DISSERTATION

Histologic assessment of two phycogenic bone graft materials used in sinus floor elevation

submitted by

Dr.med.univ. Dr.med.dent.
Alwin SOKOLOWSKI

for the Academic Degree of

Doctor of Medical Science (Dr.scient.med.)

at the

Medical University of Graz
Department of Dentistry and Maxillofacial Surgery
Division of Restorative Dentistry, Periodontology and Prosthodontics

under the Supervision of

Univ.-Prof. Dr. Martin LORENZONI

and Co-Supervision of

Univ.-Prof. Dr. Walther WEGSCHEIDER

2020

Statutory Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. Due acknowledgment has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the "Standards of Good Scientific Practice and Ombud's Committee at the Medical University of Graz."

Graz, 13.09.2020 Dr. med. univ. Dr. med. dent. Alwin Sokolowski

Disclosures

All co-authors have explicitly agreed in written form to the use of their data in this thesis. I confirm that I have obtained permission to reproduce figures and tables used in this thesis from the respective copyright holders with correct citation at the appropriate place. Throughout this thesis and in all related publications, I followed the "Standards of Good Scientific Practice and Ombuds Committee at the Medical University of Graz." Parts of this thesis have already been accepted for publication in the "International Journal of Oral Implantology," Quintessence Publishing under the title "Sokolowski AA[1], Sokolowski AA[1], Schwarze UY[2], Theisen K[1], Payer M[1], Lorenzoni M[1], Wegscheider W[1]. *Phycogenic bone substitutes for sinus floor augmentation: Histomorphometric comparison of hydroxyapatite vs. biphasic calcium phosphate in a randomized clinical pilot study. International Journal of Oral Implantology* (In press)"

[1] Division of Restorative Dentistry, Periodontology and Prosthodontics, Department of Dental Medicine and Oral Health, Medical University of Graz, Graz, Austria

[2] Department of Dental Medicine and Oral Health, Department of Orthopedics and Trauma, Medical University of Graz, Graz, Austria

[3] Division of Oral Surgery and Orthodontics, Department of Dental Medicine and Oral Health, Medical University of Graz, Graz, Austria

I confirm that I have obtained permission to reproduce figures and tables published from the respective copyright holders (Quintessenz Verlags-GmbH). The reproduced Figures are labelled with the citation (Sokolowski et al., in press)

Graz, 13.09.2020 *Dr. med. univ. Dr. med. dent. Alwin Sokolowski*

Acknowledgements

First and foremost, I would like to thank my supervisor and former head of our clinic, *Univ.-Prof. Dr. Walther A. Wegscheider*, Department of Dental Medicine and Oral Health, Medical University of Graz, Austria, for his encouragement, support and inspiration over the last years. I would also like to thank the other members of my thesis committee: *Univ.-Prof. Dr.med.univ. Martin Lorenzoni*, and *Mag.ª pharm. Corina Madreiter-Sokolowski, PhD* who always supported me during the course of this study. Many thanks also to my colleagues *Erika Hafner*, *Eva Lauinger* and *Maria Hartbauer*, who organized the study appointments for our patients and assisted in various procedures. Many thanks go to *Prof. Priv.-Doz. Dr.med.univ. Dr.med.dent. et scient.med. Michael Payer* who also performed the surgical interventions of our study. Special thanks go to our Co-authors *Dr. Kerstin Theisen* and *Uwe Yacine Schwarze, MSc.* for their valuable contribution and all the *participants* of our study, who had a lot of patience during the study procedures. I wish to thank *Prof. Dieter Bosshardt* and the Robert K. Schenk Laboratory, University of Bern, Switzerland, for kindly granting permission to use their scanning microscope, *Priv.-Doz. Mag. Dr. Dagmar Kolb-Lenz* and *Dr. Alexander Behlau* and the Core Facility Ultrastructure Analysis, University of Graz, Austria for the electron micrographs, and *Mag. Irene Mischak*, University of Graz, Austria, for the statistical analysis.

I also want to thank the *Medical University of Graz* and the *Doctoral School of Dentistry and Maxillofacial Surgery* for the financial support through salary, congress fees and publication fees.

Special thanks go to *AnnaKarin Lundgren, D.D.S., Ph.D.*, Director Global Scientific Affairs, Dentsply Sirona Implants and *Carin Höstner*, Investigator Initiated Studies Manager at Dentsply Sirona who were the main contact persons of Dentsply Sirona Implants, Mannheim, Germany, who supported our study in part with materials and third-party funds (I-BI-14-077).

Finally, I would like to thank my father *Dr. Adam Sokolowski*, mother *Małgorzata Sokolowski*, my brother and co-author of our accepted manuscript *DDr. Armin Sokolowski*, my brother *Alexander Scherer-Sokolowski* and my sister *Mag. Martyna McLaren* for their love, never ending support, encouragement and hard work. Without your support none of this would be possible.

Especially I want to thank my wonderful fiancée *Mag. Carina Radler* for her love, support and for making my life special in so many aspects.

Table of Contents

Abbreviations and Definitions

90/385/EEC	European directive on implantable medical devices
ARP	Alveolar ridge preservation
BCP	Biphasic calcium phosphate
BSE	Bovine spongiform encephalopathy
CBCT	Cone beam computer tomography
CONSORT	Consolidated Standards of Reporting Trials
DMS IV	Fourth German Oral Health Study
EN-ISO	European standards - international standards
EQUATOR	Enhancing the quality and transparency of health research
FDA	Food and Drug Administration
GBR	Guided bone regeneration
GCP	Good clinical practice
GPA	Greater palatine artery
GSP	Good scientific practice
HA	Hydroxyapatite
i.a.	Inter alia
ICH	International Conference on Harmonisation
IOA	Infraorbital artery
KAKuG	Federal Hospitals Act
KALG	Styrian Hospitals Act
MPG	Austrian Medical Devices Act
newBA	Newly formed bone area [mm²]
OHIP	Oral Health Impact Profile
oldBA	Old bone area [mm²]
PERV	Porcine endogenous retrovirus
PRF	Platelet-rich fibrin
Proc.	Processus
PSAA	Posterior superior alveolar artery
PTFE	Polytetrafluoroethylene
RCT	Randomized controlled trial
SBB	Split bone block
SFE	Sinus floor elevation
SPA	Sphenopalatine artery
ß-TCP	ß-Tricalcium phosphate
ß-TCP/HA	ß-Tricalcium phosphate/hydroxyapatite compound
TA	Tissue area [mm²]
VA	Vidian artery
VAS	Visual analog scale for pain

List of Figures

List of Tables

Abstract in German

Ziel: Für die Sinusbodenaugmentation kann eine breite Palette an Knochenersatzmaterialien verwendet werden. Diese Studie zielt darauf ab, ein phykogenes zweiphasiges Knochenersatzmaterial, das aus 80% ß-Tricalciumphosphat und 20% Hydroxylapatit (ß-TCP/HA) besteht, im Vergleich zu nahezu reinem phykogenem Hydroxylapatit (HA) hinsichtlich ihrer histologischen Merkmale drei und sechs Monaten nach der Sinusbodenelevation in einer randomisierten klinischen Studie zu bewerten.

Methoden: 20 Patienten, die eine zweistufige Sinusbodenaugmentation benötigten, wurden in die Studie aufgenommen und in eine ß-TCP/HA- und eine HA-Gruppe randomisiert. Die Sinusbodenelevation wurde unter örtlicher Betäubung und unter Anwendung der lateralen Fenster Methode und des gruppenspezifischen Transplantationsmaterials durchgeführt. Die Biopsien wurden drei Monate nach dem Knochenaufbau und während der Implantatinsertion sechs Monate nach der Augmentation in zwei benachbarten Bereichen entnommen. Zur histologischen Bewertung wurde pro Biopsie je ein nicht entkalkter Dünnschliff hergestellt und gemäß Laczko & Levai 1975 gefärbt. Alle 40 Schnitte wurden zur weiteren Bildanalyse mit einem Rasterlichtmikroskop gescannt. Die Parameter neu gebildeter Knochen, alter Knochen, Gewebe, Knochenersatzmaterial, Knocheninfiltration im Knochenersatzmaterial, Kontakt zwischen Knochen und Knochenersatzmaterial und Eindringtiefe wurden gemessen.

Ergebnisse: ß-TCP/HA zeigte nach sechs Monaten eine größere Menge an neu gebildetem Knochen als HA. HA zeigte nach sechs Monaten ein höheres relatives Verhältnis von Ersatzmaterial. Der Kontakt von neuem Knochen zu Knochenersatzmaterial war in der HA-Gruppe größer. Das von neuem Knochen umgebene und infiltrierte Knochenersatzmaterial zeigte in der ß-TCP/HA-Gruppe im Vergleich zur HA-Gruppe eine stärkere Veränderung und eine geringere Partikelgröße. Eine stärkere Resorption in der ß-TCP/HA-Gruppe führte zu einer geringeren Kontaktfläche des neuen Knochen zu Knochenersatzmaterial, jedoch zu einem höheren Infiltrationsverhältnis.

Conclusio: Die histologische Bewertung ergab in beiden Gruppen Zeichen einer vitalen Heilung. Das Augmentationsmaterial diente als Gerüst für die Knochenneubildung. Insbesondere konnte beobachtet werden, dass neu gebildeter Knochen bevorzugt durch die zerkleinerten ß-TCP/HA-Partikel wächst, was einen hohen Grad an Osteokonduktivität und Resorption des Transplantatmaterials zeigt. HA zeigte andererseits eine hohe Materialstabilität ohne signifikante Anzeichen einer Resorption, aber eine größere Kontaktfläche zwischen dem Ersatzmaterial und dem neu gebildeten Knochen nach sechs Monaten.

Abstract in English

***Aim**:* A wide range of bone substitute materials is used for sinus augmentation. This study aimed to evaluate a phycogenic biphasic bone graft material consisting of 80% ß-tricalcium phosphate and 20% hydroxyapatite (ß-TCP/HA) versus almost pure phycogenic hydroxyapatite (HA) in sinus floor elevation regarding their histologic features 3 and 6 months after surgery in a randomized clinical trial.

***Methods**:* 20 patients requiring two-stage sinus grafting were included in the study and randomized into a ß-TCP/HA and an HA group. Sinus floor elevation was performed under local anesthesia, utilizing the lateral window approach and the group-specific grafting material. Biopsies were taken at two adjacent areas 3 months after sinus augmentation and 6 months after augmentation during implant placement. For histological evaluation, one undecalcified thin ground section was obtained per biopsy and stained according to Laczko & Levai 1975. All 40 slices were scanned with a scanning light microscope for further image analysis. The parameters measured were newly formed bone area, old bone area, tissue area, bone substitute area, new bone infiltration area in bone substitute, new bone to bone substitute contact and penetration depth.

***Results**:* ß-TCP/HA showed a higher amount of newly formed bone after 6 months than HA. HA showed a higher relative ratio of residual material after 6 months. The contact area of new bone to bone substitute was higher in the HA group. Bone substitute surrounded and infiltrated by new bone showed a more severe alteration and smaller particle size in the ß-TCP/HA group compared to the HA group. More resorption in the ß-TCP/HA group led to a smaller new bone-to-bone substitute contact length but a higher infiltration ratio.

***Conclusion**:* The histological evaluation showed signs of proper healing in both groups; the augmentation material served as a guide for new bone formation. In particular, it could be observed that newly formed bone preferred to grow through the crushed ß-TCP/HA particles, showing in this case a high degree of osteoconductivity and resorption of the graft material. HA, on the other hand, showed high material stability without significant signs of resorption but a high contact area between the substitute material and newly formed bone after 6 months.

