
74	F	55	N	MAX (p)	26-27	oct-14	vert+ hor	ramus	Y	2	4	
75	M	65	Y	MAND (p)	35-36	dic-14	vert+hor	ramus	N	4	1	

Legend: mand, mandible; max, maxilla; (p), partially edentulous; (t), totally edentulous; vert, vertical; hor, horizontal; SL=sinus lift; Y= yes; N= no.

Table 5 - Patients' demographic and clinical data related to implant placement and prosthetic restorations

No	*Site of defect*	*Missing teeth*	*Date of implant placement*	*Pre-implant bone resorption (horizontal)*	*Pre-implant bone resorption vertical)*	*N impl*	*N impl mand*	*N impl max*	*Vestibuloplasty*	*Complications*	*Impl loading*	*Type prost*	*Lost to follow up*
1	MAX (p)	14-17	jul-97	0		3	0	3	Y		nov-97	FPP	2014
2	MAND (p)	45-47	apr-01	0		3	3	0	N		sept-01	FPP	2006
3	MAND (p)	44-47	jun-02	0		3	3	0	N		may-03	FPP	2010
4	MAX (p)	14-17	oct-02	0,5		3	0	3	N		mar-03	FPP	
5	MAND (p)	34-37	mar-03	0	0	3	3	0	Y		jul-03	FPP	2010
6	MAX (p)	26-27	may-03	0	0	2	0	2	N		oct-03	FPP	
7	MAND (p)	35-36	jun-03	0	0	2	2	0	N		oct-03	FPP	
8	MAND (p)	45-47	jul-03	0	0	3	3	0	N		oct-03	FPP	
9	MAND (p)	44-47	jun-03	0		4	4	0	N	perimplantitis 45-46-47 in 2008; implantoplasty	oct-03	FPP	2015
10	MAND (p)	33	jan-04	0	0	1	1	0	N		nov-04	FPP	
11	MAX (p)	25-26	nov-03		0	2	0	2	Y		feb-04	FPP	
12	MAX (p)	14-17	set-04		0	2	0	2	Y	perimplantitis 1 impl; regenerative technique	mar-05	FPP	
13	MAX (p)	24-27	oct-04	0	0	3	0	3	Y		febr-05	FPP	
14	MAND (p)	43	sept-04	0		1	1	0	N		dic-04	FPP x 2	
		32		0		1	1	0	N				
15	MAX (p)	24-26	oct-04	0		2	0	2	N		jan-05	FPP	
16	MAND (p)	36-37	nov-04	0	0	2	2	0	N		apr-05	FPP	
17	MAND (p)	45-47	mar-05		0	2	2	0	N		jul-05	FPP	2012
18	MAND (p)	34-37	apr-05		1	2	2	0	N		sept-06	FPP	
19	MAX (p)	11	may-05	0	0	1	0	1	Y	impl remov 21 in 2017 - loss of integration without resorption	sept-05	FPP	
		21		0	0	1	0	1	Y				
20	MAX (p)	25-27	may-05		0	2	0	2	N		oct-05	FPP	deceased (2017)
21	MAND (p)	45-47	set-05	0	0	2	2	0	N	peri-implantitis implantoplasty in 2011	jan-06	FPP	
22	MAX (p)	12--22	nov-05	0	0	2	0	2	Y		mar-06	FPP	

23	MAND (p)	35-37	oct-05	0		3	3	0	N		feb-06	FPP	
24	MAND (p)	34-37	mar-06	0		3	3	0	N		jul-06	FPP	
25	MAX (p)	11	feb-06	1	1	1	0	1	N		jun-06	FPP	
26	MAND (p)	44-47	apr-06	0	0	3	3	0	N		jan-07	FPP	
27	MAND (p)	34-35	apr-05	0		2	2	0	N		sept-05	FPP x 2	
		44-45		0		2	2		N				
28	MAX (p)	26-27	jul-06		0	2	0	2	N		dec-06	FPP	
29	MAX (p)	15-17	jul-06		0	2	0	2	N		nov-06	FPP	
30	MAX (p)	11	oct-06	0	0	1	0	1	Y		jan-07	FPP x 2	
		21		0	0	1		1	Y				
31	MAX (p)	11- - 22	sept-06	0	0	2	0	2	Y		jan-07	FPP	
32	MAND (p)	35-37	nov-06	0	0	6	6	0	N	perimplantitis 47 solved with regenerative treatment in 2016	apr-07	FPP x 2	
		44-47		0	0				N				
33	MAND (p)	44-47	oct-06		0,5	3	3	0	N		feb-07	FPP	2011
34	MAND (p)	35-37	oct-06	0		3	3	0	Y		feb-07	FPP	
35	MAND (p)	44-47	dec-06	0	0	3	3	0	N		may-07	FPP	
36	MAX (p)	12- - 22	mar-08	0	0	2	0	2	N		sept-08	FPP x 2	
	MAND (p)	36-37			0	2	2	0	N				
37	MAX (p)	11	jul-07	0	0	1	0	1	Y		dec-07	FPP	
38	MAX (p)	15-17	oct-07	0	0	2	0	2	Y		feb-08	FPP x 2	
		26-27		0	0	2	0	2	Y				
39	MAND (p)	34-36	oct-07	0	0	3	3	0	N		feb-08	FPP	
40	MAX (p)	25-27	nov-07	0	0	2	0	2	N		mar-08	FPP	
41	MAX (p)	16-17	nov-07	0	0	2	0	2	N	perimplantitis 16; implantoplasty in 2011	may-08	FPP x 2	
		26-27		0	0	2	0	2	N				
42	MAND (p)	46	jan-08	0	0	1	1	0	N		jun-08	FPP	
43	MAX (p)	11	may-08		0	1	0	1	Y		oct-08	FPP	
44	MAND (p)	45-47	sept-08	0,5	0,5	2	2	0	Y	perimplantitis 45 solved with regenerative treatment in 2016	jul-09	FPP	
45	MAND (p)	31-36	nov-08		0	4	4	0	Y		mar-09	FPP x 2	

46	MAND (p)	36-37	oct-08	0	0	2	2	0	N		feb-09	FPP	
47	MAX (p)	24-26	dec-08	0	0	2	0	2	Y		may-09	FPP	
48	MAND (p)	34-37	nov-08	0		3	3	0	N		apr-09	FPP	
49	MAND (p)	31-41	mar-09	0		2	2	0	N		jun-09	FPP	
50	MAX (p)	24-26	jun-09	0	0	3	0	3	N		nov-09	FPP	
51	MAND (p)	45-46	oct-09	0	0	2	2	0	Y		feb-10	FPP	
52	MAND (p)	45-47	oct-09	0	2	2	2	0	Y		feb-10	FPP	
53	MAND (p)	45-47	nov-09	0	0	2	2	0	N		may-10	FPP x 2	
		35-37		0	0	2	2	0	N				
54	MAX (p)	12;11; 21	feb-10	0	0	2	0	2	Y		may-10	FPP	
55	MAX (P)	15-16	oct-09	0	0	2	0	2	Y		feb-10	FPP	
56	MAND (p)	35-37	jun-10	0	0	3	3	0	N		oct-11	FPP	
57	MAND (p)	35	apr-11	0		1	1	0	Y		jul-11	FPP	
58	MAX (p)	26-27	dec-10		1	2	0	2	N		apr-11	FPP	
59	MAX (p)	26-27	oct-10		0	2	0	2	N		mar-11	FPP	
60	MAX (p)	13-14	dec-10	0	0	2	0	2	N		may-11	FPP	
61	MAND (p)	35	nov-10	0	0	1	1	0	Y		mar-11	FPP	
62	MAX (p)	12	jun-11	1		1	0	1	Y		oct-11	FPP x 2	
		22		1		1	0	1	Y				
63	MAND (p)	41	oct-11	0	0	1	1	0	N		jan-12	FPP x 2	
		35		0	0	1	1	0	N				
64	MAX (p)	11	sept-12	2	0	1	0	1	N		jan-13	FPP	
65	MAX (p)	21	nov-12	0		1	0	1	Y		mar-13	FPP	
66	MAND (p)	47	oct-13		0,5	1	1	0	N		febr-14	FPP	
67	MAND (p)	45-47	sept-13	0	0	2	2	0	N	perimplantitis 45 and 47 solved with regenerative treatment in 2017	jan-14	FPP	
68	MAND (p)	36-37	oct-13	1	1	2	2	0	N		jan-14	FPP	
69	MAND (p)	35-37	may-14	0		2	0	2	N		oct-14	FPP	
70	MAND (p)	45-46	jun-14	1,5		2	2	0	N		nov-14	FPP	
71	MAND (p)	35-37	jun-14	1,5	1,5	3	3	0	N		oct-14	FPP	

72	MAX (p)	12--22		0	0	2	0	2	Y		feb-15	FPP x 3	
		14-15		0	0	2	0	2	Y				
		24-25		0	0	2	0	2	Y				
73	MAND (p)	45-47	nov-14	1	1	2	2	0	N		mar-15	FPP x 2	
		35-37		1	1	2	2	0	N				
74	MAX (p)	26-27	apr-15	0	0	2	0	2	N		sept-15	FPP	
75	MAND (p)	35-36	apr-15	0	0	2	2	0	N	implant removal 35	jul-15	FPP	

Legend: mand, mandible; max, maxilla; (p), partially edentulous; (t), totally edentulous; vert, vertical; hor, horizontalimpl= implant; Y= yes; N= no; Type prost= type of prosthesis; FPP, fixed partial prosthesis; FCP, fixed complete prosthesis; ISO, implant-supported overdenture .

- ***Clinical Protocol***

All patients were treated by the same surgeon and the same prosthetic team. Bone grafting and subsequent implant placement were performed in sterile conditions under local anaesthesia or under general anaesthesia with naso-tracheal intubation, according to both patient's preference and defect extent**.** In all patients, re-opening of submerged implants was performed under local anaesthesia. The following steps were adopted during treatment.

- *Pre-operative Patients Preparation*

All patients were prepared to both the reconstructive procedure and the second stage implant surgery according to the following protocol: (a) professional oral hygiene 2 weeks prior to surgery; (b) local antisepsis with 0.2% chlorhexidine mouthwashes starting 3 days before surgery; and (c) antibiotic prophylaxis consisting of oral administration of amoxicillin and clavulanate (2 g) 1 hour before surgery. In case of general anaesthesia, antibiotics were infused intra-venously at the time of induction.

- *Reconstructive Procedure*

The following protocol was adopted:

1. Access to the mandibular ramus for bone harvesting was obtained through an incision following the "linea obliqua externa" of the ascending ramus and the elevation of a full thickness flap. A cortico-cancellous bone block was harvested using piezo-electric instruments or fine fissure burs, combined with fine chisels. Moreover, autogenous bone chips were locally collected with a manual bone scraper. Care was taken to avoid any damage to the inferior alveolar nerve, whose tri-dimensional position had been evaluated preoperatively through computed tomography scans. The harvested bone was kept into sterile saline and the access was closed with simple interrupted sutures (silk or PGA 4/0).
2. The defect site was exposed through a full-thickness crestal incision with mesial and distal releasing cuts. After careful defect debridement and perforations of the buccal cortical walls of the defect with a small round bur, to promote the access of bone marrow cells to the defect, the bone blocks were modelled and adapted to the local anatomy, to correct the horizontal and/or vertical bone deficiency. All sharp edges of the blocks which could increase the risk of dehiscence were accurately smoothed with diamond burs. A sterilised prosthetic stent was utilised in the majority of cases to optimize the reconstruction and allow the subsequent placement of implants in a prosthetically driven way. The blocks were fixed with titanium micro-screws (1.5 mm in diameter) and all remaining gaps between the bone blocks and the recipient sites were filled with autogenous bone particles obtained with scrapers. A mild over-correction of the defect was always performed both during vertical and horizontal augmentations.
3. A layer of slowly resorbable xenograft (Bio-Oss ®, Geistlich Pharma, Wolhusen, Switzerland) mixed with autogenous bone chips was placed over the grafts and stabilized with collagen membranes (Bio-Gide®, Geistlich Pharma, Wolhusen, Switzerland) to eliminate sharp edges, to harmonize the shape of the reconstructed area, and to reduce the risk of bone resorption (Proussaefs, & Lozada, 2003; Von Arx, & Buser 2005; Cordaro, et al., 2011; Wiltfang, et al., 2012; Sanz-Sánchez, et al., 2018; Chiapasco, et al., 2018, Elnayef, et al., 2018).
4. Periosteal releasing incisions of the flaps were performed to obtain a tension-free and water-tight closure with horizontal internal mattress and simple interrupted sutures using 4/0 or 5/0 silk or PGA.

Based on case variability within the study population, patients could receive, during the same surgical session, adjunctive surgical procedures, as follows:

5. Patients presenting with bone atrophy of the posterior maxilla requiring sinus grafting in addition to a tri-dimensional bone augmentation, received a sinus floor elevation with a lateral approach. Sinuses were grafted with a mixture of particulated autogenous bone and bovine bone mineral (Bio-Oss®, Geistlich Biomaterials,) with a one-to-one ratio, approximately. The bone blocks were then adapted over the lateral access to the sinus, according to local anatomy.

6. Patients presenting with bone atrophy in the posterior edentulous mandible received a single surgical access through an extended trapezoidal flap, which allowed both the harvesting and grafting procedures.

7. Patients presenting with impacted mandibular third molars at the site of harvest, with clinical and/or radiographic signs indicating the need for an extraction (signs of pericoronitis, history of recurrent infections, unfavourable contact with the second molar), underwent tooth removal during the harvesting procedure.

The following postsurgical instructions were prescribed: (a) application of ice packs over the reconstructed surgical site for 6–8 hours after surgery; (b) oral antibiotics (amoxicillin and clavulanate - 3 g per day for 6 days); (c) nonsteroidal anti-inflammatory (NSAIDs) therapy for 3–5 days after surgery (most commonly ketoprofen 80mg or ibuprofen 600mg twice a day); paracetamol 1000 mg was prescribed to patients unable to assume NSAIDs; (d) cold and liquid diet for the first 2 days after surgery and a soft diet until suture removal; (e) 0.2% chlorhexidine mouth rinses, twice per day for 21 days, combined with normal oral hygiene manoeuvres on the remaining dentition (if present); (f) when a concomitant sinus elevation was performed, patients were instructed to avoid any pressure peak in the upper respiratory tract which could have damaged the elevated Schneiderian membrane. This included sneezing with their mouth open, avoiding to blow their nose for the first 15 postoperative days, or to fly, to make underwater sports, and recreative activities for 3–4 postoperative weeks.

Sutures were removed 14–21 days after surgery. For 6 postoperative weeks, patients were not allowed to wear any type of removable prosthesis that could traumatize the reconstructed area. Vacuform retainers including resin teeth, Maryland bridges, or conventional bridges on available natural teeth (when available abutments on the adjacent dentition were already present in the patients mouth), were fabricated to provide a purely cosmetic restoration, taking care to avoid any form of occlusal trauma and any compression on the operated area.

- *Implant Placement*

Four to 12 months (mean = 6 months) after the reconstructive procedure, implants were placed in the reconstructed areas. A surgical template was used to optimize implant positioning according to the prosthetic treatment plan. Titanium screws placed at the time of bone grafting were removed only if their position interfered with implant placement. All implants were placed according to a submerged healing protocol. Postoperative instructions and therapy were equivalent to the ones of the reconstructive procedure.

According to case variability within the study population, patients could receive the following co-exposures:

1. Patients presenting with a lack of keratinised tissue buccal to the implant abutments and/or a reduced vestibule depth in the reconstructed area, received a vestibuloplasty in association with a free gingiva graft harvested from the palate, to improve local anatomy and to create an adequate width of the keratinized mucosa around the implants.

2. Patients presenting with an adequate amount of keratinised mucosa in the reconstructed sites, but a lack of soft tissue volume buccal to the implant abutments, received a bilaminar soft tissue augmentation procedure without vestibuloplasty. An autogenous connective tissue graft obtained through the extra-oral de-epithelisation of a free gingival graft, was placed under the buccal flap elevated for implant re-opening, and was stabilised with resorbable 6/0 PGA sutures.

- *Prosthetic Phases*

Patients started the prosthetic phases 3 to 10 months after implant placement (mean=4.5 months, SD=1.5). Provisionals were maintained for 3–9 months, to achieve optimal soft tissue adaptation and conditioning. Finally, fixed definitive prostheses (cemented or screw-retained) were delivered.

- *Follow-up*

During the time following the bone grafting procedure, patients were re-evaluated at suture removal, 1 month after surgery, 3 months after surgery and 2 weeks before implant placement. After implant placement, clinical controls were carried out at suture removal, 1 month after surgery and 2 weeks prior to implant re-opening. After implant uncovering, patients were controlled at the time of suture removal, abutment connection, and at the time of prosthetic loading.

After the start of prosthetic loading, patients were followed for 3 to 16 years, to assess peri-implant hard and soft tissue conditions, the stability of the reconstructed bone, and the onset of implant-related and/or prosthesis-related complications. The recall frequency for patients' maintenance and clinical evaluation was established through a continuous multilevel assessment of the patients risk profile for periodontal and peri-implant disease (Lang, & Tonetti, 2003). The frequency of recall ranged from 3 to 12 months.
Frequency of patients recall prior to the publication of the article by Lang & Tonetti (2003) (13 patients) was arbitrarily set every 3 to 6 months, based on patients previous history of caries and periodontal disease, patients compliance to oral hygiene instructions, and the overall complexity of the treated case.
Peri-apical radiographs were taken immediately after implant placement, at provisional prosthetic load, at prosthetic finalization, and annually thereafter. Radiographic examinations through periapical or panoramic radiographs were performed annually after implant placement.

- ***Analysis***
  - *Complication rate of the reconstructive procedure*

During the time following bone grafting and prior to implant placement, short-term complications regarding the donor and/or grafting site were recorded in the patients' chart.

  - *Bone resorption before implant placement*

The volumetric resorption of the grafted sites were estimated intra-operatively at the time of implant placement, adopting the titanium micro-screws used in the reconstructive procedure as the reference. Using a UNC15 periodontal probe, the distance between the screw head and the bone profile was measured after flap elevation at the mesial and distal aspect of each screw, to asses which length of the screw was located at a supra-bony level after graft resorption. Measurements were rounded at the nearest half millimeter, and the arithmetic mean of the mesial and distal values was recorded for each screw.

- *Peri-implant bone resorption*

Marginal peri-implant bone loss was evaluated on routine annual periapical radiographs using a dedicated image processing software (ImageJ® 1.38v U. S. National Institutes of Health, Bethesda, MD, USA). The analysis was performed on digital radiographs or digitalized conventional films, by measuring the distance between the implant shoulder and first bone-to-implant contact, at the mesial and distal aspect of each implant. Dimensional distortion was corrected by setting the known length and diameter of each implant in the software. Radiographs taken immediately after implant placement were considered as baseline. All measurements were rounded to the nearest half millimetre. A mean bone resorption value was then calculated for each implant and for each patient. All measurements were evaluated by one examiner (GT). Calibration was performed on repeated measurements collected on the first 10 patients (27 implants) and a Kappa intra-class correlation coefficient was calculated to estimate the presence of intra-examiner the consistency among different measurement sessions.

- *Implant-related complications*

Cases of peri-implant mucositis and peri-implantitis in which disease resolution was achieved by non-surgical and/or surgical means were considered as implant-related complications.

- *Implant survival/success rates*

Criteria used to determine implant survival were the following: (a) absence of persistent pain or dysesthesia; (b) absence of mobility; (c) absence of continuous peri-implant bone loss.

Failed implants were considered those presenting: (a) continuous peri-implant bone loss; (b) mobility or (c) fractures; (d) symptoms of persistent paresthesia/dysesthesia or (e) chronic pain; (f) presence of peri-implant infection not responding to medical/surgical treatment.

Successful implants were considered those presenting: a) absence of persistent pain or dysesthesia; b) absence of mobility; c) absence of continuous bone loss; d) absence of peri-implant infection; peri-implant bone resorption < 2 mm at the end of the observation period.

- *Patient's reported outcome measures (PROMS)*

PROMS were collected to explore the patients complaints, perceived benefits, and overall satisfaction towards the treatment that was delivered. The questionnaire was the same of the project 1 (see table 3)

- ***Statistical Analysis***

Statistical evaluation was performed with the IBM SPSS Statistics software (Version 24, SPSS Inc., Chicago, IL, USA).

Implant survival data were expressed in percentages and analysed by means of Kaplan–Meier curves to determine the cumulative survival rates. Implants were considered the statistical unit of analysis. Pearson's chi-square or Fisher's exact test, as appropriate, were used to evaluate: (a) the effect of patients' gender, smoking, area of edentulism on the occurrence of adverse events related to implants, considering either the patient, the implant, or the prosthesis as the unit of analysis; (b) the effect of the defect morphology (horizontal, vertical, combined) on implant survival and implant related complications, considering the implant or the prosthesis as the unit of analysis; (c) the effect of co-interventions (sinus lift, vestibuloplasty with free gingiva grafts, buccal soft tissue augmentation with a bilaminar technique) on implant survival and implant related complications, considering the implant or the prosthesis as the unit of analysis; (d) the effect of cemented or screw-retained final prosthetic restorations, on implant survival and implant related

complications, considering the implant or the prosthesis as the unit of analysis. Where possible, odds ratio and 95% confidence intervals (CI) were estimated.

A generalized estimating equation (GEE) with the patient as the analysis unit was performed to take into account the clustering of implants into patients, and to evaluate the simultaneous effect of various co-factors and co-interventions on the occurrence of adverse events like implant failure and biological complications (peri-implantitis). Implant failure and biological complications were set as dependent variables in different GEEs. The model was based on the following predictors: (a) smoking habits (smoking less than 10 cigarettes a day and non-smoking patients); (b) gender; (c) area of implant placement (maxilla or mandible); (e) follow-up duration; (f) sinus augmentation; (g) vestibuloplasty with free gingival grafts. The number of implants per patient was considered as the offset variable. The follow-up duration was the weight scale variable. The main factors considered for the final analysis were the following co-interventions: sinus floor elevation and grafting, vestibuloplasty, smoking habits, maxillary or mandibular arch and patients' gender. Significance threshold was set at $p = 0.05$.

Peri-implant bone resorption data were expressed as means (± *SD*). Descriptive statistical methods were adopted for this outcome. Mean data were calculated utilizing patients as the statistical unit of analysis, because of the vast heterogeneity of clinical scenarios and number of implants placed among the different cases.

4.3 – GUIDED BONE REGENERATION WITH CUSTOMIZED TI-MESH – PROJECT 3

- ***Study design***

This prospective, single arm study included patients presenting with atrophic ridges and asking for fixed implant-supported restorations. They were treated with customized titanium meshes obtained through a CAD-CAM technology (Yxoss CBR® by Reoss, Filderstadt, Germany) used in association with autogenous bone particles, deproteinized bovine bone mineral (Bio-Oss®- Geistlich Biomaterials AG- Wolhusen – Switzerland), in a 1:1 ratio, and covered with collagen membranes (Bio-Gide®- Geistlich Biomaterials AG- Wolhusen – Switzerland). Patients received dental implants after a mean period of 7.3 months (range: 5-12 months; median: 7.3; SD: 1.45), to obtain a good consolidation of the grafted material. Implants were uncovered after a mean period of 3.5 months (range: 2-5 months; median: 3.45; SD: 0.89) and were rehabilitated with implant-supported prostheses after another mean time of 1.8 months (range: 1-3.5 months; median: 1.78; SD: 0.71).

An informed consent was obtained from all patients prior to treatment, including: a) the purpose of the treatment; b) a detailed description of the procedure; c) the potential side effects and complications; c) reconstructive alternatives with advantages and disadvantages (GBR with non-resorbable membranes, autogenous bone blocks, and distraction osteogenesis); d) the necessity of clinical and radiographic controls; e) and the patients' authorization to use their data for statistical analysis.

The study was performed in accordance with Guidelines ISO Standard 14155:2011 (Clinical Investigation of Medical Devices for Human Patients with the Appendixes VIII and X of the Medical Devices Directive 93/42/EEC) and following the principles stated in the Declaration of Helsinki.

The study was approved by the Ethics Committee of the University of Milan, Italy (Registration No. 2019/ST/197; Protocol of Final Approval No. 47470/2019).

This study was registered in clinicaltrials.gov with number NCT04480073. The registration was done after the beginning of patients' enrollment. This manuscript was prepared according to the STROBE guidelines.

- ***Study Population***

In a 2- year period (January 2018- December 2019), 41 patients (10 males, 31 females) aged between 20 and 81 years (mean: 53.98 years, SD 14.32), presenting with 53 severely atrophic edentulous sites in the upper and lower jaw, and requesting implant-supported prosthetic restorations, were enrolled in this study. Information of demographic data and main clinical features (dates of GBR procedure, implant placement, loading, etc) are reported in table 6. Details of type of defects and distribution in the jaws are reported in table 7.

Initial patient screening included: a) evaluation of the residual dentition and periodontal status (Plaque Index – PI; Probing Depth – PD; and Bleeding On Probing BOP); b) intra-oral photographs; c) panoramic and peri-apical radiographs; d) preoperative impressions, study models, and manufacturing of a diagnostic wax-up to optimize the final prosthetic restoration.

Inclusion criteria included: a) systemically healthy patients; b) a minimum age of 18 years; c) relevant or severe bone atrophy at the edentulous sites incompatible with placement of even short (≤6 mm) or narrow (<3 mm) implants in an appropriate and prosthetically guided position; d) adequate compliance of patients, both in terms of oral hygiene and respect the follow-up recalls; and e) ability to understand the proposed surgical treatment and to understand and sign the informed consent.

Exclusion criteria included: a) severe kidney and/or liver disease; b) congenital or acquired immunodeficiency; c) ongoing antiblastic chemotherapy at the time of first examination; d) sequelae of

radiotherapy in the head and neck area; e) oral mucosa disease, such as lichen planus; f) full mouth plaque score (FMPS) and full mouth bleeding score (FMBS) > 20%; g) non-compliant patients; h) tobacco (>10 cigarettes per day) or alcohol abuse; i) non compensated diabetes; j) active periodontal disease at the time of first examination (in these cases, patients underwent etiologic therapy and motivation in personal oral hygiene and were re-evaluated for surgical treatment); k) bisphosphonate chemotherapy in progress; and, l) pregnant women.

