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Implant-supported restorations following horizontal ridge reconstructions by means of autologous or homologous bone block grafts: 5 - year clinical results of a multi centric RCT.

ovvero

Riabilitazioni implanto-protesiche in seguito a ricostruzione alveolare orizzontale per mezzo di innesti di osso autologo o omologo: risultati clinici di uno studio multicentrico randomizzato e controllato dopo 5 anni di follow-up

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…in verità vi dico,
che se il chicco di grano caduto nella terra non muore,
allora resterà solo.
Ma se muore,
allora porterà gran frutto…

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Abstract

Nowadays the use of autologous bone block as grafting material is considered the gold standard in case of horizontal ridge augmentation. Nevertheless, autologous bone grafts are also associated with some pitfalls which are mainly relative to the necessity for a second surgical site. In an effort to overcome these limits, it seems clear the necessity of alternative graft materials. In this regard, homologous bone seems to provide a reasonable source for grafting material without the need for a second surgical area. Unlimited availability and reduced surgical time are additional benefits which make this material a plausible clinical alternative. In every case, the validity of homologous bone as feasible alternative to autologous bone is still a matter of discussion in the literature. In this regard, there is a paucity of evidence for what concerns both the biological and clinical behavior of homologous bone grafts, since the major part of the studies are case reports and case series. Above all, data are still lacking as regards clinical outcomes over a follow-up of medium-long term. The aim of this study was to evaluate the clinical performance of fresh-frozen bone allografts (FFB) derived from tibial hemiplateau of cadaver donors when compared to intraoral autologous bone grafts (AB) for the treatment of maxillary horizontal atrophies (Cawood and Howell IV) after 5 years of follow-up. In order to reach this goal, a series of implant-related biometric parameters were recorded during follow-up program relative to each patient. Twenty-one out of 24 originally treated patients were considered as three patients dropped out because they refused to participate to the entire follow-up period. Two of them belonged to the control group whereas 1 belonged to the test group. Initially all patients were examined monthly for the first sixth months after provisional prostheses were delivered. From that moment on, the follow-up program continued with visits every 6 months in subsequent years. The following biometric parameters were evaluated and recorded at each recall appointment: peri-implant probing pocket depth

(PPD), bleeding on probing (BOP), amount of keratinized tissue (KT) and peri-implant mucosal recession (PMR) at the mid-buccal side. At the end, a total of 72 implants were taken into consideration for the statistical analysis. Forty were relative to the test group and 32 to the control group. Unexpectedly, at the time of implant exposure 16 peri-implant defects > 3mm were revealed in the FFB group. The management of these complications accounted for a surgical treatment by means of guided bone regeneration (GBR). Since it was assumed that the presence of biomaterials (e.g. DBBM, collagen) would have influenced the medium-long term data upon implant-related biometric parameters, a third group relative to homologous bone which needed regrafting (FBR) was introduced for further statistical analyses. Otherwise, the cumulative implant survival rate after 5 years of follow-up was 82% in the FBR group, 96% in the FFB group and 100% in the AB group. When considering the original homologous group (FFB+FBR) the survival rate was 90%. Every implant failure was encountered before the provisional loading. Regarding implant-related biometric parameters, no statistically significant differences were found as regards bleeding on probing, peri-implant mucosal recession and keratinized tissue. Instead, probing pocket depth was significantly lower in the FBR group. However, the interpretation of this finding remains unclear to date.

On the basis of the reported data and within the limits of this study, it can be concluded that fresh-frozen bone allografts should not be considered a feasible alternative when compared to intraoral autologous bone grafts after 5 years of follow-up. The higher resorption rate, necessity for regrafting and implant failures are shortcomings that might prevent their application for alveolar ridge augmentation in the next future. Further studies are warranted to confirm these results and the reliability of FFB block allografts in horizontal alveolar ridge reconstruction.

Riassunto

Le atrofie ossee alveolari, localizzate o generalizzate, possono essere secondarie a molteplici cause quali estrazioni dentarie, malattia parodontale o traumi. Tali condizioni possono esitare in un volume osseo insufficiente al fine di un inserimento implantare protesicamente guidato. In caso di ricostruzione alveolare orizzontale, l'utilizzo di osso autologo in blocchi è tutt'oggi considerato il gold standard. Questa tecnica presenta però molti limiti tra i quali: la morbidità di un secondo sito chirurgico e la scarsa quantità di osso prelevabile. Per tali motivi, la ricerca di materiali alternativi nel campo della rigenerativa ossea risulta essere di massima importanza. A tal proposito, nell'ultima decade è stato introdotto l'utilizzo di osso omologo da donatore non vivente per le procedure odontoiatriche. Tale biomateriale è stato ampiamente studiato e vanta una vasto utilizzo in chirurgia ortopedica e ricostruttiva.E' importante premettere che le procedure di incremento osseo pre-implantare mediante osso omologo sono supportate da evidenza scientifica molto limitata, essendo gli studi in merito prevalentemente case report e case series che non forniscono informazioni esaurienti sull'efficacia a medio/lungo termine. La presente tesi rappresenta il proseguimento di un protocollo clinico randomizzato e controllato che ha valutato le caratteristiche istologiche, istomorfometriche e radiologiche, e mira a valutare l'efficacia clinica di innesti in blocchi di osso omologo fresco e congelato (FFB) per la ricostruzione ossea orizzontale del mascellare superiore dopo un periodo di osservazione di 5 anni. Il gruppo di controllo (AB) fa riferimento a pazienti trattati con innesti in blocchi di osso autologo prelevati intraoralmente. Gli innesti di osso omologo, prelevati dall'emipiatto tibiale di donatori non viventi, sono stati forniti dalla Banca del tessuto muscolo-scheletrico (IOR, Bologna, Italy). Entrambi i gruppi erano costituiti da 12 pazienti i quali sono stati trattati tra Maggio 2008 ed Agosto 2009. In seguito alle procedure di esposizione della testa implantare e carico protesico, i pazienti sono stati visitati mensilmente per i primi sei mesi e con cadenza semestrale da allora in avanti. L'indagine ha previsto la rilevazione di parametri biometrici relativi allo stato di salute dei tessuti peri-implantari quali la profondità di sondaggio, il sanguinamento al sondaggio, la quantità di tessuto cheratinizzato e la presenza di recessioni della mucosa peri-implantare. Vengono inoltre riportati dati relativi alla necessità di reinnesto al momento della scopertura implantare ed al tasso di sopravvivenza dopo 5 anni di follow-up. Tre dei 24 pazienti inizialmente arruolati nello studio, 2

relativi al gruppo di controllo ed 1 al gruppo test, non hanno completato l'intero programma di osservazione clinica. In ultima istanza sono stati considerati 72 degli impianti inseriti, 40 appartenenti al gruppo test e 32 al gruppo di controllo. Dopo essere stati raccolti dalle diverse unità operative, i dati sono stati organizzati per l'analisi statistica. Il risultato maggiormente sorprendente di questo studio riguarda la necessità di reinnesto. Sedici dei 40 impianti nel gruppo test, al momento della esposizione implantare, ha presentato deiscenze ossee > 3 mm necessitando reinnesti. Al contrario nessuno degli impianti nel gruppo controllo è stato trattato con tale procedura. L'introduzione di una nuova variabile e la sua possibile influenza sui parametri clinici di interesse, ha portato a condurre l'analisi successiva considerando un terzo gruppo relativo ad osso omologo reinnestato (FBR). Il tasso di sopravvivenza implantare dopo 5 anni di follow-up è risultato essere 82% nel gruppo FBR, 96% nel gruppo FFB, 100% nel gruppo AB. Considerando l'originale gruppo trattato con osso omologo (FFB+FBR) la sopravvivenza è risultata del 90%. Gli impianti inseriti in osso omologo hanno dunque mostrato una sopravvivenza inferiore in modo statisticamente significativo. L'analisi dei parametri clinici non ha mostrato differenze rilevanti fatta eccezione per la profondità di sondaggio peri-implantare che è risultata inferiore nel gruppo FBR in modo statisticamente significativo. L'interpretazione di tale dato rimane poco chiara anche se è possibile ipotizzare che i limiti intrinseci del sondaggio peri-implantare quale test diagnostico possano aver influenzato tale risultato. E' interessante comunque notare come il riassorbimento degli innesti, dopo 6 mesi di guarigione, sia stato significativamente superiore nel gruppo test rispetto al controllo (FFB 52 % vs AB 25%; Lumetti et al. 2014). Tale processo di riassorbimento è proseguito nel tempo tanto da esitare in un elevato numero di difetti peri-implantari > 3mm dopo 12 mesi dal posizionamento degli innesti, che ha poi richiesto ulteriori procedure chirurgiche. Sulla base dei dati ottenuti dalla seguente indagine è possibile concludere che l'osso omologo fresco e congelato non dovrebbe essere considerato un' alternativa comparabile all'osso autologo in caso di ricostruzione alveolare orizzontale del mascellare superiore. Ulteriori studi di qualità metodologica adeguata sono comunque necessari in futuro al fine di confermare tali risultati.

Introduction

Bone is a specialized tissue of the human body which is relevant for its hardness, rigidity, and biological potential for regeneration and repair. Its main functions are the protection of the vital organs and bone marrow, but it also explicate some metabolic tasks acting as mineral reservoir for calcium homeostasis and growth factors and taking part in acid–base balance (Taichman 2005). On the basis of the origin of the cellular lineage, three different skeletons are usually described:

1. axial skeleton
2. limb skeleton
3. cranio-facial skeleton

The axial skeleton is generated by the somites, the limb skeleton is derived from the lateral plate mesoderm whereas the cranial neural crest yields to the branchial arch and craniofacial bones and cartilage. These structures have different pattern of ossification (or osteogenesis) that is the process of formation of new bone. It occurs in two different ways: endochondral ossification and intramembranous ossification. The long bones, skull base, vertebral column and pelvis tipically undergo endochondral ossification. In the first phases, this kind of ossification is characterized by a cartilage template. Soon after its formation, chondrocytes become hypertrophic thus leading to matrix erosion. The residual cartilage matrix mineralizes while the chondrocytes progressively deteriorate until apoptosis. At this point the calcified cartilage model is penetrated by newly-formed blood vessels which are able to introduce mesenchymal stem cells. These cells may differentiate into osteoblasts so that bone formation can be started. Instead, in intramembranous ossification bone is synthesized without such previous cartilage phase (Shapiro et al. 2008). This ossification pattern is a peculiarity of flat bones of the skull, the clavicle and the mandible (excepting the condyle). At the beginning, mesenchymal cells condense into a connective tissue layer

which is highly vascularized. Molecular signals lead to the differentiation of primitive mesenchymal cells into osteoblasts which start producing a trabecular pattern of early bone matrix. At a later stage, bone matrix undergo a maturation process by way of synthesis and secretion mediated by the viable cellular component. Hence, hydroxy-apatite (HA) crystals are deposited at the bone matrix site. One after the other, bone trabeculae are formed in an intricate network which is called woven bone. Finally, woven bone is replaced by lamellar bone which is a well-organized pattern of anatomical units called osteons. These basic structures are closely interconnected by Haversian and Wolkmann's canals. During life the bone is subjected to a large number of micro fractures. For this reason it requires to be renewed continuously and replaced by new bone. Altogether, the amount of newly-formed bone under normal conditions is the same as the amount of bone resorbed, thus the total bone mass remains almost unchanged.

By and large, it has been stated that embryological bone derivation seems to be a key factor regarding the osteogenic potential of the periosteal surfaces. Likewise, clinical studies upon the field of cranio-maxillofacial reconstruction performed on either animals or humans, prompt that intramembranous grafts are more resistant as far as resorption is concerned when compared to endochondral ones. Therefore, it has been stated that they are favored for harvesting when it is possible (Zins e Whitaker 1983). In this scenario the alveolar process may be considered a distinct entity of the cranio-facial skeleton. It is defined as the bone tissue that surrounds a fully erupted tooth and is formed in harmony with the development and eruption of the teeth. In fact, in a condition called "anodontia" the development of the alveolar process is impossible to occur properly because it must form in response to the tooth germs in the area. This phenomenon is supposed to be genetically determined. Noteworthy, it exhibits quite different histological peculiarities and remodeling patterns in respect to the basal bone. Alveolar bone, teeth and periodontal ligament are anatomically and physiologically connected. The alveolar bone continuously

undergo renewing and remodeling pursuant to functional needs such as mastication or other tooth contacts. Consequently the “dento-alveolar” complex can be view as a biological unit. The morphologic characteristics of the alveolar process are related to: size and shape of the tooth; site of tooth eruption; and inclination of the erupted tooth. Likewise, although some features concerning the bone tissues are strongly genetically predisposed there are a large number of epigenetic factors which may influence alveolar bone formation, remodeling and reparative mechanisms. This has been indicated as the explanation for bone resorption following teeth-loss. It is worth noting that no other skeletal structures shows similar biological behavior. How it could be easily assumed, one of the most important epigenetic factor that is closely related to both teeth and bone is the mechanical load. The relationships between mechanical usage and bone metabolism are well known since more than one century, when Wolff described in 1892 how bone modeling during growth was determined by local strains, to evolute towards the most adapted structure to resist mechanical stress. Consequently, mechanical loads and masticatory forces applied by the teeth stimulate the alveolar bone remodeling and contributes in maintaining bone trophism whilst disuse causes bone loss. To take these observations into account, Frost proposed a model to explain bone adaptation under mechanical loads which has been named Frost’s mechanostat theory (Frost 1989) . It consists of 4 different levels:

1) Pathologic unload zone: If no forces are applied on the bone it starts losing mineralized tissue in a process called atrofia ex non usu
2) Adaptation zone: A proper mechanical stimulation acts on the bone and allows its maintenance
3) Overload zone: If strains overcome the adaptation zone, bone tissue reacts by bone apposition to neutralize external stimuli
4) Pathologic overload zone: If mechanical forces exceeds tolerance range osteoblasts

are inhibited. As a result osteoclast activity is more pronounced and bone resorption prevail on bone apposition. Otherwise, if the elastic limit is overtaken, bone fractures may occur.

Incidentally, almost all physiological and pathological processes affecting teeth may, in one way or another, produce any effect upon alveolar bone structure. (Wolff 1892; Frost 2003; Frost 2004).

In line with this finding, the loss of one or more teeth in the alveolar ridge provokes marked contraction in both vertical and horizontal directions (Atwood 1973; Atwood 2001; Reich, Huber et al. 2010). Nevertheless prior to take into account the implant therapy in the true sense of the word, it is pivotal to know which phoenomena do occur after teeth avulsion.