1 | Introduction

Natural human tooth loss is related to involution processes, geochemical and genetic factors, but the most important factors are still behavioral and environmental reasons, which a person has the most ability to impact during his or her entire lifetime (Hildebolt, C., Molnar, S., Elvin-Lewis, M. & McKee, J. 1988; Tiwari et al. 2016). Human permanent dentition loss has a very individual character. Factors such as nutrition, oral hygiene, facial injuries, and microevolution have been changing in a short period (Bołtacz-Rzepkowska 1987; Burt & Ismail 1986). The current demographic development is characterized by the aging of populations. The fourth German Oral Health Study (DMS IV) found that about 22.6% of 65- to 74-year-old patients are edentulous. Seniors have an average of 14.2 remaining teeth while younger adults have an average of 2.7 missing teeth; therefore, the replacement of lost teeth remains a high priority in modern dentistry (DMSIV 2006; Micheelis & Reich 1997). This need will be especially great on the European continent, where by the year 2025 every seventh citizen may be older than 60 (Irmen & Litina 2016; Kandelman et al. 1986; Künzel 1989; Mikulasek, Fuchs & Wisbauer 2018).

This development will lead to the growth of age groups with a higher need for social and medical care. Since the risk of oral diseases and tooth loss rises over a lifetime, these age groups will also place particularly high demands on dental clinicians (Cullinan, Ford & Seymour 2009; *Links between oral health and general health - the case for action* 2011; Rozas, Sadowsky & Jeter 2017). Data on the oral health status of adults are very dependent on the geographical population and may change with increasing health awareness and better health care (Heinrich-Weltzien 1997). It is assumed that dental health status is mainly correlated with personal lifestyle (Szulgan, Kurlej & Łagowska 2005). Additional variables affecting the time of tooth loss relate to gender, tooth class, and morphology of the teeth (Kurlej et al. 2006). In a recent cross-sectional study performed at the Medical University of Graz, we found that approximately 29% of adults aged 40 to 59 have experienced tooth loss in the posterior maxillary region (Sabitzer et al. 2018). In the age group of 60 to 91 years, more than 63% suffer from tooth loss in the maxillary posterior region. Due to their vital role in transmitting bite forces from occlusion to the skull base, the prosthodontic treatment of these regions is of great importance for the adjacent tissues, the patients' stomatognathic functions, and the physiological condition of the mandibular joint (McNeill 1997). Tooth loss after surgical extraction, trauma or periodontal inflammation, as well as inadequate prosthetic solutions

resulting in insufficient force distribution, can lead to increased levels of atrophy in the human jawbone and thereby negatively affect the long-term stability of the prosthesis and the functionality of the stomatognathic system (Brugnami, Caiazzo & Leone 2009). In many patients needing implant treatment in the maxilla posterior region, a sufficient bone width and height are missing due to progressive alveolar ridge atrophy or bone defects. Before implant placement, these deficient alveolar bone volumes must be enhanced with either autogenous bone grafts or bone graft substitutes (Tonelli et al. 2011). Due to the proximity to adjacent anatomic structures of the sinus maxillaris, sinus floor augmentation has been developed and found its way into the clinical routine as the standard technique to create a sufficient vertical bone volume in the posterior maxillary region (Michael S. Block & Kent 1997; Riben & Thor 2012; Smiler et al. 1992; Tatum 1986; Testori et al. 2010). Other techniques, including all kinds of different bone substitution materials, are used to augment lost bone volume. The appropriate treatment should be chosen after evaluation of the patient's age, medical history, and the individual morphology of the gap (Bechara et al. 2017; Jin et al. 2019; Küçükkurt, Alpaslan & Kurt 2017).

The following chapters will discuss causes of bone volume loss, different approaches for augmentation and regeneration of lost tissue in the posterior maxillary region, as well as different bone substitution materials, and will lead into a prospective study based on a randomized clinical trial comparing two promising bone substitution materials. In addition, older and recent national and international literature will be examined to gain an overview of materials and methods used in this field of medicine.

1.1 | Causes of bone loss

Bone tissue is one of the most fascinating composite materials in nature, consisting of a collagenous matrix with a network of living bone cells embedded in a hard extracellular tissue of hydroxyapatite crystals, which give bone its rigidity and strength, allow it to be sculpted into complex shapes, and enable it to be superbly sensitive to stress (Henderson & Kaiser 2018). The cells communicate via cell processes and are supplied via their blood vessel system (Schroeder 2000). The remodeling of bone is a fundamental process by which the mammalian skeleton tissue is continuously renewed to maintain the structural, biochemical, and biomechanical integrity of bone and to support its role in mineral homeostasis. Like any other bone of the body, the alveolar ridge is subject to constant remodeling. These processes are achieved by the cooperative and sequential work of groups of functionally and morphologically distinct cells, termed bone remodeling units, and vary within and among the different bone locations. They may change with age, underlying the mechanism of age-related bone loss (Orwoll & Adler 2018). This renewal and bone metabolisms are based on the activity of three morphologically and functionally distinct cells, the osteoblasts, osteocytes, and osteoclasts. Osteoblasts are always located on the bone surface above the osteoid and produce the collagenous and non-collagenous bone matrix components. They arise from stromal stem cells and progenitor cells that occur in the bone marrow, on bone surfaces, and also perivascular (Schroeder 2000).

Osteocytes exhibit protein-synthesizing activities and regulate the maturation and mineralization of the newly formed bone matrix. The young osteocytes arise from osteoblasts that are trapped in their freshly formed product. The more bone is newly embedded, the deeper the osteocytes get, becoming older, mature osteocytes that are responsible for osteolysis and osteoplasia and are involved in mineral metabolism (Aarden, Burger & Nijweide 1994).

Osteoclasts lie directly on the non-osteoid-covered bone surface, usually within the Howship's lacuna, and are responsible for the resorption processes in bone tissue (Schroeder 2000). They are produced by the fusion of hematopoietic, mononuclear progenitor cells derived from the bone marrow and are delivered to the bone via the bloodstream (Ash, Loutit & Townsend 1980). The differentiation lasts about 1-2 weeks, and their lifespan is less than 6 weeks (Marks & Seifert 1985).

These three types of cells perform bone remodeling, which is also regulated by many other factors, including hormones, nutritional status, humoral factors, biomechanical stress, and the autonomic nervous system's involvement, and it can lead to bone

attachment or breakdown (Ji-Ye, Xin-Feng & Lei-Sheng 2013).

Bone calcification is induced by calcitonin and estrogens; parathyroid hormone, osteoclast activating factor and prostaglandins promote bone resorption (Schroeder 2000).

Bone remodeling plays a particularly important role in alveolar processes, jaw growth, tooth eruption, and tooth replacement. Bone regeneration, which originates from both the periosteum and endosteal bone surfaces, leads to the restructuring of osteons and interstitial bones. The rate of renewal of the alveolar process seems to be higher than that of other bones (Covani et al. 2011).

Atrophy is generally understood as a reduction in the size of an organ or tissue. A distinction is made between simple atrophy, in which there is a decrease in cell size, and numerical atrophy, in which there is a decrease in the cell count. Furthermore, it is possible to differentiate between a physiological and pathological manifestation of atrophy.

After tooth loss, morphological changes and the physiological shrinkage of the alveolar ridge can be observed. This resorptive process is called inactivity atrophy (Schumacher 1994). Chewing forces are muscular forces that occur between the occlusal surfaces of the upper and lower jaw. Various values of physiological and maximal chewing forces are found in the literature. In some cases, they differ significantly from one another due to the methodology used in different study setups. In trials with various foods, chewing forces in the posterior region even reached up to 788 N. Of course, the maximum values depend on the individual characteristics of the subjects and are generally higher in men than in women (de Las Casas et al. 2007). Piezo force measuring elements are usually used as the measuring apparatus. If there is no physiological irritation due to tooth movement and chewing tension in the jawbone, periosteal osteoclastic activity gradually reduces the alveolar bone in height and width. This degradation is irreversible and is faster in the first few months to a year after tooth loss. These processes show higher values in the lower jaw than in the upper jaw (Tallgren 2003).

Under normal conditions, bone loss in the vertical direction is mainly caused by tooth loss. In contrast, absorption in the horizontal direction is primarily caused due to the influence of forces transmitted by the tongue and soft tissues.

Due to simultaneous resorption processes in the upper and lower jaw in edentulous patients, their intermaxillary relation to each other also changes. In doing so, the upper jaw tends to become narrower while the lower jaw tends to become wider; as a result, crossbite may develop over time (Setz & Körber 2007).

The severity of atrophy can be classified into six grades, according to Atwood, Cawood, and Howell (Atwood 1963; Cawood & Howell 1988). At grades 3 and 4, the bone in the lower jaw is reduced in height; in grades 5 and 6, there is already a horizontal degradation. This shows that the base of the jaw remains stable, but the alveolar ridge significantly changes. In the upper jaw, the progression of bone atrophy begins in the bucco-palatinal direction during grades 3 and 4 and ends in vertical height loss in grades 5 and 6. The progressive narrowing and the loss of height of the combs create more and more complications for the prosthetic restoration. The absence of teeth and increasing atrophy lead to a loss of the vertical dimension (Schmid-Schwap 2006).

In addition to the physiological resorptive processes after tooth loss, patients with complete dentures or partial dentures also experience further degradation of the jawbone. This is caused by the pressure of the prosthesis on its base, the alveolar ridge (Strub et al. 2011). In this so-called pressure atrophy, there is reduced blood flow through the affected tissues in the denture-bearing area so that after long-term exposure, increasing resorptions of bone can be observed (Sokolowski, Sokolowski & Wegscheider 2015). An incorrectly adjusted occlusion or a mucosa-supported restoration can cause particularly strong forces on the jawbone. The preservation of jaw bone tissue is vital for a permanent prosthetic rehabilitation and an essential indicator of the quality of the prosthetic solution.

The so-called combination syndrome serves as an example of unfavorable stress (Carlsson 2004). This concept was first introduced by Kelly in 1972. In a prospective setting over 3 years, he observed six patients, all wearing a full denture in the upper jaw and a partial denture with bilateral free-end saddles in the lower jaw (Kelly 1972). All patients showed a maxillary anterior bone loss, mandibular anterior incisor extrusion of 1-1.5 mm, and soft tissue augmentation in the maxillary tuberosity (Bassetti et al. 2010). According to Kelly, the combination syndrome begins with bone resorption at the anterior region, the weakest point in the maxillary arch. At the same time, there is bone resorption under the free-end saddles in the lower jaw-posterior area; as a result, the upper maxillary prosthesis raises anteriorly in a cranial direction, and the posterior of the prosthetic base lifts to caudal. The constant pressure of stable teeth on edentulous alveolar ridge areas in the edentulous region causes mild, moderate, and sometimes severe changes (Bassetti et al. 2010).

Animal studies have shown that continuous pressure, such as induced by a prosthesis, causes bone resorption if a specific threshold value is exceeded. The extent of bone resorption correlates with the intensity of the applied pressure (Imai et al. 2002).

In addition, absorption depends on other individual factors such as the presence or absence of teeth, the origin of tooth loss, the periodontal situation of residual dentition, the presence of parafunction such as bruxism and habits, intermaxillary jaw relation, the type of occlusion, and the type of prosthetic restoration (Ludwig & Niedermeier 2002; Tolstunov 2014).

According to Dudic & Mericske-Stern (2002), prostheses with resilient anchoring systems require significantly more relines than those with a rigid one. This suggests that resilient anchorage systems result, on average, in more bone resorption (Dudic & Mericske-Stern 2002). The progression of atrophy is also dependent on bone density. The rough estimation of the bone quality can be achieved by analyzing X-ray images. In 2003, based on Misch's classification from 1990, Engels defined four classes of bone which can be determined using the Hounsfield scale and divided into the groups D1-D4 (Engels 2003; Misch 1990). Bone density can be differentiated between the outer cortical bone layer and the inner, well-perfused cancellous layer. The relationship between compacta and spongiosa can be described by the quality classes defined by Lekholm and Zarb: There are four classes with the increasing dominance of cancellous bone. A higher class is less favorable from a prosthetic point of view than a lower one (Lekholm & Zarb 1985). Due to the hormonal osteoblast insufficiency starting at the age of around 45, osteoporotic changes in the jawbone accompanied by a physiological decrease in spongiosa density begin, and the so-called age-related osteoporosis starts (Marx et al. 2005). The cortical layer increases with age while the spongiosa layer decreases. Studies by Ulm et al. (2009) show that the bone volume and thickness of the trabecular bone in the frontal part of the jaws are significantly higher than in the posterior region. They attribute this to the fact that molars tend to get lost sooner than anterior teeth, and thus resorption processes and remodeling processes start earlier in this area (Ulm et al. 2009).