Table 6 – Demographic data and clinical features of patients treated

# Pts	*Sex 0=F; 1=M*	*Age*	*Site and Type of Defect*	*Missing dentition*	*# sites*	*T1 date*	*Donor site*	*Sinus lift*	*T2 date*	*# of implants*	*T3 date*	*Soft tissue augm*	*Complications (number)*	*T4 date*	*Lost to F-up*	*T1-t2*	*T2-t3*	*T3-t4*	*T1-nov 20*	*T2-nov 20*	*F-up (t4-nov 20)*
1	0	20	max hor+vert	1.2; 2.2	2	09/01/18	ramus	0	10/06/18	2	10/08/18	CTG		15/09/18		5,0	2,0	1,2	34,4	29,4	26,2
2	0	46	max hor+vert	2.3	1	10/01/18	ramus	0	24/09/18	1	01/12/18	CTG	exposure (1)	10/01/19		8,4	2,2	1,3	34,4	25,9	22,4
3	1	62	mand hor+vert	3.3-3.7	1	16/01/18	ramus	0	14/11/18	3	02/04/19	V + FGG	exposure (1)	05/06/19		9,9	4,6	2,1	34,2	24,2	17,6
4	0	29	max hor+vert	2.2	1	13/02/18	ramus	0	14/11/18	1	02/02/19	CTG		03/03/19		9,0	2,6	1,0	33,2	24,2	20,7
5	1	45	max hor+vert	1.5-1.7	1	20/03/18	ramus	0	01/10/18	2	23/01/19	V + FGG	exposure (1)	10/04/19		6,4	3,7	2,5	32,1	25,7	19,4
6	0	61	mand hor+vert	4.3-4.7	1	20/03/18	ramus + calvarium	0	23/10/18	2	22/01/19	V + FGG		20/03/19		7,1	3,0	1,9	32,1	25,0	20,1
7	0	64	mand hor+vert	4.4-4.7; 3.4-3.7	2	28/03/18	ramo	0	21/11/18	4	01/03/19	/		10/05/19	10/06/19	7,8	3,3	2,3	31,8	24,0	
8	0	70	max hor+vert	1.2-1.4	1	06/04/18	ramus	0	05/11/18	2	10/01/19	CTG	exposure (1)	06/03/19		7,0	2,2	1,8	31,5	24,5	20,6
9	1	62	mand hor+vert	4.5-4.7	1	09/05/18	ramus	0	01/12/18	2	12/04/19	V + FGG		09/06/19		6,8	4,3	1,9	30,5	23,7	17,4
10	0	59	max hor+vert	1.5-1.7	1	17/07/18	ramus	1	01/04/19	2	19/06/19	V + FGG		17/07/19		8,5	2,6	0,9	28,2	19,7	16,2
11	0	59	max hor+vert	1.2-2.7	1	22/09/18	ramus + calvarium	1	02/06/19	7	01/10/19	V + FGG		13/11/19		8,3	4,0	1,4	26,0	17,7	12,3
12	0	63	max hor+vert	1.5-1.6	1	18/09/18	ramus	1	13/06/19	1	16/10/19	VSCM	exposure (1); early ti-mesh removal	27/11/19		8,8	4,1	1,4	26,1	17,3	11,8
13	0	65	max hor+vert	2.6-2.7	1	03/10/18	ramus	1	14/05/19	2	24/09/19	V + FGG		20/12/19		7,3	4,4	2,9	25,6	18,3	11,1
14	0	75	mand hor+vert	3.5-3.7; 4.5-4.7	2	10/10/18	ramus + calvarium	0	07/05/19	4	10/09/19	/		15/10/19		6,9	4,1	1,1	25,4	18,5	13,2
15	0	62	max hor+vert	1.4-1.6	1	23/10/18	ramus	0	06/05/19	2	27/07/19	V + FGG		16/09/19		6,4	2,7	1,7	25,0	18,6	14,2
16	1	56	max hor+vert	1.4-1.6	1	23/10/18	ramus	1	25/06/19	2	25/09/19	V + FGG		08/01/20		8,0	3,0	3,4	25,0	16,9	10,4
17	0	56	max hor+vert	1.1-1.6	1	24/10/18	ramus + calvarium	1	14/05/19	2	14/10/19	/	exposure (1)	09/01/20		6,6	5,0	2,9	24,9	18,3	10,4
18	0	43	mand hor+vert	3.5-3.7	1	07/11/18	ramus	0	08/11/19	2	05/02/20	/	paresthesia (1)	07/03/20		12,0	2,9	1,0	24,5	12,5	8,5
19	0	67	max hor+vert	1.7-2.7	1	20/11/18	ramus + calvarium	2	25/06/19	6	09/10/19	V + FGG		13/01/20		7,1	3,5	3,2	24,0	16,9	10,3
20	0	56	mand hor+vert	4.4-4.6	1	30/11/18	ramus	0	19/08/19	2	09/12/19	V + FGG	exposure (1)	10/01/20		8,6	3,7	1,1	23,7	15,1	10,4
21	0	62	mand hor+vert	4.5-4.6	1	23/01/19	ramus	0	07/10/19	2	13/01/20	/		20/02/20		8,4	3,2	1,2	21,9	13,5	9,0

22	1	81	mand hor+vert	4.2-3.7	1	07/01/19	ramus	0	12/06/19	4	08/10/19	V + FGG		06/12/19		5,1	3,9	1,9	22,5	17,3	11,5
23	0	60	mand hor+vert	4.4-4.7	1	09/01/19	ramus	0	26/06/19	2	03/11/19	V + FGG	paresthesia (1)	27/01/20		5,5	4,3	2,8	22,4	16,9	9,8
24	0	47	max hor+vert	2.5-2.7	1	09/02/19	ramus	1	/	0	/	/	exposure (1)	/							
25	1	55	max hor+vert	1.1-1.2	1	25/03/19	ramus	0	02/10/19	1	13/01/20	VSCM		04/03/20		6,3	3,4	1,7	19,9	13,7	8,6
26	0	53	mand hor+vert; max vert+hor	1.4-1.7; 2.4-2.7; 3.5-3.7; 4.5-4.7	4	28/05/19	ramus + calvarium	2	28/11/19	8	20/04/20	V + FGG	exposure (2 max)	15/07/20		6,0	4,7	2,8	17,8	11,8	4,2
27	0	28	max hor+vert	2.3	1	30/05/19	ramus	0	21/01/20	1	15/04/20	VSCM		19/05/20		7,8	2,8	1,1	17,8	10,0	6,1
28	0	24	max hor+vert	1.2; 2.2	2	12/06/19	ramus	0	25/02/20	2	15/05/20	VSCM		06/07/20		8,5	2,6	1,7	17,3	8,9	4,5
29	0	53	mand hor+vert	4.6-4.7	1	25/06/19		0	17/12/19	2	18/05/20	CTG		23/07/20		5,7	5,0	2,2	16,9	11,2	4,0
30	0	69	mand hor+vert	3.2-3.6	1	17/07/19	ramus	0	25/02/20	3	26/06/20	/		01/09/20		7,3	4,0	2,2	16,2	8,9	2,7
31	1	70	mand hor+vert; max vert+hor	2.5-2.7; 3.6-3.7	2	15/07/19	ramus + calvarium	1	03/02/20	4	/	/	exposure (1 max)	/		6,7			16,3	9,6	
32	0	57	mand hor+vert; max vert+hor	4.6; 2.4-2.7	2	18/07/19	ramus + calvarium	0	05/03/20	3	08/07/20	CTG		15/09/20		7,6	4,1	2,3	16,2	8,6	2,2
33	0	32	max hor+vert	1.2; 2.2	2	25/07/19	ramus	0	18/02/20	2	08/07/20	CTG		15/09/20		6,8	4,6	2,3	15,9	9,1	2,2
34	1	43	max hor+vert	2.1-2.2	1	19/08/19	ramus	0	04/02/20	1	05/05/20	/		09/06/20		5,6	3,0	1,1	15,1	9,6	5,4
35	0	30	max hor+vert	1.4-1.5; 2.4	2	12/09/19	ramus	0	02/03/20	3	05/05/20	/		05/06/20		5,7	2,1	1,0	14,3	8,7	5,6
36	1	38	max hor+vert	1.1-1.2	1	16/10/19	ramus	0	27/04/20	1	05/07/20	CTG		07/08/20		6,4	2,3	1,1	13,2	6,8	3,5
37	0	50	max hor+vert	1.4-2.2	1	26/10/19	ramus	0	05/06/20	3	06/10/20	CTG		/		7,3	4,0		12,9	5,6	
38	0	53	mand hor+vert	4.5-4.6	1	07/11/19	ramus	0	09/06/20	2	08/09/20	V + FGG		02/10/20		7,1	3,0	0,8	12,5	5,4	1,6
39	0	57	mand hor+vert; max vert+hor	1.5-1.7; 2.5-2.7	2	19/11/19	ramus + calvarium	1	07/07/20	4	20/10/20	CTG		/		7,6	3,4		12,1	4,5	
40	0	64	mand hor+vert	3.4-3.7	1	20/11/19	ramus	0	12/05/20	3	15/07/20	/		07/08/20		5,7	2,1	0,8	12,1	6,3	3,5
41	1	67	max hor+vert	2.3-2.7	1	04/12/19	ramus	0	07/07/20	4	04/11/20	CTG		/		7,1	3,9		11,6	4,5	

Legenda: F= female; M= male; T1= date of regeneration with Ti-mesh; T2= date of Ti-mesh removal and implant placement; T3= implant re-opening; T4= start of prosthetic loading; max= maxilla; mand= mandible; hor= horizontal; vert= vertical; CTG= connective tissue graft; V+FGG= vestibuloplasty + free gingival graft; VSCM= volume stable collagen matrix

Table 7 - Distribution and type of edentulism in the upper and lower jaws

	No. missing teeth: 1	No. missing teeth: 2	No. missing teeth: 3-5	No. missing teeth: >5	No. missing teeth: edentulous arch
Maxilla 1 site	3+ 1 §	5+1 §	7 + 1# + 1¶	3	1
Maxilla 2 sites	3	0	1+1*	0	0
Mandible 1 site	1 #	3+ 1¶	8	1	0
Mandible 2 sites	0	0	2 + 1*	0	0

Legenda:
* one patient presented at the same time 4 sites (2 mandibular and 2 maxillary) with more than 3 teeth missing per site
# one patient presented at the same time 2 sites (1 mandibular and 1 maxillary) with 1 and 3 teeth missing, respectively
¶ one patient presented at the same time 2 sites (1 mandibular and 1 maxillary) with 2 and 3 teeth missing, respectively
§ one patient presented at the same time 2 maxillary sites with 1 and 2 teeth missing, respectively

- ***Clinical Protocol***
  - *Pre-operative planning*

In every patient, dental impressions were taken and study casts constructed: on these latter, a diagnostic wax-up with ideal position and dimension of the teeth to be prosthetically substituted was performed and a diagnostic template including radiopaque teeth was created.
All patients received a CBCT wearing the radiological templates, and the DICOM files obtained were sent to the Ti-mesh manufacturer (Yxoss CBR® by Reoss, Filderstadt, Germany). These digital files were used to create a three-dimensional model reproducing both the edentulous and the neighbouring dentate areas. These CBCTs were considered the reference point (time 0=T0) to which the following ones were compared to calculate bone volume changes over time.
A digital model of the Ti-mesh was provided by the manufacturer for final approval. Any modification of the mesh shape, if needed, was communicated by the surgeons to obtain the appropriate morphology.
After final approval, it was possible to produce with CAD/CAM technology a precise three-dimensional class IV titanium scaffold by means of laser sintering (Yxoss CBR® by Reoss, Filderstadt, Germany).

  - *Preoperative ancillary procedures*

Two out of 41 patients presented previously failed attempts of implant placement in association with GBR procedures which were followed by implant removal because of infection, lack of osseointegration, or malposition. Healing was followed by bone defects and an insufficient quality of the residual soft tissues. It was therefore decided to create a more favourable environment by means of autogenous connective tissue grafts taken from the palate.

  - *Pre-operative Patients Preparation*

All patients were prepared to both the regeneration procedure (T1) and the second stage implant surgery (T2) according to the following protocol: a) professional oral hygiene (1-2 weeks prior to T1-T2); b) local antisepsis with 0.2% chlorhexidine mouthwashes starting 3 days before T1-T2; c) antibiotic prophylaxis

consisting of oral administration of amoxicillin and clavulanate (2 g), 1 hour before T1-T2; and d) an antioedema medication (Bromelina Plus® Solgar Italia Multinutrient SpA – Padova - Italy) to be assumed 3 days before T1-T2 (3 tabs per day). In case of intravenous sedation or general anaesthesia, antibiotics were infused at the time of induction. In case of allergy or intolerance, amoxicillin was substituted with macrolids or cephalosporins.

- *Reconstructive Procedure*

All patients were treated by the same surgical and prosthetic team. The regeneration procedure was performed in sterile conditions under local anaesthesia, intravenous sedation, or general anaesthesia, according to patient's compliance and defect extent**.**

A mid-crestal incision with mesial and distal releasing incisions at least 1.5 cm from the area to be reconstructed and the elevation of a full-thickness flap was performed to expose the edentulous area. Any residual soft tissues over the site to be regenerated were accurately removed to avoid interposition of connective tissue between the recipient site and the grafting material.

As a relevant augmentation was expected, periosteal releasing incisions on the buccal and lingual sides were performed in the mandible and only on the buccal side in the maxilla.

Bone harvesting was performed with disposable bone scrapers (SafeScraper Twist®-C.G.M. SPA Divisione Medicale Meta, Reggio Emilia, Italia) from the mandibular body and ramus in the majority of patients. In the case of atrophy in the posterior mandible, one single incision allowed access to both the harvesting and recipient sites. In the other cases, two independent incisions were performed. The amount of bone particles needed was calculated taking into account that approximately 50% of the grafting material was represented by BBM. It is worth noting that the Ti-mesh manufacturers were able to calculate pre-operatively in cubic centimetres the volume of grafting material.

In 9 patients, presenting with extended severe atrophy, autogenous bone was collected with the same scraper from the parietal bone of the calvarium through a short full-thickness, parasagittal incision.

The Ti-mesh was checked to control adequacy of shape and fitting to the atrophic site, filled with the grafting material, and placed over the defect area.

Stabilization of the mesh was obtained with dedicated titanium micro-screws, 1.4 mm in diameter and 5 to 13 mm long, according to surgical needs.

A collagen membrane (Bio-Gide®- Geistlich Biomaterials AG- Wolhusen – Switzerland) was applied over the mesh to reduce the risk of soft tissue ingrowth through the mesh during the early phases of healing.

Patients presenting with bone atrophy of the posterior maxilla requiring sinus grafting in addition to bone augmentation, received a sinus lift with BBM via a lateral approach.

The surgical procedure was concluded with a water-tight and tension-free closure with polyglactin resorbable 5/0 sutures, alternating horizontal mattress and single sutures.

The following post-surgical instructions were prescribed: a) application of ice packs for 6–8 hours after surgery; b) oral antibiotics (amoxicillin and clavulanate - 3 g per day for 6 days) or macrolids / cephalosporins in case of allergy; c) nonsteroidal anti-inflammatory (NSAIDs) or paracetamol for 3–5 days after surgery; d) an antioedema medication (Bromelina Plus® Solgar Italia Multinutrient SpA – Padova - Italy) - 3 tabs per day for 5 days; e) cold and liquid diet for the first 2 days after surgery and a soft diet until suture removal; f) 0.2% chlorhexidine mouthrinses, twice per day for 21 days, combined with normal oral hygiene manoeuvres on the remaining dentition (if present). When a concomitant maxillary sinus floor elevation was performed, patients were instructed to avoid any pressure peak in the upper respiratory tract for at least 15 days not to potentially damage the Schneiderian membrane (i.e. sneezing with their mouth closed, blow their nose, flying in the post-operative day, etc). Patients were not allowed to wear removable prostheses that could traumatize the reconstructed area for at least 6 weeks. Vacuform retainers including resin teeth, Maryland

bridges, or conventional bridges, were fabricated to provide a purely cosmetic restoration and to avoid any compression on the operated area.
A CBCT was done in all patients immediately after T1, to control the outcome of the surgery and to obtain a reference point for the following measurements. A dedicated software (Plamneca Romexis Viewer® Planmeca OY – Helsinki - Finland) was used to measure bone volume changes. Sutures were removed 2-3 weeks after surgery.

- *Ti-Mesh removal and implant placement*

After a mean waiting time of 7.3 months (median: 7.3; range: 5-12 months; SD: 1.45), patients were re-evaluated with a new CBCT (T2) performed with the aid of the same radiological templates to check bone volumes of the regenerated tissues and to plan implant placement according to the prosthetically guided project. An average waiting period of 7 months was judged a congruous time for obtaining a good integration of the graft, also on the basis of available literature on this type of technique (Sumida, et al., 2015; Sagheb, et al., 2017; Seiler, et al., 2018) and the available literature on horizontal/vertical GBR with non-resorbable barriers (Chiapasco et al., 2009; Milinkovic & Cordaro, 2014; Urban et al., 2019). Longer waiting times were determined only because a very limited number of patients did not respect the recall schedule for personal reasons. Variations in waiting times were not related to site (maxilla/mandible) or type (horizontal/vertical) of the defect.
Removal of the meshes and implant placement was performed under local anaesthesia, sedation, or general anaesthesia according to patients' compliance and extent of surgery. Access was obtained following the previous incisions. Ti-meshes and fixation screws were exposed and removed, surgical templates applied and implant site preparation started.
A total of 106 pure titanium or titanium zirconium alloys, root-form, and bone level implants were placed in the regenerated areas and they were left to heal in a submerged fashion. Post-operative instructions and medications were the same ones used after T1.

In 20 mandibular sites, one implant site was prepared with a 2.5 mm trephine drill to obtain a bone biopsy for histomorphometric analysis of the regenerated tissue.
Grid and bone carrot were immediately fixed in 10% buffered formalin for a maximum of 5 days and sent to the histological laboratory to start the processing.
All laboratory procedures were carried out according to the standard protocol for hard tissues (Donat, et al., 1982; Canullo, et al., 2016)
The specimens were immersed in a jar with PBS (Phosphate Buffered Saline 1%) for 10 minutes, and then immersed in increasing ratio alcohol-water solution to start the dehydration process (70%, 80%, 90%, 96% and 100%). Completed the dehydration process, the samples were infiltrated with a blend of resin/alcohol in increasing ratios in order to prepare the biopsy to ending inclusion in pure methyl methacrylate resin (Technovit 7200 VLC, Exact Kultzer, Bio-Optica, Milano, Italy) by light polymerization.
Each polymerized resin block was cut in two halves with a diamond blade mounted on a cutting machine. The two sections were glued with resin on a plastic support to be abraded with a grinding machines until reaching a thickness of about 100µm. Finally, the sections were treated with 0.1% alumina to remove any scratches or roughs, and then stained with Toluidine Blue and Pyronin Yellow in order to differentiate the bone tissue components.

- *Implant re-opening and prosthetic phases*

After a mean waiting time of 3.5 months (range 2-5 months; median: 3.45; SD: 0.89), implants were uncovered and healing abutments placed (T3). At this time, the quality and quantity of peri-implant soft tissues were evaluated.

Following the regeneration procedure, relevant releasing incisions were needed with modification of the position of the residual band of the keratinized mucosa and reduction of the buccal sulcus. If these latter were judged inadequate to guarantee a sufficient seal around implant abutments, a vestibuloplasty in association with a free gingival graft harvested from the palate was performed.

In patients presenting with adequate keratinized mucosa, but with insufficient thickness of peri-implant soft tissues, residual concavities were corrected with autogenous connective tissue taken from the palate or with porcine-derived collagen matrices (FibroGide® - Geisltlich Biomaterials AG- Wolhusen – Switzerland).

After a mean waiting time of 1.8 months (range: 1-3.45; median:1.78; SD:0.71) patients started the prosthetic phases. Provisional restorations were delivered first (T4), and after adequate conditioning of peri-implant soft tissues, the final prostheses (screw-retained or cemented) were delivered.

- *Follow-up*

After the start of prosthetic loading, patients were recalled 3, 6, 12 months later, and annually thereafter, to assess peri-implant hard and soft tissue conditions, the stability of the reconstructed bone, and the onset of implant-related complications. The recall frequency for patients' maintenance and clinical evaluation was established through a continuous multilevel assessment of the patients risk profile for periodontal and peri-implant disease (Lang, & Tonetti, 2003).

Peri-apical radiographs were taken immediately after implant placement (T2), at the time of implant loading with prosthetic restoration (T4), six (T5) and 12 (T6) months after the start of prosthetic loading, and annually thereafter. A flowchart describing the timing of planning and treatment is shown in figure 1.

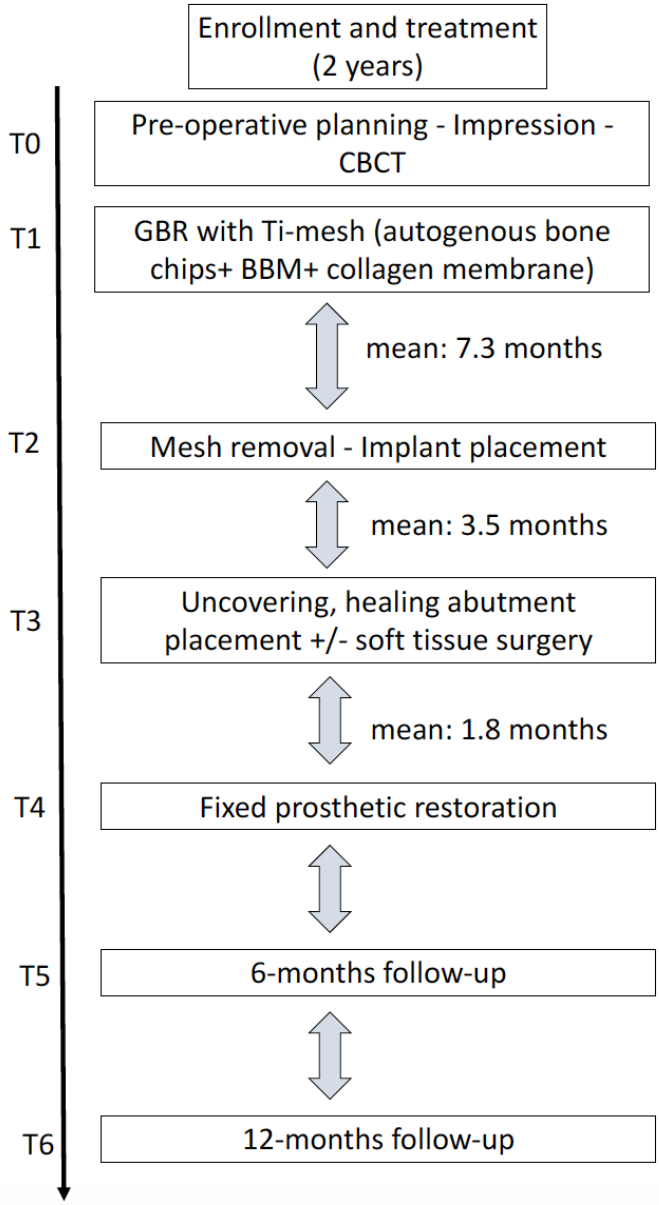


Figure 1 - flowchart explaining the different times of treatment

- ***Analysis***

The following parameters were analysed after the regeneration procedures.

- *Bone gain*

Vertical and horizontal bone gain were evaluated on a CBCT (Planmeca ProMax 3D Max® - Planmeca OY – Helsinki - Finland) taken immediately after surgery with a dedicated software (Planmeca Romexis Viewer® Planmeca OY – Helsinki - Finland) using as reference points the internal margins of the Ti-mesh and the profile of the residual bone before the reconstruction. More in detail, it was possible to identify the different radiolucency between residual bone and the regenerated one on CT scans. Only the maximum value (expressed in mm) of horizontal and vertical augmentation was calculated. In order to repeat measurements in the same points at T1 and T2, the final, computer guided position of “virtual” implants obtained by the

dedicated software according to the indication of the diagnostic template, was used as a reference point. In such a way, 2 independent examiners were able to measure vertical and horizontal bone gains (in mm) in repeatable and comparable points. Intra-examiner calibration was performed on the first ten consecutive patients using the kappa intra-class correlation coefficient. The arithmetic mean of the two examiners measurements was done. Six months later, at the time of implant planning, a new CBCT, as well as the same measurement following the previously described modalities, was performed. Any difference between the two CBCTs as regards height and width of bone gains was recorded for comparison.
Since the Ti-mesh thickness was very thin, the scattering effect given by the titanium was almost absent: for this reason, no elimination for radiographic distortion was performed.

As regards bone volume modifications, the residual basal and alveolar bone of the atrophic edentulous areas were not measured, but considered as baseline (T0). Only the volumes of the regenerated areas were measured at T1 and T2 in cubic centimetres.
As regards bone volume gains, CBCTs were segmented at T0, T1 and T2 with a dedicated software (3d Slicer 4.1 - Free Open Source Software https://www.slicer.org). After segmentation, 3D models were obtained, exported as stl files and imported on Blender (free and open source software - https://www.blender.org). Thanks to the plug-in Object Alignment (Author Plug-In: Patrick Moore, DDS), a superimposition of the 3d models at T0, T1 and T2 was performed. Then, a Boolean difference between volume at T0 and T1, and from T0 and T2 was performed to obtain only the bone volume increase at T1 and T2. The two different volumes obtained through this Boolean subtraction (T0-T1 and T0-T2) were imported again on 3d Slicer (3d Slicer 4.1 - Free Open Source Software https://www.slicer.org) and measured in cubic centimetres. A figure showing this superimposition is shown in figure 2.

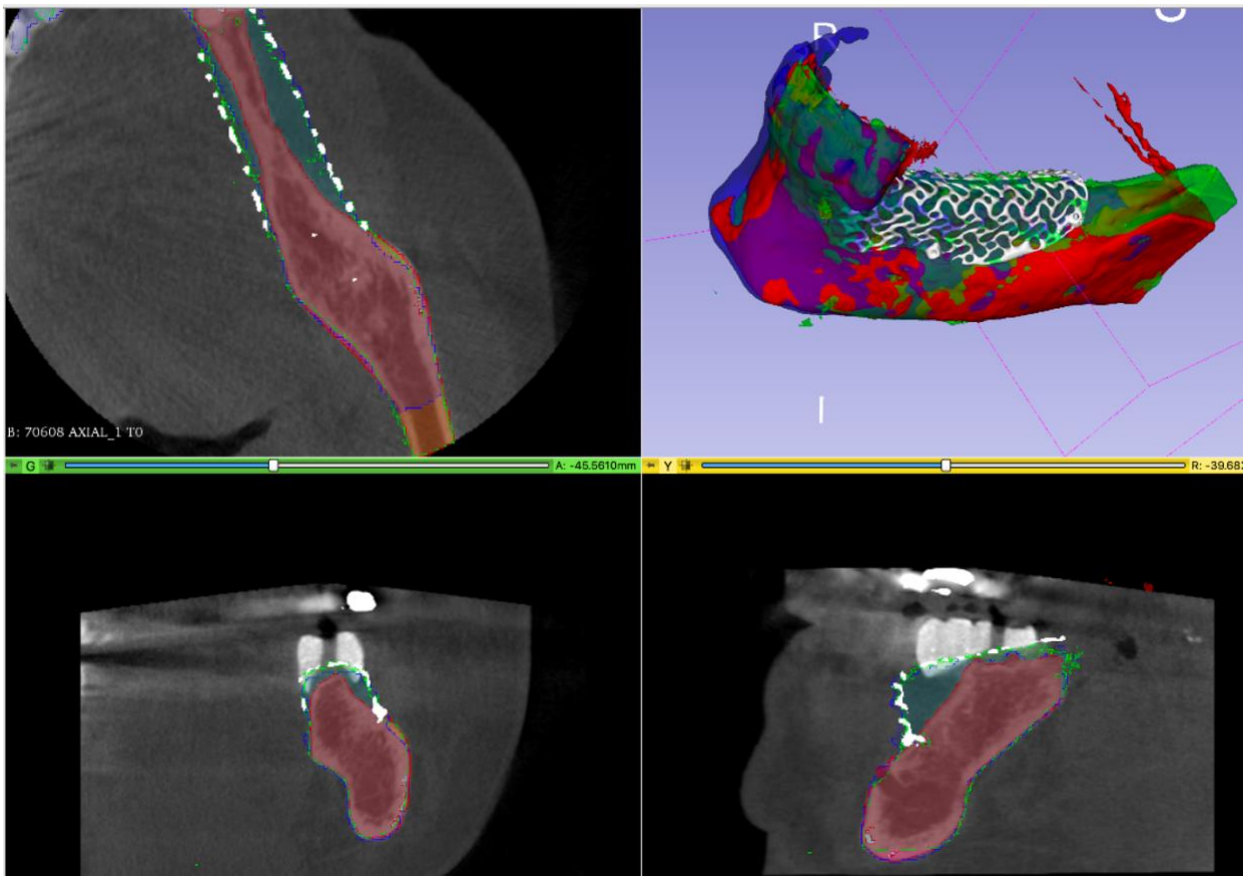


Figure 2- method of superimposition

- *Complication rate of the reconstructive procedure*

During the time following bone grafting and prior to implant placement, short-term complications regarding the donor and/or grafting sites were recorded in the patients' chart.