Alveolar ridge alterations occuring after teeth extraction can be schematically divided into two series, precisely intra-alveolar and extra-alveolar processes (Lindhe 2008)

1) INTRA-ALVEOLAR PROCESSES

Socket healing following teeth extraction has been studied by Amler 1969 who took samples from volunteer patients. It represents the first research upon biological events occurring at healing socket. Here the process is described in its different phases:

A) Presence of bleeding and formation of a blood clot immediately after tooth extraction. Blood vessels are closed by thrombi and a fibrin network is formed. B) Already 48-72 hours later, neutrophil granulocytes, monocytes and fibroblasts begin to migrate along the fibrin network.C) After 96 hours the blood clot is slowly replaced by granulation tissue.D) Granulation tissue forms predominantly in the apical third of the alveolus. Increased density of fibroblasts may be seen at this stage. After 4 days, the contraction of the clot and the initial proliferation of the oral epithelium take place. After 7 days, osteoclasts are visible at the margins of the alveolus while osteoblasts and osteoids seem to appear at the bottom of the alveolus. E) Reorganization of the granulation tissue through formation of

osteoid trabeculae. The top of the young connective tissue is covered by the epithelial proliferation from the wound margins. Next, the formation of osteoid trabeculae is evident from the wall of the alveolus in a coronal direction. After 3 weeks some of the trabeculae start to mineralize.F) Radiographically, bone formation may be visible. The soft tissue wound is completely epithelialized after 6 weeks. However, bone fill in the alveolus takes up to 4 months and does not seem to reach the level of the neighboring teeth. Unfortunately, solely events occurring at the edge of the healing socket are taken into account by this study. Furthermore, data are lacking upon the late phase of healing, when modeling and remodeling take place. Results from a longer-term study which has been performed recently seem to be more useful in such a scenario (Cardaropoli et. al 2003). The experiment consisted of morphometric measurements to determine the volume occupied by different types of tissues in the marginal, central and apical compartments of the extraction socket at different intervals. Nine mongrel dogs had their fourth mandibular premolars divided into one mesial and one distal portion. The distal root was removed and the socket with its surrounding soft and mineralized tissue was denoted "experimental unit". The dogs were killed 1, 3, 7, 14, 30, 60, 90, 120 and 180 days after the root extractions. To summarize, through mesio-distal sections authors reached these conclusions:

- The socket is filled by spongy bone by one month after tooth-extraction
- Cortical bone made by both woven and lamellar bone is formed within 3 months
- Spongy bone start to be replaced by lamellar and midollar bone within the third month

2) EXTRA-ALVEOLAR PROCESSES

One more histological and histometric study was conducted using mongrel dogs as animal model (Araujo e Lindhe 2005). After a minute full-thickness flap was elevated at buccal and lingual side, distal root of 3rd and 4rd premolars in both quadrants of the mandible were carefully removed using elevators and forceps. At the same time mesial root was endodontically treated and filled with guttapercha. In the end, gingival tissues were mobilized and sutured to cover the extraction sites. The animals were sacrificed after 1,2,4 and 8 weeks of healing. Sections representing the central portion (in mesial– distal direction) of each extraction socket were stained with haematoxyline-eosine and examined in the microscope. The purpose was to study dimensional alterations of the alveolar ridge that occurred following tooth extraction as well as processes of bone modeling and remodeling associated with such change. Based on data presented, the authors claimed that the resorption of the buccal/lingual walls of the extraction site occurred in two overlapping phases. During phase 1 the bundle bone, having lost its function, was resorbed and replaced with woven bone. Since the crest of the buccal bone wall was comprised solely of bundle bone this modeling resulted in a substantial vertical reduction of the buccal crest. On the other hand phase 2 is characterized by resorption of both lingual and buccal walls but it appeared much more pronounced in the latter one. In this regard, since the lingual wall was used as reference for buccal wall alterations, measurements are most likely underestimated because of the fact that it undergoes changes as well. Phase 2 bone loss is not completely understood. There seem to play a role:

- Elevation of the flap (as above-mentioned)(Fickl et al. 2008).

- Bone thickness: buccal bone plate is considerably thinner (or even partially absent) than the lingual plate. Januario et al. 2011 analyzed 250 subjects through CBCT measurements and stated that none patient had buccal bone thickness >1 mm and that close to 50% of sites had a bone plate thickness that was ≤0.5 mm. It is pivotal to

consider that the thinner is the buccal plate, the greater is the relative percentage of bundle bone.

- Adaptation to continued lack of function at the extraction site

- Tissue adjustments in which ridge geometry could be determined by genetic factors after tooth loss.

Noteworthy, authors stated that the relative reduction of the height of the buccal bone wall between the 1- and 8-week intervals was about 45 micron/day. This bone resorption rate is similar to that reported at fractures sites in the long bones of dogs that is 50/60 micron/day. Nonetheless these processes are not fully comparable because it must be acknowledged that the remodeling process in dogs is much quicker than in humans by three to five times (National Research Council 1980). Moreover, the regeneration of the bone external profile may be altered by deficiencies in inter-cellular signals, especially in those cases where some delimiting walls of the defect are missing (i.e. as a consequence of alveolar bone fracture during tooth extraction). From a macroscopic point of view it has been reported that alveolar bone at the extraction site undergoes a 40% to 60% reduction in height and width within the first 6 months following the extraction (Ashman 2000). Other authors stated that the major part of the alterations take place during the first 3 months of healing and that usually the vestibular side of the alveolar bone is more interested in resorption than the lingual/palatine side (Johnson 1969; Pietrokovsky and Massler 1967; Schropp et al. 2003). The rate of reduction tends to decrease after the first year and proceeds at an average of 0.5% to 1% per year so that it may be considered as a life-long process (Sennerby, Carlsson et al. 1988). These modifications have been attributed to disuse atrophy, decreased blood supply and localized inflammation. However it is now apparent that alveolar bone resorption is a complex process involving structural, functional and physiologic components. As instance, flap elevation for teeth extractions

might greatly influence this process by disrupting blood supply to the bony walls and by reducing the ability of the periosteum to provide osteogenic cells (Melcher 1969, 1971, 1976; Bragger et al. 1988). In addition, post-extraction wound healing is strictly dependent on molecular and cellular events which must occur appropriately. Therefore, it seems logical assuming that the final healing outcome after tooth extraction might be influenced by factors that affect such events (Bartee 2001). Furthermore, a variety of factors might play a role such as systemic factors including the patient's general health, the gender and the habits (e.g. smoking). Local factors include the reasons for extraction, the number and proximity of the teeth to be extracted, the conditions of the socket before and after tooth extraction, the influence of tissue biotype on healing, local differences between sites in the mouth and the type of interim prosthesis used (Chen et al. 2004). Morever several authors have been claimed that bone defects presented more often in the posterior than in the anterior segment of both jaws. This may be due to the fact that usually posterior teeth are lost at an earlier age than anterior teeth. However, it has been widely reported that alveolar bone resorption between maxilla and mandible is rather different since the maxilla follow a pattern of resorption defined as centripetal whilst the mandible is characterized by a centrifugal pattern. In both cases intermaxillary relation is strongly impaired as these anatomical discrepancies may compromise functional and structural aspects of a prosthetic rehabilitaton. Infact, localized or generalized bone defects of the alveolar ridge might prevent implant placement in a prosthodontically driven position which is considered as essential from a functional point of view. Last but not least, one has to bear in mind that bone resorption may deeply jeopardize the esthetic outcome of the final rehabilitation. In order to simplify the arrangement of individual treatment plans, the use of classification systems has become of the utmost utility. Indeed, it has been stated that the changes of the alveolar process generally follow a predictable pattern. In order to categorize bone resorption at different

stages, many classification systems of the edentulous jaws have been developed over the years. Such classifications are useful since they simplify the description of the residual ridge and give a “common language” for clinicians during case discussions. Moreover classifications help select the surgical-prosthetic treatment and can be used for comparisons with respect to baseline.

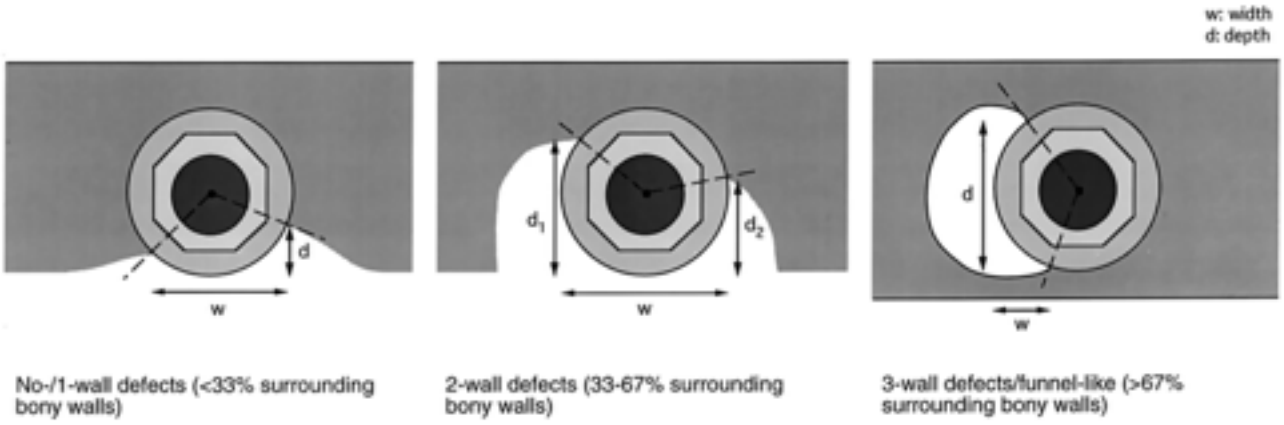
First of all it has to be cleared that rebuilding the alveolar ridge, when necessary, can be realized at different time points during treatment and is commonly classified as simultaneous or staged.

- Simultaneous or one stage approach consists in bone grafting at the same time of implant placement

- Staged or even two stage approach consists otherwise in alveolar bone reconstruction prior to implant placement, which takes place 2 to 6 months later (von Arx & Buser 2006).

As it reduces treatment time, costs and consists exclusively of one surgery, simultaneous approach is clearly the preferred choice by the patient and the clinician alike. Howbeit when the residual bone is too insufficient and primary implant stability cannot be obtained or precludes an adequate prosthodontically-driven implant positioning, the staged approach represents the best choice. There have been proposed three different classifications for horizontal defects which have to be treated by one stage approach (Zitzmann et. al 1999; Tinti & Parma-Benfenati 2003; Hammerle & Jung 2008). The simultaneous approach was taken into consideration in this study at the time of implant placement (6 months after bone grafting surgery) to determine wheter or not regrafting was necessary. In every case, since all dehiscence type defects were evaluated as dichotomous variable (yes/no) when at least 3 or more millimeters of the implant body

were not covered by bone, irrespective of the number of residual bony walls, the Zitzmann classification is presented here just as example:



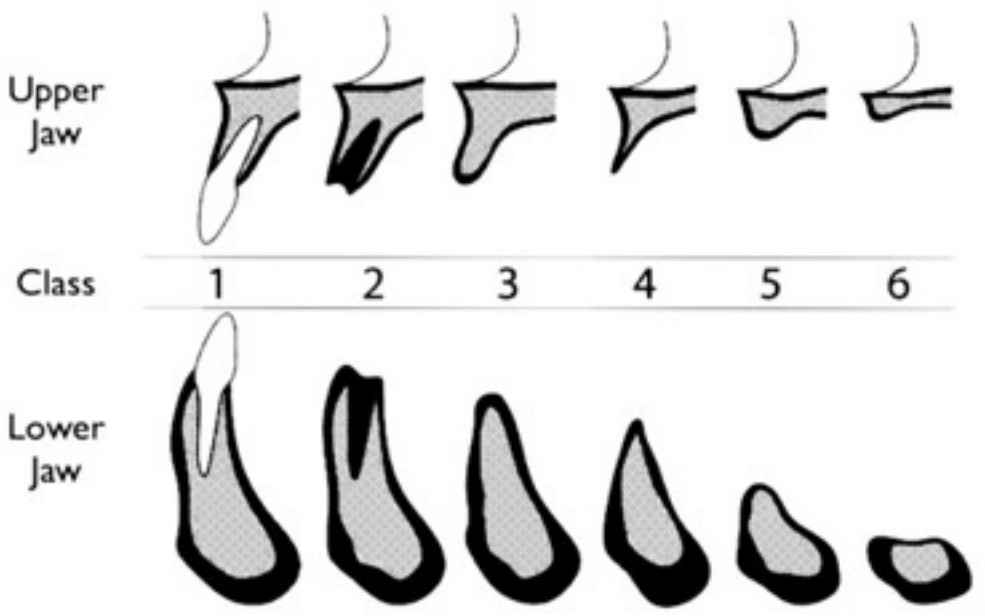
It is divided in 3 classes:

Class 1: one wall defect

Class 2: two walls defect

Class 3: three walls defect (Zitzmann et. al 1999)

Cawood and Howell in 1988 proposed a classification system which became famous over the years and is still adopted by many clinicians all over the world.The authors investigated the remodeling pattern of anterior and posterior segments of both mandible and maxilla and considered the residual width and height of the ridge as main criteria to develop their classification. Results were extrapolated from randomised cross-sectional study from a sample of 300 dried skulls. At the end, six principal types of edentulous jaws were identified and classified following a temporal and ingravescent order:



Cawood and Howell classification of the edentulous jaws

1) Dentate

2) Immediately after extraction

3) Well-rounded ridge form, adequate in height and width

4) Knife-edge form, adequate in height but inadequate in width

5) Flat ridge form, inadequate in height and width

6) Depressed ridge form, with some basal loss evidence (Cawood and Howell 1988)

Fenlon et al. in 1999 found that this classification had showed high rates of agreement between both clinicians and researchers. The authors concluded that it may be used as a good diagnostic and research tool. The present study encompasses patients with maxillary partial edentulism. Since the behavior of two different bone grafts in horizontal ridge augmentation was investigated, the inclusion criteria accounted for patients with alveolar ridges lacking in width which, in turn, are classified as Cawood and Howell class IV atrophies.

As a matter of fact, different bone atrophies require different prosthetic approaches. At the same time, once evaluated the entity of the alveolar atrophy, careful and individual based therapy should be planned before starting the treatment. Nowadays, the rehabilitation of partially or totally edentulous patients by means of oral implants and implant-supported prostheses is a routine and well-documented treatment option (Lambert et al. 2009). Besides, several randomised controlled clinical trials with long-term follow-up confirmed that implant-supported complete prostheses either fixed or removable are more advantageous when compared to conventional dentures (Boerrigter et al. 1995; Meijer et. al 2003). These benefits include a decreased bone-resorption rate, enhanced prosthetic retention and stability, improved masticatory efficacy and chewing ability, decreased soft-tissue trauma (giving the patient less decubitus problems) and reduced hindrance for tongue and other muscles during oral functions (Carlsson 2004). Since accurate three-dimensional implant positioning represents one of the key factors to reach biological, functional and aesthetic success (Grunder et. al 2005), an adequate bone quantity and quality are paramount requirements in view of placing implants. In this regard there is a general consensus in implant dentistry that the presence of a bone width of at least 1-1.5 mm is required on both the buccal and the lingual/palatal sides of the implant to achieve a predictable treatment outcome (Baffone et al. 2013). Likewise, also mesio-distal and apico-coronal dimensions need to be carefully considered (Buser e von Arx 2000; Kan et al. 2003; Piattelli et al. 2003). On the whole, it is widely recognized that alveolar bone volume should be commisurate to dental implants which in turn have to be chosen in accordance with the prosthetic demands. An adequate quantity of bone is also paramount in order to obtain primary stability and predictable survival rates of the implants. Anyhow, in patients with atrophy of high degree of the alveolar ridge, especially in the maxilla, implant treatment is more dangerous and sometimes impossible to be carried out. In an effort to overcome the anatomical difficulties, different bone-grafting

procedures to the maxilla have been introduced. The ultimate goal for these pre-prosthetic surgeries is to correct and to normalise the vertical and horizontal relations of the jaws and thereby create adequate jaw bone volumes and bone quality. In the matter in question, bone augmentation techniques are essentially used for extraction socket defect grafting, horizontal ridge augmentation, vertical ridge augmentation and sinus augmentation. The present RCT aimed at evaluating medium-long term results of horizontal ridge reconstructions by means of autologous or homologous bone block grafts. Thus, a brief overview upon these grafting materials and their clinical outcomes is presented as follows.