Good bone quality is essential in implant surgery to achieve excellent primary and secondary stability of the implants (Tanaka et al. 2018). Also, the method of bone augmentation has a severe impact on bone density (Kühl et al. 2012).

Bone atrophy is not the only reason for bone loss. Head and neck cancers are responsible for about 3% of all malignant neoplasms diagnosed in humans and may affect different anatomical structures such as soft tissue, bone tissue, and teeth (Mourad et al. 2017). The deformity and functional defects caused by neoplastic diseases and their surgical treatment may lead to different stages of bone loss accompanied by dysfunction and the impairment of vital functions like mastication, swallowing, and speaking (Rolski et al. 2016). Surgery and the supplementary use of radiation or chemotherapy, the

contemporary methods of treating tumors in the maxillofacial region, have become more and more effective (Budach et al. 2006; Zackrisson et al. 2003). Nevertheless, the treatment of resulting defects is a constant challenge for maxillofacial surgeons' rehabilitation of tissues and functions.

In addition, other surgical interventions for enucleations of jaw cysts and odontogenic tumors, as well as root resections, can result in only partial or no bone regeneration at all and create complications for a later implant treatment (Lalabonova & Daskalov 2013). Recent studies have shown that periodontal diseases with different types of pathological periodontal bacteria can significantly reduce total alveolar bone volume (Di Benedetto et al. 2013; Yang et al. 2018). In an animal study performed at the University of Washington, USA, seven 3-month-old pigs were periodically inoculated with four types of periodontal bacteria while fitted with a ligature around the last maxillary deciduous molar to induce periodontal disease. Segmentation of 3D cone-beam CT images was performed to quantify volumes of the total alveolar bone. A significant reduction of total alveolar bone volume could be measured after 8 weeks (Yang et al. 2018).

Periodontal disease is of polymicrobial and multifactorial pathogenesis since different types of bacteria are the initiators of the inflammatory process. Host innate immunity operates through recognition of the conserved molecular patterns on pathogenic bacteria. A network of cytokines leads to the activation of lymphocytes, but the progression of periodontal lesions is caused by dysregulation of molecules released by specific cell populations. Many of these secreted factors are involved in bone regulation and maintenance, and their imbalance leads to altered periodontal bone remodeling. Thus, enhanced osteoclast activity without an increase in bone formation occurs and drives the alveolar bone loss (Di Benedetto et al. 2013).

Likewise, it has been shown that several bacterial proteins from bacteria implicated in bone-remodeling pathology can inhibit osteoclastogenesis, which can also interfere with bone remodeling processes (Henderson & Kaiser 2018).

Another factor in bone loss is maxillofacial trauma. Bone tissue experiences the same atrophic processes after tooth loss through trauma, such as after tooth extraction. However, tooth loss is often accompanied by more severe destruction of the periodontium and the surrounding tissues. Especially after complex traumas, including bone fractures and injuries of adjacent structures like the sinus maxillaris or nasal cavity, the quality of following treatment options can be reduced and result in complications (Kim, Choi & Kim 2018).

1.2 | Anatomy of the maxilla and sinus maxillaris

The maxilla, also known as the upper jaw, has a central location and provides structural support to the viscerocranium. It has functional and aesthetic significance because of its fundamental role in facial architecture (Kühnel & Reichert 2015). It is a vital viscerocranial structure of the skull, and a paired bone that develops from a cover bone plate laterally attaches to the cartilaginous nasal capsule and is connected via the intermaxillary suture with its contralateral part. It is involved in the formation of the orbit, nose, and palate and holds the upper teeth. This bone is composed of a body, the corpus maxillae, and four projections: the proc. frontalis, proc. zygomaticus, proc. palatinus and proc. alveolaris. The body has a pyramidal shape and is the most significant part of the maxilla (Rohen & Lütjen-Drecoll 2006).

The maxilla contributes to the anterior margin and floor of the bony orbit, the anterior wall of the nasal cavity, and the inferior part of the infratemporal fossa. It contains the maxillary sinuses, which extend from the orbital ridge to the alveolar process and drain to the middle meatus of the nose. The infraorbital foramen is located underneath the orbital ridge and serves as a pathway for the infraorbital nerve and vessels.

The alveolar process is an inferior extension of the maxilla with a slightly porous structure and forms the maxillary dental arch, containing eight alveoli where the upper teeth are held. The frontal process has a vertical ridge that constitutes the medial border of the orbit. Together with the lacrimal bone, it forms the lacrimal groove posteriorly and is in close contact with the anterior ethmoidal sinuses mediocranially (Docherty 2012; Soriano & M Das 2020). The zygomatic process is located laterally and meets the zygomatic bone. The palatine process is a horizontal extension on the medial side of the bone constituting the roof of the mouth and the nasal cavity floor. Together with the palatine bone, it forms the hard palate that features a small process, the anterior nasal spine. The incisive foramen can be found on the median line just posteriorly to the incisor teeth where the incisive nerve and greater palatine vessels pass through. The raphe median divides the left and the right sides.

The maxilla articulates with numerous bones: superiorly with the frontal bone; posteriorly with the sphenoid bone, the palatine and lacrimal bones, and the ethmoid bone; medially with the nasal bone, vomer, and inferior nasal concha; and laterally with the zygomatic bone. All five parts of the maxilla undergo intramembranous ossification through two ossification centers.

Maxillofacial development starts in the fourth week of gestation during embryonic development with the formation of the five facial prominences around the stomodeum,

the primordial mouth, and the topographical center of the face (Wilson 1979). The first pharyngeal arch and neural crest cells form the paired maxillary, paired mandibular, and frontonasal prominence (Baxter & Shroff 2011). The stomodeum is limited cranially by the frontonasal prominence, laterally by the maxillary prominence, and inferolaterally by the mandibular prominence. The maxillary prominences give rise to the secondary palate, the majority of the maxilla, and the lateral upper lip (Mossey et al. 2009). The lower half of the frontonasal prominence gives rise to the nasal placodes, which develop into paired lateral and medial nasal processes divided through the nasal groove. At the end of week 6, the medial nasal processes fuse to form the philtrum. At the end of the eighth week, they combine with both maxillary processes to create the intermaxillary segment, the upper lip, and the primary palate. Specifically, the primary palate forms from a deep structure of the intermaxillary part known as the median palatine process. The lateral nasal processes form the nasal alae (Baxter & Shroff 2011; Soriano & M Das 2020; Wilson 1979).

The secondary palate begins to develop during the sixth week of fetal life. In the seventh week, one can differentiate between the maxilla and premaxilla, the jaw elongates, the tongue descends, and the palatal shelves acquire a horizontal position. In the third month, both parts fuse around the area of the alveolar process, after which the premaxilla becomes the anterior part of the maxilla.

The midline forms the secondary palate and fuses anteriorly to the primary palate and nasal septum, followed by replacement of the palatal mesenchyme by muscle and bone that correspond to the hard and soft palate. At the central fusion point in between the primary and secondary palate, the nasopalatine canal forms and posteriorly becomes the incisive canal (Lake et al. 2018). The fusion of the palate is completed by the tenth week and fully forms by the twelfth week of embryonic development. The paranasal sinuses and maxillary sinuses are relatively small in newborns and become larger during the development of the maxilla and other skull bones.

The maxilla's blood supply leads through branches of the maxillary artery, which is a terminal branch of the external carotid artery. It originates posterior to the upper portion of the ramus mandibulae, runs anteriorly through the inner side of the mandibular ramus, enters the pterygopalatine fossa, and terminates as the pterygopalatine artery. It consists of three major segments, the mandibular, pterygoid, and pterygopalatine parts. The pterygopalatine segment is the main blood supply of the maxillary region. It is in close relation to the pterygopalatine fossa, where it branches into five vessels: the posterior superior alveolar artery (PSAA), infraorbital artery (IOA), greater palatine

artery (GPA), sphenopalatine artery (SPA) and Vidian artery (VA) (Tanoue et al. 2013). The PSAA runs toward the zygomatic process, has a prominent curve on its inner surface and courses toward the maxillary tuberosity with branches supplying the upper molars and premolars. The VA is a recurrent branch and courses posteriorly to enter the Vidian canal, supplying the mucosa of the pterygopalatine fossa and nasopharyngeal cavity. The IOA runs along the posterior wall of the maxillary sinus and enters the inferior orbital fissure and infraorbital canal, supplying the lacrimal sac, upper incisors, canines, and mucous membrane of the maxillary sinus. The GPA emerges near the PSAA and descends through the greater palatine canal to exit through the greater palatine foramen and supply the hard palate. The SPA is the terminal branch of the pterygopalatine segment and enters the nasal cavity posterior to the nasal turbinates to supply the nasal septum and turbinates (Alvernia et al. 2017; Otake, Kageyama & Mataga 2011; Tanoue et al. 2013). The posterior septal branch of the SPA courses through the incisive canal to form an anastomosis with the GPA. The maxillary sinus is supplied by the PSAA, IOA, GPA, and SPA, so it is essential to determine the sites of the IOA and PSAA for surgical planning as they anastomose and form a double arterial arcade that surrounds the maxillary sinus (Danesh-Sani, Loomer & Wallace 2016). Damage to these vessels can lead to excessive bleeding during surgery, so detailed knowledge of maxillary anatomy is essential for all kinds of surgical interventions involving these structures. Severe bleeding after sinus floor elevation using the transcrestal technique is a rare but clinically significant complication that can be handled accordingly during surgery. But it could lead to a potential risk if left unnoticed (Jensen, Eriksen & Schiodt 2012; Solar et al. 1999). The maxillary sinus is the largest of the paranasal air-filled spaces and has a pyramid-shaped form with a mean volume of 12.5 ml (Gosau et al. 2009). The size, shape, and thickness of the buccal bone wall vary among the population and also between the two sides of an individual.

The respiratory mucous lining that covers the inner part of the maxillary sinus, the Schneiderian membrane, is named after Conrad Victor Schneider (1614-1680), a German physician and anatomist from Wittenberg, Germany (Schneider 1660). Histologically, it is a bilaminar membrane with pseudostratified ciliated columnar epithelial cells on the inner side and periosteum on the osseous side (Kalyvas et al. 2018). Mesenchymal stem cells of the sinus membrane have the potential to form bone, which plays a significant role in sinus floor elevation procedures (Kim et al. 2009). The thickness of the Schneiderian membrane is approximately 1 mm; however, in everyday clinical practice, variations are commonly found and can reach up to 4 mm

physiologically (Kalyvas et al. 2018; Monje et al. 2016). Adjacent periodontitis, chronic inflammations, and smoking, as well as the presence of maxillary septa, can also result in a thickening of the sinus membrane. These are frequent anatomic variations of the maxillary sinus that present as walls of cortical bone that reach into the maxillary sinus and, while mostly asymptomatic, may increase the difficulty of surgical interventions and the risk of perforation (Rancitelli et al. 2015). The septa shape has been described by Underwood as an inverted gothic arch arising from the inferior or lateral walls and can divide the sinus into two or more cavities (Underwood 1910).

Due to the anatomical relationship between the maxillary sinus floor and the posterior teeth, this area is a constant challenge for the dental practitioner, in particular for nonsurgical and surgical endodontics, but also for extraction, surgical removal, prosthodontic treatment and regenerative surgery, as many patients who require implants in the posterior maxilla lack horizontal and vertical bone quantity due to advanced ridge resorption or bone defects (Von Arx, Fodich & Bornstein 2014; Fry et al. 2016).

1.3 | Bone augmentation techniques

Successful prosthetic therapy with dental implants relies on adequate bone quality and quantity to ensure long term stability (Brugnami, Caiazzo & Leone 2010). Since in many cases a minimum bone width and height for implant placement do not exist, these deficient alveolar bone volumes must be enhanced with either autogenous bone grafts or bone graft substitutes before implant placement (Tonelli et al. 2011). Depending on the progression and severeness of the bony defect, different methods and techniques may be used in modern dentistry.