- *Implant survival rate and implant-related complications*

Criteria used to determine implant survival were absence of: (a) persistent pain or dysesthesia; (b) mobility; (c) continuous peri-implant radiolucency.
Failed implants were considered those presenting: (a) continuous peri-implant radiolucency; (b) implant mobility or fracture; (c) symptoms of persistent paresthesia/dysesthesia or chronic pain; (d) presence of peri-implant infection not responding to medical/surgical treatment.
Cases of peri-implant mucositis and peri-implantitis in which disease resolution was achieved by non-surgical and/or surgical means were considered as implant-related complications.

- *Peri-implant bone resorption*

Marginal peri-implant bone loss was evaluated on periapical radiographs using a dedicated image processing software (ImageJ® 1.38v U. S. National Institutes of Health, Bethesda, MD, USA). The analysis was performed on digital radiographs by measuring the distance between the implant shoulder and the first bone-to-implant contact, at the mesial and distal aspect of each implant. Dimensional distortion was corrected by setting the known length and diameter of each implant. Radiographs taken immediately after implant placement were considered as the baseline. All measurements were rounded to the nearest half millimetre. A mean bone resorption value was then calculated for each implant and for each patient. All measurements were evaluated by the same examiners and with the same modalities used for bone gain measurements.

- *Patient's reported outcome measures (PROMS)*

PROMS were collected to explore the patients complaints, perceived benefits, and overall satisfaction towards the treatment that was delivered. The questionnaire was the same of the project 1 (see table 3).

- *Histomorphometric analysis*

  - *Whole slide image and morphological analysis*

Whole slide images were obtained for each plastic slide using high resolution digital imaging scanner (NanoZoomer S360 Digital slide scanner, Hamamatsu, Japan). All sections were acquired with a resolution that allows to observe morphological features with a total magnification of 400x until a digital magnification of 800x.
Using digital pathology tools of Ndp view software, the morphological and morphometrical assessments were performed zooming at different magnifications.
A qualitative analysis was performed following the indications of ISO-10993-6:2007 annex E to evaluate cell type response by observing the presence of necrosis and inflammatory infiltrate and tissue response, and by detecting the number of blood vessels in the medullary spaces, and the eventual fibrosis and fatty infiltrate associated with phlogosis both on grid and bone samples.
Furthermore, each section was observed at total magnification of 100x-200x to assess the biomaterial and grid osseointegration, the presence of Howship's resorption lacunae and the remodeling fronts between biomaterial remnants and the newly formed bone. Finally, a morphological evaluation of the bone cell populations was made at higher magnifications in order to identify osteoblasts, osteocytes, and osteoclasts (ISO-10993-6:2007 annex E).

- *Histomorphometry*

The tissue composition of the regenerated area was assessed by means of stereological principles. Briefly, a digital counting grid was applied on each acquired section in order to evaluate the volume fractions occupied by mature bone tissue, immature bone tissue/ woven bone, biomaterial residual particles and medullary spaces. Similarly, the percentage of the regenerated area occupied by new blood vessels was then calculated. The software used for the computation allowed to maintain the correct proportion between the grid and the anatomical structure also when zooming.

- ***Statistical analysis***

Sample size was calculated using the free tool at https://clincalc.com/stats/samplesize.aspx, considering as outcome variable the vertical bone gain. As the present was a prospective single cohort study, a previous study was taken as comparison (Troeltzsch, et al., 2016). For augmentation procedures with a mixture of autogenous bone with allogeneic/xenogeneic grafting materials, that study provided a mean vertical gain of 3.7±1.4 mm. It was assumed to achieve a mean value of 4.4mm in the present study (about 15% better than the literature control). Considering a power of 80% and an alpha =0.05, 31 patients would be required to observe a difference of 0.7mm. Assuming a dropout rate of 15%, it was established to enroll at least 36 patients.

Statistical evaluation was performed using GraphPad Prism 5.03 (GraphPad Software, Inc., La Jolla, CA, USA), and IBM SPSS Statistics software (Version 24, SPSS Inc., Chicago, IL, USA).

Descriptive statistics of data were done using means, medians, ranges, and standard deviation (SD) for quantitative variables normally distributed. Normality of distribution was evaluated through the d'Agostino and Pearson omnibus test. Occurrence of complications were expressed in percentages and analysed by means of Kaplan–Meier curve. Implants and cases (reconstructed sites), respectively, were considered the statistical unit of analysis. Fisher's exact test was used to evaluate the effect of patients' gender, age (> or <60y at the time of surgery), area of edentulism, number of sites involved per patient, co-interventions (e.g. maxillary sinus augmentation), harvesting site, on the occurrence of complications. Either the patient or the reconstructed sites (cases) were considered as the unit of analysis. Fisher's exact test was used because the incidence of complications was less than five in at least one subgroup. Descriptive statistical methods were used for vertical and horizontal bone changes at the reconstructed site, and peri-implant marginal bone level (MBL) changes at mesial and distal aspect after 6 months of loading.

Means ± SD, median, minimum and maximum values were calculated utilizing the patient as the statistical unit of analysis. The effect of mesh exposure on bone changes was evaluated by comparing exposed versus non-exposed groups of patients, by means of unpaired Student's t-test. Significance threshold was set at $p = 0.05$.

A multilevel mixed-effects linear regression model was used to evaluate the relationship between exposure, vertical bone loss, horizontal bone loss and the following variables, using the patient as the unit: age, number of sites per patient, number of implants per patient, sinus lift. For vertical and horizontal bone loss, also the occurrence of exposure was considered. The estimates of coefficients' standard errors and confidence intervals were adjusted using a robust variance-covariance estimator. The same model was used to evaluate the relationship between bone volume loss and the following variables, using the case as the unit: n. of implants per case, exposure, sinus lift. The analyses were performed using Stata 16.1 (StataCorp LLC, 4905 Lakeway Drive College Station, TX 77845, USA)

5 - RESULTS

5.1 – RECONSTRUCTIVE PROCEDURE WITH CALVARIUM – PROJECT 1

Out of 72 patients treated and 330 implants placed, 69 patients and 323 implants were available for a recall evaluation and were included in the study for statistical analysis (see table 1 and 2 for further details).

- ***Complication rate of the reconstructive procedure***

Post-operative recovery after the reconstructive surgery was uneventful in the majority of patients. None of them developed severe surgical complications. One patient developed a postoperative sub-cutaneous hematoma in the parietal region which resorbed spontaneously within two weeks (pt. #8). Six patients underwent a dehiscence of the intra-oral flap with consequent graft exposure, between 2 and 4 weeks after suture removal (pts #1, #8, #26, #29, #35, #60). Treatment consisted of local curettage to promote secondary healing: a spontaneous closure of the dehiscence occurred 4 to 7 weeks later. In these 5 patients, bone loss was extremely limited and implant placement was performed according to the original, prosthetically driven project.

- ***Bone resorption before implant placement***

Bone resorption before implant placement was very limited ranging from 0 mm to 1 mm (mean: 0.13 mm; SD ± 0.71; median: 0).

- ***Implant survival rate and implant related complications***

No implants were lost before the start of prosthetic loading. The follow-up after the start of prosthetic loading ranged from 3 to 19 years (mean: 8 years).
Three implants in 3 patients (pts #31, #44, #60) were removed due to the presence of a continuous peri-implant radiolucency and mobility 4, 6, and 9 years after the start of prosthetic loading, respectively.
Nineteen implants in 7 patients (pts # 7, #31, #32, #33, #37, #50, #70), developed peri-implantitis without implant mobility, 38 to 110 months after the completion of the rehabilitation (see table 2 for details).
These implants were maintained and peri-implantitis was treated with an open flap technique. More in detail, 7 implants in 4 patients (pts #31, #32, #33, #50) presenting with a non-contentive vertical bone defect ranging from 3.2 mm to 5.4 mm, were treated with debridement, implantoplasty performed with diamond polishing burs, decontamination with 1% chlorhexidine gel, and closure with an apically repositioned flap. Radiographic controls performed 2 and 3 years later, demonstrated no further progression of bone resorption and implants stability with no need to modify the prosthetic supra-structures. The remaining 11 implants in 6 patients (pts # 7, #32, #33, #37, #50, #70), presenting with an infra-bony defect, were treated with open flap debridement, topical application of a 1% chlorhexidine gel for 2 minutes followed by application of EDTA 24% (Straumann® PrefGel®) for 2 minutes, rinsing with sterile saline, regeneration of the peri-implant defects with a collagen linked bovine bone mineral (Bio-Oss Collagen®, Geistlich Biomaterials, Switzerland), and closure of the flap around the implants neck/abutment with a 6/0 resorbable suture. Radiographic controls performed 2 and 3 years later demonstrated an almost complete regeneration with disappearance of the infra-bony defects.

The overall survival rate of implants at the end of the observation period was 98.5%. No major prosthetic complication requiring the manufacturing of a new prosthetic restoration was reported during the observation period, thus leading to a 100% survival rate of the restorations (see table 2 and 8 for details).

Table 8 – Life table analysis of implants (standard actuarial method)

TIME	Number of implants at risk at start of interval	Number of dropped out implants (number of pts)	Failed implants (number of pts)	survival rate per year (%)	Cumulative survival rates (%)
PLC-LOAD	330	0	0	100,00	100,00
0-1"	330	2 (1)	0	100,00	100,00
1-2"	328	5 (2)	0	100,00	100,00
2-3"	323	0	0	100,00	100,00
3-4"	323	5 (1)	0	100,00	100,00
4-5"	298	6 (2)	0	100,00	100,00
5-6"	273	0	1 (1)	99,63	99,63
6-7"	245	6 (1)	0	100,00	99,63
7-8"	193	4 (1)	1 (1)	99,48	99,11
8-9"	155	4 (1)	1 (1)	99,35	98,46
9-10"	115	0	0	100,00	98,46
10-11"	109	3 (1)	0	100,00	98,46
11-12"	84	5 (1)	0	100,00	98,46
12-13"	52	0	0	100,00	98,46
13-14	18	0	0	100,00	98,46
14-15	14	0	0	100,00	98,46
15-16	14	0	0	100,00	98,46
16-17	12	0	0	100,00	98,46
17-18	12	0	0	100,00	98,46
18-19	3	0	0	100,00	98,46

Figure 3 shows the Kaplan-Meier curve considering the implant as analysis unit.

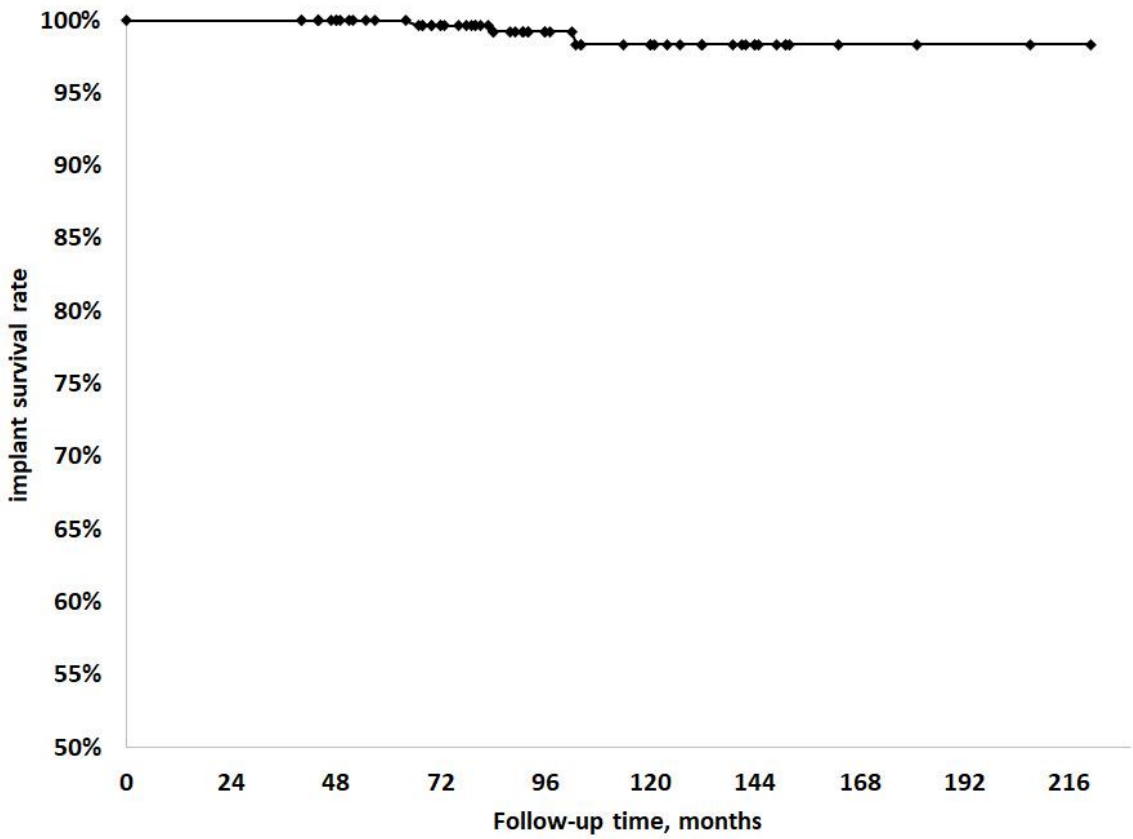


Figure 3. Kaplan Meier method for determining implant survival rate. The analysis of implant failure for 330 implants in 72 patients showed that the 19-year cumulative survival rate of the implant fixtures was 98.5%.

A synthesis of the distribution of patients, prostheses, and implants according to the main individual factors (e.g. smoking habits, gender, area of edentulism), with details on the number of adverse events (implant loss and peri-implantitis) occurred during the follow-up is presented in Table 9. From this table one may observe that biological complications like peri-implantitis occurred more frequently in the upper arch of female patients. No individual factors affected implant survival, also due to the very low number of implant failures recorded.

Table 10 reports post-reconstruction complications (dehiscence and bone resorption) per patient as a function of the use or not of the pericranium, with indication of sinus lift. cases. Table 11 reports post-reconstruction complications according to the edentulous jaw (maxilla or mandible) and in correlation to the use or not of pericranium, These tables showed no statistically significant differences between cases using and not using pericranium, although dehiscences and post-reconstruction bone resorption appeared to be more frequent in the latter cases.

In Table 12 are presented implant complications per patient as a function of the use of vestibuloplasty and free gingival graft in the areas which received implants. Data seem to demonstrate a beneficial effect of the use of vestibuloplasty and free gingival grafts, because a higher number of implant failures and peri-implantitis were observed in cases not treated with vestibuloplasty and gingival grafts. However, the difference was not statistically significant probably due to the limited number of cases.

The main results of the Generalized Estimating Equations are reported in Table 13. Among the different co-interventions taken into account, sinus lift and vestibuloplasty associated with free gingival graft showed a significant effect on the occurrence of implant failure, peri-implantitis, and on marginal bone loss. The effect of vestibuloplasty was significantly positive on all those outcome variables. No other factor showed to have a significant impact on outcomes.

Table 9 – Data distribution per patient, prostheses, and implants, and significance of individual factors for implant failures and peri-implantitis occurred during the follow-up.

	Patients (imp failure/ peri-implantitis)	**P-value**	**Prostheses (imp failure/ peri-implantitis)**	**P-value**	**Implants (imp failure/ peri-implantitis)**	**P-value**
No total	69 (3/7)		92 (3/8)		323 (3/19)	
Smokers	10 (0/2)		13 (0/2)		48 (0/5)	
Non smokers	59 (3/5)	0.34/0.24	79 (3/6)	0.63/0.23	275 (3/14)	0.32/0.15
Male	16 (1/0)	0.73/0.04*	22 (1/0)	0.42/0.10	67 (1/0)	0.59/0.002*
Female	53 (2/7)		70 (2/8)		256 (2/19)	
Maxilla	40 (2/6)	0.60/0.10	48(2/7)	0.40/0.03*	199 (2/18)	0.84/0.002*
Mandible	36 (1/1)		44 (1/1)		124 (1/1)	

* statistically significant

Table 10 – Occurrence of pre-implant complications according to reconstructive procedures, using or not the pericranium. The patient was the unit of analysis.

Interventions	**N. patients**	**Pre-implant complications**	
		dehiscence	Bone resorption
Calvarial graft alone (8 SL)	32	4	9
Calvarial graft+pericranium (14 SL)	37	2	7
O.R. (95% CI), p-value		2.5 (0.43, 14.66), p=0.30	1.68 (0.54, 5.18), p=0.37

SL=sinus lift, O.R.=odds ratio, CI= confidence intervals

Table 11 – Occurrence of pre-implant complications according to the area of edentulism, using or not the pericranium. The prosthesis (case) is the unit of analysis.

	Interventions	**N. cases**	**Pre-implant complications**	
			dehiscence	Bone resorption
Mandible	**Calvaria alone**	14	1	3
(n=44)	**Calvaria+pericranium**	30	0	4
	O.R. (95% CI), p-value		NE, p=0.32*	0.56 (0.11, 2.95), p=0.49
Maxilla	**Calvaria alone (10 SL)**	23	3	6
(n=48)	**Calvaria+pericranium (17 SL)**	25	2	3
	O.R. (95% CI), p-value		0.56 (0.09, 3.82), p=0.57	0.39 (0.08, 1.77), p=0.21

*Fisher exact test; O.R.=odds ratio, CI= confidence intervals, NE=not estimable, as one cell was =0

Table 12 – Incidence of implant complications using or not vestibuloplasty and free gingival graft procedure. The patient is the unit of analysis.

Interventions	**N. patients**	**Complications after implant placement**	
		Implant failure	Peri-implantitis
No vestibuloplasty+FGG	53	3	6
Vestibuloplasty+FGG	16	0	1
O.R. (95% CI), p-value		NE, p=0.45*	0.52 (0.06, 4.69), p=0.56

*Fisher exact test; FGG= free gingival graft; O.R.=odds ratio, CI= confidence intervals, NE=not estimable, as one cell was =0

Table 13 – Main results of the Generalized Estimating Equations using the Generalized Linear Model for estimating the effect of main co-interventions and individual factors on implant failure, peri-implantitis, and bone loss. The patient is the unit of analysis.

Dependent variable	Co-intervention	OR (95%CI)	Standard error	p-value
Implant failure	Sinus lift	25.79 (5.64, 119,1))	0.78	<0.001
	Vestibuloplasty+FGG	5.26 (1.62, 17.12)	0.60	0.006*
	Iliac bone chips added	1.28 (0.20, 9.12)	0.95	0.79
	Smoking habits	0.58 (0.13, 2.69)	0.78	0.49
	Jaw (mandible)	0.73 (0.24, 2.23)	0.57	0.58
	Gender (female)	2.25 (0.60, 8.50)	0.68	0.23
Peri-implantitis	Sinus lift	25.03 (5.05, 122,7)	0.81	<0.001
	Vestibuloplasty+FGG	5.81 (1.67, 20.29)	0.64	0.006*
	Iliac bone chips added	0.63 (0.05, 8.67)	1.34	0.73
	Smoking habits	0.44 (0.09, 2.14)	0.80	0.31
	Jaw (mandible)	1.14 (0.35, 3.71)	0.60	0.83
	Gender (female)	1.32 (0.34, 5.16)	0.69	0.69
Peri-implant bone resorption	Sinus lift	22.42 (5.10,98.49)	0.76	<0.001
	Vestibuloplasty+FGG	5.47 (1.73, 17.46)	0.59	0.004*
	Iliac bone chips added	1.11 (0.17, 7.24)	0.96	0.92
	Smoking habits	0.35 (0.08, 1.52)	0.76	0.16
	Jaw (mandible)	0.46 (0.16, 1.34)	0.55	0.15
	Gender (female)	1.97 (0.52, 7.39)	0.68	0.32

*vestibuloplasty associated with free gingival graft (FGG) has a significant positive effect; OR=odds ratio; CI=confidence interval

- ***Peri-implant bone resorption***

The mean peri-implant bone resorption per patient was 1.11 mm (range: 0.00 – 4.87 mm; SD: 1.14; IQR: Q1 0.00 – Q2 0.90 – Q3 1.49). Frequency distribution and interquartile ranges of resorption are reported in table 14.

Table 14 – Peri-implant bone resorption at the end of the observation period

mean value MBL (mm)	1,11
median value MBL (mm)	0,90
Standard deviation	1,14
min value (mm)	0,00
1st quartile	0,00
2nd quartile	0,90
3rd quartile	1,49
max value	4,87

Frequency distribution of peri-implant bone resorption	**Number of patients (implants)**
< 1 mm	59 (166)
1 – 1.9 mm	44 (101)
2 – 2.9 mm	14 (28)
3 – 3.9 mm	6 (13)
> 4 mm	8 (12)
Implants removed	3 (3)

- ***Patient satisfaction***

Overall, patient's satisfaction outcomes were positive. On a scale from 1 (none) to 5 (high), the post-operative pain at the grafted and the donor sites, during the 2 weeks after surgery, presented a median value of 2 and 1, respectively; no patients reported a level 5 pain assessment. The median value of discomfort due to the inability to use provisional prostheses for the first 6 weeks was 1. The median values of discomfort following the second postoperative week and the one related to the dietary limitations prescribed during the healing phases were both 2. The median value of discomfort related to necessity of meal interruption following the second postoperative week as well as the discomfort /limitations during working activities 2 week after surgery onward was 1. The median value of pain/discomfort immediately after the delivery of the final prosthesis was 1.

The median value related to the time after which all forms of pain and discomfort disappeared according to Likert scale was 1.

Overall, a successful cumulative score (≤ 25 out of 50 points) was reached in 89.9% of the treated patients and the same percentage (89.9%) of them would had accepted to undergo the procedure again (see table 15 for details). The mean successful cumulative score was 16.4.

Table 15 –Patients' satisfaction

	Q. No. 1	**Q. No. 2**	**Q. No. 3**	**Q. No. 4**	**Q. No. 5**	**Q. No. 6**	**Q. No. 7**	**Q. No. 8**	**Q. No. 9**	**Q. No. 10**
min value	1	1	1	1	1	1	1	1	1	1
1st quartile	1	1	1	1	1	1	1	1	1	1
2nd quartile	2	1	1	2	2	1	1	1	1	1
3rd quartile	3	2	1	3	2	2	2	2	1	1
max value	4	4	5	5	5	5	5	5	5	5
Number of fully satisfied patients (%of pts)	36 (52.2)	46 (66.7)	55 (79.7)	21 (30.4)	31 (44.9)	43 (62.3)	40 (58)	38 (55.1)	60 (87)	61 (89.9)
Number of very satisfied patients (%of pts)	12 (17.4)	12 (17.4)	3 (4)	21 (30.4)	21 (30.4)	18 (26.1)	20 (29)	21 (30.4)	7 (10.1)	
Number of partially satisfied patients (%of pts)	16 (23.2)	7 (10.1)	3 (4)	19 (27.5)	6 (8.7)	4 (6)	3 (4)	6 (8.7)	0	
Number of partially unsatisfied patients (%of pts)	5 (7)	4 (6)	6 (8.1)	5 (7)	4 (6)	0	2 (2.9)	2 (2.9)	1 (1.4)	
Number of totally unsatisfied patients (%of pts)	0	0	2	3 (4)	7 (10.1)	4 (6)	4 (6)	2 (2.9)	1 (1.4)	8 (10.1)

Legend: Q No., question number; pts, patients. Only 69 patients answered to question no. 3 because they had not a provisional prosthesis during the healing phase: the others had a provisional prosthesis.

A representative case treated with calvarial grafts and implants is reported from figure 4 to figure 13.