Autologous bone grafts

At present date, autologous bone grafts are still considered the gold standard since they lack of immunologic rejection and are provided with stem cells and growth factors. It has been widely claimed that it is unique among the plethora of biomaterials since it is characterised by ostoconductive, osteoinductive and osteogenetic properties. As the major challenges of alveolar bone reconstruction through autologous bone grafts are the incorporation in the recipient site and the resulting bone volume, a thorough understanding of the healing process of the grafted bone is of pivotal importance. First of all, it has to be pointed out that there are some differences in biological events during the healing of cortical vs cancellous grafts. In fact, because of its porous nature cancellous bone is revascularized more rapidly than cortical bone thus resulting in a better incorporation and, in some circumstances, a total replacement. Some studies reported that new bone formation on transplanted trabeculae occurs shortly before resorption activities. As a whole, it is worth noting that cancellous bone tends to repair entirely with time whereas cortical grafts persist as admixtures of necrotic and viable bone (Goldberg et al. 1993). Anyway, it is supposed that the initial phases of incorporation are the same for both cancellous and cortical grafts. At the beginning, blod clot forms around the graft. Subsequently, the graft's cellular component undergo necrosis and stimulates an inflammatory response which give rise to a fibrovascular stroma. This temporary matrix conveys blood vessels and osteogenic precursor from the recipient bed to the transplanted graft which in turn provides with space keeping, osteoconduction and osteoinduction. In the last phase remodeling takes places, therefore the bone morphogenetic units progressively replace the graft in a process called creeping substitution. Although lots of biomaterials can be used, it reimains one of the most popular material for pre-prosthetic

augmentation procedures. This fact is mainly justified by quality, quantity and high predictability of uneventful healing at the recipient sites. Either way autologous bone harvesting, to a variable extent, is obviously associated with donor site morbidity. Morbity, in turn, is a major concern for patients who appreciate the maximum reduction of annoyances when implants have to be placed. A first distinction among autografts may be done as regards the donor site. They can be retrieved from intraoral or extraoral sites. These grafts mainly differ from bone quantity and quality apart from specific pros and cons relative to each one. The decision-making relative to the donor site is dependent on the size of the alveolar bone defect to be treated. Small defects may be augmented by intraoral bone from the maxillary tuberosity, mandibular symphysis, retromolar, and ramus area. Larger defects necessitate bone harvesting from extraoral sites, for example, the rib, tibia, calvarium, fibula, and iliac crest; the latter three are the most used pertaining to this group. The harvesting from intraoral donor sites in characterised by a higher patient acceptance (Cordaro et al. 2011). It has been reported that chin grafts supply with sufficient volume of cortico-cancellous bone (around 10 ml, the same quantity as mandibular ramus) but it is associated with greater morbidity, mainly represented by neural disturbances (as paresthesia or hypersensitivity/necrosis of the inferior teeth), ptosis of the chin and lip incompetence (which may occur as a result of complete degloving). On the other hand, the mandibular ramus offers mostly cortical bone and is associated with less postoperative morbidity. Temporary and permanent postoperative sensory disturbances of the skin and mucosa are significantly higher in symphyseal grafts with respect to ramus grafts (Nkenke et al. 2014). Instead, the most common extraoral donor site is the iliac crest which provides a great amount of cortical, cortico-cancellous and cancellous bone. Major causes of postoperative complications include gait disturbances, hematomas, seromas, infection, chronic donor-site pain, lateral femoral cutaneous nerve injury, sensory disturbances, etc… even if the general morbidity is

described to be moderate to low (Boven et al. 2014). It has to be distinguished between harvesting from anterior and posterior region of the iliac crest. In fact, when using posterior iliac crest as donor site, fewer complications have been described even though a longer operation time is often necessary. In regard to anterior iliac crest, Dimitriou et al. in 2011 reported a complication rate which amounted to 16.73%. Another interesting source of bone for harvesting is the calvaria which is a reliable source of cortical bone for both horizontal and vertical ridge augmentation (Monje et al. 2014). These grafts can be used in a single or a multilayer technique (as istance the “double-barrel technique), on account of the quantity of bone required. Although donor-site morbidity after calvarial harvesting is considered to be low as the most common complications are pain, edema, epidural hematoma, infection, hemorrhage, vertigo, and alopecia, several authors reported more serious complications such as: meningitis, entry into the sagittal sinus, coup/contrecoup lesions, dural exposure and tear. Provided that these complications tend to occur very seldom they have to be carefully considered as they might endanger the patient’s life. In 2014 Nkenke et al. performed a systematic review of the literature in order to delineate a “state of the art” regarding autologous bone grafts. Their analysis was carried out following major key points which are discussed below:

I. *Patient’s acceptance of bone harvesting:* The least accepted donor site is the chin which was considered inferior to both ramus and iliac crest bone grafts. It is explained by major concerns relative to aesthetic changes that arise when chin bone harvesting is planned. In reference to the patients’ acceptance of calvarial and tibial grafts, no conclusive data are available in literature.

II. *Characteristics of bone graft harvesting:* One of the main differences between intraoral and extraoral donor sites is that the first ones can be performed under local anaesthesia thus leading to fewer risks than harvesting from distant donor sites. Moreover, they can be realized on an outpatients basis differently from extraoral sites

which often imply hospitalization and increased costs. Briefly, according to the data presented in the literature, mandibular ramus harvesting should be preferred by expert surgeons whereas when extraoral bone is needed it should be retrieved through trephine burs in order to simplify the surgery and reduce the patients' morbidity.

III. *Bone graft volume and density:* The highest bone density is provided by chin grafts which shows this property whether at graft positioning or after the completion of the healing. Since this site offers a modest quantity of bone, extraoral donor sites are preferred for vast defects.

IV. *Donor site morbidity*: Morbidity is of primary interest when assessing a specific donor site in elective pre-prosthetic surgery. As above mentioned ramus is considered superior to chin since it is characterized by a lower percentage of superficial skin sensitivity disorders and altered sensations in the mandibular incisors. Regarding extraoral donor site, there is a paucity of evidence in literature for morbidity of tibial and calvarial grafts. By contrast it has been extensively documented that iliac crest is well accepted by patients.

V. *Graft resorption:* As mentioned above, there seems to be a tendency for a lower resorption rate of membranous grafts than endochondral grafts. Worthy of note, it has been reported that these differences progressively decrease with an increasing follow-up interval (Carinci et al. 2005). It was found that interpositional grafts lead to more predictable outcome than onlay grafts. Either way, there are not differences in final outcome and graft resorption after implants are placed. However, Chiapasco et al. in their review (2006) found that horizontal bone resorption ranged from 10 to 50%. Moreover, analyzing only iliac crest grafts they found a resorption rate ranging from 12 to 60% after 1-5 years of follow-up.

VI. *Implant survival and success:* There seems to be no differences in implant survival and success rates among different donor sites. The global survival rates vary between 90% and 100% even if particulated bone from chin or iliac crest showed a lower percentage in a few cases. These results are comparable to those reported for implants placed in pristine bone. Noteworthy even in cases of extensive bone resorption high implant survival rates may be obtained (Sbordone et al. 2009). It has to be pointed out that when considering implant survival and success rate at augmented site, the implant type might play a significant role which has not been completely investigated so far. To this purpose Lambert et al. in 2009 reported statistically significant results regarding higher survival rates of rough implants versus machined implants over a long follow-up period.

In light of what it has been written, two considerations have to be made:

1. The osteogenic potential of autologous bone grafts is still a matter of debate. In fact, the major part of osteocytes which are harvested undergo necrosis at recipient site. Following their behavior over time, it has been observed that autologous grafts are characterized by non viable bone which is progressively substituded by viable bone in a healing time not inferior to 7 months.

2. Many studies and systematic reviews have pointed out that there is not a clear evidence which shows the superiority of Autologous bone as the best bone grafting biomaterials. Al Nawas et al. (2014) in a recent meta-analysis reported that there is no statistically significant difference among Bone Substitute Biomaterials (BSM), BSM mixed with AB and AB alone in horizontal ridge augmentation. However, this statement is refferred to small defects since for larger defects solely AB has been sufficiently investigated.

These considerations highlight that the role of AB as gold standard in the field of bone regeneration might be revised in the next future.

Fresh-frozen bone allografts

Analysis of the literature regarding the use of FFB shows that its application in oral and maxillofacial surgery began in 1992 with Perrot. Since then, many studies have been carried out with the purpose to demonstrate the reliability of allografts to assist in bone regeneration. Anyway, this techniques had a real development and increased interest only in the last decade. The primary reason for which attention has been focused on allograft is the necessity to overcome some pittfails related to autogenous grafts. In fact, homologous bone is free from many drawbacks and furnishes some advantages, such as:

- No need for second surgical area (donor site), thus resulting in a much lower risk of comorbidities (bleeding, infections, post-surgical pain, etc…)
- Time of surgery is noticeably shortened so that the entire procedure is easier from a clinician viewpoint. For the same reason it is much better accepted by the patients.
- There are far less concerning about bone availability. As it is harvested from cadavers there are not limitations for preserving the donor sites, therefore its amount is virtually unlimited.
- On the whole, costs are lower compared to both bone substitutes, bone-derivated materials and autografts. As far as the latter is concerned, if the graft site is extraoral, general anesthesia is often recommended, which then increases both the risks for the patient and the costs for the surgery.

Otherwise, major drawbacks which have hindered its vast application are the risks for infections, rejection (mainly due to its assumed antigenicity) and limited literature support in terms of inadequate patient populations and follow-ups as compared to autologous grafts. At present day, indeed, the risk of bone resorption before or after implant placement/loading remains unclear. Despite that, within the family of allograft, FFB is the least processed and maintains a better osteoinductivity and osteoconductivity. Apart from

cases belonging to jaw reconstruction after cyst or tumor removal (Macedo et al. 2009; Albanese et al. 2011), it has been mainly used in implant dentistry for:

- sinus floor augmentation (Xavier et al. 2014; Shanbhag et al. 2014; Sbordone et al. 2014)
- alveolar ridge preservation (Eskow & Mealey 2014; Avila-Ortiz et al. 2014)
- alveolar bone reconstruction, meaning either horizontal (Perrott, Smith et al. 1992; D'Aloja, Santi et al. 2008; Gomes KU 2008; Barone, Varanini et al. 2009; Contar, Sarot et al. 2009; Franco, Viscioni et al. 2009; Carinci, Brunelli et al. 2010; Contar, Sarot et al. 2011; Orsini, Stacchi et al. 2011; Spin-Neto, Landazuri Del Barrio et al. 2011; Araujo et al. 2013; Lumetti et al. 2014; Monje et al. 2014), vertical (Monje et al. 2014) or both (Chiapasco et al. 2015)

However, allografts can be used in form of either blocks or granules. In this regard a recent systematic review of the literature performed by Araujo et al. claimed that most of the studies reporting the use of FFB have employed morcellized grafts (Petrungaro et al. 2005; Keith et al. 2006). Thus, the clinical evidence about efficacy of block allografts for vertical and horizontal augmentation is limited so far. In addition, authors reported that there is a lack in clinical studies evaluating mandible rather than maxilla.

Generally speaking, as far as allografts are concerned, outcomes reported in the literature are controversed as some authors apparently claimed good results while others reported inconsistent results. This controversy may be due to the heterogeneity of the studies. In fact sometimes it is difficult, if not impossible, to make efficient comparisons between them or to draw unequivocal conclusions. For instance the type of bone, meaning the anatomic area from which the bone is harvested, is quite influential. In fact, structural and microarchitectural features including the relative percentage of cortical or

cancellous component remarkably vary from one to another. Concerning the type of grafted bone, the main donor sites are the iliac crest, the head of the femur (D'Aloja, Santi et al. 2008; Barone, Varanini et al. 2009) and tibia(Contar, Sarot et al. 2009; Contar, Sarot et al. 2011) with a preference for the iliac crest. Regrettably in several studies the type of bone used is not reported. Likewise, the employed surgical technique (inlay, onlay, veneers; intramarrow penetration, use of titanium screws and/or osteosynthesis bar; with/without membrane etc…) may generate diversification among studies as well as the extent of the reconstruction and the timing for graft healing or implant loading (Deluiz et al. 2015). For these reason, data gathered from different studies are barely comparable. When comparing the clinical success of the graft it is worth noting that this outcome is variously intended. In some studies it is considered as the absence of complications during the healing period such as dehiscences or graft exposures, whilst in other cases as the clinical appearance of the graft at re-entry procedure (using bleeding after perforation as equivalent of integration), or even as the ability of providing appropriate stability for dental implants. Furthermore, the absence of standardization in clinical studies might be highlighted by different modality of result evaluation with regard to clinical outcomes and histological/histomorphometrical analyses. Indeed, available studies evaluating the amount of graft resorption have not used standardized methodologies and mostly have not compared different time intervals. The majority of the studies have performed two-dimensional measurements using either a reference point (i.e. bone reduction relating to the screw head) or linear measurements on radiographs or CT scans. However, it is well known that the resorption of bone grafts occurs in a three-dimensional and nonuniform pattern. Linear measurement techniques for the assessment of bone graft resorption can easily under- or overestimate the process, depending on the reference point and the region of interest (ROI) considered. Thus, three-dimensional evaluation of morphological changes is an important tool in the

analysis of the resorption of bone grafts (Orsini, Stacchi et al. 2011). Based on the existing literature, only few studies have addressed the behavior of FFB allografts for alveolar ridge reconstruction. Hence, there is a paucity of evidence for applying this biomaterial since it has been mainly based on case reports and case series. According to Monje et al. 2014, only 15 studies out of 109 previously selected fulfilled all inclusion criteria for their systematic review. At the end the authors detected 14 perspective case series and just 1 randomised controlled trial which have been performed by Lumetti et al. which represents the first part of the same study treated by the present thesis. Thus, authors inferred interesting results:

1) **Failure rate of allogeneic bone blocks:** The cumulative survival rate of the block grafts was 98% after a period of 4 to 9 months from the surgery. Only 9 out of 361 considered grafts failed, 7 of which were combined with the use of membrane and the other 2 were not. As a matter of fact Kusiak et al. found that barrier membrane has a limited effect on the onlay block. Interestingly, other authors claimed that the use of membranes might lead to a higher prevalence of complications, such as membrane exposure and subsequent infection (Widmark et al. 1997) even if it is thought that bioadsorbable membranes have overcome these drawbacks which were mainly related to non-bioadsorbable ones. Due to the limited number of failed cases, the effect of the graft type and the membrane use have not permitted to perform a meta-analysis.