Alveolar ridge preservation (ARP) therapy is used to prevent the extensive alveolar ridge resorption after tooth extraction that is initiated by a cascade of biological events that result in alterations of the homeostasis and structural configuration of the existing periodontal tissues. ARP has been described either as a part of immediate implant placement interventions or a means of reducing the need for complicated ridge augmentation prior to or at the time of delayed implant placement (Avila-Ortiz et al. 2020).

Osburn first described this method in 1974, and since then numerous modalities have been described and tested in different clinical settings (Avila-Ortiz et al. 2020; Osburn 1974). During a necessary tooth extraction, trauma should be minimized, and bone preservation should receive careful attention. Literature has shown that early bone loss can be significantly reduced by socket grafting. However, failure or success of this technique is dependent on revascularization and remodeling of the grafted material into a vital, load-bearing bone (Allegrini et al. 2008). In most described cases, patients receive grafting material into the alveolar socket of the extracted tooth, which is then covered using xenogeneic resorbable collagen membrane or autologous soft tissue gathered using the palatal gingival graft technique (Kim et al. 2017; Puri et al. 2019).

While this technique can enhance the bone situation in patients with planned tooth extractions, patients with healed ridges and bone deficiencies require more drastic therapies. For horizontal and small vertical defects, guided bone regeneration (GBR) is commonly used before, or in combination with, the installment of titanium implants (Elgali et al. 2017). GBR is a reconstructive procedure in which a bone graft material is packed onto a bone defect and stabilized using a membrane that may be held in place using titanium pins or tight suturing. The principles of guided bone regeneration include cell exclusion of gingival fibroblasts and epithelial cells, tenting of a space in which the bone graft material can be filled, scaffolding characteristics of the graft material, and

stabilization and protection of the clot from disturbance of movement (Dahlin et al. 1989; Farzad & Mohammadi 2012; Schenk et al. 1994; Wang & Carroll 2001).

To exclude non-osteogenic tissues from interfering with bone regeneration, the bone graft is covered by the application of a membrane. Different modifications of the physicochemical and mechanical properties such as thickness and porosities of membranes may promote bone regeneration. The desired characteristics of the membrane used in GBR therapy include biocompatibility, cell-occlusion properties, clinical manageability, space-making ability, and compression of the bone graft material (Liu & Kerns 2014; Sam & Pillai 2014). In general, non-resorbable synthetic materials such as polytetrafluoroethylene (PTFE) or titanium-enhanced membranes, as well as resorbable membranes such as porcine or bovine collagen, are widely used for this purpose. However, non-resorbable membranes bear the disadvantage of requiring a second surgical intervention for membrane removal (Elgali et al. 2017). The evolution of membranes used in GBR ranges from barrier membranes with antimicrobial activity, bioactive calcium phosphate incorporation, membranes with growth factor release, and multilayered barrier membranes (Sam & Pillai 2014). Also, autologous membranes that are obtained using the platelet-rich fibrin (PRF) technique developed by Choukroun et al. have found their way into GBR (Choukroun et al. 2006).

So-called three-dimensional alveolar ridge defects that show combined horizontal and vertical bone loss are challenging procedures in dental implantology. However, surgical procedures involving autogenous bone grafts offer a predictable and low-risk treatment option to these patients. Harvesting autogenous bone grafts intraorally became a common and safe surgical technique within the last 15 years (Khoury & Hanser 2015; Khoury & Khoury 2006; Sakkas et al. 2016). Autogenous bone block grafts are utilized with or without membranes using different techniques and approaches to reconstructing missing alveolar volume (Khoury & Hanser 2015; Stimmelmayr et al. 2014), However, soft tissue necrosis with graft exposure as well as inadequate revascularization of the mandibular cortical graft, leading to increased resorption of the grafted area, is still a common risk of these procedures (Khoury & Hanser 2015). Good coverage of the augmentation site with tension-free soft tissue and proper suturing technique are of great importance to avoid wound dehiscence and possible loss of augmentation (Kang et al. 2019).

For favorable long-term prognosis after vertical ridge augmentation, a sufficient recovery period is recommended before implant placement to ensure proper bone formation (Kang et al. 2019). However, recent studies evaluating the split bone block (SBB) technique in combination with sinus floor elevation for vertical bone augmentation in the posterior

maxilla suggest using simultaneous implant placement, which allows an acceleration of transplant revascularization, graft regeneration, and a shortening of the patient treatment time (Khoury & Hanser 2019). In this technique, autogenous bone blocks are harvested from the mandibular retromolar region and split longitudinally with a diamond disk. After performing a sinus floor elevation, the bone blocks are stabilized horizontally and vertically using microscrews. Gaps and the remaining crest are filled with autogenous bone chips. The authors suggest a healing period of at least 3 months before implant placement (Khoury & Hanser 2019).

To create a sufficient vertical bone volume for implant placement in the posterior maxillary region in patients with enough horizontal bone volume, the sinus floor augmentation was developed and became part of clinical routine as the standard technique (Michael S. Block & Kent 1997; Riben & Thor 2012; Testori et al. 2010).

Boyne and James reported the first approaches to the sinus floor elevation technique in the 1960s to 1980s. They described a lateral window procedure with a two-stage setup in which the augmentation was performed using autogenous particulate iliac bone. Blade implants were placed 3 months later to support fixed or removable reconstructions (Beaumont et al. 2008; Boyne & James 1980).

In 1976, Tatum initially presented the lateral window technique at a congress in Birmingham, Alabama (Chanavaz 1990; Pjetursson & Lang 2014; Tatum 1986). The transcrestal approach with subsequent placement of implants, in which a set of tapered osteotomes with increasing diameters is used to elevate the sinus floor and increase bone density and thus results in higher primary stability of the inserted implants, was described by Summers in 1994 (Summers 1994). This technique was described with and without bone substitution but should be reserved for those patients with enough horizontal bone volume and a reduced residual bone height of 5 mm or more (Felice et al. 2013; Pjetursson et al. 2008; Tan et al. 2008).

The primary surgical principle and technique of the lateral window approach have not changed significantly, although variations exist (M S Block & Kent 1997; Riben & Thor 2012; Smiler et al. 1992; Testori et al. 2010). Intraoral access to the maxillary sinus is gained through the oral mucosa in the region of the anterior maxillary sinus wall by performing trapezoid crestal and vertical incisions. A bony window is prepared using diamond burs. The sinus membrane is dissected and lifted from the sinus floor to enable the insertion of a graft into the newly created secluded space (Artzi et al. 2001; Riben & Thor 2012). The buccal window size is analogous to the area needing augmentation and to the planned number of implants (Saccardin & Kühl 2018). Due to the different buccal

bone thickness of the maxillary sinus, the cranial osteotomy often needs to be deeper than the caudal osteotomy (Yang et al. 2009).

The bony window can be kept attached to the membrane and elevated superiorly or removed completely using the wall-off technique (Saccardin & Kühl 2018). The surgery is commonly performed under local anesthesia. There are two main lateral sinus augmentation procedures to be distinguished: A one-step procedure followed by immediate implantation and a two-step procedure followed by delayed implantation. The requirement for a single-stage procedure is a minimum bone height of approximately 5 mm or more in the region of the planned implantation to ensure a high success rate (Felice et al. 2013). In the two-step procedure, on the other hand, the maxillary sinus membrane is lifted in a first engagement through a buccal bone window. The resulting space is filled with a bone substitute material. After a healing period of about 3 to 6 months, the implantation is carried out in the augmented area as the second engagement (Beaumont et al. 2008; Wildburger et al. 2014). The surgical technique of sinus floor elevation has developed over time, and several minor variations now exist. Special elevation tools and instruments for piezo surgery have been developed to reduce the risk of membrane perforations (Jordi et al. 2018).

In a biomechanical comparison, Küçükkurt et al. evaluated sinus floor elevation and alternative treatment methods using the finite element method. The results of this study indicate that LSE should be the first choice among treatment options. Based on the results obtained under conditions of oblique force, vertical force, and excessive stress, short implants and tilted implants did not reach the desired values for lasting functionality. Also, every effort should be made to avoid distal cantilever extensions, which provided the least successful results among the alternative treatment methods based on the model simulations (Küçükkurt, Alpaslan & Kurt 2017).

1.4 | Types of bone augmentation material

Particular specifications are desirable for a substitution material to meet the requirements as a replacement to bone tissue. It should be biocompatible, osteoconductive, osteoinductive, bioresorbable, structurally similar to bone, porous, mechanically resistant, easy to use, safe, and cost-effective (Faour et al. 2011). Various grafting materials have been applied in regenerative surgery, and a broad majority of the available materials are osteoconductive, but only very few offer osteoinductive properties. These include allografts, animal and plant-derived xenografts, and alloplastic materials, as well as combinations of various materials (Cordaro et al. 2008; Hallman, Sennerby & Lundgren 2002; Schlegel et al. 2003). Considering the specifications of an ideal material, autogenous bone grafts are still the gold standard in bone regeneration surgery due to their osteoinductive potency (Dumitrescu n.d.; Hallman & Thor 2008; Tonelli et al. 2011). As harvesting sites, extraoral (iliac crest, skull, ribs, tibia) and intraoral (linea oblique mandibulae, ramus mandibulae, maxillary tuberosity, symphysis region) donor regions are preferred (Brugnami, Caiazzo & Leone 2010; Jakse et al. 2001; Schaller 2009). Autologous bone contains osteoinductive substances such as bone morphogenetic proteins (BMP) and osteogenic cells that accelerate new bone formation. However, the osteogenic potential may vary with patient age, systemic diseases, harvesting location, and particle size. Another significant advantage is the absence of immunologic resistance or rejection problems and disease transmission risk due to its autologous nature (Jakse et al. 2001; Saikia et al. 2008; Tilkeridis et al. 2014). Depending on the area of harvesting autogenous bone, a second surgical site may be needed, and the donor site may be accompanied by comorbidity (Pieske et al. 2009). The graft may also tend toward higher resorption during the healing period than deproteinized xenogeneic material (Yildirim et al. 2001). Long-term radiological investigations also show that, especially in larger bone defects, if an autologous bone is used alone, its influence on the success of the implants is still unclear (Kirmeier et al. 2008; Sbordone et al. 2013; Tetsch, Tetsch & Lysek 2010). Allogenic bone, though meeting the mechanical and biological requisites for a substitution material, has a minimal risk of transmitting disease such as HIV or hepatitis B and C (Delloye et al. 2014; Gunzburg et al. 2002; Khan et al. 2005; Mroz et al. 2008; Zimmermann & Moghaddam 2011). Moreover, the complex treatment, sterilization process, storage, and the resulting high cost of such materials must be considered. Another alternative is the use of xenogeneic material derived from animal sources; however, especially in bovine or porcine substitutes, the same limitations regarding the risk of immunogenicity problems and disease transmission exist, even if the risk is

estimated to be very low and generally concerns porcine endogenous retrovirus (PERV) and bovine spongiform encephalopathy (BSE) (Campana et al. 2014; Laurencin & El-Amin 2008). Additionally, ethical or religious factors, as well as patients' acceptance, may play a role when considering such materials for regenerative surgery (Offner et al. 2019; de Vries et al. 2008). The usual main component of xenogeneic bone graft materials is hydroxyapatite (HA), which is part of the apatites, crystalline compounds that are the primary mineral components of teeth and bones. HA can be derived from the mineral part of bovine or porcine bone material, which is deproteinized, cleaned with strong alkaline solutions, and sterilized at a high temperature (Aghaloo & Moy 2007; Orsini et al. 2005). Synthetic HA can be made by the precipitation of calcium nitrate and ammonium dihydrogen phosphate (Saikia et al. 2008). Coral-based substitutes were approved by the Food and Drug Administration (FDA) in 1992, and due to their structure they can be used in their original state, which allows faster resorption, or can be transformed industrially into HA (Chai et al. 2011; Chiroff et al. 1975). Coralline HA is osteoconductive, shows an excellent bone-bonding capacity, and is unlikely to promote disease transmission or the risk of deep infections (Bucholz, Carlton & Holmes 1989; Khan et al. 2005).