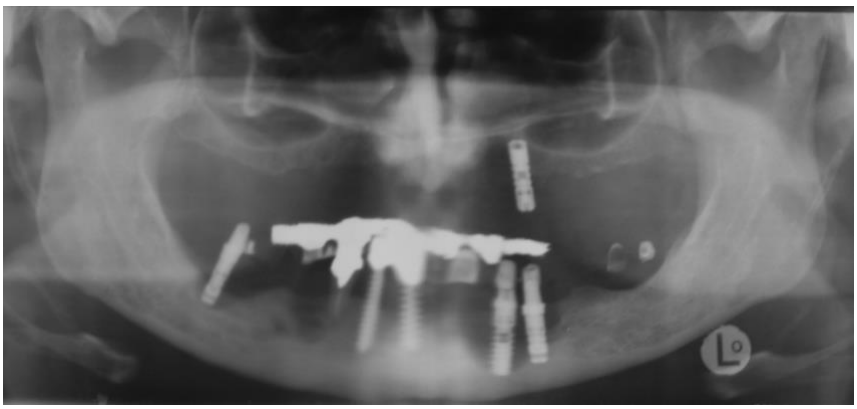


Figure 4 - Panoramic radiograph of initial situation (Feb. 2010) showing severe peri-implantitis with relevant bone resorption and infection, requiring removal of all implants

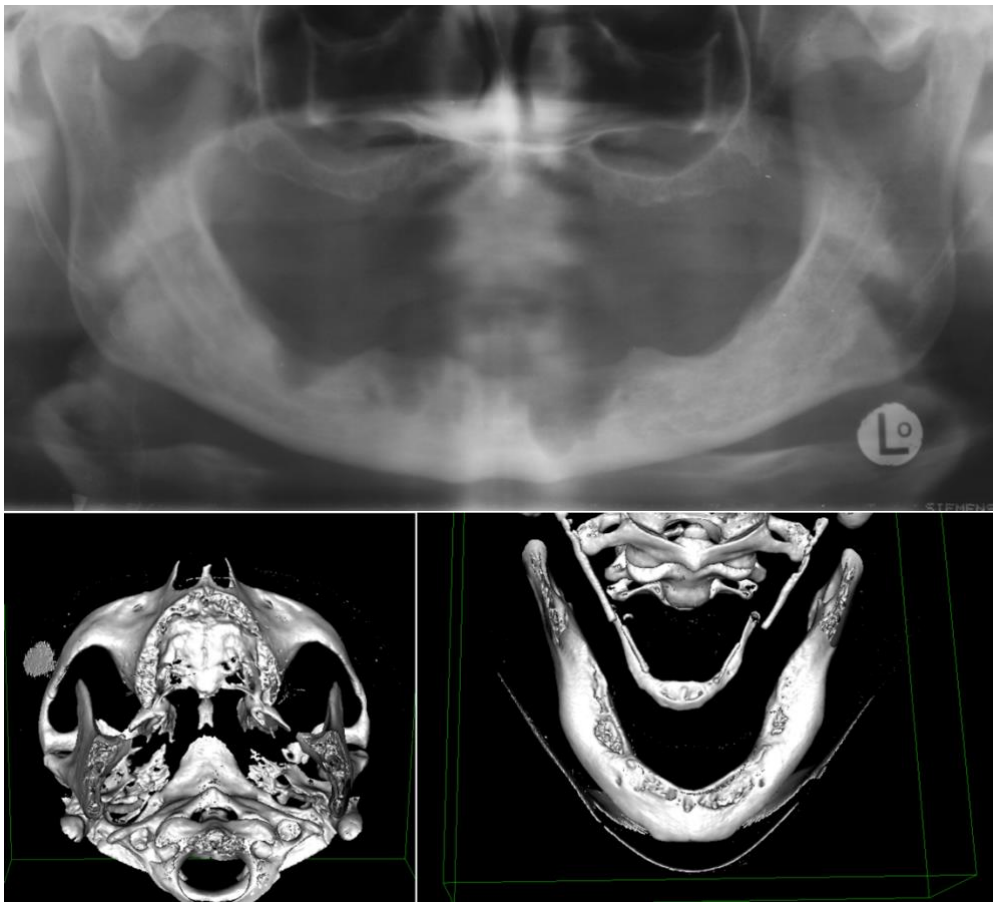


Figure 5 - Panoramic radiograph and CT scans 3 months later (May 2010) showing severe bone resorption with residual volumes insufficient to host new implants

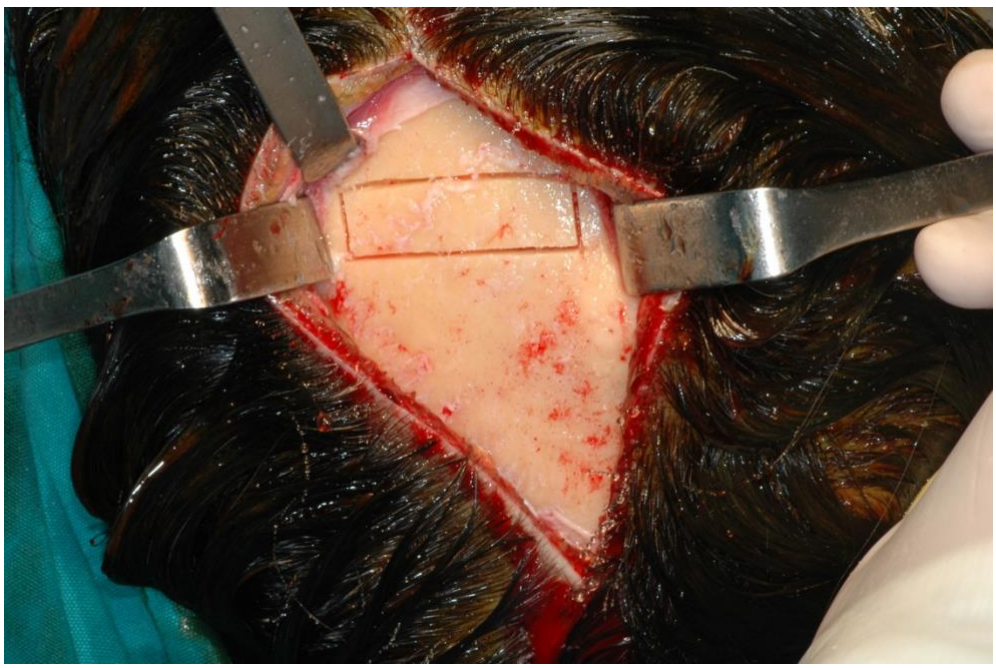


Figure 6 - Bone harvesting from the parietal calvarium (Oct 2010)

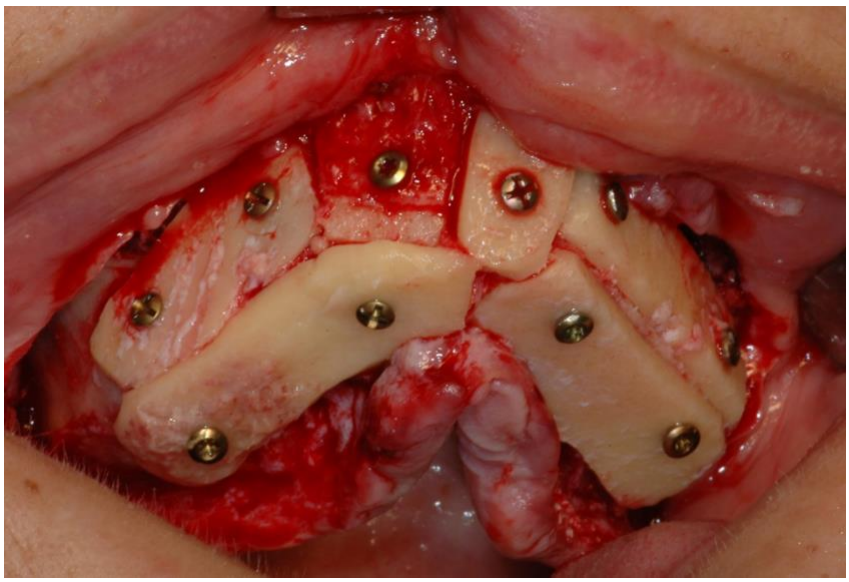


Figure 7 - Intra-operative view at the end of maxillary reconstruction (Oct 2010) showing the relevant bone augmentation obtained (in the same session the mandible was reconstructed with a similar procedure)

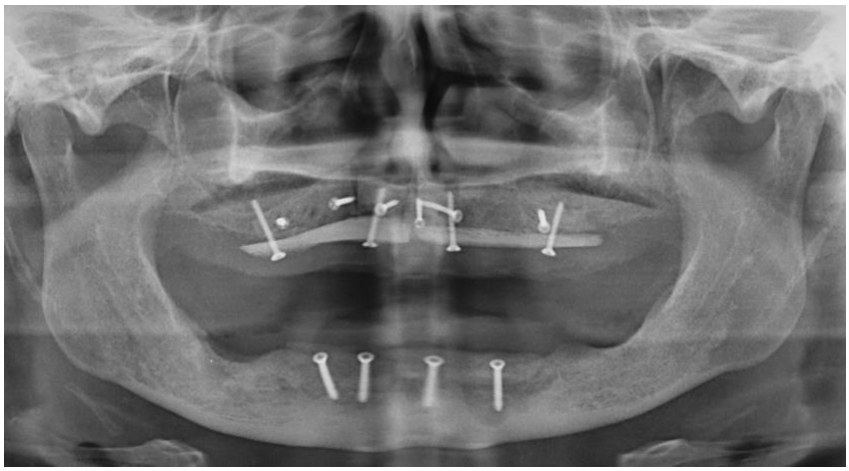


Figure 8 - Panoramic radiograph immediately after the reconstruction (Oct 2010)

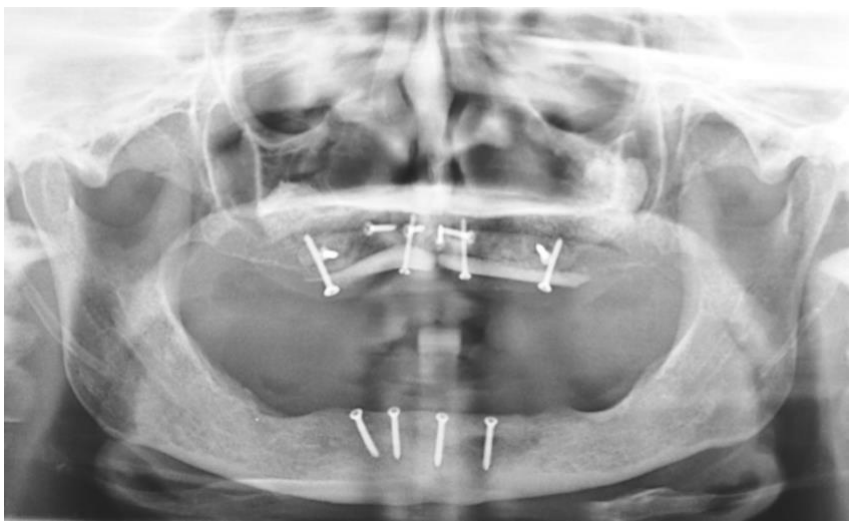


Figure 9 - Panoramic radiograph 8 months later showing good integration between the grafts and the residual jaw bone (June 2011)

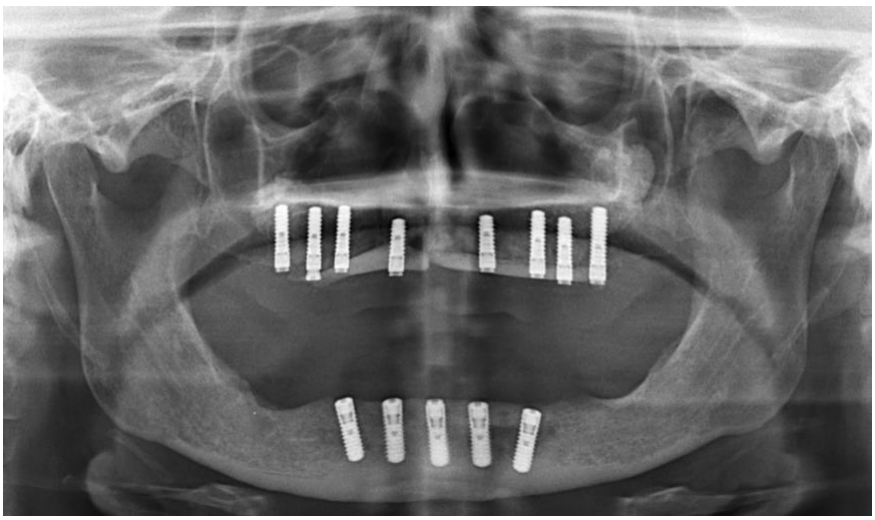


Figure 10 - Panoramic radiograph taken immediately after placement of 8 endosseous implants in the reconstructed maxilla and 5 implants in the reconstructed mandible (July 2011)

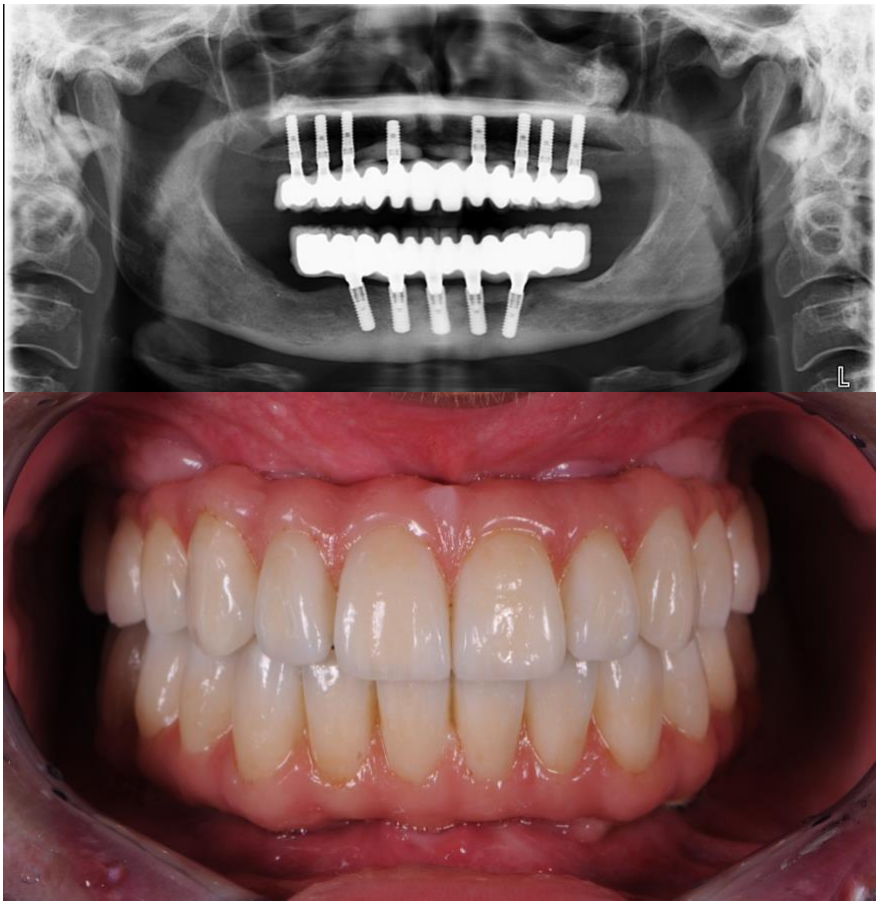


Figure 11 - Radiographic and clinical control at the end of the prosthetic restoration (May 2012)

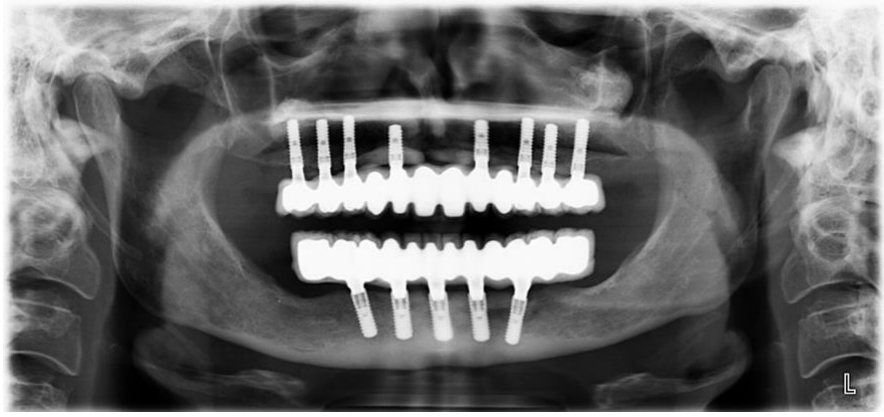


Figure 12 - Radiographic control 2 years after the completion of final prosthetic restoration (May 2014)

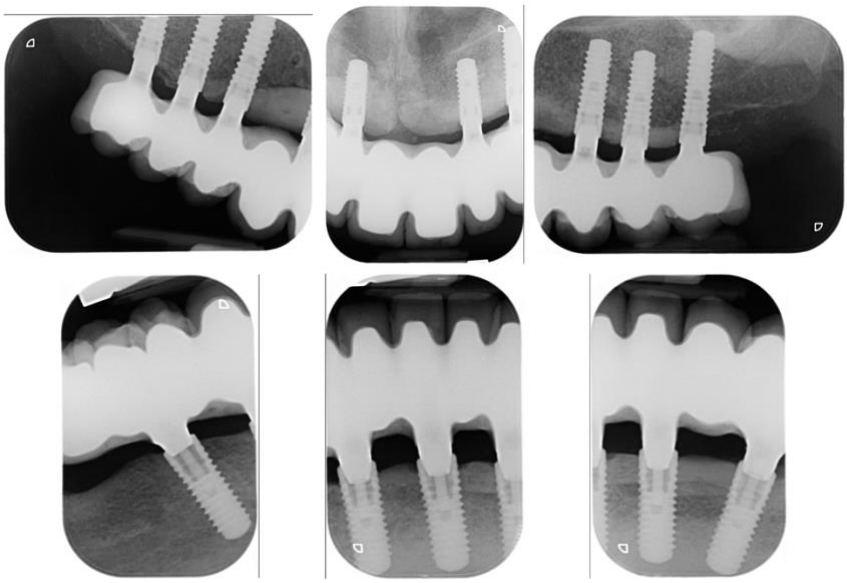


Figure 13 - Radiographic control 5 years after the completion of final prosthetic restoration (March 2017)

5.2 – RECONSTRUCTIVE PROCEDURE WITH MANDIBULAR RAMUS – PROJECT 2

Out of 75 patients (82 sites) treated and 182 rough surface implants placed in the reconstructed areas, 8 patients and 23 implants were lost to follow-up at different times of the observation period (see Table 16).

Table 16. Life table analysis of implants (standard actuarial method)

Interval, years	No. of implants	No. of patients	No. of failures	implants lost to follow-up	interval survival rate	cumulative survival rate
0-1	182	75	1	0	99.45%	99.45%
1-2	181	75	0	0	100.0%	99.45%
2-3	181	75	0	0	100.0%	99.45%
3-4	181	75	0	0	100.0%	99.45%
4-5	181	71	0	14	100.0%	99.45%
5-6	167	63	0	18	100.0%	99.45%
6-7	149	61	0	2	100.0%	99.45%
7-8	147	58	0	8	100.0%	99.45%
8-9	139	50	0	15	100.0%	99.45%
9-10	124	45	0	12	100.0%	99.45%
10-11	112	38	0	18	100.0%	99.45%
11-12	94	31	0	19	100.0%	99.45%
12-13	75	22	1	26	98.67%	98.12%
13-14	48	15	0	17	100.0%	98.12%
14-15	31	8	0	15	100.0%	98.12%
>15	16	5	0	16	100.0%	98.12%

Follow-up from the start of prosthetic loading ranged from 3 to 16 years (mean= 10 years, SD= 3.5). Distribution of type of initial defects, reconstructive procedures, surgical co-interventions, and number of implants placed in the reconstructed areas are reported in tables 17-18-19-20.

Table 17 - incidence of complications occurring before and after implant placement according to type of reconstruction (horizontal, vertical, combined). The site reconstruction was the unit of analysis (n=82)

Interventions	**N. grafting procedures (patients/implants)**	**Pre-implant complications**			**Post-implant complications**	
		paresthesia	Vertical bone graft resorption (0.5 to 2mm)	Horizontal bone graft resorption (0.5 to 2mm)	Implant failure (patients)	Peri-implantitis (patients)
Combined vertical+horizontal defects	48 (44/107)	2	6	6	2 (2)	7 (6)
Only horizontal defects	20 (19/46)	1	Not measured	3	0	3 (1)
Only vertical defects	14 (14/29)	0	4	Not measured	0	0
p-value*		0.25	0.04	0.45	0.34	0.04

*Fisher exact test

Table 18 - Data distribution per patient, prostheses, and implants, and significance of individual factors for implant failures and peri-implantitis occurred during the follow-up.

	Patients (imp failure/ peri-implantitis)	**P-value**	**Prostheses (imp failure/ peri-implantitis)**	**P-value**	**Implants (imp failure/ peri-implantitis)**	**P-value**
No total	75 (2/7)		89 (2/7)		182 (2/10)	
Smokers	10 (1/2)	0.23/0.19	11 (1/2)	0.24/0.19	25 (1/2)	0.24/0.27
Non smokers	65 (1/5)		70 (1/5)		157 (1/8)	
Male	21(1/2)	0.41/0.33	26 (1/2)	0.42/0.33	50 (1/2)	0.59/0.40
Female	54 (1/5)		63 (1/5)		132 (1/8)	
Maxilla	33 (1/1)	0.50/0.10	48(1/1)	0.50/0.04*	75 (1/1)	0.49/0.03*
Mandible	44 (1/6)		44 (1/6)		107 (1/9)	

* statistically significant

Table 19 - Occurrence of pre-implant complications, using ramus graft in combination or not with sinus augmentation procedure. The patient was the unit of analysis (n=75).

Interventions	**N. patients**	**Pre-implant complications**		
		paresthesia	Vertical bone graft resorption (0.5 to 2mm)	Horizontal bone graft resorption (0.5 to 2mm)
Ramus graft alone	60	2	9	8
Ramus graft+sinus augmentation	15	1	1	1
O.R. (95% CI), p-value*		2.07 (0.18, 24.51), p=0.49	2.47 (0.29, 21.19), p=0.27	2.15 (0.25, 18.69), p=0.31

*Fisher exact test; O.R.=odds ratio, CI= confidence intervals

Table 20 - Incidence of implant complications using or not vestibuloplasty and free gingival graft procedure. The patient was the unit of analysis (n=75).

Interventions	**N. patients (implants)**		**Complications after implant placement**	
		Failed/unsuccessful implants*	Implant survival/success	Peri-implantitis
No vestibuloplasty+FGG	70 (168)	2/27	97.85%/84.12%	6
Vestibuloplasty+FGG	5 (12)	0/0	100%/100%	1
O.R. (95% CI), p-value**		p=0.87/p=0.13	--	2.67 (0.26, 27.86), p=0.40

*Implants with marginal bone resorption >2mm were considered unsuccessful; **Fisher exact test; FGG= free gingival graft; O.R.=odds ratio, CI= confidence intervals

- ***Complication rate of the reconstructive procedures***

The majority of patients recovered from the reconstructive procedure uneventfully (69 out of 75 patients. None developed severe surgical complications.

Six out of 75 patients underwent early complications. Out of these, 3 patients reported a transient paresthesia of the inferior alveolar nerve at the mandibular side that received the harvesting procedure (Table 2). All signs and symptoms of nerve disfunction regressed spontaneously within 2 to 8 weeks.

Two patients underwent a dehiscence of the flap at the grafting site, with consequent graft exposure, between 2 and 4 weeks after suture removal. Treatment consisted of local curettage with manual curettes and perforations of the graft surface with 0.2mm round burs mounted on a low-speed straight handpiece under constant irrigation with sterile saline until spontaneous bleeding was detectable through the perforations, to promote secondary healing thanks to the formation of granulation tissue followed by re-epithelization over the previously exposed bone. A spontaneous closure of the dehiscence occurred 5 to 8 weeks later. In these patients, a minor bone loss occurred during wound healing, which was still compatible with the placement of implants according to the original, prosthetically driven project. One patient presented with suppuration but without any clinically detectable dehiscence in the area of the grafted site three weeks after surgery which was solved with systemic antibiotic therapy for 10 days. At the time of implant placement, no significant bone loss was however observed.

- ***Bone resorption before implant placement***

At time of implant placement, bone resorption around the fixation screws ranged from 0 to 2 mm (mean=0.15 mm; SD=0.42mm) and from 0 mm to 2 mm (mean=0.18 mm; SD: 0.43mm) in the horizontal and vertical dimension, respectively (see table 17 for further details) .

- ***Peri-implant bone resorption***

The mean vertical peri-implant bone loss ± standard deviation was 1.06±1.19 (range 0.00 to 5.05) at patient level, and 1.11±1.26 (range 0.00 to 5.20) at implant level. The Kappa intra-class correlation coefficient calculated on repeated measurements collected from the first 10 patients (27 implants) was 0.88 Frequency distribution and interquartile ranges of resorption are also reported in Table 21.

Table 21 - Peri-implant bone resorption at the end of the observation period. Values are in mm.

	Per patient (n=75)	**Per implant (n=180)**
mean value	1.06	1.11
Standard deviation	1.19	1.26
min value	0.00	0.00
1st quartile (25%)	0.00	0.00
2nd quartile (50%)	0.625	0.95
3rd quartile (75%)	1.45	1.51
max value	5.05	5.20

Frequency distribution of peri-implant bone resorption	**Number of patients (%)**	**Number of implants (%)**
< 1 mm	43 (57.3)	90 (49.5)

1 – 1.99 mm	21 (28.0)	59 (32.4)
2 – 2.99 mm	4 (5.3)	10 (5.5)
3 – 3.99 mm	3 (4.0)	7 (3.8)
≥ 4 mm	4 (5.3)	14 (7.7)
Implants removed	2 (2.7)	2 (1.1)

- ***Implant related complications***

None of the implants placed in the reconstructed bone was lost before prosthetic loading or presented biological complications.

After prosthetic loading, there were 12 implants in 9 patients affected by biological complications. Two implants (one per patient) were removed due to loss of integration, 11 months and 12 years after prosthetic loading, respectively.

Ten implants in 7 patients developed peri-implantitis with no implant mobility, 36 to 84 months after prosthetic loading (Table 18). In all cases, a combined non-surgical and surgical treatment protocol was delivered aiming to stop disease progression and maintain the implants.

First, non-surgical therapy with dedicated peek ultrasonic devices (PI ultrasonic insert, EMS electro medical systems SA, Nyon, Switzerland) and topical application of chlorhexidine gel was delivered at the affected site. One month later, surgical treatment was performed. Six implants in 4 patients, presenting with supra-bony defects, developed approximately 3 to 7 years after the start of loading, were treated with open flap debridement and implantplasty performed with diamond polishing burs, decontamination with 1% chlorhexidine gel, rinsing with sterile saline, and closure with an apically repositioned flap. Radiographic controls, performed yearly during the observation period (last controls ranging from 2 to seven years after treatment of peri-implantitis), showed no further progression of bone resorption. On the other hand, 4 implants in 3 patients, presenting with contentive infra-bony defects developed approximately 3 to 9 years after the start of loading, were treated with a reconstructive approach consisting of decontamination of the implant surface through an open flap debridement with dedicated titanium brushes (Ti-Brush, Straumann Institute AG, Basel, Switzerland). Then, a 1% chlorhexidine gel for 1 minute followed by EDTA 24% (Straumann® PrefGel®) for 2 minutes were applied. Subsequent reconstruction of the peri-implant hard tissues was performed using a collagen-linked bovine bone mineral (Bio-Oss Collagen®, Geistlich Biomaterials, Switzerland) within the infra-bony component of the defect, as suggested by some Authors (Roccuzzo, et al., 2011). Finally, the flap was closed around the implant neck/abutment with a 6/0 resorbable PGA suture. Radiographic and clinical controls performed 2,3, and 4 years after treatment of peri-implantitis, respectively, demonstrated an almost complete regeneration with disappearance of the infrabony defects.

Overall, the cumulative complication rate at patient level was 16.78% and at implant level it was 8.29%.

Implant complications were not associated with pre-implant bone resorption, as all implant failures and peri-implantitis cases occurred in patients that did not show bone resorption prior to implant placement. Only one patient who showed 0.5mm bone resorption in both horizontal and vertical dimension before implant placement, developed peri-implantitis.

No major prosthetic complications requiring the manufacturing of a new prosthetic restoration were reported during the observation period, thus leading to a 100% survival rate of the restorations. A synthesis of the distribution of patients, prostheses, and implants according to the main individual factors (e.g., smoking habits, gender, area of edentulism), with details on the number of adverse events (implant loss and peri-implantitis) occurred during follow-up, is presented in Table 18.

The occurrence of pre-implant complications, using ramus graft in combination or not with sinus augmentation procedure is presented in table 19. The incidence of implant complications using or not vestibuloplasty and free gingival grafts is shown in Table 20.

The main results of the generalized estimating equations are reported in Table 22. None of the co-interventions taken into account showed a significant effect on the occurrence of implant failures or peri-implantitis. No other factors showed a significant impact on outcomes.

Table 22 - Main results of the Generalized Estimating Equations using the Generalized Linear Model for estimating the effect of main co-interventions and individual factors on implant failure and peri-implantitis. The patient is the unit of analysis.

Dependent variable	Co-intervention	OR (95%CI)	Standard error	p-value
Implant failure	Sinus lift	1.63 (0.92, 2.89)	0.29	0.09
	Vestibuloplasty	0.38 (0.14, 1.05)	0.52	0.06
	Smoking habits	1.18 (0.52, 2.69)	0.42	0.69
	Jaw (mandible)	0.62 (0.34, 1.12)	0.30	0.11
	Gender (female)	0.98 (0.63, 1.53)	0.23	0.93
Peri-implantitis	Sinus lift	1.44 (0.82, 2.52)	0.29	0.20
	Vestibuloplasty	0.38 (0.14, 1.05)	0.52	0.06
	Smoking habits	1.03 (0.48, 2.22)	0.39	0.94
	Jaw (mandible)	0.65 (0.35, 1.20)	0.32	0.17
	Gender (female)	1.04 (0.68, 1.60)	0.22	0.86

- ***Implant survival/success rates***

The overall survival and success rates of implants at the end of the observation period were 98.11% and 85.16%, respectively.