2) **Timing and causes of failures of allogeneic bone blocks:** It was reported that membrane exposure is the main reason for block graft failure whilst fixation screw loosening is the second one. Most grafts generally failed within 2 months after surgery or rather in the early stages of graft healing (Barone et al. 2009; Novell et al 2012; Deluiz et al. 2013; Spin-neto et al. 2013). It could be argued that the chances for graft success increase from the third month on.

3) **Resorptive pattern and final bone gain of allogeneic bone blocks:** Based on 119 grafts in 5 studies (Acocella et al. 2012; Nissan et al.2008, 2011, 2011 ; Wallace et al. 2010) a weighted mean of 4.79 mm horizontal bone gain was estimated. Allograft resorption ranged from 10 ± 10 % (Nissan et al. 2011) to 52 ± 25.97 % (Lumetti et al. 2014) at 6 months after grafting. However the mean value was found to be relatively low (21.70 ± 30.55%). Authors also reported a correlation between healing time and graft resorption. This finding is in accordance with Deluiz et al. (2015) who performed re-entry procedures at different time point and reported a mean volume reduction of 13.02 ± 3.86 % in 4 months group, 32.77 ± 7.84 % in 6 months group and 50.78 ± 10.43 % in 8 months group. However it should be highlighted that when mean averages of resorption are presented, they often result from heterogeneous comparison of different grafting techniques.

4) **Implant cumulative survival rate:** Based on 228 implants the weighed mean implant survival rate was 96.9% over a mean follow-up period of 23.9 months (Barone et al. 2009; Contar et al. 2009; Nissan et al. 2008, 2011, 2011; Acocella et. al 2012, Deluiz et al. 2013).

5) **Histomorphometric and Histologic Characteristics of Allogeneic Bone Blocks:** It is worth noting that among these studies only 6 reported histological outcomes and just 2 had a control group. In both cases it constisted of autogenous block grafts harvested from the mandibular ramus. Acocella et al. 2012 after a healing period of 9 months showed that a high number of empty osteocyte lacunae were still present. Additionally, newly formed bone (61.96 ± 11.77%) was surrounded by nonvital bone with empty osteocyte lacunae. Contar et al. 2011 reported lamellar arrangement around Haversian canals interspersed with osteocytes in lacunae. In addition, in the center of the block grafts osteocytes with higher number of empty lacunae were noticed. Despite that, when comparisons were made with autogenous graft control

groups no agreement have been achieved. After 6 months of healing Lumetti et al. demonstrated that osteocyte lacunae were mostly empty for the allogeneic block graft group. Furthermore, it was reported that newly formed bone contained viable osteocytes at that point. In these samples, bone forming osteoblasts and fluorescent labeling were detected. Dense connective tissue with the presence of inflammatory cells and eroded areas were also observed in such a group. Minimal differences were shown for the autogenous block grafts group in which no connective tissue was found and where the presence of inflammatory cells was meaningfully lower. Contrarily, Spin-neto et al. in 2011 found major dissimilarities between the groups. For the allogeneic bone block were found large segments of necrotic bone with empty osteocytes lacunae and little osteoclastic activity, along with blood vessels invading the Haversian canals of the material. In addition, no direct contact between remodeled and grafted bone was found. For the autogenous block grafts were detected just small areas of necrotic bone with abundant presence of osteocytes. In the end no difference was noticed between the graft and the host bone.

It has to be observed that histological analysis are not exactly comparable since biopsies were performed after different times of healing period which ranged from 5 to 9 months from grafting. Moreover, the biology of allograft concerning inflammatory infiltrate or immune reaction into the grafts is still unclear. Either way, some histological differences shed light on the fact that grafts' composition may affect their biological incorporation pattern. Infact, it is well known that graft healing is strictly dependent on its composition seeing that cortical grafts are hard and resistant to vascular penetration which occurs solely after 5-6 days, so that a large number of cells (osteoblast, osteocytes etc…) undergo necrosis in the meanwhile. Several authors reported that necrotic remnants remain into newly formed viable bone up to 24-72 months (Burchardt 1983; Stevenson 1999). On the other hand cancellous bone is completely revascularized and remodeled

within 12 months but undergo a higher resorption rate. Even if data are not conclusive for FFB, it is supposed to display a similar behavior to autogenous grafts. In this regard Spin-Neto et al. 2011 reported that neither newly formed bone nor pristine bone were in contact with the FFB cortical grafts after 5 months of healing whilst Orsini et al. 2011 noticed that FFB corticocancellous grafts underwent a better integration showing close continuity with native bone and newly formed vessels into the grafts. To summarize, although cancellous grafts seem to be endowed with better biological properties, the cortical component might be responsible for obtaining a greater bone volume and density which is pivotal to guarantee a long-term support for dental implants. By the way, as above-mentioned, several authors stated that the amount of bone graft resorption over time may be dependent on its embryologic origin. By contrast, it has been stated that the resorption of bone grafts is much more related to density than origin (and consequently composition). As a consequence, the difference in bone gain achievable by mesenchymal grafts may be due to the fact that they are generally denser than endochondral ones. According to this affirmation, Lumetti et al. in 2014 found a linear correlation between FFB grafts resorption and their initial density sixth months after the baseline evaluation. Authors stated that such results are quite improbably due to embryologic origin since all FFB block grafts were harvested from tibia. Nevertheless, the relationships among FFB graft composition, density and remodelling need to be further investigated. In conclusion, it can be asserted that allografts seemed to be a feasible alternative to autografts by yielding satisfactory short-term clinical results. Unfortunately, there is no convincing evidence since clinical studies are mainly case series and case reports and the sole RCT performed by Lumetti et. al is available in literature at present day. Moreover, long-term clinical data of allografts are still missing. According to recent systematic reviews, more well-designed studies (e.g. larger sample size, longer follow-up, better controlled) are needed to determine the advantages of allografts for alveolar

ridge augmentation and to support clinical decision-making. To the best of our knowledge, there are no clinical studies reporting long-term specific outcomes as PPD, KT, BOP, REC and necessity for regrafting at 5-year follow-up visits. Besides these data, implant cumulative survival rate covering the same follow-up period will be reported. To give a better understandig regarding such parameters, a general overview is described in the following paragraphs.

Probing pocket depth (PPD)

It has long been recognized that a proper diagnosis is a prerequisite to dispense an adequate and optimal medical treatment. If this basic principle is not respected, no effective treatment can be provided for the illness (Hippocrates 460 BC- 377 BC). According to this affirmation periodontal and peri-implant diseases do not represent special cases. Already in 1882 appeared the first description of periodontal probe as diagnostic tool for periodontal disease. From that moment on, several probe designs were

developed parallel to an increasing cultural backgroup in periodontology. To date, probing represents the diagnostic test of choice for monitoring periodontal and peri-implant conditions because of its cost/benefit ratio and practical efficacy. Nonetheless it can not be considered free from drawbacks. Infact, several authors pointed out that probing may result in tissue penetration so resulting in overestimation of the actual sulcus or pocket depth. Moreover, the situation remained unclear for many years when the description of periodontal pocket was still a matter of discussion. Only in 1971, thanking to the introduction of electron microscopic techniques, Schroeder & Listgarten demonstrated that probe insertion in a periodontal pocket causes penetration within the junctional epithelium, leaving the innermost epithelial cells attached to the tooth surface. They described that in almost all specimens, epithelium was present between the probe tip and the connective tissue. On the basis of these results Listgarten in 1972 stated that it is better to differentiate between clinical and histological sulcus depth. Soon after, in order to minimize confusion Van der Velden in 1979 proposed to reserve the term sulcus for histological and pocket for clinical measurements. As above-stated probing assessment of the pocket in both teeth and implants is influenced by many variables such as: manual dexterity and tactility of the examiner, site and angle of insertion (Listgarten 1980), accuracy of reading the millimeter markings (Magnusson et al. 1988), and patient cooperation. Other factors might be related to teeth e.g. malposition, furcation sites, remaining calculus and overhanging restorations. By and large, it has been stated that major factors are probing force employed, probe thickness and the degree of inflammation of periodontal/ peri-implant tissues. Either way, many efforts have been made to determine the proper probing force to monitor implant health in time. The main focus was also the behavior of different probing forces in both periodontal health and disease. To this purpose Armitage et al. (1977) made an experiment in an animal model (beagle dogs) where they examined: clinically healthy sites, inflamed sites after experimental gingivitis and sites exhibiting

periodontitis. An experimental probe (0.35 mm diameter at tip) was inserted with a force of 25 N into the gingival sulcus, hence animals where sacrificed and block section were processed for histology. Relationships between the tip of the probe and the junctional epithelium were observed to be strictly dependent on the degree of inflammation of the gingival tissues. In health, the probe reached a point located at approximately 0.4 millimeter from the cemento-enamel junction. Junctional epithelium was pulled away by disrupting, but not damaging, the hemidesmosomal attachment. In gingivitis the probe penetrated nearer the CEJ by 0.1 mm whereas at sites suffering from periodontitis the probe tip passed beyond the apical termination of the junctional epithelium by a mean distance of 0.25 mm. Other studies confirmed these results in humans, stating that in healthy sites a force of 0.25 N do not damage the supracrestal connective tissue (Polson et al. 1980). It has been concluded that the position of the probe tip was mainly determined by the condensation of connective tissue rather than the resistence opposed by the epithelial attachment. As a consequence in presence of tissue inflammation there is a higher risk for probing pocket depth overestimation. On the basis of the same principles, several studies have been carried out in order to understand the role of probing around endosseous implants. Lang et al. in 1994 conducted a study on beagle dog aimed at evaluating the level of probe penetration after standardized probing, with respect to the histologic level of connective tissue, mucosal margin and alveolar bone crest in clinically healthy peri-implant tissues, mucositis and peri-implantitis. The probing force employed was of 0.2 N. Authors concluded that:

- in their study there was not difference between the two different probe analyzed
- In peri-implant gingival health the probe tip reach at most the junctional epithelium
- In peri-implant mucositis, although tissues are more inflamed, the probe tip remained within the junctional epithelium
- In peri-implantitis, despite the controlled force, the distance between the bone crest and

the probe tip was reduced as the probe penetrated more deeply

Another important study to evaluate probing as indicator of tissue health was conducted by Schou et al. in 2002. Authors used cynomolgus monkeys (*Macaca fascicularis*) as animal model and the design of the study was rather comparable to that performed by Lang et al. except for the fact that also probing around teeth was assessed. Authors concluded that:

- In case of periodontal or peri-implant health, probing with a medium-high pressure (0.4 N) did not reveal any significant difference in both teeth and implants. The probe was stopped into the junctional epithelium and the measurements ranged from 0.5 to 2 mm
- On the contrary, in presence of gingivitis/mucositis probing depths varied between 1 and 4mm. Hence, in some cases, the probe fell beyond the junctional epithelium
- In periodontitis/peri-implantitis the distance between the probe tip and the crestal bone was progressively reduced as probing varied between 2 and 6 mm.

The study which has allowed to complete the understanding of probing at implants was conducted by Abrahamsson and Soldini in 2006. Authors had the purpose of investigating the histological level of probe penetration in healthy periodontal and peri-implant tissues. Beagle dogs were used for the analysis and the probing force applied during the experiment was nearly the same (0.2 N) that had previously showed clinical feasibility at tooth sites (Armitage et al. 1977; Fowler et al. 1982). The conclusions of this study were that:

- In the presence of health, when using a light or moderate probing force there is not difference between the position of the probe tip at teeth and implants sites
- Probing extension is at level of the junctional epithelium
- The distance between the probe tip and the alveolar bone is approximately 1- 1,5 mm

According to these studies at sixth European Workshop on Periodontology (Lindhe et al. 2008) authors concluded that:

- Probing is essential for diagnosis of peri-implant diseases.
- Conventional probing using a light force (0.25 N) does not damage the peri-implant tissues

Bleeding on probing (BOP)

Since periodontal and peri-implant diseases are fundamentally inflammatory in nature, diagnostic indicators are of the utmost importance to detect early signs of inflammation in gingivitis and mucositis. An early diagnosis could also be seen as a factor of prognosis and is useful for assessing individual risk of the patients. Given that periodontal/peri-implant indices of inflammation may be evaluated by non-invasively (e.g gingival index Loe & Silness 1963 or its counterpart, modified gingival index Mombelli et al. 1987) or invasively methods (e.g., bleeding on stimulation or provocation) (Armitage 1996), it has been stated that clinical indices should provide the possibility of being converted into an acceptable numerical form for statistical analysis. As such, visual indices may be considered quite arbitrary whereas bleeding on probing is better associated with tissue inflammation. Moreover, it furnishes more precise quantitative measurments thus permitting the monitoring of disease over time. Bleeding on probing (BOP) evoked after inserting a probe into the sulcus with moderate pressure (0.25 N) has been shown to reveal the presence of inflammatory lesions around teeth with a normal (Lang et al. 1991) or healthy but reduced periodontum. Nonetheless it has been asserted that BOP has a limited predictive value for disease progression (Lang et al. 1986) whilst negative BOP has been related to periodontal health with a negative predictive value of 98.5% (Lang et al. 1990). Regarding the role of BOP at implants sites, at the beginning Lekholm et al. in 1986 reported no correlation among BOP, histologic, microbiologic or radiographic changes around endosseous implants. Authors assumed that results could have been distorted because of an improper force transmission from the periodontal probe tip to the peri-implant soft tissues. In a further study, Jepsen et al. (1996) followed a cohort of patients in order to investigate several diagnostic indicators. The auhors concluded that bleeding on probing

was characterized by a high negative predictive value and thus, negative score can serve as indicator for stable peri-implant conditions. To gather a global comprehension of the characteristics of a diagnostc test is useful to bear in mind the following notions:

	A	**B**
Positive test for BOP identification	True positive	False positive
	C	**D**
Negative test for BOP identification	False negative	True negative

Sensitivity is the probability that a site with disease progression will score positive.

Specificity represents the probability that a stable site will score negative.

Positive predictive values represent the probability of disease progression to occur in a subject with a positive test result.

Negative predictive values correspond to the probability of stability when the test result is negative.

These determiners for diagnostic test may be calculated as follows:

- Sensibility: A/ (A+C)

- Specificity: D/ (B+D)
- Positive predictive value: A/ (A+B)
- Negative predictive value: D/ (C+D)

Another crucial study was performed by Luterbacher et al. (2000). The authors followed 19 patients which were enrolled in a program of supportive periodontal treatment. These patients had previously received partial fixed implant-supported restoration so that comparison with tooth sites was possible. In addition, different treshold regarding percentage of BOP were set and microbiological samples at both implants and teeth were taken. At the end, the authors reached some interesting conclusions:

- All implant sites showing a BOP frequency of more than 50% underwent loss of attachment with specificity of 100%
- BOP has higher positive and negative predictive values for implant sites compared to tooth sites

Based on these findings Lindhe et al. (2008) concluded that:

- Bleeding on probing indicates the presence of inflammation in the peri-implant mucosa.
- Bleeding on probing may be used as a predictor for loss of tissue support.
- An increase in probing depth over time is associated with the loss of attachment and supporting bone.
- The probing depth, the presence of bleeding on probing and suppuration should be assessed regularly for the diagnosis of peri-implant diseases.