Phycogenic (algae-derived) HA is a nonanimal, biological material derived from the calcium-encrusted marine algae *Corallina officinalis*. It is prepared by hydrothermal conversion of calcium carbonate in the presence of ammonium phosphate, which helps to preserve the algae's columnar structure and porosity (Thorwarth et al. 2007). Even though all organic components are eliminated in this process, samples of adherent human bone have nevertheless revealed evidence of resorption, presumably due to fragments of residual molecules getting incorporated into the crystals (Ewers et al. 2004). HA is highly biocompatible and does not show any inflammatory reaction when implanted (Ghosh et al. 2008; Koshino et al. 2001; Nandi et al. 2008; Okazaki et al. 2000). The interconnecting pores of natural HA show a porous trabecular structure that acts as a scaffold for the penetration of vital bone without being resorbed but lacks the properties of osteoinductivity. It requires a longer healing time before the recommended implant placement (John & Wenz 2004; Schlegel et al. 2003; Yildirim et al. 2001). The resorption speed is generally considered very slow, which allows slow bone ingrowth and cell colonization. The material is usually still intact 3 years after implantation (Spivak & Hasharoni 2001). HA offers excellent mechanical properties and high compression resistance and is considered basically non-resorbable (Fujita et al. 2003; Liljensten et al. 2003). However, studies have demonstrated higher levels of bioactivity for its phycogenic variant, including gradual but complete replacement by new bone despite its apatite

nature (Ewers et al. 2004; Spassova et al. 2007). HA can be used alone for small bone defects with low loading conditions (Fernandez de Grado et al. 2018). *β*-tricalcium phosphate (*β*-TCP) found its way into orthopedic and dental regenerative surgery more than 25 years ago and has been used as a bone substitute ever since (Galois & Mainard 2004; Galois, Mainard & Delagoutte 2002; Malhotra & Habibovic 2016; Sai & Fujii 2005). Its specifications show high biocompatibility and bioresorption that is similar to the inorganic phase of bone and is therefore meant to be wholly resorbed over time by osteoclasts (Campana et al. 2014; Chai et al. 2011; Daculsi et al. 1990; Gaasbeek et al. 2005; LeGeros et al. 1988). Calcium phosphate materials are not per se osteoinductive but can be made to behave that way through certain geometrical and topographical features and combinations of macro- and microporosity, with concavities favoring entrapment and concentration of circulating growth factors or osteoprogenitor cells (LeGeros 2008). *β*-TCP has a porous structure which depends on the processing conditions and which plays a vital role in its osteoconductive characteristics (Frayssinet et al. 2000). Its interconnected pores may accelerate bone remodelling by facilitating the colonization of osteogenic cells and nutrients via enhanced capillarity, and it seems to have the potential to influence angiogenesis (Gunzburg et al. 2002; Malhotra & Habibovic 2016). Although it has the desirable qualities of fast remodelling and high biocompatibility, its mechanical properties are inferior to cancellous bone or other substitute materials.

Additionally, its resorption rate appears to be too high for persistent volume stability, especially during the first weeks after implantation. Therefore β-TCP should be used selectively (Gouin et al. 2010; Roberts & Rosenbaum 2012). By combining HA with β-TCP, it becomes possible to capitalize on the advantages offered by both materials, as the more osteoconductive HA has been shown to yield significantly larger amounts of new bone in conjunction with the more soluble β-TCP than pure HA alone (Galois & Mainard 2004; Schopper et al. 2005). These so-called biphasic calcium phosphates (BCP) offer a bone substitute material that combines the positive effects of the two materials (Bansal et al. 2009). Studies suggest that BCP provides an added regenerative impact in promoting the clinical resolution not only in regenerative bone surgery but also in patients with intrabony defects with periodontitis (Bansal et al. 2014). Different HA/*β*-TCP ratios can be associated with different bone regeneration responses. A model of corticocancellous defects in sheep's ribs has been used to compare almost pure phycogenic HA and two BCP biomaterials with HA-to-*β*-TCP ratios of 50:50 or 30:70, all having the same three-dimensional geometry (Schopper et al. 2005). Both the formation of new bone and

scaffold resorption were significantly more pronounced in the BCP groups than in the HA group. Still, no significant differences were seen between the two BCP variants. The study did confirm the utility of HA/*β*-TCP compounding in improving bone formation and scaffold resorption while, at the same time, preserving the osteoconductive properties of the scaffolds. Effects from the porosity of a graft material have been shown to arise from pore geometry, pore size, and material density (Rateitschak, KH., Wolf 2012; Spassova et al. 2007). Different preparation methods such as a compact form or a porous form with interconnected macropores, equivalent to cancellous bone, are available, which provide enhanced osteogenic properties to the material (Jarcho 1981).

Histomorphometric comparisons between bone grafts concerning new bone formation, resorption characteristics, and other parameters are always useful to gain more insights into the broad diversity of substitution materials (Nishimoto et al. 2019). Few studies have compared the histological behavior of different calcium phosphate-based materials of phycogenic origin in SFE grafts. In SFE, the fundamental question arises as to whether filling materials should be used at all. Certain techniques suggest the use of platelet-rich fibrin or absorbable gelatin sponge alone for the stabilization of the membrane in SFE (Aoki et al. 2018; Sohn et al. 2010). By maintaining the prepared cavity through the inserted implants, in the sense of tenting the Schneiderian membrane, a space between the crestal bone and the periosteal layer is created where blood coagulum and new bone formation can develop (Chen et al. 2007; Hatano, Sennerby & Lundgren 2007; Linde et al. 1993). The osteogenic potential of the Schneiderian membrane may be the main reason for the successful forming of bone with this augmentation technique (Riben & Thor 2012). Nevertheless, an overview with a direct comparison indicates that implant placement after sinus floor elevation without the use of bone augmentation material shows a lower survival rate (96.0%) after 48 to 60 months than in those in whom graft material was used (99.6%) (Silva et al. 2016). Whether autologous bone or bone substitution material was used had no significant influence on the survival rate of the implants (Chiapasco, Casentini & Zaniboni 2009; Jensen & Terheyden 2009). However, the delayed bone regeneration in the first 9 months when using bone substitution material and the healing period of up to two times longer in the two-stage procedure must be taken into account (Handschel et al. 2009). Independent of the selected augmentation material, it was also observed that the augmentation volume could shrink up to approximately 15 to 20% after 6 months (Kühl et al. 2014, 2015).

1.5 | Histologic considerations

In optical microscopy, various tissue components can be identified by a wide variety of selective staining techniques, which can give the different parts characteristic colors (Hayat 1993). Generally, the specimen is stained more than once to achieve adequate differential between the various components. In any attempt to analyze the factors that may influence staining results, one must consider all of the treatments the tissue has been given before or following staining. The outcome is also dependent on the type of fixation and dehydration employed before staining (Hayat 1993). For staining cartilage and bone, the procedure described by Laczko and Levai is widely used to differentiate the components of specimens (Laczko & Levai 1975). Bone morphometry allows a quantitative evaluation of bone microarchitecture, bone remodeling, and bone formation and provides insight into cellular changes. The activity of bone cells and the amount and distribution of bone tissue can be evaluated (Rauch 2014). Therefore, it plays a vital role in the study of particular metabolic disturbances and their treatment and in monitoring changes in bone properties in certain skeletal diseases like osteoporosis (Fritsch 1989). Studies of fracture healing, effects of biomaterials, or drug treatments also depend on quantitative evaluation of biopsies (Malhan et al. 2018). Especially in dentistry, histomorphometric analysis is an essential tool toward understanding specific physiological and pathophysiological processes (Deguchi et al. 2008; La Monaca et al. 2018). Morphometry may also be helpful in the evaluation of individual biopsies. Several factors determine the quality of a bone biopsy, such as the selected site, the ease of clinical availability, and having an adequate amount of the specimen (Revell 1983). Care must be taken to ensure the reproducibility of techniques, and though methods have changed with the development of new technologies, bone histomorphometry remains time-consuming and complicated to this day. Teeth and bone biopsies are usually decalcified before making sections for histologic analysis in daily routines.

In some cases, however, bone or dental research sections of undecalcified bones or crownless teeth are used for histologic studies (Donath & Breuner 1982). Even with specialized equipment, it is hardly possible to cut sections of undecalcified teeth or bone embedded in acrylate if the bone contains alloplastic implants of hard, metallic materials (Gross & Strunz 1977). Gross and Strunz achieved the first step in solving this problem in 1977 by developing a method of fixating and sawing the specimens as thin as 50 to 200 µm (Gross & Strunz 1977). Donath adjusted their approach using an improved staining method and developed the sawing and grinding technique (Donath 1988; Donath & Breuner 1982).

The biopsies are usually fixed using formaldehyde. After dehydration with ascending concentrations of alcohol solutions, the samples are infiltrated with ascending levels of light-curing resin Technovit 7200 (KULZER GmbH Leipziger Straße 2 63450 Hanau, Germany) and embedded into it. Undecalcified ground sections with a thickness of approximately 50 µm are prepared and stained according to the Laczko & Levai 1975 dye procedure with azure II and methylene blue in alkaline solution and counterstaining with basic fuchsin in water (Laczko & Levai 1975). To qualitatively describe the histological bone formation and resorption of the bone substitute as well as for the histomorphometric analysis, the slices are scanned using a scanning light microscope and analyzed using image analysis software. A range of programs is available by which morphometry of bone sections can be performed. However, the process of coloring and marking the different sections is usually still done by hand or semi-automated because automated software using the threshold method cannot yet provide exact values, as the stained color of slices, osteoid, osteoclasts, bone, and fibroblasts can vary throughout and between the slices and labels cut at an oblique angle are not well recognized and therefore require manual editing (Clermonts & Birkenhager-Frenkel 1985). Nevertheless, the implementation of specialized software for semi-automated detection of the stained areas resulted in a substantial decrease in analysis time compared to manual drawing of the labels (van ’t Hof et al. 2017).

1.6 | Aim

Calcium phosphate-based materials are increasingly used in biomedical applications, usually for tissue regeneration within the skeletal system (Albulescu et al. 2019; LeGeros et al. 1988). Alternatives to xenogeneic materials do matter in a time of climate change and cultural diversity. A recent survey of the French population's acceptance of bone graft materials has revealed that animal-derived xenografts are the least popular modality compared to autografts, allografts, and alloplastic materials (Offner et al. 2019). Fears of treatment failure, pain, and infection accounted for most of the nonacceptance, but ethical and religious considerations also played a role. A promising alternative to minimize these concerns are plant-based or, more specifically, phycogenic materials of the kind manufactured from the calcium-encrusted red seaweed *Corallina officinalis*. This species of marine algae is both a nonanimal renewable resource and has a carbonate apatite structure very similar to human bone and is thus well-suited not only for patients with ethical or religious concerns but for any patients with bone defects that require augmentation (de Groot 1983; LeGeros et al. 1988). Histomorphometric comparisons between bone graft materials regarding new bone formation, resorption characteristics, and other parameters are essential for gaining more insights and making it easier for clinicians to select appropriate materials for specific augmentation procedures (Nishimoto et al. 2019). However, few studies have compared the histological behavior of different calcium phosphate-based materials of phycogenic origin in SFE grafts. The method of taking biopsies from maxillary sinus augmentation for histomorphometric analysis is well-described, though, and in the research of new bone regenerative materials, it is often performed (Deguchi et al. 2008; Moy, Lundgren & Holmes 1993). Our study aimed to evaluate two algae-derived bone substitution materials using bone histomorphometry.

2 | Material and Methods

We designed a prospective randomized clinical trial to learn more about plant-based materials for bone grafting by evaluating the histological footprints of two different phycogenic variants of calcium phosphate in biopsy specimens obtained 3 and 6 months after SFE. The two bone substitutes for comparison included a biphasic material consisting of 80% β-TCP and 20% HA (Symbios® Biphasic Bone Graft Material; Dentsply Sirona Implants, Mannheim, Germany) and an almost pure HA (Symbios Algipore®; Dentsply Sirona Implants).