Fig. 14 shows the Kaplan–Meier curve as regards success rate, considering the implant as analysis unit.

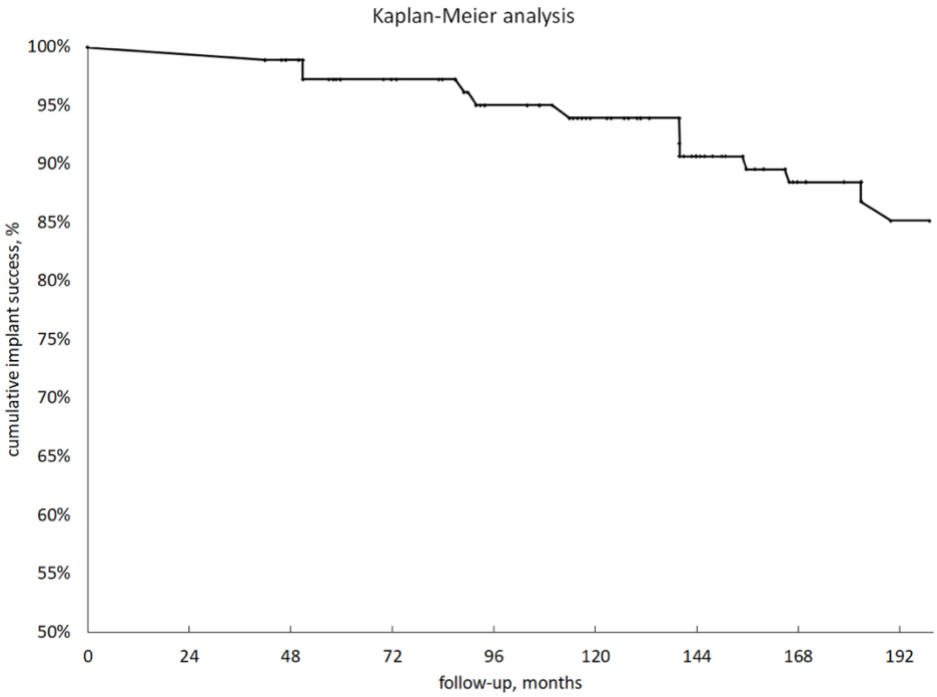


Figure 14 - The Kaplan–Meier curve, considering the implant as analysis unit

- ***Patient satisfaction***

Out of 75 patients treated, 67 were available for answering to the dedicated questionnaire. Overall, patient's reported outcome measures showed a high level of acceptance and satisfaction as regards the received treatment. Overall, a successful cumulative score (≤25 out of 50 points) was reached in 94% of the patients and 95.5% of them would have accepted to undergo the procedure again (Table 23 for details). The mean successful cumulative score was 16.5.

Table 23 - Patients' satisfaction

	Q. No. 1	**Q. No. 2**	**Q. No. 3**	**Q. No. 4**	**Q. No. 5**	**Q. No. 6**	**Q. No. 7**	**Q. No. 8**	**Q. No. 9**	**Q. No. 10**
min value	1	1	1	1	1	1	1	1	1	1
1st quartile	1	1	1	1	1	1	1	1	1	1
2nd quartile	2	2	1	2	1	1	1	1	2	1
3rd quartile	3	3	2	2	2	1,5	2	2	2	1
max value	5	5	5	5	5	3	5	5	5	5
Number of fully satisfied patients (% of pts)	24 (35.82)	19 (28.36)	35 (61.4)	32 (47.76)	44 (65.67)	50 (74.63)	46 (68.66)	43 (64.18)	28 (41.79)	64 (95.50)
Number of very satisfied patients (% of pts)	22 (32.84)	23 (34.33)	13 (22.8)	20 (29.85)	20 (29.85)	14 (20.90)	13 (19.40)	13 (19.40)	33 (49.25)	
Number of partially satisfied patients (% of pts)	14 (20.90)	21 (31.34)	4 (7)	13 (19.40)	2 (2.99)	3 (4.48)	6 (8.96)	8 (11.94)	0	
Number of partially unsatisfied patients (% of pts)	4 (5.97)	2 (2.99)	4 (7)	1 (1.49)	0	0	1 (1.49)	1 (1.49)	3 (4.48)	
Number of totally unsatisfied patients (% of pts)	3 (4.48)	2 (2.99)	1 (1.8)	1 (1.49)	1 (1.49)	0	1 (1.49)	2 (2.99)	3 (4.48)	3 (4.50)

Legend: Q No., question number; pts, patients; 10 patients didn't answer to question no. 3 because they had a provisional prosthesis during the healing phase.

A clinical case is reported from figure 15 to figure 21.

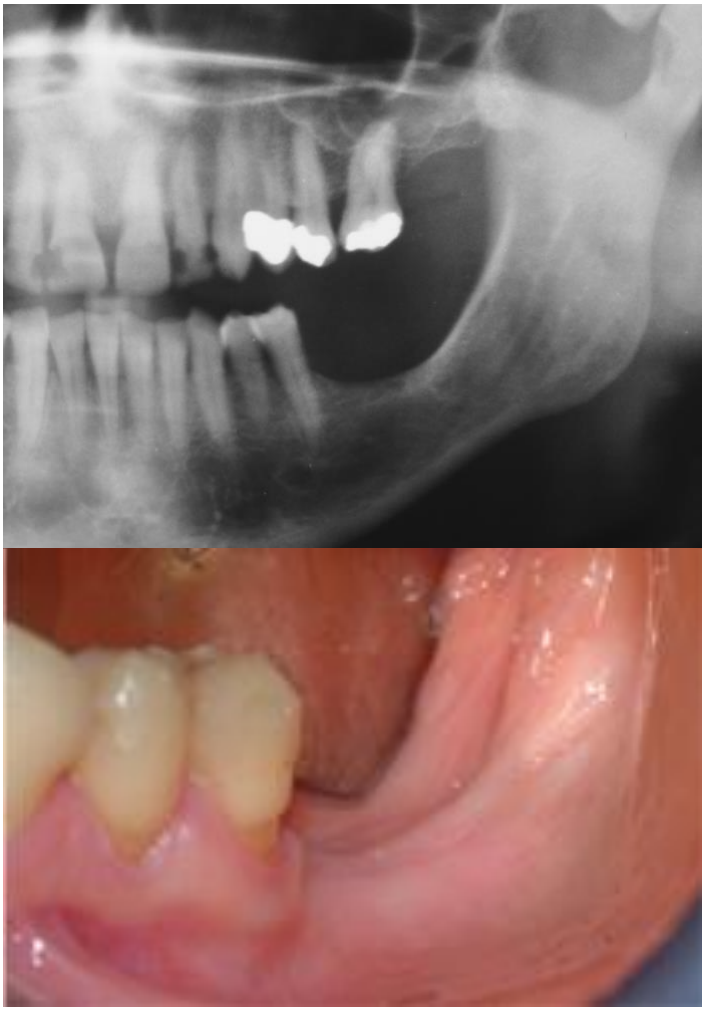


Figure 15 - panoramic radiograph and intra-oral view showing initial situation with vertical resorption of the mandible distal to tooth 3.5

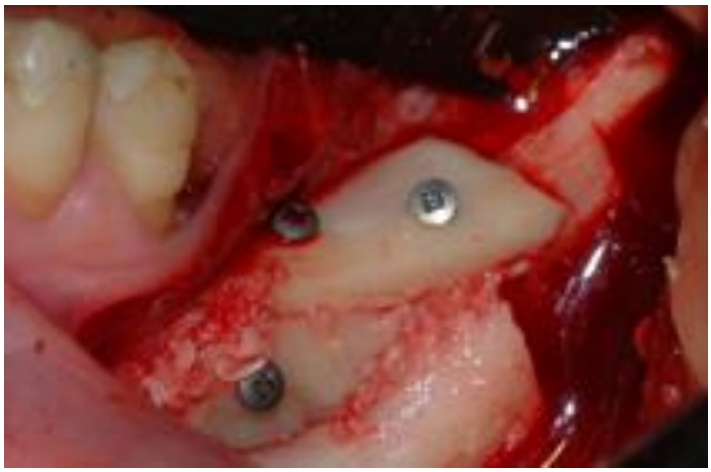


Figure 16 - Intra-operative view at the end of the reconstruction showing the bone augmentation obtained

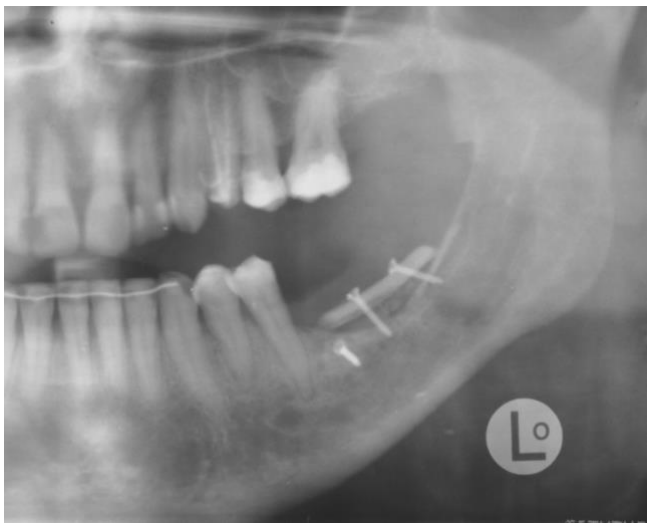


Figure 17 - panoramic radiograph immediately after surgery showing vertical augmentation with autogenous blocks taken from the mandibular ramus

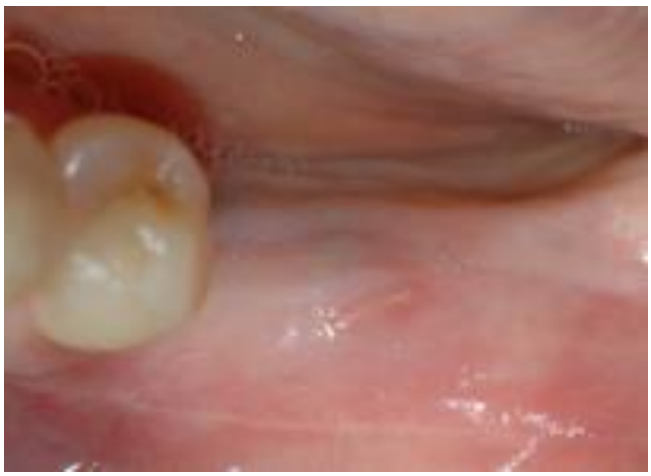
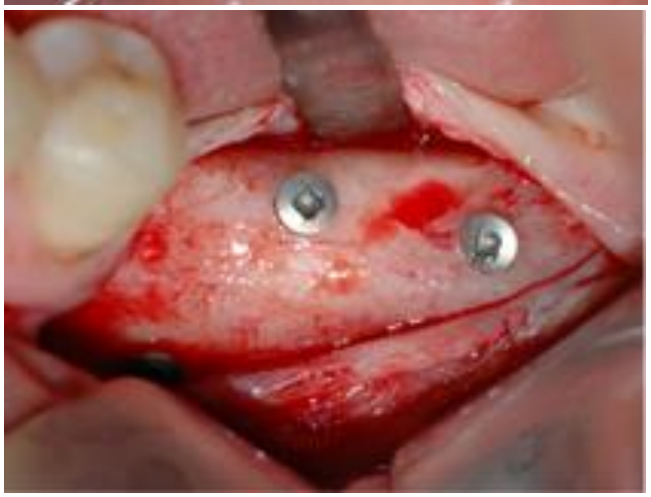


Figure 18 - clinical view before screws removal and implant placement, 7 months after the bone reconstruction



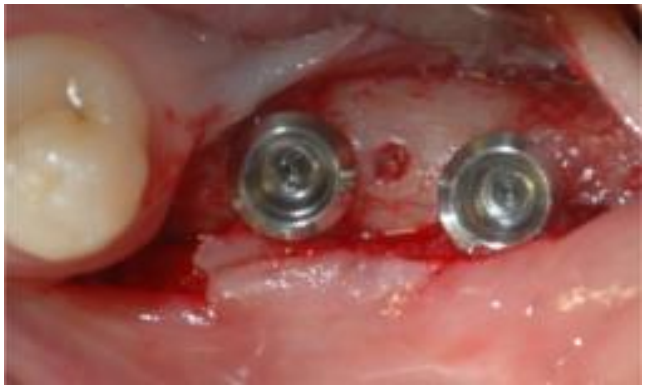
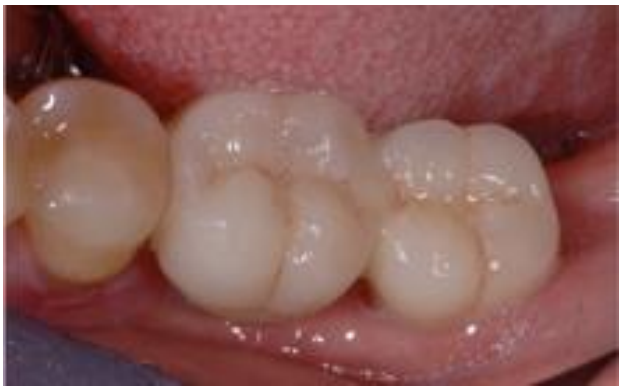


Figure 19 - intra-operative views showing the optimal integration of the graft, the adequate vascularization of the bone and implants placed after screws removal



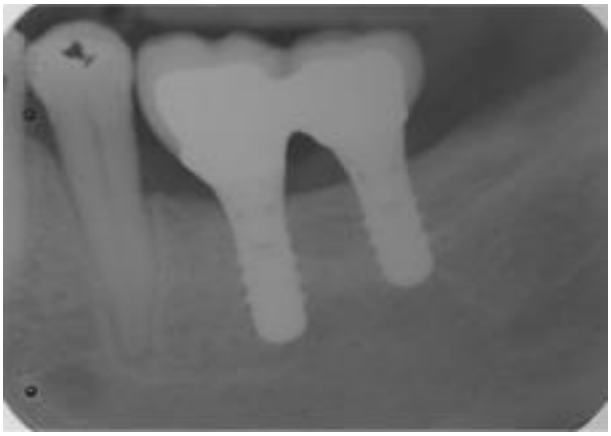


Figure 20 clinical and radiographic images showing implant-supported restoration after 1 year of loading

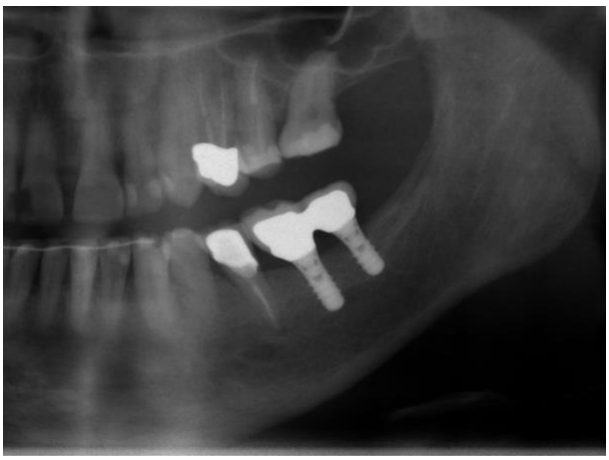


Figure 21 - Ten-year radiographic control showing stability of the augmented bone with no peri-implant bone resorption

5.3 – GUIDED BONE REGENERATION WITH CUSTOMIZED TI-MESH – PROJECT 3

- ***Bone gain***

Changes in vertical and horizontal dimensions at T1 and T2, measured both in mm and in percentage, are reported in table 24.

Volume changes between T1 and T2 measured in terms of variations of cubic centimetres and in percentage are reported in table 2. More details are available in table 25 and, as box plots, in figures 22-23-24-25-26.

On average, the comparison between initial bone gain and the one at the time of implant placement demonstrated very limited contraction of bone volumes.

It is worth noting that in 5 sites, an overgrowth of bone covered the mesh in some areas. The growth of bone through and even over the "open spaces" of the meshes may also explain the relative complexity in removing them at T2. In these patients, the areas of the meshes completely covered by new bone were left "in situ" as they never interfered with implant placement, as shown in the clinical case presented.

Table 24 - Changes in bone height and width of the regenerated areas at T1 and T2; volume changes at T1 and T2 .

	N. pat	mean	SD	median	min	max
Vertical bone gain (T1), mm	41	4.78	1.88	5.12	1.00	8.90
Horizontal bone gain (T1), mm	41	6.35	2.10	6.24	2.14	11.48
Vertical bone resorption (T2), mm	40	-0.39	0.64	-0.27	+0.80	-3.10
Vertical bone resorption (T2), %	40	-8.09%	12.27%	-5.00	+12.54%	-48.66%
Horizontal bone resorption (T2), mm	40	-0.49	0.83	-0.30	+0.40	-3.70
Horizontal bone resorption (T2), %	40	-6.17%	11.11%	-4.40	+14.76%	-42.05%
Volume at T1 (cc)	41	1.49	1.36	1.15	0.20	6.86
Volume at T2 (cc)	40	1.37	1.27	1.12	0.20	6.80
Volume changes between T1-T2 (%)	40	- 6.34%	10.14	-2.86	-41.67	+ 5.00

Legenda:
Pat= patients; T1= values of bone gains at the end of reconstructive procedure; T2 = values of bone gains at the time of mesh removal and implant placement

Table 25 - Data related to bone volume augmentation

# pts	site and type of defect	missing dentition	Dim. in mesiodistal direction (mm)	volume augmentation T1	volume augmentation T2	bone volume variations T1-T2 (%)
1	max hor	1.2	3,5	0,21	0,20	-4,76
	max hor	2.2	3,5	0,20	0,20	0,00
2	max hor+vert	2.3	6	0,46	0,44	-4,35
3	mand hor+vert	3.3-3.7	38	2,21	2,21	0,00
4	max hor	2.2	4	0,23	0,22	-4,35
5	max hor+vert	1.5-1.7	21	2,51	2,38	-5,18
6	mand hor	4.3-4.7	21	1,24	1,25	0,81
7	mand hor+vert	3.4-3.7	28	1,39	1,37	-1,44
	mand hor+vert	4.4-4.7	32	1,47	1,44	-2,04
8	max hor+vert	1.2-1.4	12	0,70	0,60	-14,29
9	mand hor+vert	4.5-4.7	29	2,01	2,00	-0,50
10	max hor+vert	1.5-1.7	21	1,17	1,16	-0,85
11	max hor+vert	1.2-2.7	62	4,58	4,52	-1,31
12	max hor+vert	1.5-1.6	15	0,56	0,51	-8,93
13	max hor+vert	2.6-2.7	22	1,85	1,82	-1,62
14	mand hor+vert	3.5-3.7	27	1,15	1,12	-2,61
	mand hor+vert	4.5-4.7	27	1,15	1,13	-1,74
15	max hor+vert	1.4-1.6	23	1,51	1,48	-1,99
16	max hor+vert	1.4-1.6	26	2,35	2,35	0,00
17	max hor+vert	1.1-1.6	28	3,21	3,02	-5,92
18	mand hor	3.5-3.7	24	0,59	0,56	-5,08
19	max hor+vert	1.7-2.7	94	6,86	6,80	-0,87
20	mand hor+vert	4.4-4.6	19	2,42	2,30	-4,96
21	mand hor+vert	4.5-4.6	17	0,67	0,65	-2,99
22	mand hor+vert	4.2-3.7	44	2,75	2,71	-1,45
23	mand hor+vert	4.4-4.7	31	0,89	0,90	1,12

24	max hor+vert	2.5-2.7	28	1,08	--	--
25	max hor+vert	1.1-1.2	9	0,40	0,39	-2,50
26	max hor+vert	1.4-1.7	32	4,12	2,51	-39,08
	max hor+vert	2.4-2.7	27	3,24	2,43	-25,00
	mand hor+vert	3.5-3.7	25	1,66	1,41	-15,06
	mand hor+vert	4.5-4.7	23	1,30	1,04	-20,00
27	max hor+vert	2.3	7	0,33	0,29	-12,12
28	max hor	2.2	4	0,20	0,21	5,00
	max hor	1.2	4	0,20	0,21	5,00
29	mand hor	4.6-4.7	21	0,46	0,47	2,17
30	mand hor+vert	3.2-3.6	31	2,03	2,03	0,00
31	max hor+vert	2.5-2.7	32	1,97	1,32	-32,99
	mand hor+vert	3.6-3.7	24	0,90	0,85	-5,56
32	mand hor+vert	4.6	16	0,34	0,32	-5,88
	max hor+vert	2.4-2.7	23	3,57	3,28	-8,12
33	max hor+vert	2.2	6	0,35	0,34	-2,86
	max hor+vert	1.2	6	0,34	0,31	-8,82
34	max hor+vert	2.1-2.2	13	0,77	0,70	-9,09
35	max hor+vert	1.4-1.5	6	0,65	0,63	-3,08
	max hor+vert	2.4	15	1,16	1,14	-1,72
36	max hor+vert	1.1-1.2	10	0,67	0,66	-1,49
37	max hor+vert	1.4-2.2	56	4,02	3,88	-3,48
38	mand hor+vert	4.5-4.6	18	0,71	0,68	-4,23
39	max vert+hor	1.5-1.7	28	1,92	1,88	-2,08
	max vert+hor	2.5-2.7	22	1,52	1,43	-5,92
40	mand hor+vert	3.4-3.7	33	2,90	2,88	-0,69
41	max hor+vert	2.3-2.7	29	3,62	2,92	-19,34

Legenda:; max= maxilla; mand= mandible; hor= horizontal; vert= vertical; dim= dimensions; T1= date of regeneration with Ti-mesh; T2= date of Ti-mesh removal and implant placement

Figure 22 - Box plot showing bone gain (in mm) in the vertical and horizontal dimension at T1

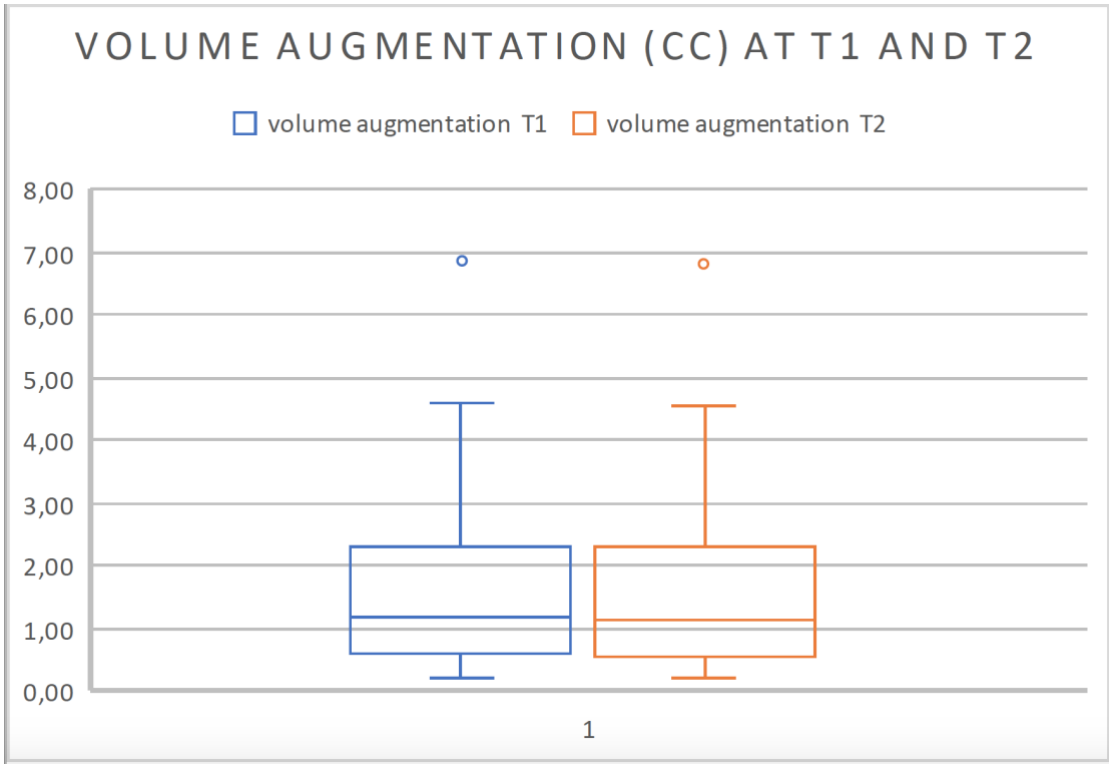


Figure 23 - Box plot showing bone volume augmentation (in cubic centimetres) at T1 and T2

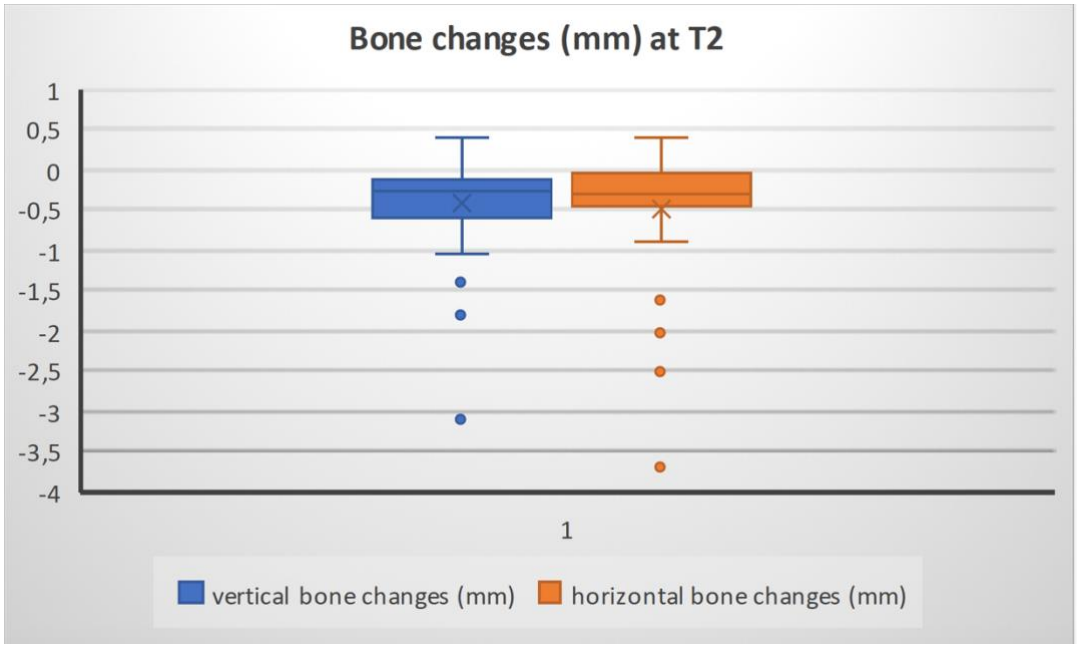


Figure 24 - Box plot showing bone changes (in mm) in the vertical and horizontal dimension at T2