Kertinized tissue (KT)

Many authors have asserted that the stability of the peri-implant mucosa is a keystone for the general stability of dental implants and the maintenance of bone health. Whether this goal is assured by non-keratinized mucosa or keratinized attached mucosa is still a matter of debate. On the one hand some studies were unable to reveal any difference in the preservation of peri-implant bone levels (Linkevicious et al. 2009). On the other hand, other reports showed an increased risk for peri-implant bone loss when the implant is surrounded by alveolar mucosa (Cairo et al. 2008). The absence of agreement to what concern peri-implant soft-tissue health is due to multifactorial issues. It has been stated that implant design, position in the mouth, local anatomy, surgical technique, prosthetic design, oral hygiene, host immunologic responses, function, etc… make it arduous to delineate clinical studies of appropriate quality to supply with conclusive answers. Nevertheless, the convenience of keratinized attached mucosa around dental implants is less arguagle. Infact, several clinicians have taken position in matter, asserting that it is preferable to surround the implant with an adequate band of keratinized mucosa. The assumed benefits are an improved tissue health, greater patient satisfaction, and fewer complications over time (Bouri et al. 2008). The mucosal stability should give a better support for the underlying connective tissue so that the junctional epithelium could produce a stable seal around the implant. Moreover, it may simplify and make more precise the prosthetic procedures since challenges to the soft tissue during this phase are better absorbed by keratinized tissue (Linkevicious et al. 2009). As far as final esthetics is concerned, at third EAO consensus conference the authors reported that a wide band of keratinized mucosa is of the utmost importance to reach this outcome (Klinge et al. 2009). In line with this statement Jung et al. in 2008 found that a thick biotype offers the opportunity to hide the shine-through of the underlying structures (Jung et al. 2008)

Peri-implant mucosal recession (PMR)

In respect to the chewing function it is well established in the dental literature that the majority of inserted implants show an excellent prognosis with survival rates of up to 95% (Buser et al. 1997). Since demand for aesthetics has been increasing always more often, it should be considered that peri-implant gingival recession is an implant-related complication which deserve more awareness. Firstly, peri-implant gingival recessions have multifactorial etiology even though it has been reported that gingival biotype plays a key role. In this regard, Kan et al. in 2003, analyzing post-extraction implants over a period ranging from 2 to 8 years, found that peri-implant mucosal recession is more frequent for thin gingival biotype when compared to thick biotype. Therefore, Lin et al. reported that implants surrounded by lining mucosa show significantly higher plaque scores, more peri-implant mucosal recessions, more inflammation, and more attachment loss. Standing to these studies, it may be argued that nonkeratinized mucosa associated with other etiological factor for gingival recessions at teeth (trauma, plaque, malposition, etc…) could be risk factors for peri-implant mucosal recessions. In addition, even bone remodeling around two-piece implants and buccal malpositioning or malangulation have been mentioned. Contrarily to gingival recessions, the prevalence of peri-implant soft tissue recessions is fairly limited. It has to be pointed out that since dental implants are supposed to support prostheses for many years (or maybe decades), peri-implant recessions might be observed frequently in a long-term perspective (Fickl 2015). Besides the exposure of implant shoulder and implant threads, which can make oral hygiene difficult if not impossible, peri-implant mucosal recession generate a mismatching colour between implants and the surrounding hard and soft tissues. This finding strongly affect the patient's perspective especially in cases of high smile line, thus resulting in an overall success impairment of the final prosthesis.

Materials and Methods

Aim

The present multicenter randomized controlled clinical trial aimed at evaluating the clinical performance of fresh frozen bone (FFB) block grafts (test group) as compared to autologous bone (AB) block grafts (control group), reporting on implant-related biometric parameters at 5-years follow-up visits. The main purpose is to provide with long-term clinical data which are currently lacking in the scientific international literature.

Operative Units

This multicenter RCT was conducted by the University of Parma, Modena and Bologna.

Patient selection

Twenty-four sistematically healthy patients (max 10 cigarettes/day) presented with inadequate bone volume were included in the study. They needed one or several implants in the interest of restoring partial or total edentulism in the upper maxilla. Patients were treated between May 2008 and August 2009. All of the eligible patients received comprehensive written and verbal information and signed informed consent forms before enrollment. The study was conducted in full accordance with the World Medical Association Declaration of Helsinki and it was approved by the ethics committee of the participating institutions.

Primary endpoints were implant-related biometric parameters recorded at follow-up visits which were scheduled every 6 months from the delivery of final prostheses. Measurements were taken and collected for both AB and FFB patient groups at baseline

and repeated over time so that data reported refer to the clinical evaluation after 5-years of follow-up. Data regarding previous check-points will not be reported. This study constitutes the prosecution of an earlier protocol which also encompassed histological, histomorphometrical and radiological data. These outcomes are not taken into consideration but are available in previously published works.

Inclusion criteria were:

- at least 18 years of age
- Cawood and Howell class IV atrophy
- adequate oral hygiene, i.e. full mouth plaque score and full mouth bleeding score ≤25%. Oral hygiene was improved until periodontal status complied with the inclusion criteria.

Exclusion criteria were:

- ASA score ≥III
- presence of active clinical periodontal disease as expressed by probing pocket depths ≥4mm and bleeding on probing
- a history of radiotherapy to the head and neck region
- medical conditions requiring prolonged use of steroids
- history of leucocyte dysfunction
- history of bleeding disorders
- history of renal failure
- patients with metabolic bone disorders
- patients with uncontrolled endocrine disorders
- physical handicaps that would interfere with the ability to perform adequate oral hygiene

- use of any investigational drug or device within the 30-day period before implant surgery
- alcoholism or drug abuse
- HIV infection
- smoking >10 cigarettes a day or cigar equivalents
- conditions or circumstances that would prevent completion of study participation.
- mucosal diseases
- lack of primary implant stability
- conditions requiring chronic use of antibiotics;

Prior to surgery, all patients were submitted to clinical observation, which included the execution of panoramic radiographs, impressions and bite registrations. Preoperative, photos were also taken. Hence, dental study casts were mounted on articulator in order to provide an ideal prosthetic set-up to arrange the restoration of missing teeth.

Randomization

A specifically designed locked computer software program (Minitab 1.5, Minitab, State College PA, USA) was used to randomly assign patients to one of two study groups to receive either autologous bone block grafts (AB group) or fresh-frozen homologous bone block grafts (FFB group). The allocation result was kept in a locked computer file that was not accessible for the examiner and the practitioners. The dentist who had to perform surgical procedures was informed about the allocation on the day scheduled for the intervention.

Operative procedures

Pre-medication

All patients were pre-medicated with 2 g of Amoxicillin as antibiotics prophylaxis 1 hour prior to surgery. Shortly before surgery, patients were instructed to rinse with Chlorexidine 0,2% for one minute and Diazepam 1.2mL (Valium, F.Hoffmann-La Roche SA) was administered sublingually.

Surgery

Autologous bone grafts were retrieved from intraoral sites, either from the symphysis or the mandibular ramus. The choice of donor site, either symphysis or ramus, was determined preoperatively based on defect morphology and recipient site location. Panoramic radiographs were used to assess the donor sites as regards anatomic relationships with the inferior alveolar canal, tooth apexes etc…The recipient site was completely healed prior to graft surgery forasmuch as a period of at least 8 weeks from the last tooth extraction had passed.

Symphysis donor site

Access to the symphysis was obtained via a vestibular incision paying attention to the mental nerve which might have crossed the area. The incision was made in the mucosa between the canine teeth areas at least 1 cm beyond the mucogingival junction; dissection was continued across the mental muscle until the periostium, which was cut horizontally. The mucoperiosteal flap was reflected toward the base of the mandible to the level of pogonion to expose the bone surface comprised between the mental foramina. After the symphysis was exposed, the osteotomy for graft harvest was planned. Block size was determined in view of the defect to be filled. A rectangular bone area was drawn with a fissure bur in a surgical handpiece under copious saline irrigation. The superior osteotomy was made at least 5 mm below the tooth apices in order to maintain tooth vitality and avoid

damages to the mental nerve. The inferior osteotomy was executed parallel to the inferior border of the mandible leaving at least 2 mm of healthy bone so that the inferior cortex was maintained. Vertical osteotomies were made to link those last. The depth of the osteotomies was extended only through the outer cortex. Hence the required bone dowels were delivered by means of bone chisels which were tapped along the osteotomy. Auxiliary bone chips were obtained with a rongeur or chisel. In the end the donor area was medicated with oxydate cellulose (Tabotamp, Johnson&Johnson Medical S.p.A.)*(Figure).* Soft tissues were elevated to reduce tension on the flap, then a two-layered closure was carried out using Vicryl 3-0 (Johnson & Johnson International, Belgium).

Mandibular ramus donor site

To gain access to the ramus area an oblique sagittal incision was made in the buccal vestibule medial to the external oblique ridge and vertical releasing incisions were performed beyond the retromolar pad and on the mesial side. A mucoperiosteal flap was elevated from the mandibular body thus exposing the retromolar region and the lateral aspect of the ramus. The flap was raised along the upper part of the external oblique ridge to the base of the coronoid process with a notched ramus retractor. Osteotomic lines were created via fissure and round burs mounted on a straight handpiece under copious saline irrigation. These cut were intentionally left shallow in order to create a line of fracture by way of chisels which were tapped along the osteotomic cut. The amount of bone harvesting was proportional to the defect in the recipient site. After bone grafts were obtained, sharp edges around the ramus were smoothed with a bur or file. At last, soft tissues were managed with periosteal releasing incisions at the base of the flap to allow stretching of the mucosa and primary intention closure without tension. At this point AB grafts were rounded and fixed so that they accomplished a well-suited shape which was compatible with the recipient site. During the preparation of the recipiet site, the bone blocks were kept in sterilized gauze soaked in saline solution.

Fresh-frozen bone (FFB)

The FFB was obtained by Banca del tessuto muscolo-scheletrico (Istituti Ortopedici Rizzoli, Bologna, Italy). Bone grafts were retrieved from the tibial hemiplateau by 12 hours of donor death. Immediately after completion of all safety tests, eventual tendinous or periostic remnants were eliminated. At this point the bone underwent disinfection for at least 72 hours at -4°C in an antibiotic solution of vancomycine, polymyxine, glazidine and lincomycine. As FFB was not irradiated it was lacking of lipid oxidation. Then, the samples were moistened by way of a sterile saline solution at 37°C, cut into blocks, packed in double sterile casing, and frozen at the standard temperature of -80°C. Immediately before surgery, they were thawed in a 600 mg/L Rifampicine and saline solution (Rifadin, Lepetit Lainate, Italia) at 37°C, according to the manufacturer's instructions. The portions that were supposed to offer the best quality were selected and isolated.Thus, FFB grafts were modeled with burs and bone forceps aiming at obtaining the best fit in the recipient area. The blocks need to be adjusted so that they sit flat upon the receipient site without rocking and with intimate contact with the underlying host bone. Cortico-cancellous frustules and shreds remaining after these procedures were retained in saline solution in order to be availble for filling gaps when needed. Eventual residual periostium was removed from the blocks. During the grafting procedures cancellous chips were used in combination with blocks. When improving their firmness was necessary, it was obtainend by mildly compacting bone pieces with blunt instruments.

Grafting technique

Under aseptic conditions, local anesthesia composed of articaine 4% with adrenaline 1:100.000 (Optocain, Molteni Dental S.p.A.) was injected in the recipient site. To gain access to the surgical area a beveled full-thickness incision slightly palatal to the alveolar

ridge was made. The incision proceeded into the gingival sulcus of the adjacent teeth and vertical releasing incisions were made at line angle of these last, when needed, to improve the mobility of the flap. In this way, a trapezoidal mucoperiostal flap was reflected with a periostal elevator, avoiding damage to the anatomic structures and the periosteum, thus allowing a full view over the surgical area. Hence, the bone block grafts were shaped and adapted to the recipient site. They needed to be adjusted to sit flat upon the receipient site avoiding wobble so as intimate relationship with the underlying host bone could be reached. Next , intramarrow penetration were performed using a small diameter twist drill to generate multiple communication with the marrow space and optimize blood supply in order to achieve easier new bone formation around the graft. Therefore, bone block grafts were penetrated in a lagged fashion with a twist drill which was larger than the final screw diameter so that the fixation screw threads did not engage the block, but rather only the cortical bone of the underlying recipient site. At least two titanium screws to block the grafts in the proper place were used (Cizeta Surgical, Bologna, Italy) so as bone block rotation was hindered and immobility could be warranted. Any gap between the grafts and the recipient sites was filled using bone chips. At this time, collagen membranes (Osseoguard, Biomet 3i, Indiana, USA) were placed so as to cover the grafts prior to perform releasing incisions to obtain a better mobility and passivity of the flap. The primary intention wound closure was obtained by means of monofilament sutures (Prolene 3-0 and 5-0, Ethicon, Johnson & Johnson, Amersfoort, Netherlands). A double layer technique was employed using horizontal internal mattress and interrupted sutures. Finally, antibiotics (Amoxicillin, 2 g/day for 10 days) and pain medications for a maximun of 3 times a day were given. After 10 days from surgery, sutures were removed and patients were scheduled for implant surgery.

Implant placement

At stage-two surgery, six months after surgical augmentation, all patients were submitted to a second surgical session in order to remove the microscrews and place implants in the reconstructed areas. After the augmentation surgery, implant placement was possible in all cases. On the basis of available bone volume and prosthetic needs, which were evaluated clinically and radiographically, implant lenghts and diameters were chosen at each implant site. The surgical procedure was accomplished under local anesthesia (articaine 4% with adrenaline 1:100.000 Optocain, Molteni Dental S.p.A.) which extended according to the number of implants to be placed and patient's characteristics. To get access to the surgical field a full-thickness flap was raised following the same incision line used for the reconstructive surgery. The incision was performed by means of a 15C blade (Hu-Friedy, Chicago, USA). The cut was executed slightly palatal with respect to the alveolar crest in order to obtain at least 1 mm of keratinized buccal wound margin. The incision was extended through the sulci of the facial aspects of the adjacent teeth and followed by vertical releasing incisions made at the distal line angles of these teeth. At this point the mucoperiosteal flap was raised with a fine tissue elevator to expose the surgical site. Hence, to ensure a sufficient surgical access the flap was pulled aside by means of retractors. Firstly, any residual titanium microscrew which hindered implant placement was removed. Therefore, implant site preparations were carried out; a brief description is presented as follows: the preparation of the osteotomic site began using a small round bur to mark the position of the implant and ensure the correct position of the following drills. The series was composed by a sequence of progressively wider drills in diameter according to the manufacturer's instructions. Osteotomies were realized considering a distance of at least 1,5 mm from tooth and 3 mm from implants. They were performed under copious saline irrigation with a contrangular handpiece. Thus, the implants (NANOTITE CILINDRO Certain, Biomet3i, Indiana, USA) were positioned using an insertion device attached to a contra-angle handpiece at 35 rpm. In certain cases implant

insertion was accomplished by manual devices. Site analysis with the implants in place was then carried out by means of a periodontal probe (UNC 15, Hu-Friedy, Chigago, USA). At the end of the insertion procedure cover screws were attached to cover the implants. Releasing incisions were made to mobilise the flap when needed. Suture closure of the flap were initiated at the mesial papilla. Hence, primary wound closure was finished by horizontal mattress and interrupted sutures using monofilament sutures (Prolene 3-0 and 5-0, Ethicon, Johnson & Johnson, Amersfoort, Netherlands). Once this point was reached, implants were left to integrate in a submerged protocol and patients were discharged with the same postoperative instructions given after grafting procedures.