The primary objective of this study was to investigate the effectiveness of these two bone substitution materials by assessing and monitoring various parameters of a successful implant-osseointegration and through comparison of the histologic features by obtaining biopsies of both materials 3 and 6 months after regenerative surgery. Symbios OsteoShield® Collagen Resorbable Membrane (Dentsply Sirona Implants) was used as the guided bone regeneration membrane. As a secondary objective, the patients' comfort and acceptance of the surgical procedure was determined using standardized questionnaires. While constructing the study protocol, the following questions arose for consideration in this clinical trial:

- Does the biphasic graft material have any benefits over the HA substitution material?
- What are the clinical differences between the two bone substitution materials?
- What are the histologic differences between the two bone substitution materials?
- What are the macroscopic differences between the two bone substitution materials?
- Is the biphasic bone graft material a suitable bone substitute material for the two-step sinus augmentation procedure?

2.1 | Study design

The institutional ethics committee approved the study protocol at the Medical University of Graz (ref. 27-224 ex 14/15). The study procedures were performed in line with the Helsinki Declaration, ICH-GCP, and CONSORT EQUATOR guidelines, and following the relevant provisions of Austrian Medical Devices Act (MPG), the European directive on implantable medical devices (90/385/EEC), the Styrian Hospitals Act (KALG), the Federal Hospitals Act (KAKuG), the EN-ISO standards 14155-1 and -2, EN ISO 14971, 10993 and all other EN-ISO eligible relevant legislation.

2.1.1 | Patient recruitment

A power analysis preceding recruitment showed the need for a sample size of 20 patients for statistically significant results. Accordingly, we recruited 14 female and six healthy male participants, all nonsmokers with noncontributory medical histories and a mean age of 59.5 (40–75) years. The patients were recruited from the regular pool of patients seeking implant therapy at the outpatient clinic and prosthodontic consultation service at the Department of Dental Medicine and Oral Health of the Medical University of Graz, Austria. No additional efforts for recruitment were undertaken.

Participation in this study was voluntary. The patients gave consent to the use and interpretation of the resulting data. The therapy-related risks of implant surgery were equivalent in all individuals. There were only standard therapy-related costs for the patients that fell within the normal range. Individual patient identification numbers were assigned according to the time of entry into the study. On termination of a patient from the study, another participant had to be included so that the minimal number of participants did not fall below 20.

The investigators explained to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits, and any discomfort that might ensue. Each subject was informed that participation in the study was voluntary and that he/she could withdraw from the study at any time and that withdrawal of consent would not affect his/her subsequent medical treatment. The subject was informed that his/her medical records might be examined by authorized individuals other than the treating dentist. All participants in this study were provided with an information sheet and a consent form describing the study procedures and providing sufficient information to make an informed decision about their participation in this study. The subject information sheet and the consent form were submitted and approved by the

ethics committee. The formal consent of each subject was obtained before he or she submitted to any study procedure. The subjects read and considered the statement before signing and dating it and were given a copy of the signed document. The consent form was signed and dated by the investigator as well and was retained as part of the study records.

2.1.2 | Inclusion and exclusion criteria

Specific inclusion and exclusion criteria were established before the recruitment period to avoid any unpredictable complications or misinterpretations of the acquired data. Patients aged 20 to 75 years who fulfilled all of the following inclusion criteria were enrolled in the study:

- capable of giving informed consent
- good health as defined by the subject's medical history (no contraindications as described in the exclusion criteria below)
- missing teeth in the posterior maxilla requiring implant therapy for reconstruction
- a residual vertical bone height of 3-5 mm requiring a 2-staged sinus floor elevation and implant placement. Measurements were performed using cone-beam computed tomographies (CBCTs) that were not yet part of the study.

The presence of any of the following exclusion criteria would have led to the exclusion of the subject:

- Homelessness
- Smoking
- Medication with a contraindication for implant therapy
- Skeletal immaturity or any active malignancy or ongoing treatment for malignancy
- An active infection at the operative site
- Persistent compartment syndrome or neurovascular residua of compartment syndrome
- Pathological fractures such as those observed in (but not limited to) Paget's disease or in metastatic bone
- Contraindications to the class of drugs under study, e.g., known hypersensitivity or allergy to the type of drugs or the investigational product
- Pregnancy or intention to become pregnant during the study
- Breastfeeding
- Lack of safe contraception

All patients considered for the study underwent a detailed oral examination and were reviewed according to the inclusion and exclusion criteria mentioned above. If eligible, they were scheduled for study visits as well as the staged surgical treatment, including SFE with bone reconstruction and implant surgery. The patients were comprehensively informed about alternative treatment options, the planned surgical procedures, the study-related additional step of biopsy collection, and the scheduled recall plan. Patients needing SFE on only one side were randomized to either a β-TCP/HA or an HA group using a web-based tool developed at our university (Randomizer for Clinical Trials, www.randomizer.at). Bilateral cases were also randomized to one of these groups, and the bilateral patients' right or left side was selected at random for analysis (Ofner, P; Errath 2004).

2.1.3 | Course of study

Visit 1: Patient recruitment and screening: During the first visit, about 1 week before the planned sinus lift, the participants were informed about the course of the study, the procedures, and the examinations. The patients' medical history was taken and current health status recorded. All relevant demographic, medical, and dental data of the patients were recorded, and the agreement to participate in the study was obtained. For preoperative, radiological diagnostics such as the evaluation of the sinus maxillaris and measuring the residual bone height, a panoramic X-ray and a digital volume tomography were performed. Randomization was performed to determine the assigned group (β-TCP/HA or HA).

Visit 2: Two-step sinus augmentation with delayed implant placement: All surgical procedures were performed by one of three experienced bone-augmentation and implant surgeons on an outpatient basis under local anesthesia and standard conditions. Before surgery, the patient's mouth was rinsed with a local antiseptic chlorhexidine gluconate solution (Chlorhexamed Forte 2 mg/ml; GlaxoSmithKline, Bühl, Germany), and local anesthesia was applied with articaine (Ultracain with epinephrine 1:100.000; Sanofi-Aventis, Frankfurt, Germany). A crestal incision was performed in a slightly palatal position, with mesial and distal releasing incisions on the buccal aspect. Full-thickness flaps were raised to expose the alveolar crest and lateral sinus wall. High-speed rotating surgical burs were used under cold, sterile saline irrigation to create a trap door in the lateral sinus wall. The door was turned inward and upward with a top hinge to a horizontal position. The elevation of the Schneiderian membrane up to the medial wall of the sinus

was accomplished using straight and angled elevators (Frios SinusSet, Dentsply Sirona Implants). The formed cavity between the bone and the Schneiderian membrane was then filled with either HA or β-TCP/HA crushed and soaked in autologous blood according to the randomization protocol. Figure 1 shows the clinical steps of the surgical procedure, including the freshly packed bone substitute in the cavity after elevating the sinus membrane. The augmentation site was covered with Symbios OsteoShield Collagen Resorbable Membrane (Dentsply Sirona Implants) for guided bone regeneration and fixed with two fixation pins (Symbios Membrane Tacks, Dentsply Sirona Implants), and the mucoperiosteal flap was repositioned and sutured tension-free with non-resorbable monofilament polyamide (Resolon 5-0; Resorba Medical, Nuremberg, Germany). All patients received antibiotics, anti-inflammatory medication, and painkillers. Cefalaxine or, in the case of intolerance, Clindamycine was administered as an antibiotic and Dexibuprofen as a painkiller. For post-operative radiological diagnostics, exclusion of membrane perforations, and evaluation of the sinus augmentation material, a panoramic X-ray and CBCT were taken directly after the surgical procedure.

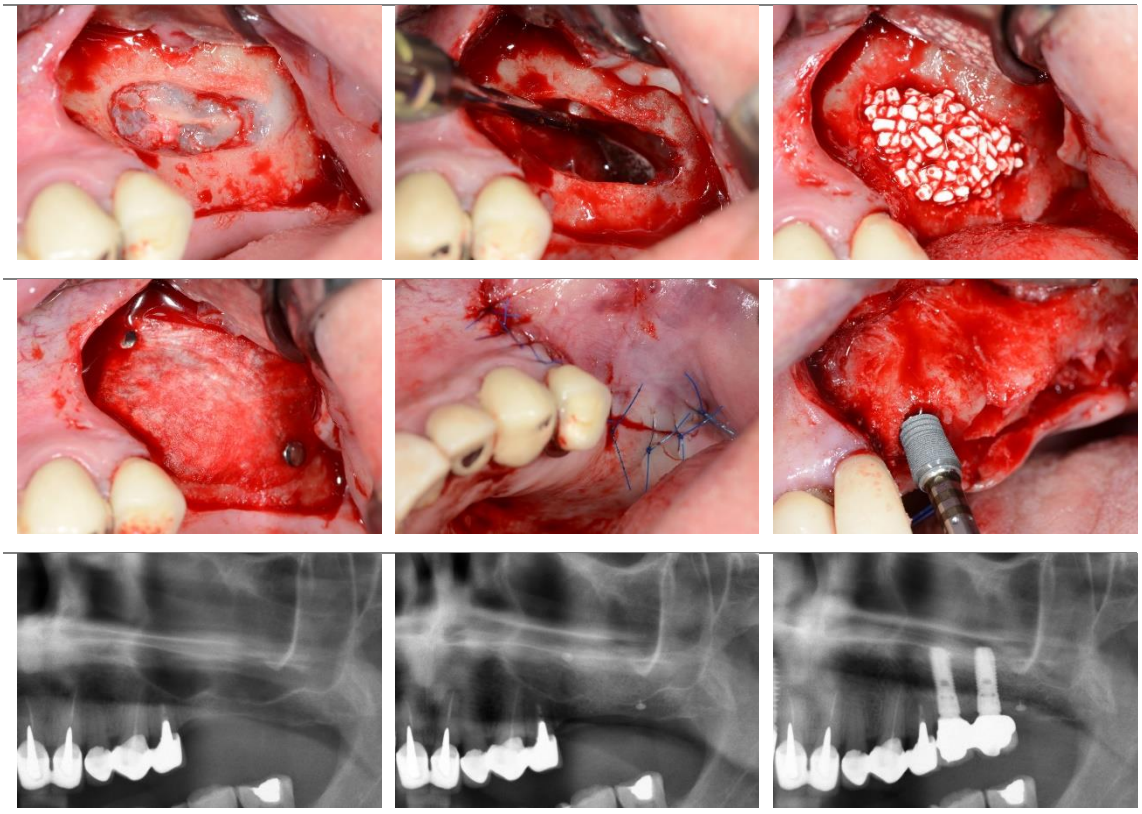

Figure 1: Clinical photographs of the surgical procedure of SFE as well as implant placement after 6 months (Lorenzoni, M., 2017, Photographs from private collection)

Visit 3: Suture removal: 7 days after surgery, all sutures were removed, and the patients' perception and acceptance were assessed using the visual analog scale (VAS) assessing pain and swelling after augmentation surgery (Bijur, Silver & Gallagher 2001).

Visits 4-5: Healing period: A 3-month healing period of the sinus floor augmentation followed. Regular clinical checks were carried out at intervals of about 4-5 weeks with sensitivity tests, patient comfort tests (VAS) and questionnaires on general patient satisfaction (oral health impact profile: OHIP-14 [Slade 1997]).

Visit 6: Bone biopsy I: A biopsy of the augmented region was taken 3 months after the SFE with the help of a surgery guide to assess bone quality histologically and histomorphometrically. The biopsy was performed from a crestal direction, parallel and with a minimum safety distance of at least 5 mm to the nearest planned implant position. The biopsies were taken with a trephine bur after three-dimensional planning of the location and length to avoid perforations into the sinus (Figure 2).