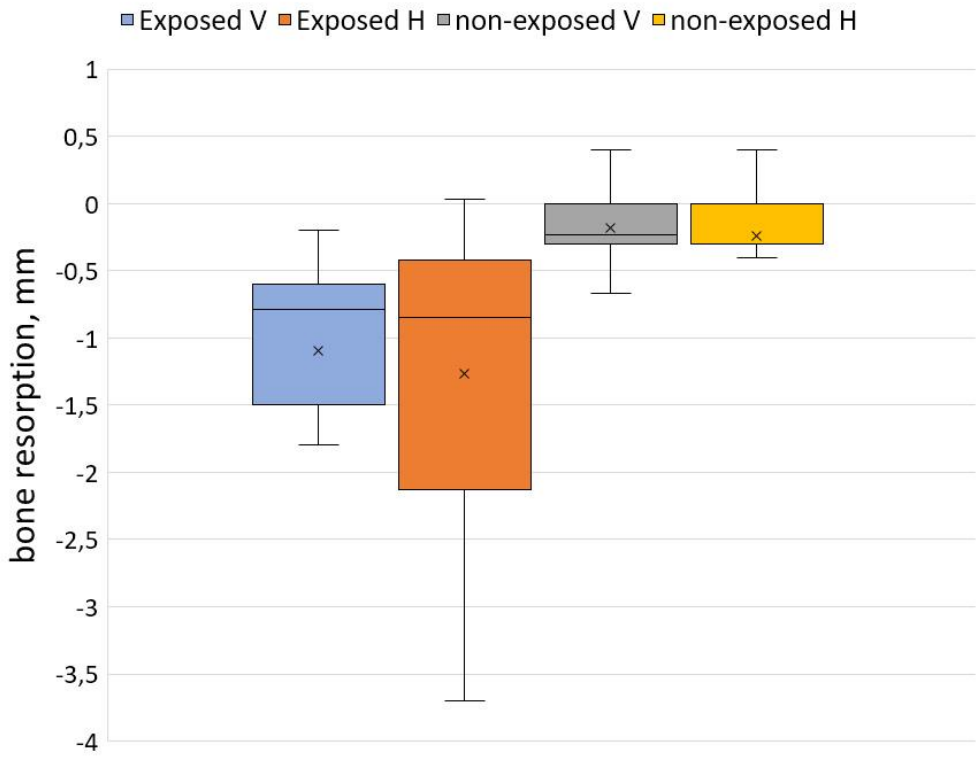


Figure 25 - Box plot showing modifications of the initial bone gain (bone resorption), expressed in mm, in the vertical (V) and horizontal (H) dimension at T2, in exposed and non-exposed sites

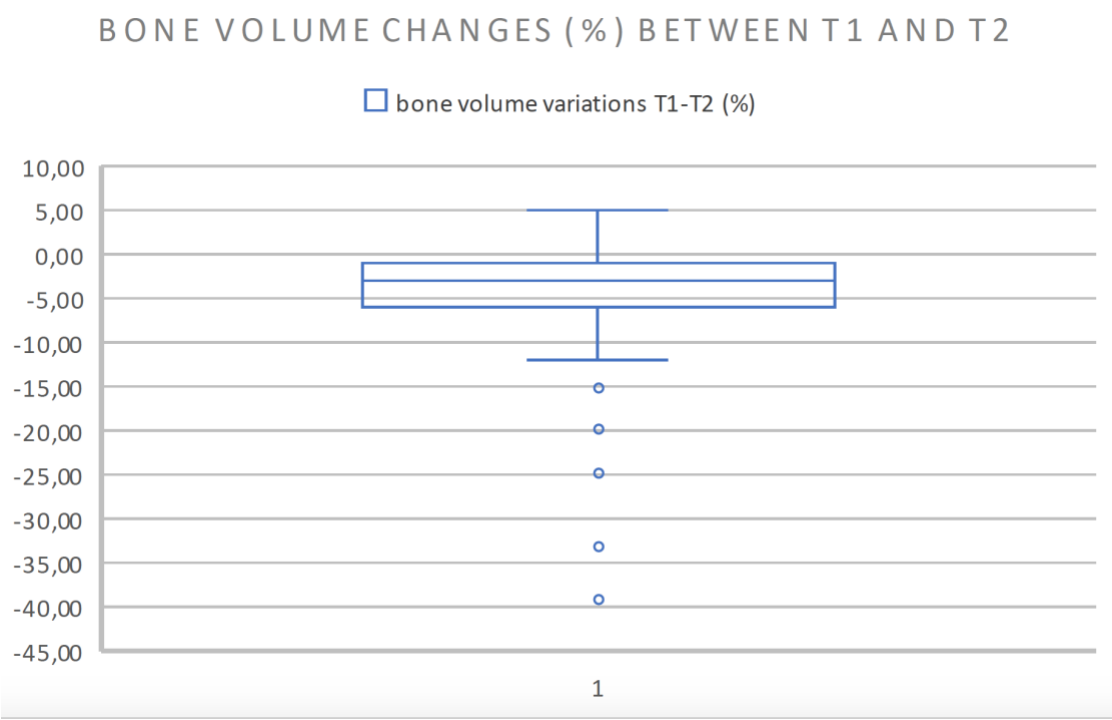


Figure 26 - Box plot showing bone volume changes (in %) between T1 and T2

- ***Complication rate of the reconstructive procedures***

In none of the patients the Ti-meshes had to be adapted as they fitted perfectly.

The number of treated sites was considered the unit of statistical analysis, as many patients presented with multiple atrophic sites at the same time. Out of 53 sites (33 in the maxilla and 20 in the mandible), 42 healed uneventfully, while 11 presented with a variable degree of Ti-mesh exposure which occurred 15 to 150 days after surgery.

Out of these, in 3 sites the exposure was very limited and no reduction of the initial bone gain was observed at T2.

In another 3 sites, exposure was followed by a minimal reduction of initial bone gain, but implant-supported restoration was completed according to the initial program.

Another 3 sites in 2 patients underwent partial bone loss, but it was still possible to deliver the planned prosthetic restorations, although supported by shorter implants.

In one site, the Ti-mesh had to be removed prior to the planned timing, but it was still possible to complete restoration because of limited bone loss.

Finally, one site, treated with GBR and sinus lift, underwent dehiscence and relevant infection 30 days after surgery, not responsive to local and systemic antibiotic therapy. It was decided to remove the Ti-mesh and all the infected graft. The patient healed by secondary intention with preservation of the sinus graft only and the more apical part of the residual ridge. This patient has been considered the only one failure of the procedure and prosthetic rehabilitation has not been started yet.

The follow-up after the reconstructive procedure (T1-November 2020) ranged from 13 to 34,20 months (mean and median=22.24 months; SD=6.56).

Overall, a successful outcome of the regeneration procedure was obtained in 42 out of 53 sites. Occurrence of distribution of dehiscences and number of cases without complications are reported in fig. 27 (Kaplan-Meier curve).

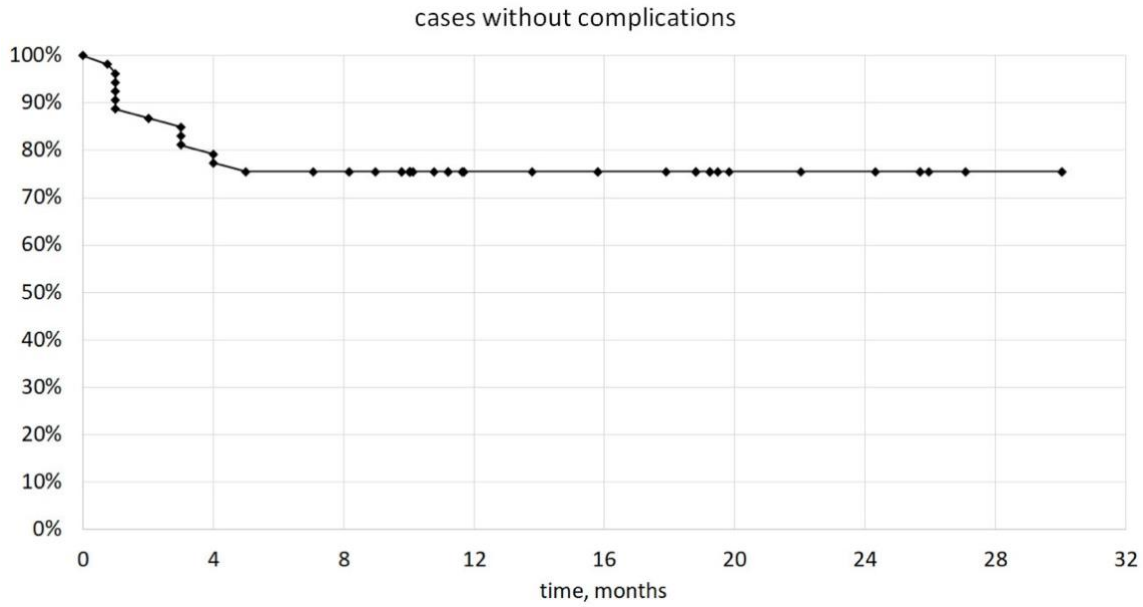


Figure 27 - Distribution of complications analyzed by means of Kaplan–Meier curve.

No statistically significant correlation was found between exposures and: a) gender; b) age; c) number of sites treated; d) mandibular or maxillary sites; e) GBR in association with sinus lift; and f) the harvesting site (ramus vs calvarium), as shown in table 26.

Table 26 - Effect of different variables on the occurrence of mesh exposure (Fisher's exact test).

Variable (unit)	Feature	No exposure	Exposure	P-value
Gender (patient)	Male	7	3	0.28
	Female	24	7	
Age at reconstructive surgery (patient)	<60y	18	6	0.29
	>60y	13	4	
Number of sites treated (patient)	1	23	8	0.32
	>1	8	2	
Site location (case)	Mandible	19	2	0.08
	Maxilla	23	9	
Site location (case)	Anterior (13-23, 33-43)	13	3	0.28
	Posterior	29	8	
Sinus lift (maxillary case)	Yes	7	6	0.10

	No	16	3	
Harvesting site (patient)	Ramus	25	7	0.25
	Calvarium	6	3	

It is however worth noting that Ti-mesh exposures were more frequent in the maxilla, in particular when maxillary reconstruction was associated with sinus lift. On the contrary, no correlation was found between the extent of the defect in mesio-distal direction and the quantity of augmentation and the incidence of exposures.
Moreover, Ti-mesh exposures were positively correlated with bone resorption in the regenerated areas and the difference as compared to non-exposed sites was statistically significant ($p=2.66x10^{-6}$ and $2.94x10^{-4}$ for vertical and horizontal bone resorption respectively) as it is shown in table 27.

Table 27 - Effects of mesh exposure on bone parameters change.

Parameter		N. pat	Mean(SD)	median	min	max	P-value*
Vertical bone resorption, mm	Exp	9	-1.10(0.79)	-0.84	-0.2	-3.10	$2.7x10^{-6}$
	Non-exp	31	-0.19(0.26)	-0.23	0.40	-0.80	
Horizontal bone resorption, mm	Exp	9	-1.27(1.18)	-0.85	0.03	-3.70	$2.9x10^{-4}$
	Non-exp	31	-0.25(0.49)	-0.23	0.40	-2.04	
MBL at 6m post-load, mm	Exp	7	0.25(0.32)	0.00	0.00	0.75	0.185
	Non-exp	17	0.42(0.26)	0.44	0.00	1.00	
MBL at 1y post-load, mm	Exp	4	0.56 (0.43)	0.63	0.00	1.00	0.915
	Non-exp	9	0.59 (0.42)	0.50	0.00	1.25	

*unpaired Student's t-test; Pat= patients; Exp= exposed; Non-exp= non-exposed; SD=standard deviation

Finally, two patients, treated in two mandibular sites, reported a transient paraesthesia of the inferior alveolar nerve: all signs and symptoms disappeared spontaneously within 4 to 12 weeks.
The multilevel linear regression analysis showed a significant relationship between exposure and bone graft linear horizontal/vertical dimension loss, and volumetric loss. Sinus lift also had a significant effect on vertical bone loss (for details see tables 28-29-30-31).
Table 32 compares bone loss in sites with/without exposure and with/without sinus lift. Data are expressed as mean values and standard deviation.

Table 28 - Multilevel mixed linear regression model of the effect of the variables on exposure. The unit of analysis was the patient (n=41).

Parameter	Coefficient	Robust Standard Error	95% CI	P-value
Age	0.008	0.005	(-0.001; 0.02)	0.11
n. of sites/patient	0.372	0.184	(0.01; 0.73)	0.04
n. of implants/site	-0.063	0.050	(-0.16; 0.36)	0.21
Sinus lift	0.264	0.195	(-0.12; 0.65)	0.175
Time graft-implant	0.038	0.051	(-0.06; 0.14)	0.45

Table 29 - Multilevel mixed linear regression model of the effect of the variables on vertical bone loss. The unit of analysis was the patient (n=41).

Parameter	Coefficient	Robust Standard Error	95% CI	P-value
Age	-0.006	0.004	(-0.01; 0.002)	0.15
n. of sites/patient	-0.051	0.086	(-0.22; 0.12)	0.55
n. of implants/site	0.141	0.082	(-0.02; 0.30)	0.09
Sinus lift	-0.519	0.251	(-1.01; -0.03)	0.04
Exposure	-0.606	0.152	(-0.91; -0.31)	<0.001
Time graft-implant	0.069	0.035	(0.001; 0.14)	0.05

Table 30 - Multilevel mixed linear regression model of the effect of the variables on horizontal bone loss. The unit of analysis was the patient (n=41).

Parameter	Coefficient	Robust Standard Error	95% CI	P-value
Age	0.003	0.007	(-0.01; 0.02)	0.68
n. of sites/patient	0.135	0.188	(-0.23; 0.51)	0.47
n. of implants/site	0.099	0.118	(-0.13; 0.33)	0.40
Sinus lift	-0.502	0.343	(-1.17; 0.17)	0.14
Exposure	-0.798	0.218	(-1.23; -0.37)	<0.001
Time graft-implant	0.111	0.056	(0.001; 0.22)	0.05

Table 31 - Multilevel mixed linear regression model of the effect of the variables on bone volume loss. The unit of analysis was the site (n=52).

Parameter	Coefficient	Standard Error	95% CI	P-value
n. of implants/site	0.477	0.936	(-1.36; 2.31)	0.61
Sinus lift	-3.99	2.786	(-9.45; 1.47)	0.15
Exposure	-8.95	2.748	(-14.33; -3.56)	0.001

Table 32 - bone loss in sites with/without exposure and with/without sinus lift. Data are expressed as mean values and standard deviation.

	exposure	No exposure	Sinus lift	No sinus lift (maxilla+mandible)	No sinus lift (only maxilla)
Vertical loss, mm	-1.10±0.84	-0.19±0.25	-0.89±0.91	-0.23±0.29	-0.27±0.25
n	10	31	11	30	16
P-value	<0.001		0.001		0.015
Horizontal loss, mm	-1.27±1.18	-0.24±0.49	-1.01±1.27	-0.30±0.52	-0.42±0.63
N	10	31	11	30	16
P-value	<0.001		0.014		0.12
Volume loss, mm3	-14.07±13.54	-3.61±5.30	-10.79±14.44	-4.23±5.54	-4.76±5.84
N	10	42	11	41	20
P-value	0.0002		0.02		0.11

- ***Histomorphometric analysis***

  - *Qualitative and histomorphometric evaluation*
    - ***Mesh***

Evaluated meshes resulted biocompatible in all cases without signs of adverse reactions. The tissue surrounding the grid was well-organized and characterized superiorly by mucosa and inferiorly by mineralized bone well in contact with the mesh of the grid. No signs of necrosis, fibrosis or ectopic adipose tissue were detected.

Bone was in close contact with the mesh and was characterized by medullary spaces populated by blast-like cells, rare inflammatory cells and blood vessels. Bone showed high level of organization and mineralization and its margins presented remodeling fronts with a large amount of osteoid (Fig. 28).

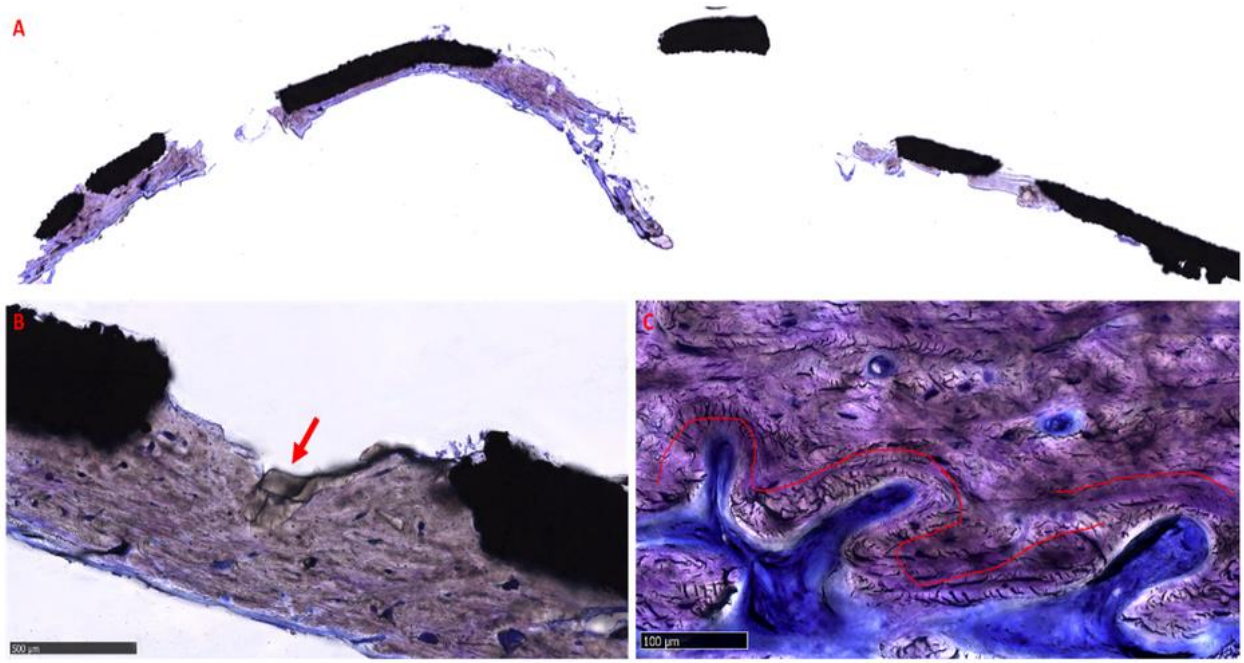


Fig 28 - Osseointegration of the mesh: A) Overview of the grid that shows how mineralized tissue surrounds and links all rods of the mesh, total magnification 50x. B) At higher magnification, numerous bone bridges appear to integrate small granules of biomaterials (red arrow) by connecting and surrounding the rods of the grid, total magnification 70x; C) A detail of the bone that shows an area with a lower level of mineralization. It is characterized by newly formed osteons, spaces in which a very dense osteoid-like matrix is detectable indicating bone in active phase of remodeling and organization process, total magnification 100x. Staining used was Toluidine Blue and Pyronin Yellow, acquisition by scanner at high resolution. 338x190mm (72 x 72 DPI)

In the 3 exposed sites, mucosa was characterized by the presence of biomaterial blocks immersed in dense connective tissue populate
d by numerous fibroblast-like cells (Fig. 29).

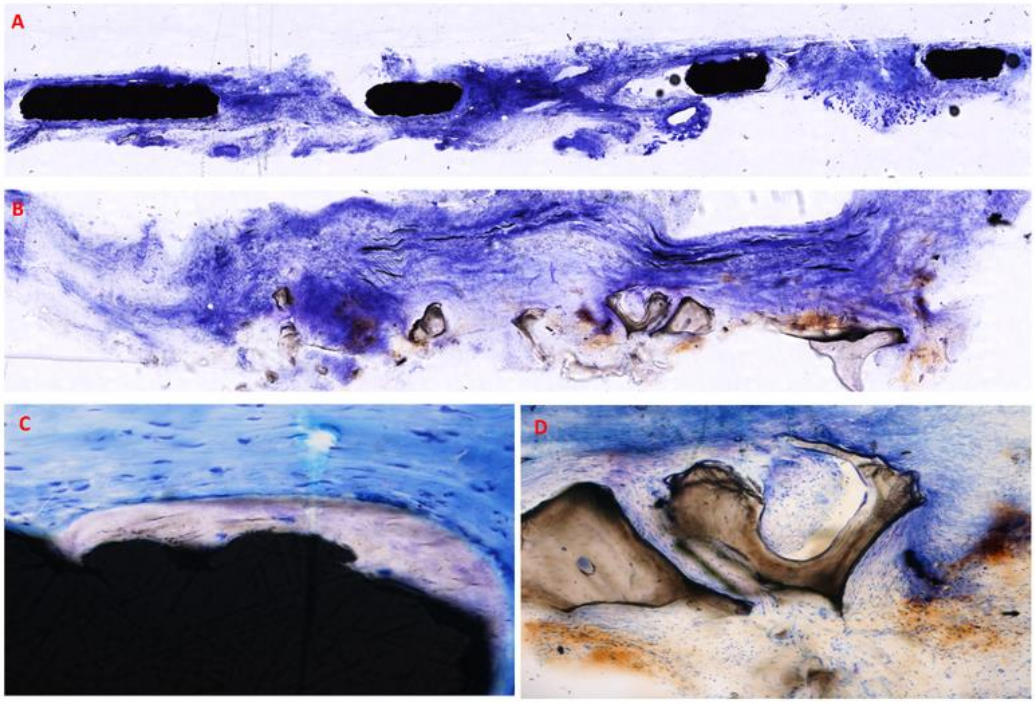


Fig. 29- Connective tissue- mesh interface in sites with late exposure: A) Overview of the "grid trabeculae" immersed in gingival connective tissue, total magnification 70x; B) Overview of the mucosal connective tissue growing over the grid. It appears characterized by irregular fibers that form dense bundles in the areas surrounding biomaterial blocks (stained in brown), total magnification 100x; C) Highly mineralized bone characterized by physiological lacunae containing osteocytes is observable in contact with the grid, total magnification 400x; D) The image shows blocks of biomaterial dispersed in the connective tissue which presents metachromatic areas, indicating a greater concentration of peptidoglycans and glycosaminoglycans, typical of a tissue that is preparing for the mineralization phase, total magnification 200x. Staining used was Toluidine Blue and Pyronin Yellow, acquisition by scanner at high resolution. 338x190mm (72 x 72 DPI)

- **Bone samples**
- *Tissue and cellular reaction in bone samples*

In all sites no signs of necrosis, fibrosis, and ectopic infiltration of fatty cells were observed. Only few inflammatory cells were detected in the samples.
New vessels were spread on the whole section of all specimens. Some capillaries appeared in focal buds (Fig. 30 A) where matrix was in phase of calcification while in medullary spaces (Fig. 30 B) capillaries were wide and organized in groups of 4-7 microvessels supported by a fibroblastic structure. Histomorphometric analysis revealed that the regenerated areas were occupied by 4.06±1.26% of blood vessels.

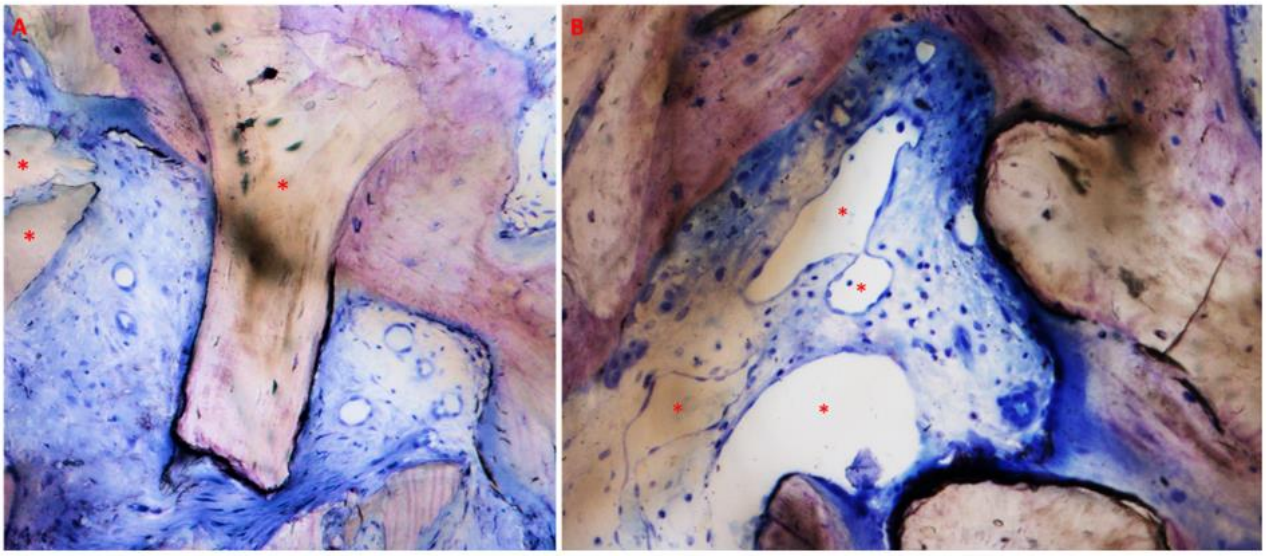


Fig. 30- Vascularization and inflammation: Medullary spaces are characterized by the presence of numerous blood vessels. A) Red asterisks indicate graft blocks surrounded by large medullary spaces and new bone matrix. Small blood vessels can be detected close to osteoid (in blue) and new regenerated bone (in violet); B) A detail shows numerous and large blood vessels in the medullary spaces indicated by red asterisks. Some inflammatory cells resembling lymphocytes are recognizable morphologically. Staining used was Toluidine Blue and Pyronin Yellow, acquisition by scanner at high resolution at total magnification of 200x. 338x190mm (72 x 72 DPI)

- Tissue regeneration and remodeling in bone sample

Histological analysis at T2 showed a highly mineralized and well-organized new bone. Figure 4 shows a high interindividual variability in the amount of mineralized matrix regenerated. Bone samples were characterized by highly mineralized (in brown) alternating with less calcified areas (in violet) still in the remodeling phase and surrounded by dense osteoid matrix, mainly located in the coronal portion of the biopsies (in blue) (Fig. 31).