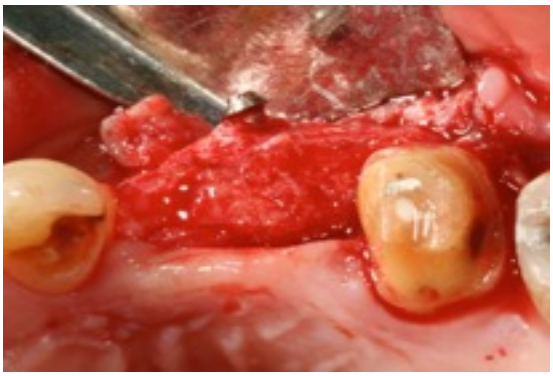


Figure 1

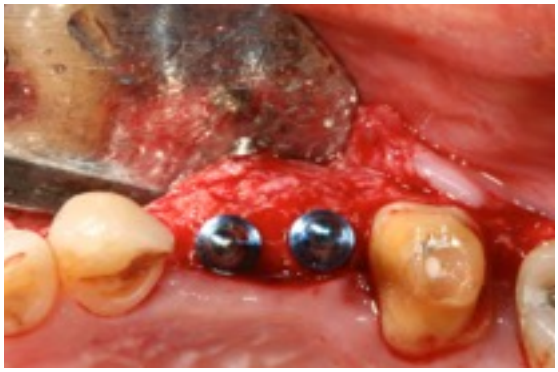


Figure 2

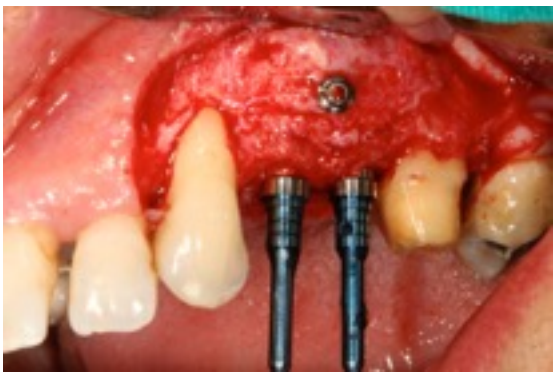


Figure 3

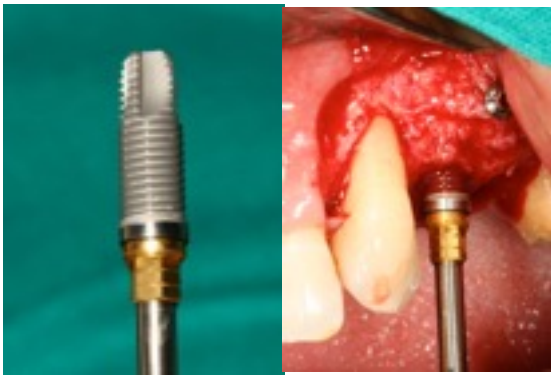


Figure 4

Implant exposure and Prosthetic loading

After 6 months of healing, implants were uncovered and healing abutments connected. In case any implant thread was uncovered by bone tissue (dehyscence type defect) re-grafting procedures were carried out. All these defects measured at least 3 mm in lenght. Each defect was treated by means of deproteinized bovine bone matrix (Bio-oss Geistlich Biomaterials, Wolhusen,Switzerland) covered by a collagen membrane (Bio-gide Geistlich Biomaterials, Wolhusen,Switzerland). Hence, they underwent a transmucosal healing for 3 months before the provisional loading. Instead, as far as the non-regrafted groups (AB & FFB) are concerned, final impressions were taken 4 weeks after the attachment of healing abutments. Custom impression trays were fabricated with BEGO resin (BEGO Medical GmbH, Bremen, Germany) which was photopolymerizated in accordance with the manufacturer's instructions. The impression trays were created with windows to grant access for coping screws. They had been previously sprinkled with Impregum polyether adhesive (3M ESPE). Before every impression procedure, an impression transfer was secured to the implant, and the transfers were splinted with resin (Duralay Reliance Dental, Worth, Illinois- USA). The impression material (Impregum Penta, 3M ESPE) was machine-mixed (Pentamix, 3M ESPE) and then partly syringed all around the transfers in order to guarantee the complete coverage by the impression material. Once the impression material had completed the polymerization, the transfers were unscrewed and the impressions were removed from the patients' mouths. Hence, an implant analogue was screwed upon the impression coping, and the impression were poured with type IV artificial stone according to the manufacturer's instructions. All laboratory procedures were performed by the trusted laboratory of each operative unit. To conclude, implant-supported provisional prostheses remained in place for a period ranging from 3 to 6 months on the basis of the treated area. Thereafter, implant-supported prostheses were delivered and patients were scheduled for follow-up visits.

Follow-up program:

A follow-up program was carried out for each patient. In this way, patients were examined monthly for the first sixth months. After this checkpoint, the follow-up program continued with visits every 6 months in subsequent years. The following biometric parameters were evaluated and recorded at each recall appointment: peri-implant probing pocket depth (PPD), bleeding on probing (BOP), amount of keratinized tissue (KT), peri-implant mucosal recession at the mid-buccal side *(see next paragraph).*

Biometric parameters:

As mentioned above, at recall visits clinical measurements were carried out for monitoring peri-implant conditions over time. All these data were collected using a UNC-15 periodontal probe with markings up to 15 mm (Hu-Friedy, Chicago, USA) (figure 5) as follows:

-Probing pocket depths (PPD) were measured from peri-implant gingival margin to the most apical penetration of the probe into the peri-implant crevice using a force of 0,25 N. Six values per every implant (specifically mesio-buccal, disto-buccal, middle-buccal, and mesio-palatal, disto-palatal, middle palatal) were taken and recorded to the nearest millimeter.

-Bleeding on probing (BOP) was evaluated by inserting a probe into the sulcus with light pressure (0,25 N). It was estimated as positive if bleeding occurred within 30 seconds after measuring the peri-implant probing depth. It was considered irrespective of the amount of bleeding and recorded dichotomously.

-Width of keratinized tissue (KT) was measured in millimeter at midfacial of the implant site and recorded to the nearest millimeter. It was considered as the distance from the most apical point of the gingival margin to the mucogingival line.

-Gingival recessions (GR) were measured in millimeter positioning the probe at the mid-buccal point of the implant and measuring the distance between the gingival margin and the implant shoulder. In case of absence of gingival recession, it was scored as 0.

All peri-implant related measurements were repeated at various observation times but only data at 5 years follow-up were considered for the statistical analysis.

- 15-mm-long probe with markings at each millimeter
- Colour coding at the 5th, 10th and 15th marks

Figure 5

Regrafting was evaluated as the necessity for performing additional GBR procedures at the time of implant placement or implant exposure. It was considered unavoidable if any implant showed bone defects of at least 3 mm (Figure 6,7,8 & 9) . This variable was recorded dichotomously.

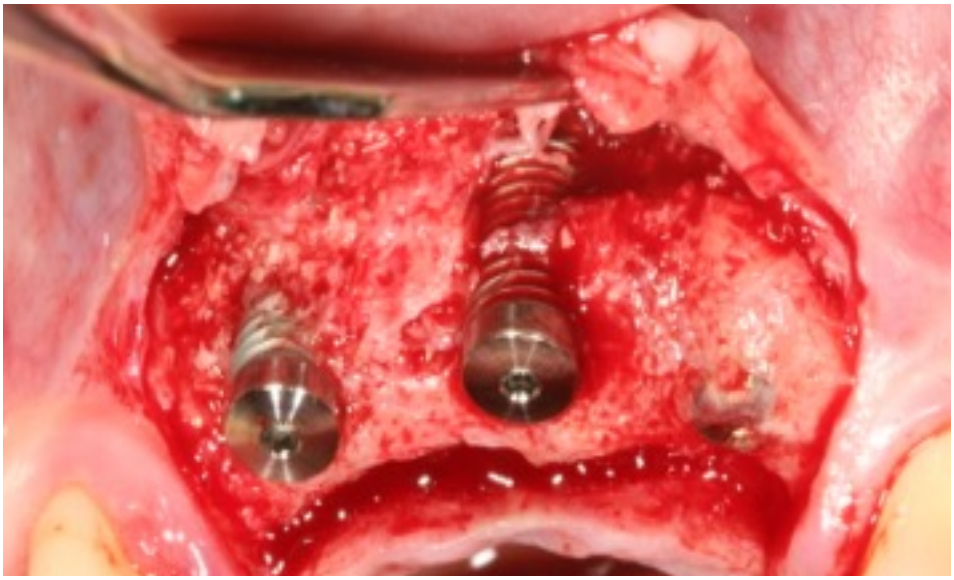


Figure 6

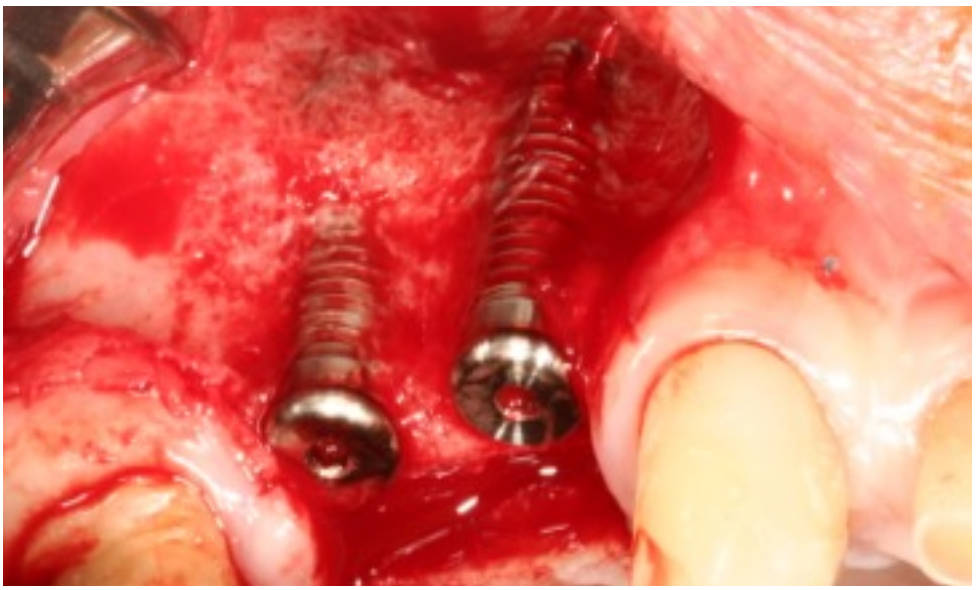


Figure 7

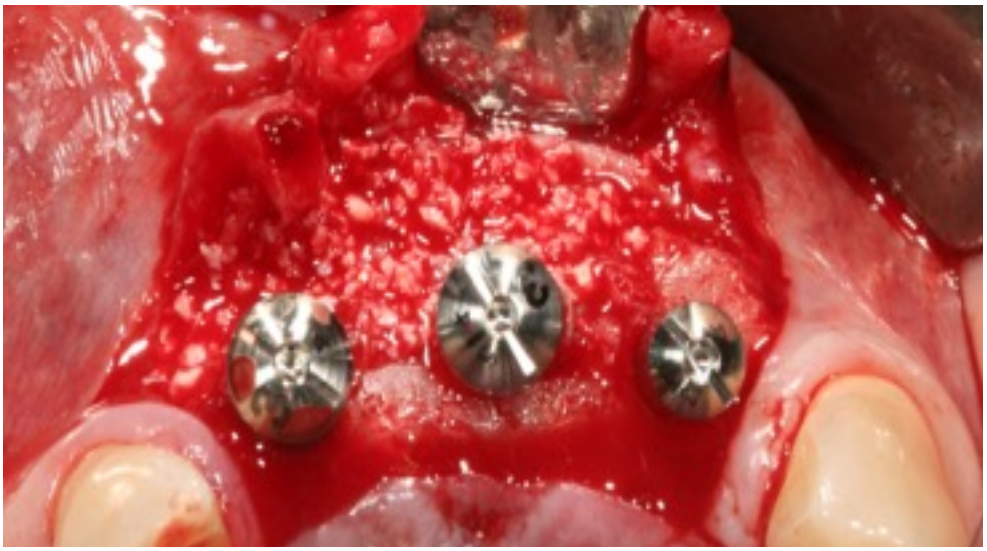


Figure 8

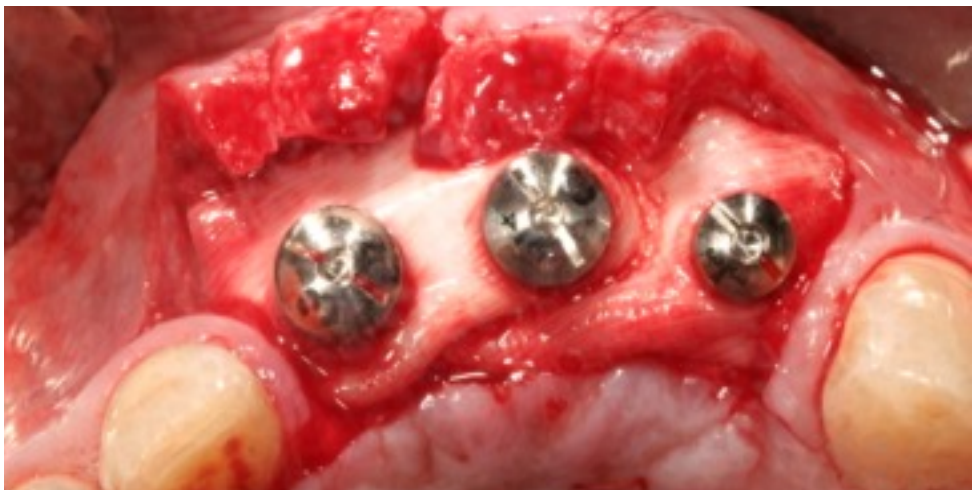


Figure 9

Criteria used to determine implant survival were:

- (*1*) absence of persistent pain or dysesthesia
- (*2*) absence of peri- implant infection with suppuration
- (*3*) absence of mobility
- (*4*) absence of continuous peri-implant radiolucency

Implant cumulative survivale rate was calculated and reported as percentage.

Statistical analysis

Descriptive statistics including mean values and standard deviations were calculated for each variable. Continuously-distributed clinical parameters (keratinized tissue amount, buccal recession and probing depth) were analyzed using the ANOVA for non-normally distributed data. Categorical variables (re-grafting and bleeding on probing) were compared using the Fisher's exact Test applying three-by-two tables. The level of significance was set at $p<0.05$. The implant cumulative survival rate was obtained as a percentage.