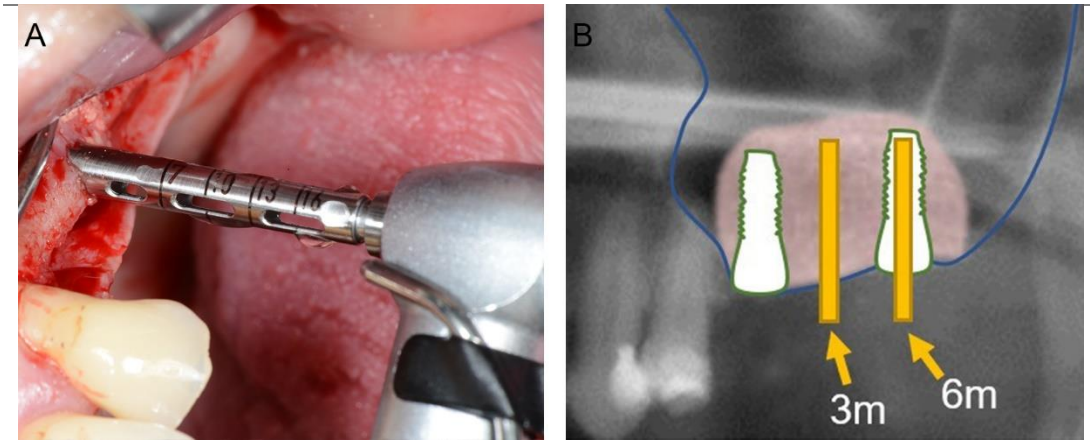

Figure 2: Trephine bur used for biopsies (A), positions of 3-month and 6-month biopsies (B) (Sokolowski et al., in press)

Visit 7: Implant placement and bone biopsy II: The implant placement in the augmented sinus was performed after a healing period of 6 months. The incision was made in the crestal region of the missing teeth to the neighboring teeth to get an overview of the horizontal bone expansion. The needed number of implants were placed in the area of the sinus augmentation. Before implant placement, a second biopsy of the augmented region was performed from a crestal direction at a planned implant position within the augmented area. All patients received implants from the same company and of the same design (Astra Tech Implant System, OsseoSpeed EV; Dentsply Sirona Implants). One week after implant placement, all sutures were removed, and each patient's perception

and acceptance was assessed using the visual analog scale (VAS).
Subsequent to visit 7, all patients underwent and continued their regular therapy. During this period, impression taking and various try-ins and elective soft-tissue augmentation surgery took place until the insertion of the final reconstruction was performed.

Visits 8-11: Follow-up after implant placement: During the follow-up time, regular radiological and clinical recall check-ups were carried out. Oral hygiene and periodontal or peri-implant conditions were controlled and documented, and professional tooth cleaning was performed in intervals adjusted to the patients' needs. Radiographic check-ups were scheduled for 12 and 24 months after the first surgery (sinus augmentation).

2.2 | Approach of histologic and histomorphometric analysis

In both groups, a biopsy of the augmented region was taken 3 and 6 months after the SFE and used to assess the bone quality histologically and histomorphometrically. All biopsies were taken with a trephine bur after three-dimensional planning of position and length. The biopsies were processed according to the Karl Donath method for undecalcified thin ground sections (Donath 1988; Donath & Breuner 1982; Gross & Strunz 1977). One slice per biopsy was obtained. After dehydration with ascending concentrations of alcohol solutions, the samples were infiltrated with ascending levels of light-curing resin Technovit 7200 (KULZER GmbH Leipziger Straße 2 63450 Hanau, Germany) and embedded into it. Undecalcified ground sections with a thickness of approximately 50 µm were prepared and stained according to the Laczko & Levai 1975 dye procedure with azure II and methylene blue in alkaline solution and counterstained with basic fuchsin in water (Laczko & Levai 1975). To qualitatively describe the histological bone formation and resorption of the bone substitute and to conduct the histomorphometric analysis, all 40 slices were scanned using a scanning light microscope (Zeiss Axio Imager.M2) and a high-resolution digital camera (AxioCam MRc, ZEN 2017). For the histomorphometric evaluation, all images were converted into tiff files. The autologous bone and the most crestal bone substitute were identified; from that point on, a whole burr width zone of 1 mm length was analyzed in an apical direction as Zone 1. In the 6-month group, a further zone 1 mm apical of the first zone and the width of another 1 mm was analyzed as Zone 2. In Photoshop CS6 (13.0.1; Adobe, San Jose, CA, USA), the different tissues in every image were marked on layers with specific colors consecutively by two blinded investigators (Figure 3).

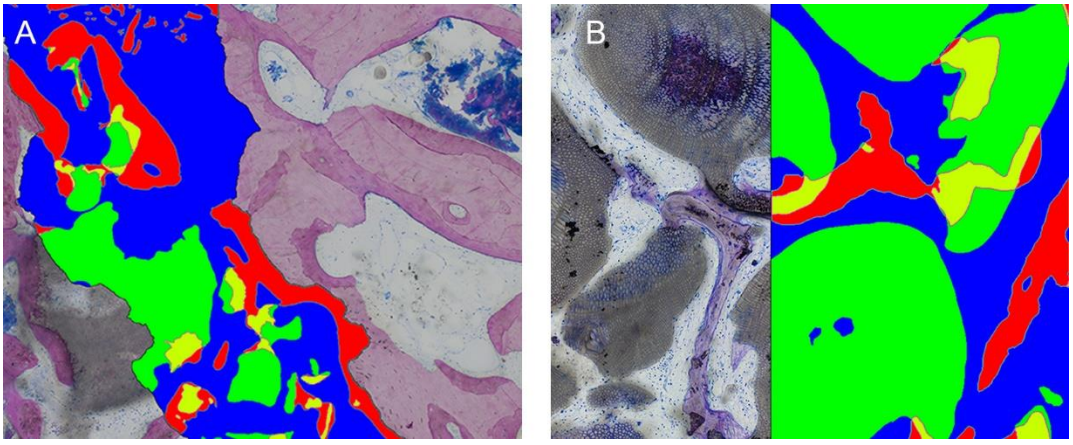

Figure 3: Colorization of histologic structures of β-TCP/HA (A) and HA (B) – blue: connective tissue, red: new bone, green: substitution material, yellow: old bone

The number of pixels of the different colors was automatically counted, scaled, and their percentage of the whole tissue area computed. The following parameters were calculated: Newly formed bone area (newBA [mm²], [%]), old bone area (oldBA [mm²], [%]), tissue area (TA [mm²], [%]), bone substitute area (BsA [mm²], [%]), new bone infiltration area in bone substitute (newBA in BsA[mm²], [%]), new bone to bone substitute contact (NewB-BsC [mm], [%]) and penetration depth (Pd [mm]).

2.3 | Statistical considerations

Based on former studies we performed a sample size estimation (Ewers 2005). A sample size of 9 in each histologic group would have had 80% power to detect a difference in means of 6% assuming that the common standard deviation was 5% using a student t-test for independent samples with a 0.05 two-sided significance level. Based on these statistical calculations and preceding power analysis as well as considerations on possible dropouts, a patient sample size of 20 was determined for statistically significant outcomes, resulting in 10 histologic slices per bone substitute for the 3 month and 6 month biopsies.

Descriptive and explorative statistics was performed using IBM SPSS (version 24; SPSS, Chicago, IL, USA).

In order to investigate the differences between newly formed bone area, bone substitute area, new bone infiltration area in bone substitute, new bone to bone substitute contact and penetration depth, student t-test for independent samples was performed to compare the means of the values obtained from the HA and β-TCP/HA groups. The tested null hypothesis proposed that there were no differences between both materials in regards of new bone formation and bone substitute resorption.

In the resulting diagrams, that were created using Excel (Microsoft Office 2019), the standard error of the mean is shown. Differences between the groups were considered significant at $p < 0.05$.

2.4 | Investigated phycogenic grafting materials

The HA granules of the tested material range from 0.3 to 2 mm and have pores in the range of 0.5–10 µm (Schopper et al. 2003; Simunek et al. 2005). Small perforations interconnect the pores in regular septation with a mean length of 50–100 µm and a mean diameter of 1–3 µm (Schopper et al. 2003, 2005). The β-TCP/HA granules range from 0.25 to 1 mm and have similar pores to the pure hydroxyapatite in the range of 0.5–10 µm and a mean length of 50–100 µm (Schopper et al. 2003; Simunek et al. 2005). The surface area (m^2/g), estimated by mercury porosimetry, shows similar values for both materials: 22.9 m^2/g for HA and 23.10 m^2/g for β-TCP/HA (Spassova et al. 2007).

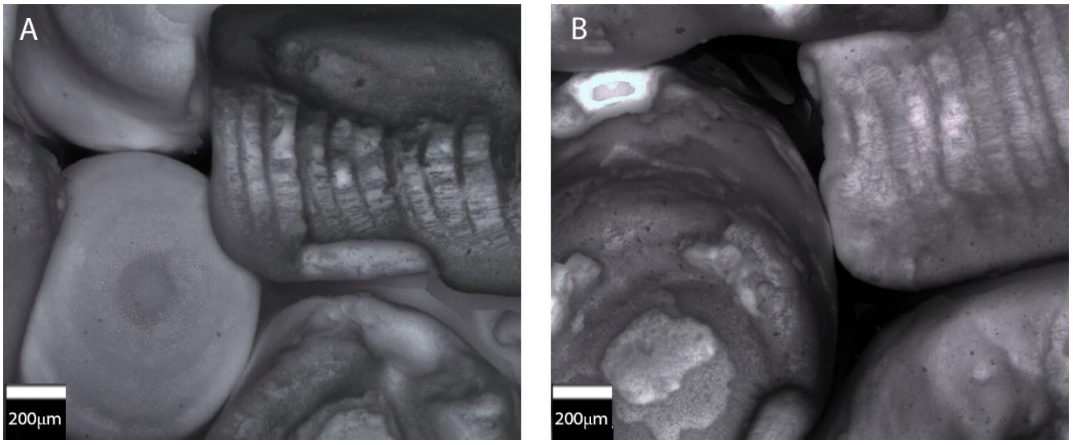

Figure 4: Microscopic images of β-TCP/HA (A) and HA (B)

The microscopic images show no significant difference between the materials, although the unique three-dimensional morphologic structure of the algae calcite scaffold with interconnecting pores can be recognized (Figure 4).

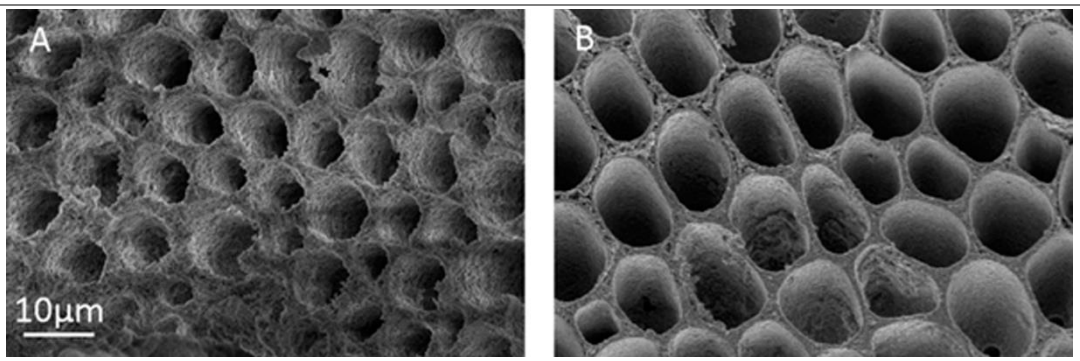

Figure 5: Electron micrograph images of β-TCP/HA (A) and HA (B) (Sokolowski et al., in press)

When looking at the scanning electron microscope (SEM) images, one can recognize the periodically septate micropores (Figure 5). The compound material shows rough edges that are shaped by the coarse-structured crystalline β-TCP grains. The HA material presents regularly arranged homogenous pores divided by sharp, clean edges of bilayer separating walls.