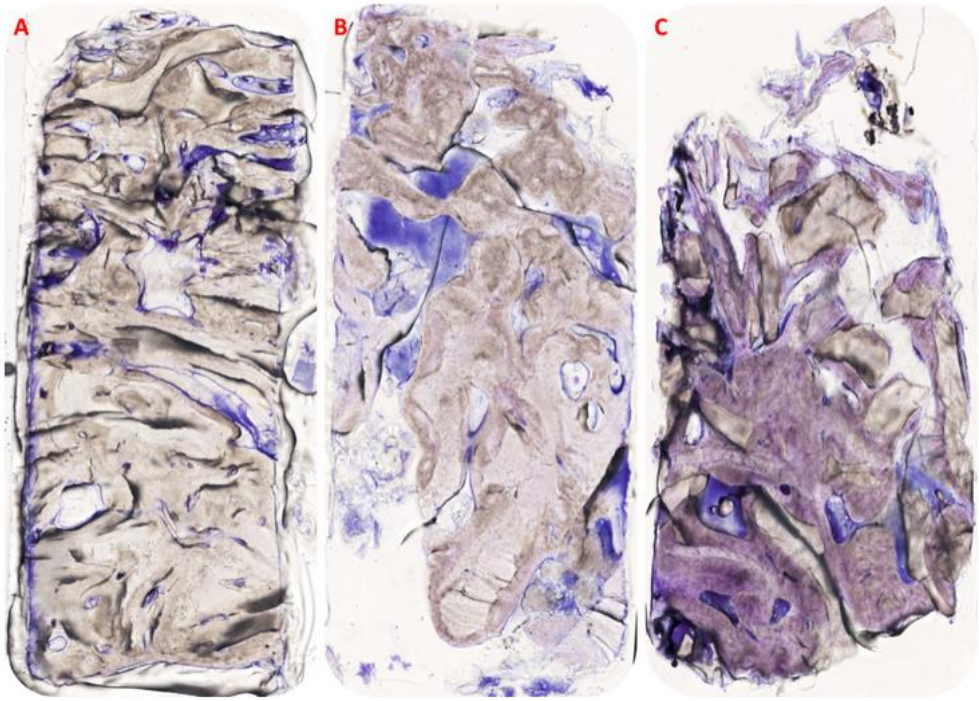


Fig. 31 - Overview of the bone samples: A) A highly mineralized sample where biomaterial blocks seem well osseointegrated and hardly differentiable from newly calcified bone; B) A highly mineralized sample presenting some regenerated areas in phase of organization (dark brown) surrounded by osteoid matrix (in blue); C) A sample moderately mineralized where biomaterial particles are distinguishable from new deposed bone in violet due to the higher concentration of collagen fibers compared to samples A and B. Staining used was Toluidine Blue and Pyronin Yellow, acquisition by scanner at high resolution at total magnification of 25x. 338x190mm (72 x 72 DPI)

Bio-Oss® remnants were perfectly osseointegrated and surrounded by marrow spaces populated by numerous blood vessels without inflammatory infiltrate (Fig. 30). Many fronts of bone remodeling rich in osteoblasts deposing new matrix and in osteoclasts hosted in a resorption lacuna confirm the vitality of the regenerated bone (Fig. 32).

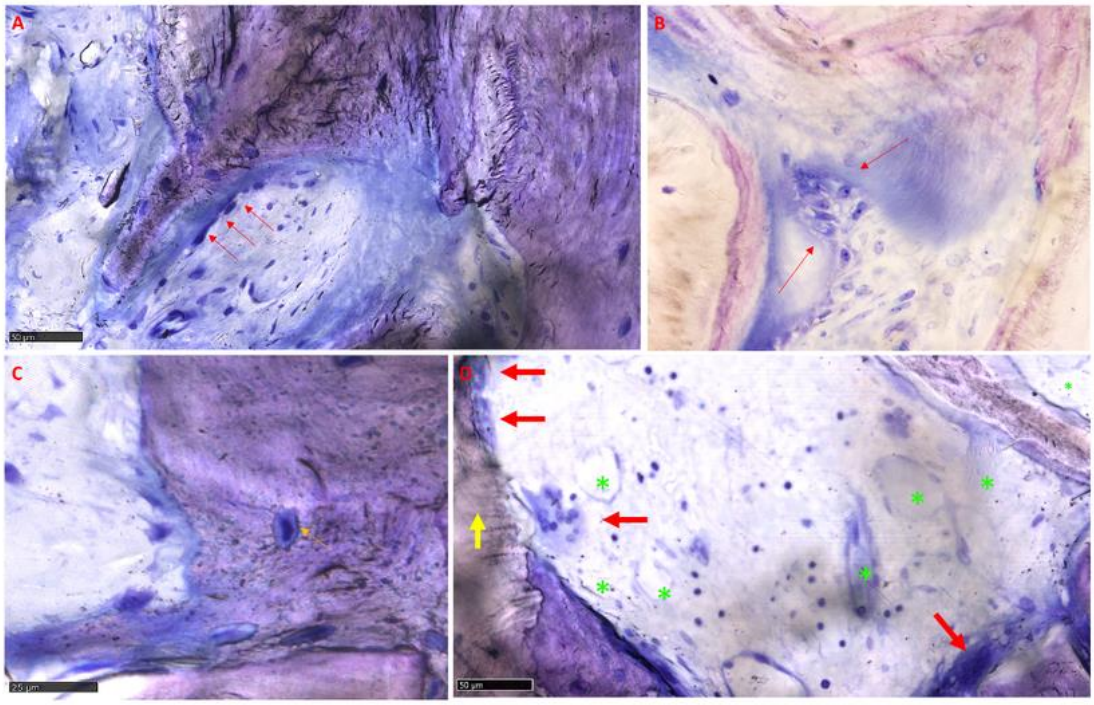


Fig. 32 - Bone cells and remodeling fronts: A) Numerous bone cells are observable, probably osteoblasts that are thickening a new bone trabecula, depositing osteoid matrix (red arrows), total magnification 400x; B) Numerous osteoblasts trapped in the osteoid matrix in phase of mineralization (red arrows); C) Osteoblasts in active phase of deposition. The yellow arrow indicates an osteocyte incorporated into new bone matrix, total magnification 800x; D) Large multinucleate osteoclast like-cells (red arrows) are observed in Howship's lacunae close to a block of biomaterial (yellow arrow), total magnification 400x. Staining used was Toluidine Blue and Pyronin Yellow, acquisition by scanner at high resolution. 338x190mm (72 x 72 DPI)

Histomorphometric analysis revealed that a 77% of the tissue was completely calcified or in phase of mineralization or occupied by biomaterial particles divided in the following proportion: 35.88%±13.53% of new lamellar bone, 16.42%±6.41% of woven bone, 10.88%±6.41% of osteoid matrix and 14.10%±7.54% of grafted remnants. The 22.72%±9.72% of the regenerated area was occupied by medullary spaces.

- *Sites with membrane exposure*

In sites where soft tissue dehiscence occurred, morphological features of the newly formed bone were resembling as sites healed in a physiological way. At morphometric analysis, tissue was composed on average by 32.67% of lamellar bone, 11.80% of woven bone, 10.46% of osteoid matrix, 16.64% of biomaterial and 28.42% of medullary spaces. Mean percentage of the regenerated area occupied by vessels was 3.64%.

- ***Implant survival and implant related complications***

Out of 106 implants placed in the regenerated areas in 40 patients, one was removed in a patient who received 7 implants because of loss of integration before loading. Out of the remaining 105 implants, 90 in 36 patients were loaded with fixed prosthetic restorations. Fifteen implants in 4 patients are still waiting prosthetic restoration, as inserted more recently. One patient (2 mandibular sites and 4 implants) interrupted clinical and radiographic controls after having received the final restoration. Therefore, at the end of the observation period (November 2020), 105 implants before loading, and 86 implants in 35 patients after prosthetic restoration were available for statistical analysis. The mean follow-up after implant placement and after delivery of prosthetic restorations were 14.99 months (range: 6.6 - 29; median= 14.99; SD=6.29), and 10.6 months (median= 10.6 months; range: 2-26.02; SD=6.49), respectively. No implants developed biological complications such as peri-implantitis.

The survival rate of loaded implants at the end of the observation period was 100%. No prosthetic complications were reported during the observation period.

- ***Peri-implant bone resorption***

Peri-implant bone loss per patient before and after the start of prosthetic loading was very limited and never >1.5 mm at the end of the observation period (see Table 33).

Table 33 - Marginal Bone Loss (MBL) around implants placed in the regenerated areas

	N. pts	mean	SD	median	min	max
MBL (mesial) at 6m post-load (T5), mm	24	0.33	0.34	0.50	0.00	1.00
MBL (distal) at 6m post-load (T5), mm	24	0.37	0.41	0.50	0.00	1.50
MBL (mesial) at 1y post-load (T6), mm	13	0.54	0.34	0.50	0.00	1.00
MBL (distal) at 1y post-load (T6), mm	13	0.56	0.42	0.50	0.00	1.50

- ***Patient satisfaction***

Out of 41 patients treated, 40 were available for answering to the dedicated questionnaire. Overall, patient's reported outcome measures showed a high level of acceptance and satisfaction as regards the received treatment. Overall, a successful cumulative score (≤25 out of 50 points) was reached in 39 out 40 patients (97.5%) and 92,5% of them would have accepted to undergo the procedure again (Table 34 for details). The mean successful cumulative score was 14.98.

Table 34 - Patients' satisfaction

	Q. No. 1	Q. No. 2	Q. No. 3	Q. No. 4	Q. No. 5	Q. No. 6	Q. No. 7	Q. No. 8	Q. No. 9	Q. No. 10
min value	1	1	1	1	1	1	1	1	1	1
1st quartile	1	1	1.25	1	1	1	1	1	1	1
2nd quartile	2	1	2	2	2	1	1	1	1	1
3rd quartile	3.25	3	3	2	3	1	1	1	1	1
max value	4	3	5	4	5	2	3	3	4	5
Number of fully satisfied patients (%of pts)	12 (30)	21 (52,5)	7 (26.9)	15 (37,5)	17 (42,5)	38 (95)	34 (85)	39 (97.5)	37 (92.5)	37 (92.5)
Number of very satisfied patients (%of pts)	15 (57,7)	15 (57,7)	7 (26.9)	24 (60)	5 (12,5)	2 (5)	5 (12.5)	0	0	
Number of partially satisfied patients (%of pts)	3 (7,5)	4 (10)	6 (23.1)	0	16 (40)	0	1 (2.5)	1 (2.5)	0	
Number of partially unsatisfied patients (%of pts)	10 (25)	0	1 (3.9)	1 (2,5)	1 (2.5)	0	0	0	3 (7,5)	
Number of totally unsatisfied patients (%of pts)	0	0	5 (19.2)	0	1 (2.5)	0	0	0	0	3 (7,5)

Legend: Q No., question number; pts, patients; 14 patients didn't answer to question no. 3 because they had a provisional prosthesis during the healing phase.

A representative case is reported in figures 33- 43.

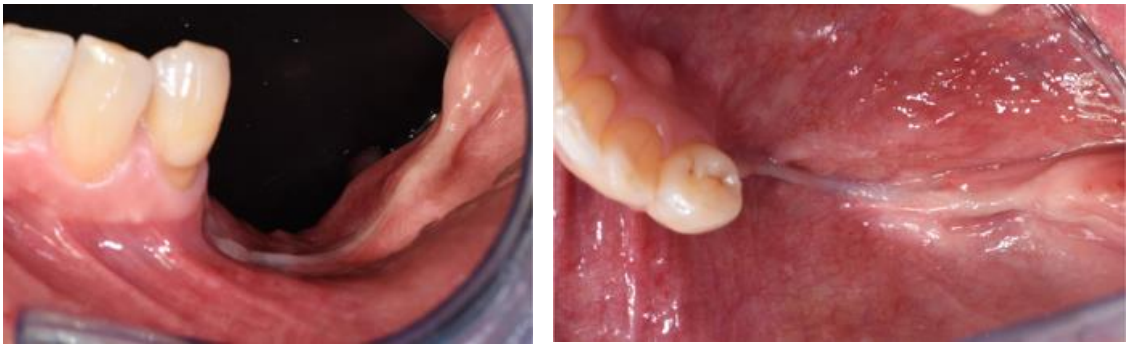


Figure 33 - Intra-oral view showing the severe vertical and horizontal bone atrophy of the left mandible

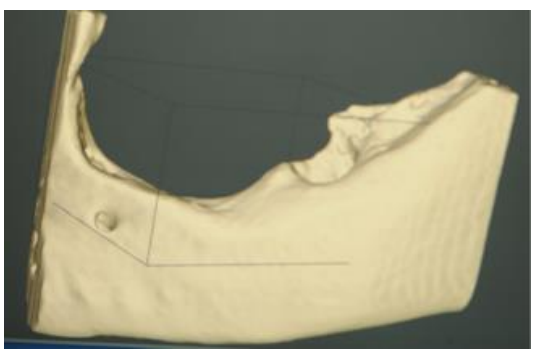


Figure 34 - The 3-dimensional reproduction of the edentulous area allows to produce a precise and customized Ti-mesh

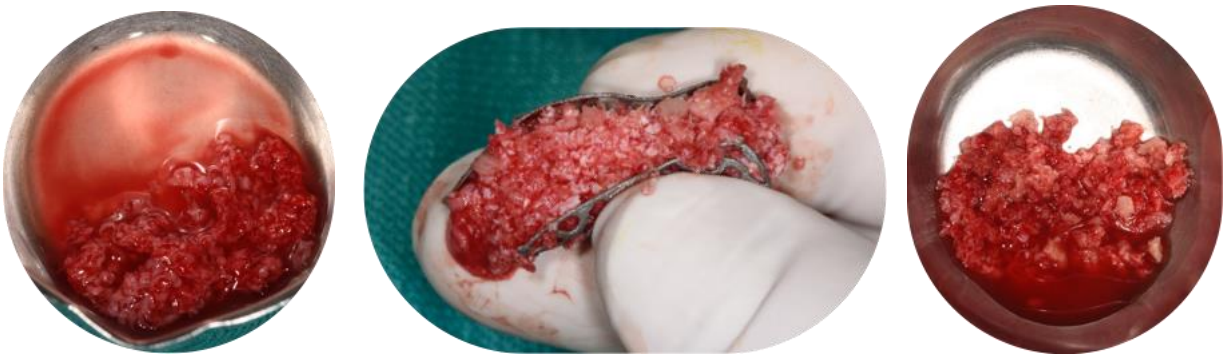


Figure 35 - The customized Ti-mesh is filled with the autogenous bone chips mixed with the DBBM granules in a 1:1 ratio

Figure 36 - Intra-operative view at the end of the reconstruction showing the bone augmentation

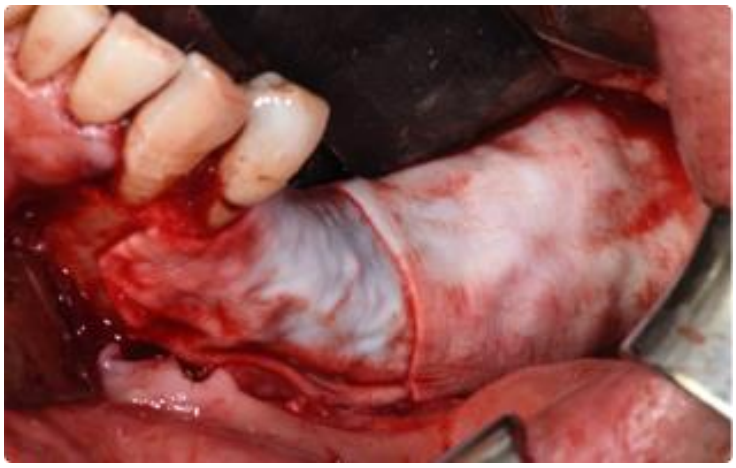


Figure 37 - A collagen membrane is used to cover the Ti-mesh in order to increase the barrier effect

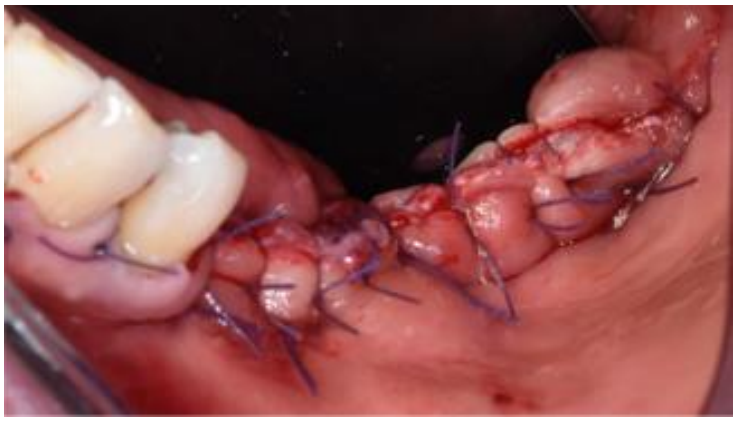


Figure 38 - Intra-operative view after primary closure of the surgical wound

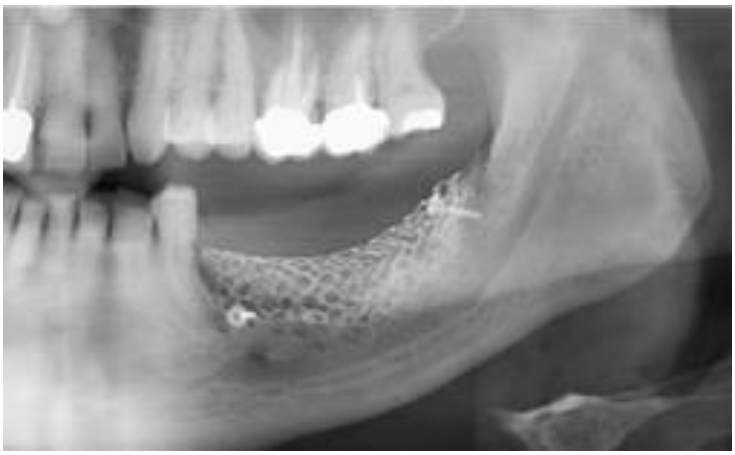


Figure 39 - Panoramic radiograph after surgery

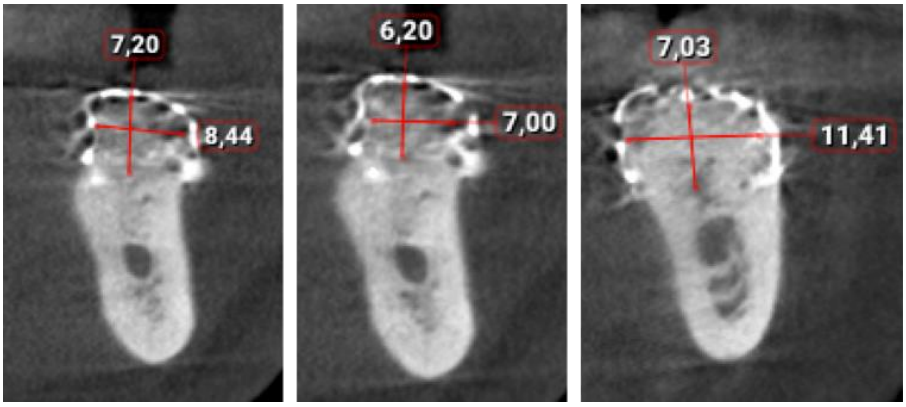


Figure 40 - CBCT scans showing the vertical and horizontal bone gain in mm

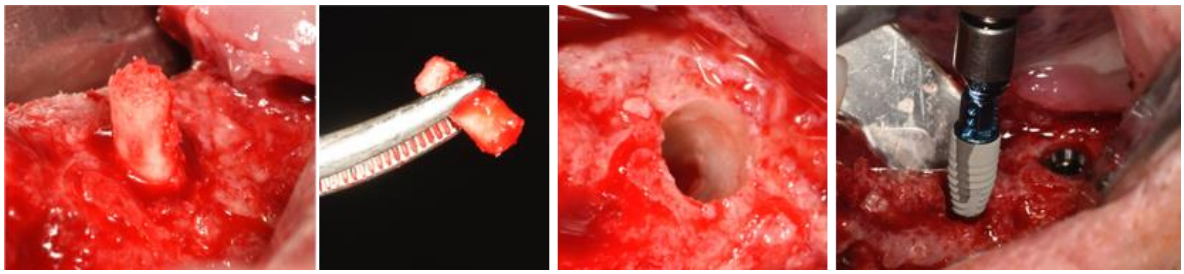


Figure 41 - During T2, one implant site was prepared with a 2.5 mm trephine drill to obtain a bone biopsy for histomorphometric analysis of the regenerated tissue. Then, implants were placed.

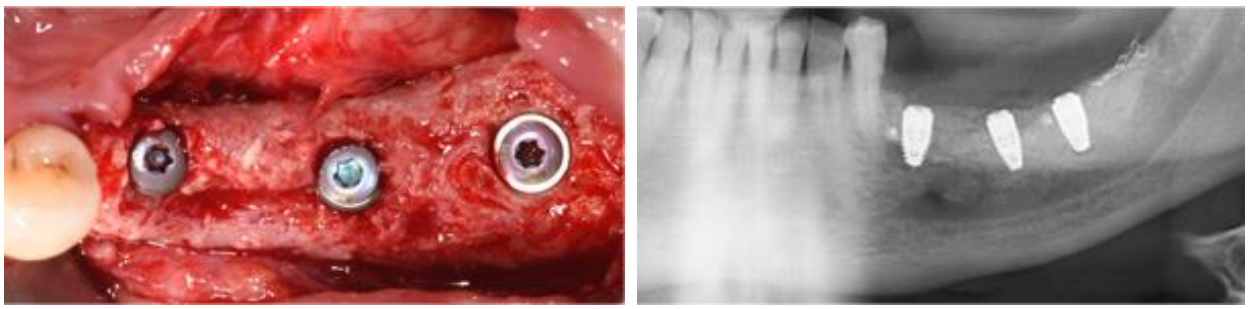


Figure 42 – Clinical images and panorami radiograph after implants placement

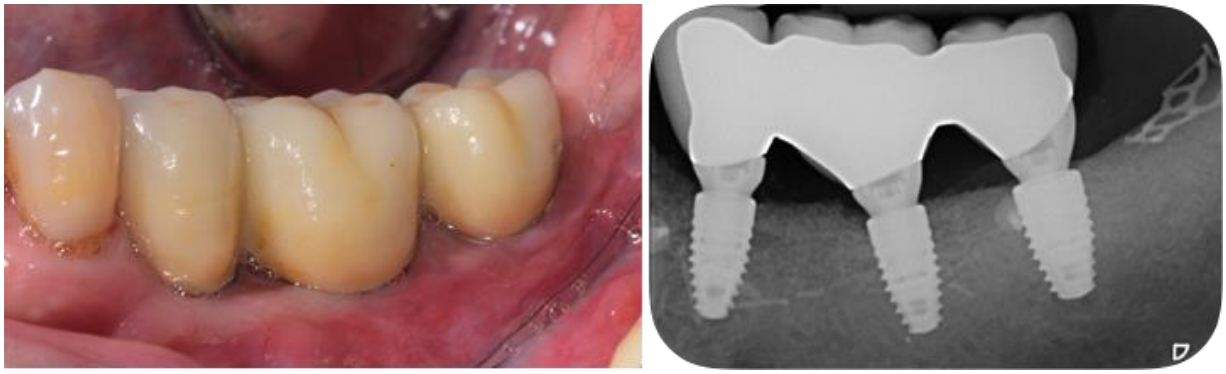


Figure 43 - clinical and radiographic control after the delivery of the final implant-supported restoration

8 – DISCUSSION

These three projects have offered, on one side, a relevant opportunity to evaluate the short, medium, and long-term outcomes of both reconstructions with autogenous bone blocks and guided bone regeneration with an innovative, customized, titanium mesh.
On the other side, "intrinsic" methodologic limits have appeared during the development of this PhD thesis, such as:

- heterogeneity of patient samples and type of defects;
- retrospective and prospective type of study;
- duration of the observation period;
- presence/absence of evaluation of bone gain and with different methods;
- different methods used to evaluate bone resorption before implant placement.

As regards heterogeneity, it must be underlined that it is almost impossible to select in reasonable times a relevant number of patients with very similar clinical situations in all studies.
Secondly, as the first 2 projects were retrospective, the sample size was not calculated before recruiting patients, but only when it was decided to recall all of them and to publish the related data. The main advantage of these two studies has been the relatively long follow-up, as the first patients were treated almost twenty years ago.
The third project, on the contrary, was prospective and had therefore the sample size calculated during the preparation of the protocol before the enrolment of patients. Conversely, as patients were enrolled in a 2-year period, the number of patients and the follow-up were limited.

In the first project, the initial bone gain was not analysed in a systematic manner, but the main focus was the behaviour of autogenous bone block grafts after implant placement in terms of peri-implant bone resorption. In the second project, this parameter has been calculated in mm on panoramic radiographs taken before and after the reconstructive procedures. On the contrary, the third project was focused on the space maintaining potential and consequent bone regeneration of these new customized Ti-meshes. During the CAD-CAM design and creation, it was possible to calculate in advance the expected volume of the regenerated bone and to compare it immediately after surgery with the actual one. This offered the chance to observe also volume modifications between the day of surgery (T1) and the time of mesh removal (T2), also thanks to updated software which allow superimposition of CT scans taken at different times.
Finally, a limit of the third project is represented by the absence of a control group treated with a different regenerative procedure. Therefore, any kind of comparison among other types of GBR procedures will be performed using the available literature (mainly systematic reviews and meta-analyses).

Despite these limits, results from these three researches offered relevant information as regards the behaviour of the transplanted /regenerated bone as well as the behaviour of peri-implant bone over time.
Although the three projects significantly differ among them as regards details in the reconstructive procedures, it must be underlined that many aspects are in common.
Therefore, to reduce redundancies it has been decided to write a "unified" discussion which will make it possible a direct comparison of pros, cons, indications and contra-indications of each of them.

More in detail, each of the following issues will be discussed reporting and comparing obtained from each study:

1. bone gain;
2. complication rate of the reconstructive procedure;
3. bone resorption before implant placement;
4. peri-implant bone resorption;
5. survival rate of implants and implant-related complications;
6. patient's satisfaction inquired with a dedicated questionnaire.

1. Bone gain

As already underlined above, the calculation of initial bone gain was performed only in projects 2 and 3. In project 2 the calculation of vertical bone gain was performed by measuring the distance between the profile of the residual crest and an ideal line which connected: a) the CEJ of neighbouring dentition in patients with partial edentulism and without CAL; b) the highest peaks of the alveolar bone of neighbouring dentition, in case of CAL. In edentulous mandibles, as reference lines were impossible to draw, the bone gain was calculated by comparing the distance between the lower margin of the mandible and the level of the alveolar crest before and after surgery. In edentulous maxillae, the maxillary sinus and the nasal floors were considered as the reference and bone gain was calculated by comparing panoramic radiographs before and after surgery. The vertical bone gain ranged from 1 to 10 mm. In project 3, vertical and horizontal bone gains were evaluated on a CBCT using as reference points the internal margins of the Ti-mesh and the profile of the residual bone before the reconstruction (for further details see M&M of project 3). The vertical bone gain ranged from 1 up to 8.9mm (mean:4.78), while horizontal bone gain ranged from 2.14 to 11.48 mm (mean 6.35) .

Both projects demonstrated that relevant vertical increases can be obtained both with blocks and GBR with Ti-meshes. These results are consistent with those reported in a recent systematic review by Urban et al. (2019), in which the mean vertical bone gains were 3.5 mm, and 4.31 mm, and 4.99 mm with bone blocks, expanded PTFE and dense PTFE membranes, respectively.

2. Complication rate of the reconstructive procedure – project 1 (calvarium)

As regards reconstructions with autogenous calvarial bone, notwithstanding the fact that this procedure is well supported by current literature (Donovan et al. 1994; Chiapasco et al. 2006, 2009; Smolka et al. 2006; Sahoo et al. 2010; Mertens et al. 2013, 2016; Putters et al. 2015; Chiapasco et al., 2018), complications albeit limited may occur. More in detail, postoperative morbidity occurred at the donor site only in one out of 69 patients (a subcutaneous hematoma that resorbed spontaneously in 2 weeks). Post-operative pain, as reported in the patient's questionnaires, was extremely limited or absent. However, it must be underlined that this donor sites is generally used only when intra-oral donor sites are insufficient in terms of quantity (i.e. fully edentulous maxilla) and that a maxillo-facial expertise is mandatory (Chiapasco et al. 2006, 2009, 2018). In fact, although extremely rare (Donovan, et al,1994; Iizuka, et al., 2004) complication may be severe and life-threatening, if the cranial content is violated. On the other hand, calvarial bone besides the large quantities it may be offer is form by a highly corticalized, dense bone which may maintain its original volume over long observation period (up to 20 years). The only limit might be represented by the slow re-vascularization of this bone (in particular if vascular condition of the recipient site are not optimal, as in the case of previously treated and failed areas, scarry tissues , etc,.