Results

This 5-year report includes 21 out of 24 originally treated patients. Three patients dropped out because they refused to participate to the entire follow-up period. Two of them belonged to the control group whereas 1 belonged to the test group. These patients were consecutively admitted into the study and randomly allocated to the control (AB) or the test group (FFB). They needed one or more dental implants in order to restore a partial or total edentulism in the upper jaw. Only horizontal defects were considered and treated. Patients who were assigned to the control group received autologous bone grafts from intraoral donor sites (chin or ramus) whereas patients assigned to the test group received fresh-frozen cortico-cancellous bone allografts derived from the tibial hemiplateau of donor cadavers. Nine patients were enrolled by the operative unit of Parma, 4 of which were allocated into the control group and 5 into the test group. The operative unit of Modena enrolled a total of 10 patients, 7 of which into the control group and 3 into the test group. The remaining 5 patients were enrolled by the operative unit of Bologna, 1 of them was allocated into the test group and 4 into the control group.

In total, 24 patients were enrolled in the study *(Table),* the two groups were composed as follows:

Test group: 7 male,5 female, mean age 52.62 median age 53

Control group: 8 male, 4 female, mean age 54.33 median age 55

Each patient received one bone graft. Bone grafting had different size since bone atrophies to be corrected presented with different extent.

Postoperative convalescence during the time preceding suture removal was uneventful in all patients. Sometimes, there was tolerable discomfort mainly represented by swelling

and face hematomas. However, they disappeared completely within 3 weeks after reconstructive surgery.

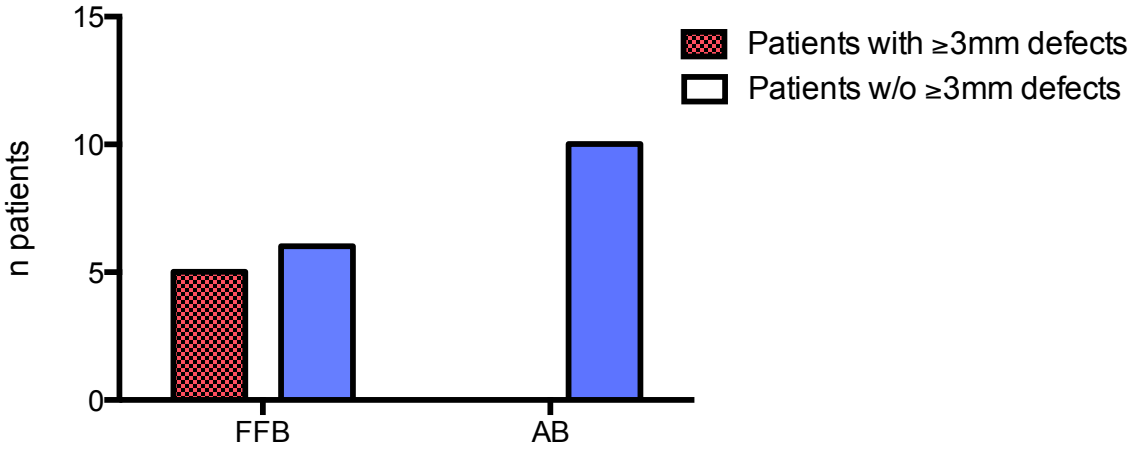
As a whole, based on 21 remaining patients at 5-years follow-up visits, a total of 72 implants were taken into consideration for the statistical analysis. Forty belonged to the test group and 32 to the control group.

	Number of patients		
	Control group + Test group	**Control group (AB)**	**Test group (FFB)**
Parma	9	4	5
Modena	7	5	2
Bologna	5	1	4
Total	**21**	**10**	**11**

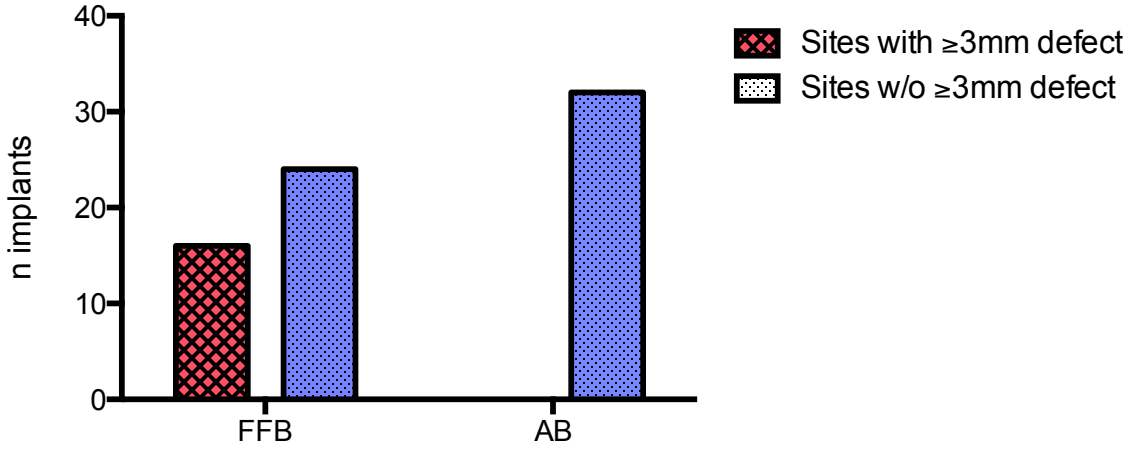
Re-graft

Necessity for re-grafting was evaluated as the presence of peri-implant defects, specifically of at least 3 mm. All these defects were detected at the time of implant exposure. As a matter of fact, a total of 16 defects were found at uncovering surgical procedures. Interestingly, all these defects referred to the FFB group. The management of these complications accounted for a surgical treatment by means of guided bone regeneration (GBR). To accomplish these procedures, deorganified bovine bone matrix

and collagen membranes were used. Another interesting finding was the absence of defects in the AB group at the time of second surgical procedures (Graph 1).This variable was analyzed using either implant or patient as statistical unit. For both, the Fisher's exact test was applied. When patient was used as statistical unit, the difference between groups was statistically significant ($p<0.035$). Worthy of note, when implant was used as statistical unit, the significativity was much more pronounced (Graph 2). Moreover, when the necessity for regrafting was evaluated in the light of bone radiological density, it was found that all re-grafts were performed when the density was inferior to 800 Hounsfield units. Since it was assumed that the presence of biomaterials (e.g. DBBM, collagen) would have influenced the medium-long term data upon implant-related biometric parameters, further analyses of data were conducted basing on 3 groups as 16 implants belonging to 5 patients were subtracted from the FFB group. In such a way, an additional group called regrafted-fresh frozen bone (FBR) was considered in the statistical analysis.



Graph 1

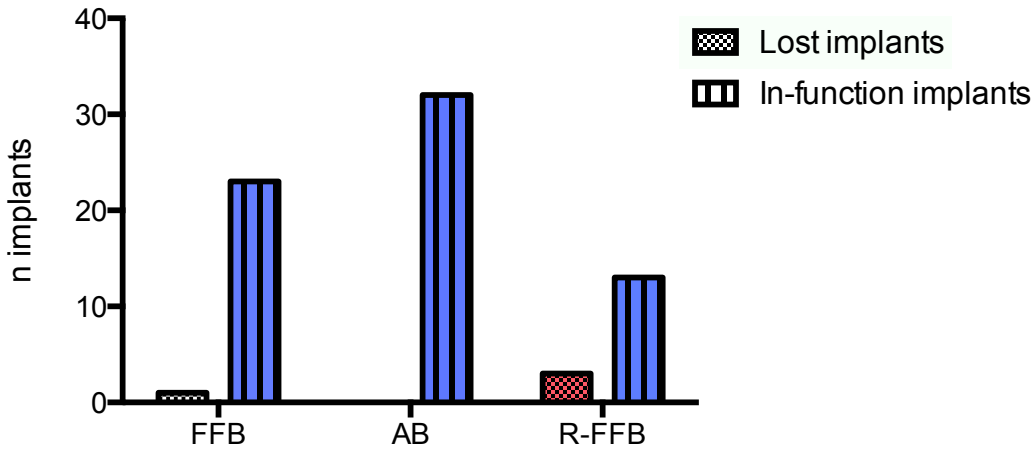


Graph 2

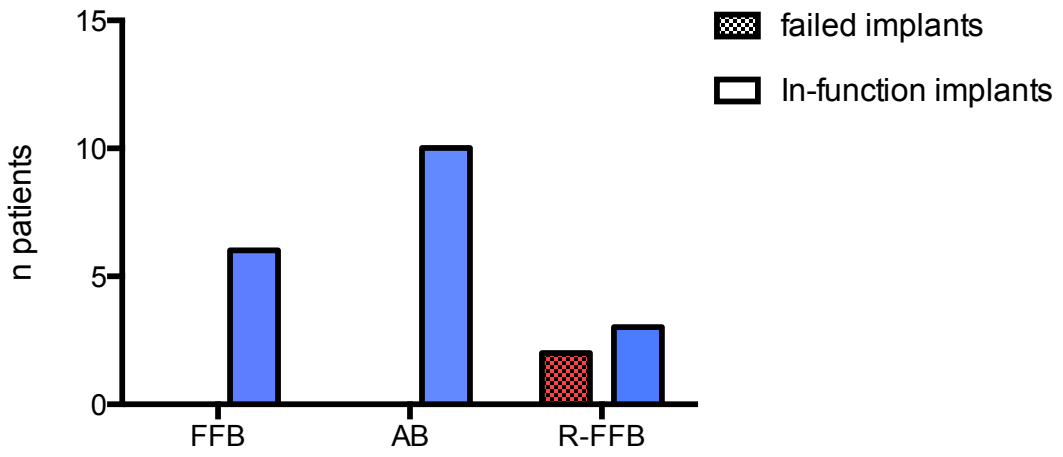
Implant survival rate:

At present day, after a follow-up period of 5 years, 4 out of 72 total implants were lost. More specifically, the whole number (4) of failed implants referred to the homologous bone. Every implant failure was encountered before the provisional loading. One lost implant, belonged to the FFB group and was removed within 6 months of implant placement as it showed clinical pain and mobility at control visits. After the removal, a small flap was advanced to obtain primary intention healing. Instead, the remaining 3 failed implants belonged to the group of regrafts (FBR). They were removed at the visit scheduled for implant loading, after 3 months from the regrafting surgery. However, it has to be highlighted that 5 out of 11 patients pertaining to the original homologous group (FFB+FBR) needed re-grafting. In particular 2 out of 5 patients relative to the FBR group lost implants (Graph 4). To be more precise, one of these patients lost 1 implant and

another one lost 3 implants ,of which, 1 was not regrafted. Interestingly, none of the remaining patients (6) in the FFB group lost implants after 5 years of follow-up. As a whole, only 1 (4%) out of 24 implants was lost in the FFB group; 3 (18%) out of 16 implants were lost in the FBR group whereas AB was characterised by no implant loss (Graph 3). The statistical analysis through the Fisher's exact test revealed a statistically significant difference among them (p=0.0251). To conclude, the cumulative implant survival rate after 5 years of follow-up was 82% in the FBR group, 96% in the FFB group and 100% in the AB group. On the other hand, when considering the original homologous group the survival rate was 90% even if the only one implant in the FFB and the 3 implants in the FBR represented,respectively, the 25% and 75% of the total implant loss. Since no failed implants have been observed in the following visits, further analyses at 5 years of follow-up were carried out on the basis of the remaining 68 implants.



Graph 3

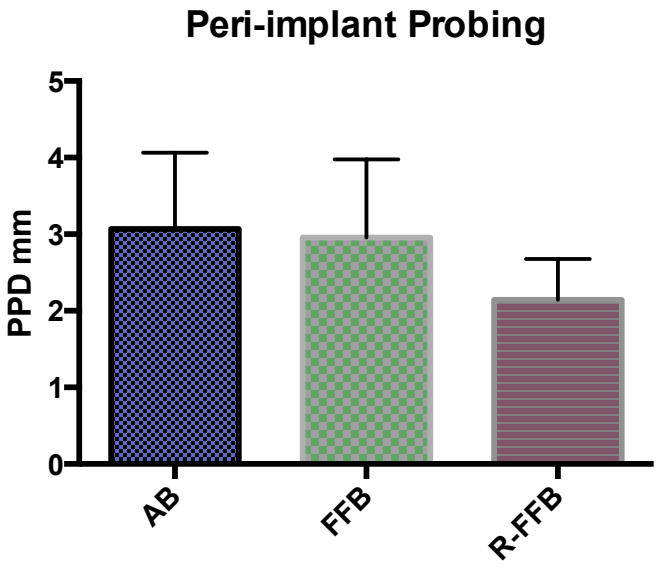


Graph 4

Probing pocket depth (PPD)

Probing pocket depth was evaluated using the technique named “midpoint probing”. Six measurements were taken for each implant. Since it was assumed that the bone grafts had mainly influenced the buccal side, only these 3 measurements were considered to calculate the average. Thus, each implant was matched to 1 probing depth which represented the mean value of 3 different measurements. The descriptive statistical analysis showed the following results: AB mean 3.07±1.00 mm (std error 0.18); FFB 2.96±1.02 mm (std error 0.21); FBR 2.14±0.53 (std error 0.14)(Graph 5). The inferential statistics was performed through ANOVA (Kruskal-Wallis test) which showed statistically

significant differences among groups. In particular, as regards FBR the difference was statistically significant with respect to both AB and FFB. On the contrary, no statistically significant difference was noticed when those last two groups were compared.

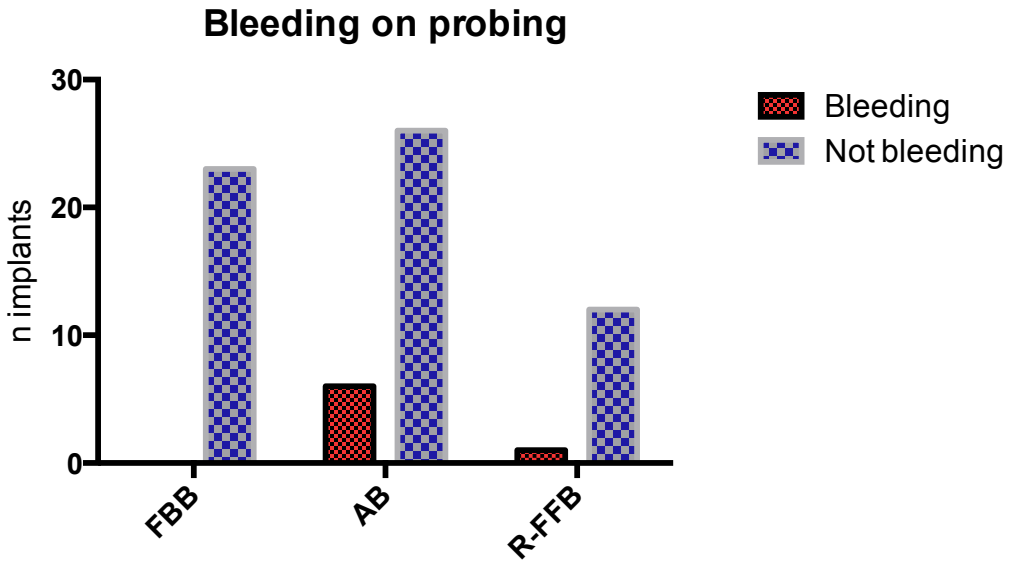


Graph 5

Bleeding on probing (BOP)

Bleeding on probing was analized using the implant as statistical unit (Graph 6). It means that in case one implant had just one affected site, it was allocated in the BOP+ cell. This parameter was considered as a dichotomous variable (yes/no); as a consequence the groups were compared using the Fisher's exact Test. (three-by-two table reported below). In conclusion, the differences between groups were not statistically significant (p= 0.06)

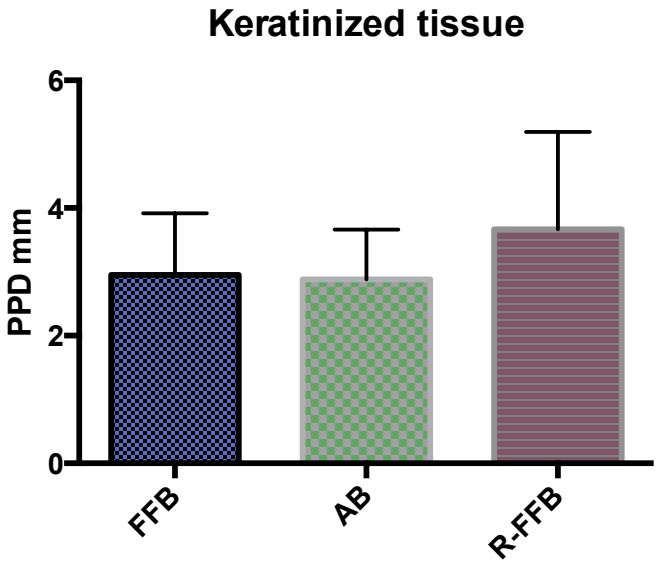
	BOP +	BOP-	total
AB group	6	26	32
FFB group	0	23	23
FBR group	1	12	13
total	7	61	68



Graph 6

Keratinized tissue (KT)

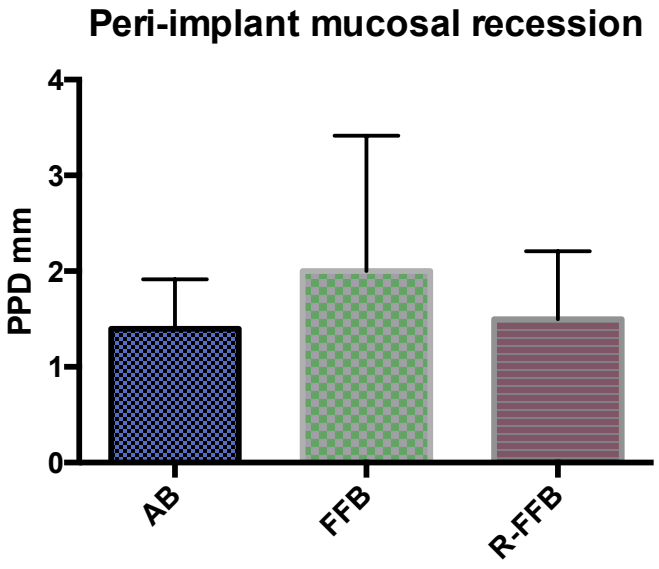
The average amount of keratinized tissue was 2.89±0.78 mm (std error 0.15) in AB group, 2.96±0.96 mm (std error 0.28) in FFB group and 3.67±1.53 mm (std error 0.88). In respect of this variable (KT) the groups were compared using the ANOVA (Kruskal-Wallis test). The statistical analysis revealed no significant differences among these groups as concerns keratinized tissue amount. (p=0.55) (Graph 7)



Graph 7

Peri-implant mucosal recession (PMR)

The peri-implant mucosal recessions were recorded as a single value relative to the most apical position of the gingival margin from the implant shoulder. In this case, implants were used as statistical units.The recession average was 1.40±0.52 mm (std error 0.16) in the AB group, 2.00±1.40 mm (std error 1.00) in the FFB group and 1.50±0.71 mm (std error 0.50) in the FBR group. The statistical analysis was performed by means of ANOVA (Kruskal-Wallis test). Finally, no statistically significant differences among groups were observed (p=0.81).



Graph 8

Discussion

Localized or generalized bone defects of the alveolar ridge, generally occur as a result of tooth extractions, periodontal disease and trauma sequelae. These events may provide insufficient bone volume or unfavorable vertical, transverse and sagittal inter-arch relationship. These circumstances might prevent implant placement in a prosthodontically driven position that, as such, is considered as correct from a functional and esthetic point of view. Nowadays the use of autologous bone block as grafting material is considered the gold standard in case of horizontal ridge augmentation (Misch & Misch 1995; Nowzari & Aalam 2007). Nevertheless, autologous bone grafts are also associated with some pitfalls at the donor site. They are mainly relative to the necessity of a second surgery such as: post-surgical pain, risk of paresthesia, and limitations in the quantity of available bone (Misch 1997; Cordaro et al. 2011). Moreover, vast graft resorption of the autogenous bone block can be another concern (Nystrom et al. 2009). In an effort to overcome these limits, it seems clear the necessity of alternative graft materials. One of the alternatives to autologous bone that has been proposed in literature is allogeneic (homologous) bone. Homologous bone is available as ready-to-use products which mainly differ one from another in respect to the processing procedures after harvesting. It seems to provide a reasonable source for grafting material without the need for a second surgical area (Goldberg & Stevenson 1987). Unlimited availability and reduced surgical time are additional benefits which make this material a plausible clinical alternative (Mankin et al. 1983; Spin-Neto et al. 2011a). In every case, the validity of homologous bone as feasible alternative to autologous bone is still a matter of discussion in the literature. In this regard, there is a paucity of evidence for what concerns both the biological and clinical behavior of homologous bone grafts, since the major part of the studies are case reports and case series. Above all, data are still lacking as regards

clinical outcomes over a follow-up of medium-long term (Contar et al. 2009). The aim of this study was to evaluate the clinical performance of fresh-frozen bone allografts derived from tibial hemiplateau of cadaver donors when compared to intraoral autologous bone grafts for the treatment of maxillary horizontal atrophies (Cawood and Howell IV) after 5 years of follow-up. To the best of our knowledge, at the present day, no studies on homologous bone grafts have reported clinical data as probing pocket depth, bleeding on probing, amount of keratinized tissue, peri-implant mucosal recession and necessity for regrafting.

First and foremost, it has to be pointed out that the previous part of the present multicentric RCT had analyzed histological and radiological features after 6 months from grafting surgery. On the basis of CT scan data, it was found that both AB and FFB groups underwent extensive resorption. The statistical analysis showed significant difference with FFB that lost twice the amount of bone with respect to AB group (52% ± 25.87 vs 25% ± 12.73). This finding is the highest resorption rate of allograft for horizontal ridge augmentation in literature. On the contrary Nissan et al. in 2011 found a resorption rate ranging from 10 ±10 % which is, in turn, the least resorption rate reported in the literature. At a first glance such high variability might not find clear explanations. However, it has to be considered that these authors do not report three-dimensional-evaluation neither after the grafting procedure nor after 6 months of healing. They based their assessment on panoramic and orthoradial periapical radiographs. Nevertheless, it should be considered that the resorption of bone grafts occurs in a three-dimensional and nonuniform pattern. Linear measurement techniques for the assessment of bone graft resorption can easily under- or overestimate the process, depending on the reference point and the region of interest (ROI) considered. Cone-beam CT scans taken at different time points with respect to the bone-grafting procedure, as performed in the current study, provide more accurate and reliable measurements of bone loss. Moreover, patients were treated by different

protocols (one stage vs two-stage) making the comparison not fully possible. As a consequence, these results should be considered with caution. Moreover, although it has been reported that the mean resorption rate among the studies amounted to 21.70±30.55 (Monje et al. 2014), the comparisons are often based on different grafts (e.g. as regards the donor site) with different surgical techniques (e.g. onlay, inlay, sinus augmentation etc…). According to these considerations, it can be stated that no conclusive data are still available upon the allograft resorption rates in horizontal ridge augmentation.

Besides, another finding of pivotal significance is the necessity for regrafting. To our knowledge, no previous studies have reported data concerning this specific complication in homologous bone grafts. Interestingly, it was found that 40 % of the implants placed in homologous bone needed regrafting procedure after 6 months of submerged healing. Such defects were observed at implant exposure without clinical signs of infection. In some way, it might be associated with the initial bone density at the time of grafting surgery. In this context, it has to be noticed that Lumetti et al. in 2014, on the basis of the same cohort of patients analyzed in the present study, reported that denser FFB grafts showed significantly less resorption than low-density grafts. Authors reported a critical value of 800 HU which might be associated with the behavior of FFB as regards resorption. Curiously, all the implants which needed regrafting were placed into FFB grafts of < 800 HU. At all events, no correlation was found in this study between radiological density of the grafts and necessity for regrafting. Nonethelsess, the density range of FFB and the sample size were relatively small. This fact may have influenced this finding. Therefore, further studies are needed to determine whether the initial graft density might influence the degree of resorption and/or ,specifically, the necessity for regrafting. Worthy of note, Deluiz et al. (2015) have recently investigated the resorption rate of FFB block allografts at several time points after the grafting procedure. The authors reported that after 8 months of healing the resorption rate could reach up to 70% (mean 50.78±10.43)

and was significantly higher when compared to 4 and 6 months of healing. At the same time we clinically found 16 vast bone defects (>3mm) at implant exposure, which means 12 months after the grafting procedure. According to this observation, we may state that the longer is the period of healing, the greater is the resorption rate of the allografts. Moreover, it seems that allograft resorption is rather unpredictable and progresses continuously over time. Since in all patients patients we did not find further implant failures and bone loss after implant loading, it may be supposed that implant loading can act on the bone trophism and maintenance over time. However, no data are available in the literature as regards the best time point to load the implants in this type of procedure.

On the other hand it has to be pointed out that in a relatively high percentage of cases (18%) even the use of bone substitutes was insufficient to modify the implant prognosis. Indeed, the efficacy of this procedure in FFB has not been investigated so far and should be considered as unpredictable until further data will be available.

Concerning the cumulative implant survival rate, we found that 90% of implants in homologous group (FFB+FBR) and 100% of implants in AB group were in function after 5-years of follow-up. These results are in line with those reported in the literature. Monje et al. in 2014 reported that the weighed mean implant survival rate was 23.9 months computed from 228 implants over a mean follow-up period of 23.9 months. However, only two studies reported results after 5 years of follow-up (Nissan et al. 2011; Novell et al. 2012). In both cases the authors reported just that implants were observed “up to” 5 years and neither important details as the number of patients or implants taken in consideration nor the criteria for their evaluation were described. Furthermore, they considered bone block allografts for both horizontal and vertical ridge augmentation. Noteworthy, Novell et al. found two cases of mucosal fenestrations between 2 and 3 years after the placement of dental implants but it is not clear wheter they considered such findings as failures. In addition, although the authors reported the percentage of implant survival, in most cases

the number and/ or the reason for failures were not mentioned. Noteworthy, when in this study the original homologous group was considered as FFB distinct from FBR the difference in survival rate was statistically significant. As a consequence we could speculate that implants requiring regrafting may have had a higher chance of failure. Unfortunately no data are still available to draw unequivocal conclusions.

In reference to implant-related outcomes, our analysis considered those diagnostic parameters that are mainly used to evaluate the health status of dental implants (Serino & Turri 2011; Schmitt et al. 2014). To the best of our knowledge, no previous studies in matter of fresh-frozen bone allograft for horizontal ridge augmentation have reported these data besides the importance of the medium-long term follow-up we considered. Specifically, probing pocket depth analysis showed a statistically significant difference among groups with FBR that showed lower probing depths with respect to both AB and FFB. However, the reason for this remains unclear as it could be somehow hazardous to affirm that different graft materials may have influenced this parameter even considering all the pitfaills connected to the measuring, recording and reporting probing pocket depth values (Lang et al. 1994; Schou et al. 2002; Abrahamson & Soldini 2006). On the other hand bleeding on probing showed no statistically significant differences among groups. BOP is well-recognized to be a valuable diagnostic indicator for monitoring the inflammation status of peri-implant tissues. Hence, this is of the utmost importance since peri-implant mucositis and peri-implantitis are essentially inflammatory disease which may cause bone loss and consequently dental implant failures. However it has to be noticed that several factors such as patient manual skills in cleansing procedures, patient habits (e.g. smoking), different types of prosthetic design, etc… may have influenced this finding. Accordig to this it is difficult, if not impossible, to draw convincing conclusions. At the same time no differences were observed regarding peri-implant mucosal recessions. It is worth noting that Henriksson et al. (2004) in a previous study performed with autogenous bone

block grafts, reported a mean decrease of the soft tissue margin of 1 mm in 55% of the cases during the first year of function. It should not be excluded that FFB block allografts may exhibit the same behavior, even if the slight differences among groups and the relatively small sample size may have played a key role in this analysis. On the other hand, keratinized tissue analysis deserve a special mention. Indeed, it has been hypothesized that a sufficient amount of attached mucosa might have a role in long-term stability of both bone and implants. According to this, Lin et al. (2013) in a recent systematic review of the literature found that implants surrounded by lining mucosa are more prone to plaque accumulation, peri-implant mucosal recessions, inflammation (considered as modified gingival index) and attachment loss. Otherwise, no statistically significant differences were found regarding bleeding on probing, modified bleeding index, GI, probing depth, and radiographic bone loss. The authors concluded that 1-2 mm of keratinized tissue are paramount for long-term maintenance. However, the relationship between keratinized tissue and peri-implant inflammation as well as peri-implant mucosal recession has not yet been demonstrated with certainty. In this study the amount of keratinized tissue was influenced by the surgical procedure as during implant exposure a slightly palatal incision associated with the buccal transposition of the flap were aimed at obtaining a sufficient amount of keratinized tissue surrounding implants. This fact may be responsible, to a variable extent, for the lack of statistical significance among groups. However, this variable showed no variations from baseline to the visit after 5 years, thus suggesting that different biomaterials might not have a role in determining this parameter. Interestingly, no correlation was found between the amount of keratinized tissue and, respectively, probing pocket depth, bleeding on probing and peri-implant mucosal recession.

Conclusions

This clinical investigation found that FFB block allograft undergo a significantly higher resorption rate after 6 months of healing when compared to autologous bone grafts. Furthermore, this resorption process continued over time resulting in a large numbers of bone defects > 3 mm to be treated by bone substitutes. Nevertheless, this further surgical procedure was unable to change the implant prognosis in a relatively high percentage of cases. Moreover all the implant failures observed in this study occurred in the group of homologous bone grafts. These shortcomings might prevent the employment of allografts for alveolar ridge augmentation in the next future. On the basis of the data reported after 5 years of follow-up and within the limits of this study, it can be concluded that fresh-frozen bone allografts should not be considered as a feasible alternative when compared to intraoral autologous bone grafts. Nevertheless, further studies are warranted to confirm these results.

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