As regards the complication rate in the reconstructed area, this study demonstrated an uneventful healing in the majority of patients (63 out of 69 patients). The excellent integration of the grafts allowed a proper, prosthetically driven implant placement. It must be stressed that these positive results might be also related

to the use of autogenous pericranium. As demonstrated by previous studies (Autelitano et al. 2008; Chiapasco et al. 2012) pericranium grafts, used to cover the bone blocks, reduce the risk of bone exposure in case of an early wound dehiscence which may occur also in case of a tension-free, water-tight suture. In this study, the majority of wound dehiscences associated with bone grafts exposures occurred in patients not receiving pericranium grafts (4 out of 6 patients). Although no statistically significant differences were found (also because of the limited number of patients), these results seem to confirm the positive effect of pericranium in preventing early graft exposures in case of dehiscence, as already shown in previous studies (Autelitano et al. 2008; Chiapasco et al. 2012).

2. Complication rate of the reconstructive procedure – project 2 (mandibular ramus)

As regards the complication rate in the donor site, paresthesia involved only 3 patients (4%), but all of them recovered within 1 to 2 months after the surgical procedure. This is in line with a systematic review by Chiapasco et al 2009, in which the incidence of neural disturbances related to bone harvesting of mandibular ramus ranged from 0 to 5%.

As regards the complication rate of the reconstructive procedure, this study demonstrated that an uneventful healing occurred in the majority of patients (72 of 75 patients). Only 3 patients reported early infection and/or dehiscence which healed after local treatment within 8 weeks, with no significant damage to the grafted bone.

In this study, the incidence of dehiscences was lower as compared to data reported by other studies (Chaushu, et al., 2010) or systematic reviews (Milinkovic & Cordaro, 2014; Chiapasco et al., 2006-2009) and no significant bone loss was reported, also in case of dehiscence. These positive results might be related to the relevant periosteal releasing incisions, to the accuracy in suturing, the systematic use of antibiotics, and chlorhexidine mouth washes.

Furthermore, the coverage of the bone graft with a resorbable collagen membrane might reduce the risk of "direct" bone graft exposure in case of an early dehiscence, as the membrane acts as a protective shield as regards the graft.

(Proussaefs, & Lozada, 2003; Von Arx, & Buser, 2005; Jensen, & Terheyden 2009; Cordaro, et al., 2011).

The second factor which may explain the "rescue" of exposed grafts is the very limited extent of these dehiscences and the prompt treatment by local curettage and perforations of the graft with small round burs to obtain bleeding form the basal bone and, consequently, formation of granulation tissue over the exposed area. This eventually evolved in secondary re-epithelization over the graft with preservation of the initial volume.

The only limit of mandibular ramus harvesting may be represented by the quantity of available bone. In case of extreme atrophy involving extensive areas of the jaws, it is often necessary to use extra-oral donor sites such as the iliac crest (Vermeeren, et al., 1996; Verhoeven, et al., 1997; Becktor, et al., 2004; Chiapasco, et al., 1999, 2009; Van der Meij, et al., 2005) or the calvarium (Donovan, et al., 1994; Iizuka, et al., 2004; Gleizal, et al., 2007, Chiapasco et al., 2013; Smolka, 2014; Chiapasco et al., 2018).

2. Complication rate of the reconstructive procedure – project 3 (GBR with Ti-mesh)

An uneventful healing was observed in 42 out of 53 sites, with minimal or no resorption of the graft at the time of implant placement (T2). In 3 of these sites, an overgrowth of bone covering the mesh was also observed in some areas (see table 4). The growth of bone through and even over the "open spaces" of the meshes may also explain the relative complexity in removing them at T2.

It is however worth noting that also in case of uneventful healing, the elevation of flaps to expose the underlying meshes was more complex and more time consuming as compared to cases treated with autogenous bone block or GBR procedures with non-resorbable membranes such as the PTFE ones.

Out of the 11 sites where an exposure occurred (20.75%), only one site, in which exposure was associated to infection not responsive to local and systemic antibiotic therapy, it was necessary to remove the Ti-mesh and all the infected graft. In the remaining sites, 6 of them were associated with no significant reduction of initial bone gain at T2 and implant-supported restoration was completed according to the initial program. Four sites underwent partial bone loss, but it was still possible to deliver the planned prosthetic restorations, although supported by shorter implants. In all these cases, a spontaneous and fast re-epithelization beneath the inner part of the mesh was observed. This phenomenon may explain the limited reduction of initial bone gain, as reported by other authors (Sagheb, et al., 2015; Sumida, et al. 2017; Sailer, et al. 2018). Moreover, the quality of the residual graft was very good as demonstrated by both the briefly described histomorphometric results and the possibility to prosthetically restore these patients.

Another interesting aspect is that exposures were never associated (besides the only one case in which the mesh was early removed) to local infection/suppuration below the mesh: this may have contributed to the maintenance of the graft (see table 4).

However, it has been shown that both vertical and horizontal bone volume changes were higher in the exposed sites, and the difference was statistically significant (see table 4).

Since the exposures occurred even months after surgery, and in the majority of cases not at the level of the incisions lines, one may presume that these exposures were not related to an insufficient flap release or suturing technique, but rather to the mechanical trauma caused by the rigid structure of the mesh on the overlying mucosa.

In accordance with other Authors (Sagheb, et al., 2017; Seiler, et al., 2018), results from this study showed higher incidence of exposures was observed in the maxilla. As the palatal mucosa cannot be released, 100% of the releasing procedure was performed on the buccal flap with consequent “thinning” and potential reduction of vascularization of the flap. On the contrary, releasing incisions in the mandible can be equally distributed on both the buccal and lingual sides. This might maintain thicker flaps, less exposed to mechanical trauma. Finally, one may speculate if the gingival phenotype (thin or thick) may influence the percentage of exposures. However, this data is lacking at present.

Despite the relatively high rate of exposures (11 out of 53 sites – 20.75 %), it is worth noting that only 4 (7.55%) were followed by significant reduction of the initial bone volume. In two systematic reviews (Milinkovic & Cordaro, 2014; Urban et al., 2019), the complication rate of GBR for the correction of vertical defects was 13.1%, and 12.1%, respectively. Therefore, results from this study are consistent with those reported in the cited literature reviews.

As regards the reconstruction with autogenous bone blocks, the complication rate from the same systematic reviews (Milinkovic & Cordaro, 2014; Urban et al., 2019) was 8.1% and 23.9%, respectively. Again, it appears that the complication rate of regeneration with customized Ti-meshes is consistent with that reported in the previously cited publications.

To summarize the issue of complications following the aforementioned procedures, it has been observed that the highest rate of early or late dehiscence/ graft exposures occurred in patients treated with customized Ti-meshes (20.75%), while these complications were 9.1% and 4% for reconstructions with calvarium and ramus, respectively.

As regards the differences between calvarium and ramus grafts, the higher incidence of dehiscences observed in reconstructions with calvarium might be justified by the fact that this material is used in the majority of cases for extremely severe defects, in particular in case of relevant vertical defects. As a consequence, the higher complexity, the more relevant releasing incisions (with potential reduction of flap vascular support) might justify this difference.

On the contrary, the relevantly high incidence of exposures of the Ti-meshes is not related to the extent of the defect, as shown by results in project 3, but rather to the rigidity and the roughness of this material. Moreover, the majority of exposures occurred in the maxilla and one may speculate this phenomenon may depend by the fact that release of the flaps can be obtained only on the buccal side. However, it is worth noting that similar conditions should be found also in case of autogenous bone grafting: in fact, a total of 5 dehiscences occurred in the maxilla (out of 48 bone grafts) versus 1 (out of 44 reconstructions) in the mandible in project 1; 2 dehiscences occurred in the maxilla vs 0 in the mandible (out of 82 maxillary and mandibular sites) in project 2. These data, even if not statistically significant in none of the three projects, are therefore slightly more relevant in project 3: it can be supposed that the type of material (the rigid and roughness Ti-mesh vs autogenous bone) may play a role.

The second relevant issue related to dehiscences is the behaviour of the underlying bone in different phases of regeneration.

As regards bone grafts, three main clinical consequences may occur:

2. bone graft exposure with no clinically detectable infection and no bone loss;
3. bone graft exposure with partial sequestration of the graft;
4. bone graft exposure with total sequestration of the graft.

As compared to Ti-meshes, the perforation of the outer part of the graft which will make it possible to the newly formed deeper vessels may potentially speed up the revascularization of the remaining part of the graft.

Briefly, the formation of granulation tissue through the perforation will act as an active tissue which will allow the survival of the graft and eventually its integration.

On the contrary, in case of Ti-mesh exposure this surgical manoeuvre is ineffective and probably contra-indicated as it will disperse the particulated graft with diffusion of infection.

Conversely, it has been observed that Ti-mesh exposure in the absence of clinically detectable infections may be followed by early re-epithelization between the inner part of the Ti-mesh and the grafted material.

This will create a new “barrier” which will protect the further phases of integration, thus leading to a potentially successful integration of the material.

On the contrary, the growth of an epithelial/connective layer between the recipient site and bone grafts in blocks is almost by definition a sign of sequestration and, eventually, total failure of the reconstructions.

Furthermore, in all cases of exposure, the use of chlorhexidine mouth rinses, chlorhexidine in gel and the use of local antibiotic may be effective to control the local infection. On the contrary, primarily in bone blocks exposures, the use of an intravenous or oral systemic antibiotic, even if used in the maximum dosage, is not indicated to limit local infection because the bone block itself has a scarce vascularization and the antibiotic would not be able to reach the infected site.

3. Bone resorption before implant placement

As reported in the results section, calvarial bone grafts covered with DBBM demonstrated a very limited resorption before implant placement (range: 0-1 mm; mean: 0.13 mm; median: 0). Although to the Authors knowledge no articles comparing bone resorption of calvarial grafts with or without DBBM are currently available, it seems that this xenograft may have a positive effect on the maintenance of initial bone volume gain. The positive effect of DBBM on bone volume maintenance over time, although obtained in cases where iliac grafts or intra-oral grafts were used, has been demonstrated also by other studies (Prossaefs & Lozada, 2003; Maiorana et al. 2005; Von Arx and Buser 2006).

As far as bone volume augmentation stability over time is concerned, also the second project showed a very limited horizontal and vertical resorption before implant placement (0 - 2 mm; mean=0.15 mm; SD=0.42mm, and 0 - 2 mm; mean=0.18 mm; SD: 0.43mm, respectively). Also in this study, as already noted in the first

project, the covering with a protective layer of DBBM and a collagen resorbable membrane seems to confirm that this "barrier" may prevent the risk of autogenous bone resorption thanks to the osteoconductive and space making properties of DBBM. These results are comparable with data from the test group of a randomized controlled clinical trial, comparing grafting with autogenous mandibular bone blocks with (test) or without (control) covering the blocks with a xenograft and a collagen membrane (Von Arx, & Buser, 2006; Cordaro, et al., 2011).

As regards the same values in project 3, at the time of implant placement, mean changes of initial bone gain were -0.39±0.64mm (range -3.1 to +0.80mm), and -0.49±0.83mm (range –3.7 to +0.4mm), in the vertical and horizontal dimensions, respectively.

To summarize, it appears that bone changes before implant placement are lower in case with reconstruction with bone blocks. It is however difficult to compare in an objective way these data as different techniques of measurement were adopted in the three protocols. In fact, in the first two projects, fixation screws were used as reference points and a UNC-15 probe allowed to measure bone changes. Values were rounded at the nearest half millimetre. In the third project, on the contrary, the volumetric changes and the differences in bone gains were measured in a different, and, perhaps, more precise and predictable way (using specific software, and measuring two decimal factors of a millimetre). A comparison in bone changes between T0, T1, and T2 was therefore possible.

Moreover, in the third project, bone changes were also compared in patients with and without early or late dehiscences. It has been shown that both vertical and horizontal bone volume changes were higher in the exposed sites, and the difference was statistically significant (p value for vertical and horizontal: $2.7x10^{-6}$ and $2.9x10^{-4}$,respectively).

4. Peri-implant bone resorption

As far as peri-implant bone resorption after the start of prosthetic loading is concerned, results from project 1 demonstrated very good values over time with a mean peri-implant bone resorption of 1.11 mm (frequency distribution range 0.00 – 4.87 SD: 1.14) at the end of the observation period. In terms of frequency distribution, 166 implants out of 323 presented < 1 mm peri-implant bone resorption, and 101 implants a peri-implant resorption < 2mm at the end of the observation period. Peri-implant bone resorption rates > 2mm at the end of the observation period involved only 56 implants.
In project 2, similar results were obtained. The mean vertical peri-implant bone loss ± standard deviation was 1.06±1.19 (range 0.00 to 5.05) at patient level, and 1.11±1.26 (range 0.00 to 5.20) at implant level. In terms of frequency distribution, 90 implants out of 182 presented < 1 mm peri-implant bone resorption, and 59 implants a peri-implant resorption < 2mm at the end of the observation period. Peri-implant bone resorption rates > 2mm at the end of the observation period involved only 33 implants.

Results from the first two projects are comparable as the same measuring method, and the same statistical analysis were performed. Moreover, very similar follow-ups were evaluated. From this comparison, it appears that no statistically significant differences in terms of peri-implant bone resorption can be found between the two groups. These results are also consistent with those reported in other publications (Chiapasco et al. 2013; Milinkovic & Cordaro 2014).
Moreover, results of both projects are also coherent with those presented by other Authors who used calvarial grafts (Quiles et al. 2015; Restoy-Lozano et al. 2015; Mertens et al. 2013, 2017), but also with those reported for implants placed in native bone (Jung et al. 2012; Pjetursson et al. 2012; van Velzen et al. 2015; De Medeiros et al. 2016; Niu et al. 2017). On the contrary, peri-implant bone resorption in patients treated

with iliac grafts was higher (Vermeeren et al. 1996; Becktor et al. 2004; Veroeven et al. 1997; Chiapasco et al. 2006-2009).
Another trends as regards peri-implant bone resorption is that, generally speaking and besides case of acute peri-implant infection, bone resorption tends to occur in the first two years after loading and, then, in the absence of complications such as peri-implantitis, it stops.
One may speculate that this phenomenon may derive by two main factors: on one hand, the revascularization of the external part of the grafted area can last several months and, on the other, the functional stimulus to the bone after implant loading might potentially explain the maintenance of the graft. In fact, if we compare the behavior of bone grafts, we can easily see that non-loaded bone will undergo almost a complete resorption, whereas grafted areas with loaded implants survive better. However, this is potentially in contrast with clinical outcomes in which, despite implant loading, bone resorption occur in the early phases of function.

As regards horizontal bone resorption after implant placement, the author is well aware that without CBCTs it is impossible to obtain annual controls. However, the irradiation dose would be unjustified and non-ethical. For this reason, only clinical controls verifying the absence of implant threads exposure in the buccal or lingual aspects, as well as the absence of soft tissues contour changes, have been done every year after the completion of prosthetic rehabilitation. Clinical evaluation demonstrated (within the limits of this type of evaluation) stability of the graft width over the years.

MBL was recorded also in project 3: the very limited follow-up do not allow any comparison with results reported in project 1 and 2. However, the mean mesial and distal peri-implant bone resorption (0.57 and 0.59 mm 1 year after loading, respectively) seem to confirm the reliability of the GBR with Ti-mesh.

5. Survival rate of implants and Implant-related complications

As already underlined in the previous point, results from projects 1 and 2 are comparable as very similar follow-ups and the same statistical analysis were used. The third project, as a prospective one, has a limited follow up and results are not comparable to the other researches.
The cumulative survival rates of implants placed in calvarial or mandibular bone blocks were 98.5% and 98.11% , respectively.

Results from these studies are consistent or slightly higher not only with those related to implants placed in calvarial bone blocks (Chiapasco et al. 2006-2009-2013; Quiles et al. 2015; Vinci, et al., 2019) and in bone blocks taken from the mandibular ramus (Chiapasco et al. 2006-2009), but also to GBR procedures with resorbable and non-resorbable membranes (Hämmerle, et al., 2002; Chiapasco, et al., 2009; Esposito, et al., 2009; Milinkovic & Cordaro, et al., 2014; Cucchi, et al., 2017; Urban, et al., 2019), and with those related to implants placed in native bone (Moraschini et al., 2015).

After prosthetic loading, there were 22 implants in 10 patients and 12 implants in 9 patients affected by biological complications in project 1 and 2, respectively. Three implants and 2 implants in project 1 and 2, respectively were removed due to the presence of a continuous peri-implant radiolucency and mobility. The other developed peri-implantitis with no implant mobility. In all these latter cases, a combined non-surgical and surgical treatment protocol was delivered aiming to stop disease progression and maintain the implants.
It is worth noting that, in project 1, according to the GEE using the Generalized Linear Model for estimating the effect of main co-interventions and individual factors, the use of vestibuloplasty in association with

palatal grafts had a positive and statistically significant effect on implant failure, peri-implantitis, and bone loss (p-value 0.006, 0.006, 0.004, respectively).
Also in project 2, it appears that the use of vestibuloplasty in association with free gingival grafts taken from the palate reduced the incidence of implant failures and peri-implantitis, although the difference is not statistically significant.

In fact, as Roccuzzo et al., (2016) stated, implants that are not surrounded by a sufficient band of keratinized mucosa seem to be more prone to plaque accumulation and recession, even in patients exercising sufficient oral hygiene and receiving adequate supporting periodontal therapy. After regenerative and reconstructive techniques, due to the releasing incisions of the flaps to guarantee a primary closure, the mucogingival line is often moved coronally. As a consequence, additional vestibuloplasty in association with free gingival grafts taken from the palate may re-create a band of tick and keratinized tissue which will favor the maintenance of oral health and will allow proper oral hygiene procedures. This seems to reduce implant related complications, as also confirmed by the literature (Sukuroglu, & Baltacioglu., 2019; Roccuzzo, et al., 2016; Gobbato, et al.; 2013).

6. Patient's satisfaction inquired with a dedicated questionnaire

With respect to patients-reported outcomes recorded on the dedicated questionnaire, results can be easily comparable because the questionnaire was the same in the three projects.
On average, it appears that the treatment received was well tolerated by the majority of patients in all the three regenerative/reconstructive techniques. Patients reported a slightly higher discomfort in the early phases of healing (15 days after surgery) and in case of mandibular bone blocks (question No. 2). Postoperative pain and discomfort related to dietary/social limitations were low in all cases. The final prosthetic restorations were judged very satisfying in terms of aesthetics and chewing capability by the majority of patients, in all the three projects.
On average, results obtained by these questionnaires showed lower values in the second, third and fourth quartile in case of GBR with Ti-meshes. Moreover, the mean successful cumulative score (maximum value 50 points) was slightly lower with the use of Ti-mesh (14.9) rather than bone blocks (16.4 with calvarium and 16.5 with ramus).
Finally, 88.4% and 95.5% of patients reconstructed with calvarium and ramus respectively, and 92.5% with GBR with Ti-mesh, knowing exactly what to be expected in terms of surgical and prosthetic phases, would have undergone the same reconstructive procedure again, if needed.

Histomorphometric results of project 3
In the third study, histological features of alveolar bone tissue regenerated by means of customized CAD/CAM titanium meshes were assessed.
At the analysis resulted that tissue regenerated under the titanium mesh was composed by newly formed vital bone tissue at different stages of mineralization that integrated and remodeled the biomaterial. In the medullary spaces or in the connective tissue adjacent the remaining xenogenic particles no inflammatory reaction was observed, but many osteoblastic and osteoclastic cells were found. These morphological assessments, together with the morphometric data, seem to indicate that the proposed customized procedure induces the regeneration of bone tissue well-structured and organized, vital and active in the remodeling and integration of the biomaterial. In literature, no histologic data are available on alveolar ridge augmented by this device. Similar results are presented in a study of non-customized Ti-

mesh filled with a 1:1 ratio of autogenous bone chips and DBBM particles, on 17 patients (Proussaefs & Lozada, 2006). These Authors observed the active remodeling pattern of bone around the grafted material and an average 36.47% of regenerated bone and 14.35% of Bio-Oss particles at about 8 months of healing. These data are in line with those reported in the present study, despite the healing period was much more variable (5 to 13 months, average 8.47) than that of patients of the present study (8 to 10 months, average 9). Recently, Cucchi et al. (2019) compared histological and histomorphometric outcomes of vertical ridge augmentation achieved by means of non-resorbable membranes or resorbable membranes with Ti-mesh. At 9 months of healing, authors observed no significant differences between the two protocols. Moreover, the proportion of bony tissue (42.1%) and residual grafted material (9.6%) was comparable to that found in the current study. In a clinical trial assessing clinical and histological outcomes of horizontal maxillary ridge augmentation using native collagen membrane vs titanium mesh, Atef and Colleagues (2020) observed a significant increase in alveolar bone width in both groups.[19] In the group treated with titanium meshes, the average bone area was 27.8%, residual particles were 23.6%, and marrow spaces were 48.5%. These data are consistent with those of this study since they are representative of the previous stage of maturation that the regenerated tissue reaches at 6 months of healing.

Vascularization is a further important histological feature of the newly-formed bone that may influence the success of implant-supported rehabilitation (Wang, et al., 2006). Angiogenesis plays a key role for the success of the regenerative procedures since it supplies the higher metabolic needs of the newly formed tissue (Lakey, et al., 2000). In a clinical study, a significant higher microvascular density was found in augmented sites compared to pristine bone (Piatteelli, et al., 2002). Furthermore, after implant placement, blood supply provides the essential cellular and molecular elements for the establishment and maintenance of its osseointegration. During the first days after surgery, blood clot organizes, new vessels invade the coagulum from the pristine bone and run even in strict contact with the implant surface leading to the formation of granulation tissue (Pellegrini et al., 2018). After that, osteoid matrix, mainly composed of collagen type I, forms around these new vascular structures (angiogenetic osteogenesis), fills the defect and will be gradually mineralized. In vivo studies on a rodent model reported that the pharmacological inhibition of angiogenesis may negatively affect the osseointegration or decrease the formation of peri-implant bone (Al-Jandan, et al., 2019; Al Subaie et al., 2015; Mair et al., 2007). Despite the importance of vascularization as indicator of tissue vitality and activity, human histometric studies on GBR rarely report the number or the area of tissue occupied by vessels, but usually focus on the amount on newly formed bone, remnants biomaterial and connective tissue. To our knowledge, this is the first study that analyzes the vascularization of tissue regenerated by means of customized Ti-mesh and 1:1 mixture of autologous and bovine bone particles. Results reported that vessels represented the 4.06% of the newly formed tissue. On the contrary, human studies related on reconstructions with bone blocks, a maximum mean value of 2.38% of vessels in the regenerated tissue at 7 to 9 months of healing was observed (Hartlev, et al., 2020; Dellavia, et al., 2016). This lower value could be explained considering that, in these trials, defects were grafted with bone blocks that may oppose more resistance against the penetration of newly formed vessels than bone particles. Otherwise, data of the present study seem to confirm that the porous structure of xenograft particles favours the vessels formation through them and the high vascularization rate of the newly formed tissue (Galindo-Moreno, et al., 2014). The marrow-derived mesenchymal stem cells contained in the autogenous bone may also have contributed to the vascularization due to their capability to differentiate into vascular endothelial and/or pericyte-like cells and to induce the expression of vascular endothelial growth factors (Hass et al 2012; Saghiri, et al., 2016).

In GBR procedure, the mesh acts as a scaffold and as a stabilizer of blood clot and graft particles. This device interfaces with the surrounding bone and mucosa and its surface micro features may play a role in bone regeneration as osteoconductive material (Almazrooa, et al., 2014). In the present study, titanium meshes

appeared well integrated in newly formed bone, confirming the osteoconductive properties of this device. The response of osteogenic cells and bone tissue to titanium mesh has been studied by Hirota et al. (2016) that proposed the photofunctionalization to improve its osteoconductive capabilities. These Authors demonstrated that surface treatment of titanium mesh is able to increase “de novo” tissue formation and to enhance the bone regeneration within the defect.

Titanium mesh interfaces with the bone tissue, the above periostium and, when exposure occurs, also with the oral cavity. In the present study, Ti-mesh exposures occurred in 3 sites: in two cases as early complication, and in one case as late complication. In all these sites, a spontaneous and fast re-epithelization of the wound dehiscence was observed, without clinical signs of infection. Therefore, the exposed meshes were left on site until the implant placement and they were incorporated within the fibrous dense connective tissue with elongated fibroblastic-like cells and few scattered inflammatory cells, confirming the growth of the soft tissue in the interface between the device and the newly formed bone. No histological sign of infection appeared and morphometric data of bone tissue in the “exposed sites” are in line with those of the uneventfully sites. The spontaneous and fast re-epithelization of the wound dehiscence may have limited the infection and the reduction of initial bone gain, as reported by other authors (Sumida, et al., 2015; Sagheb, et al., 2017; Seiler, et al., 2018). In the two cases of early exposure of the present study, the quality of regenerated tissue was not affected and implants were placed according to the original plan. Due to the limited number of cases with exposures, statistical comparison has not been done. Further studies should be designed to better understand if the use of CAD/CAM customized meshes may reduce the negative effect of wound dehiscence on quality of the newly formed tissue.

In this study, a control group treated with a different regenerative procedure has not been included. To verify if histologic features of the regenerated bone are optimal, the more appropriate control group would be the spontaneous healing which however is not feasible in this size defect. Data from this study support the use of customized CAD/CAM titanium mesh for regeneration of vital, well-structured and vascularized alveolar bone. Future studies could be designed to compare this method with different surgical approaches.

9 - CONCLUSION

Despite the descriptive nature of the first two retrospective longitudinal cohort studies and the variability of the selected patients (including different patients anamnesis, defects locations, defect morphologies, and prosthetic rehabilitations), medium- to long-term results seem to confirm the efficacy of reconstruction of atrophic ridges using autogenous bone blocks taken from both the calvarium and the mandibular ramus covered by a protective layer of bovine bone mineral and stabilized by a collagen membrane. The use of autogenous calvarial grafts may be prefer in case of severely deficient edentulous ridges, when the ramus can offer an insufficient quantity of bone. The low postoperative morbidity, the stability over time of the augmented bone, the high survival rate of implants placed in a prosthetically driven way, leading to very satisfactory prosthetic restorations, confirmed the long-term reliability of these procedures.

Preliminary results of the third prospective study, on the other hand, despite the limited number of patients, implants, short follow-up, and the non-negligible incidence of Ti-mesh exposures, seem to demonstrate that CAD-CAM customized Ti-meshes may represent a reliable GBR option for the correction severely atrophic edentulous ridges in terms of vertical bone gain, limited peri-implant bone resorption and survival rate of implants. It is however worth noting that if on one hand it is possible to simplify the reconstructive procedure thanks to the customization of Ti-meshes, on the other hand surgeons have to face the non-negligible incidence of Ti-mesh exposures and the higher complexity of their removal at the time of implant placement. Therefore, studies involving a higher sample of patients and with longer follow-ups are necessary.

PROMs seem to validate the use of all the regenerative and reconstructive procedures, with high value of patient' satisfaction.

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