3 | Results

Between February 2016 and November 2017, 20 patients (6 male and 14 female), aged 40 to 75 years (mean age 59.6), underwent sinus floor elevation within the course of the study. In eight patients, the sinus augmentation was unilateral, whereas 12 patients needed treatment on both sides. After randomization, the study's relevant site was located on the right sinus in six patients and on the left sinus in 14 patients. There was no loss of augmentation material and no loss of implants during the time of the study. The mean volume of material used was 3.8 ml of HA or 3.4 ml of β-TCP/HA per patient. Minor perforations of the Schneiderian membrane occurred in two cases and were treated with an additional layer of resorbable collagen membrane, preventing displacement of the substitution material. Both groups showed similar mean residual bone heights (HA: 2.8 mm, β-TCP/HA: 2.6 mm) and vertical heights of grafting (HA: 19.2 mm, β-TCP/HA: 17.3 mm). The clinical application of the two materials was similar. The handling characteristics of both materials showed rapid and complete hydration of the particles with autologous blood during the surgery, and due to their smooth cylindrical-conical form, they were a secure material that is unlikely to injure the Schneiderian membrane. The histomorphometric results are summarized in Table 1 and illustrated in Figures 9-14. Four samples had to be excluded from the study due to tissue inconsistencies, which would have resulted in misleading evaluation and misinterpretation of results. Two blinded investigators evaluated each image consecutively by allocating colors to the histologic structures. Each image was divided into the zones in question, and the different components were colored on layers according to the origin of the tissues (Figure 6, Figure 7).

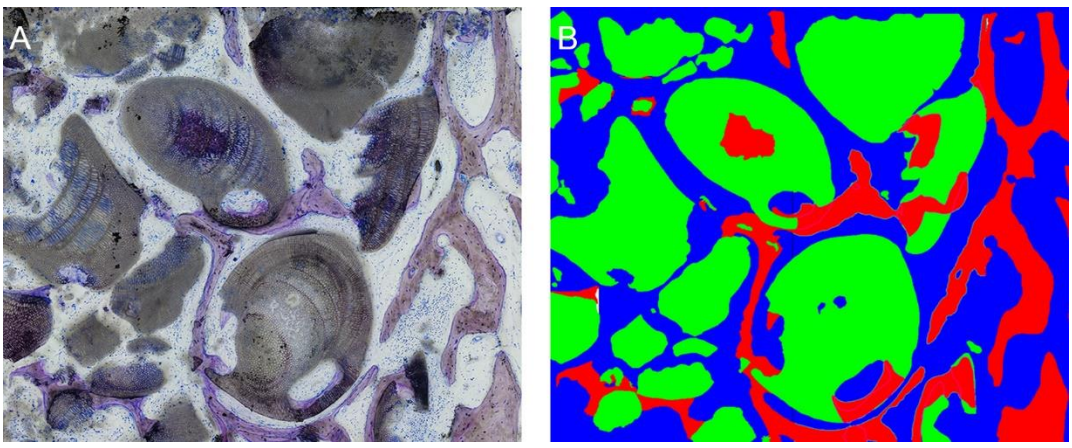

Figure 6: Schematic example of histological slide of HA (A) and with colorization (B), blue: connective tissue, red: new bone, green: substitution material

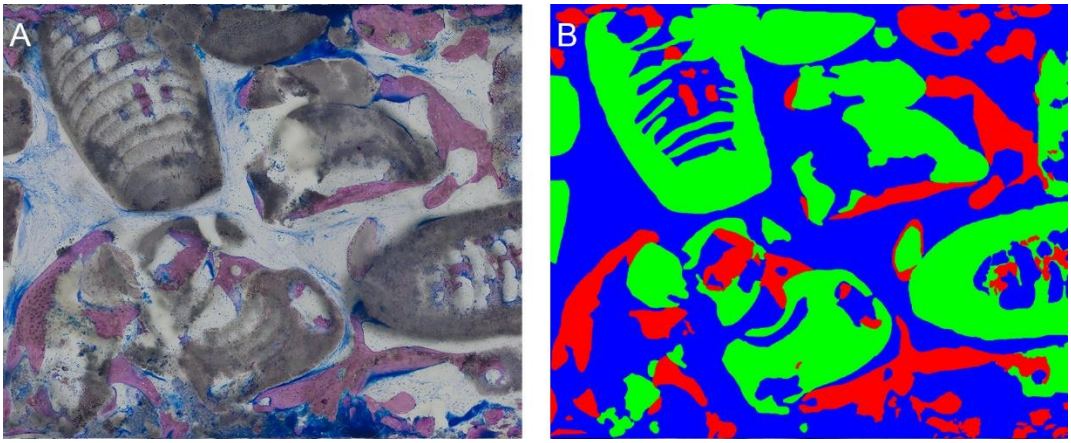

Figure 7: Schematic example of histological slide of β-TCP/HA (A) and with colorization (B) blue: connective tissue, red: new bone, green: substitution material (Sokolowski et al., in press)

Figures 6 and 7 show the two substitution materials in histological thin-ground sections stained with Laczko & Levai dye. Bone presents in shades of red, older bone is pale, newer bone is stronger stained, and soft tissue is blue. The colorization of tissues was performed by marking them in Photoshop using different colors: newly formed bone in red, bone substitute in green, soft tissue in blue. Pixels of the marked areas were automatically counted and scaled for size. After that, the percentages were computed.

HA and β-TCP/HA were similar in both morphology and behavior among the oral tissues but showed some significant differences in the resorption rate and infiltration characteristics. Newly formed bone most often surrounded the augmentation material with direct contact or was sometimes divided by connective tissue (Figure 8 c, h). Due to the pores of the substitute, newly formed bone also infiltrated the material (Figure 8 h). Three conditions of bone infiltration into the substitution material could be differentiated. In the first, pristine condition, the augmentation material was not or was only slightly stained or altered, disregarding the soft tissue situation (Figure 8 d, g). The second condition was strongly reddish-stained material; soft tissue may or may not have been present inside the pores or surrounding the bone substitute (Figure 8 c, g). The third condition showed pores that were filled with osteoid or bone material. When new bone infiltrated and surrounded the bone substitute material, it could also show signs of continuing resorption (Figure 8 c, d, g, h).

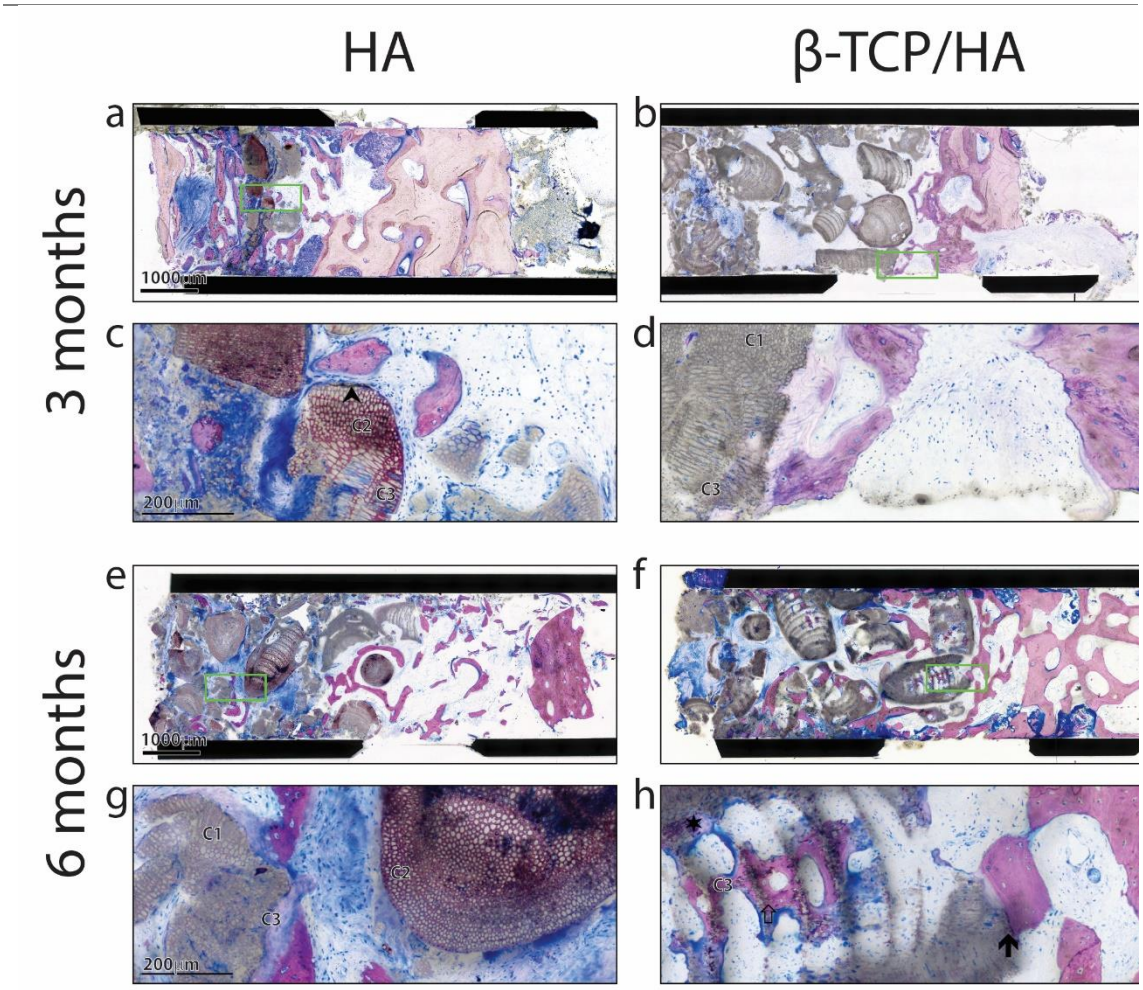

Figure 8: Histological thin ground sections stained with Laczko & Levai, bone in shades of red, older bone is pale, newer bone is stronger stained, soft tissue is blue. Images c, d, g, h are details of the images above them. The specific region is marked with a green rectangle. The black arrowhead (c) shows newly formed bone separated from the bone substitute by soft tissue, a full black arrow (h) shows direct contact with the bone substitute. The black asterisk (h) marks a region with bone infiltration in bone substitute, while the empty arrow (h) highlights bone substitute that is integrated into newly formed bone and consists of small particles. Enzymatic resorption is assumed to have taken place. C1 = condition 1 (d, g) marks pristine bone substitute without staining or tissue infiltration, C2 = condition 2 (c, g) strongly stained bone substitute, C3 = condition 3 (c, d, g, h) soft tissue and/or newly formed bone infiltrated bone substitute material. (Sokolowski et al., in press)

Table 1: Histomorphometric results of the two phycogenic bone substitutes based on sinus graft biopsies taken after 3 and 6 months. (Sokolowski et al., in press)

	HA group (almost pure HA)			β-TCP/HA group (80% β-TCP/+ 20% HA)		
Histomorphometric parameters	3 months crestal zone Means (± SEM)	6 months crestal zone Means (± SEM)	6 months apical zone Means (± SEM)	3 months crestal zone Means (± SEM)	6 months crestal zone Means (± SEM)	6 months apical zone Means (± SEM)
New bone (%)	21.4 (± 5.59)	16.4 (± 7.31)	14.0 (± 16.9)	26.4 (± 8.58)	34.0 (± 16.9)	23.0 (± 8.80)
Graft material (%)	17.1 (± 7.70)	36.4 (± 15.1)	40.0 (± 11.4)	16.2 (± 11.6)	16.4 (± 11.4)	32.9 (± 15.6)
New bone contact to graft (%)	9.47 (± 12.4)	29.4 (± 14.9)	26,2 (± 17,8)	4.20 (± 4.06)	13.6 (± 14.9)	37.1 (± 37.5)
Graft infiltration by bone (%)	2.64 (± 2.48)	7.78 (± 10.0)	6,20 (± 8,81)	2.71 (± 4.08)	10.6 (± 20.8)	6.43 (± 5.59)
Penetration depth (mm)	3.78 (± 2.10)	4.49 (± 0.78)	4,83 (± 1,33)	5.30 (± 2.31)	5.24 (± 3.01)	6.12 (± 2.44)

While newly formed bone was present in both groups, the β-TCP/HA group showed more new bone area, especially after 6 months, which could be measured in the histomorphometric analysis (Figure 9). The amount of new bone in the first biopsy group 3 months after sinus augmentation showed similar values in both groups, specifically 21.4% for HA and 26.4% for β-TCP/HA. In the healing period from 3 to 6 months, the proportion of new bone in the HA group decreased (16.4%), while β-TCP/HA showed a relative increase (34.0%) of newly formed bone ($p<0.05$) (Table 1).

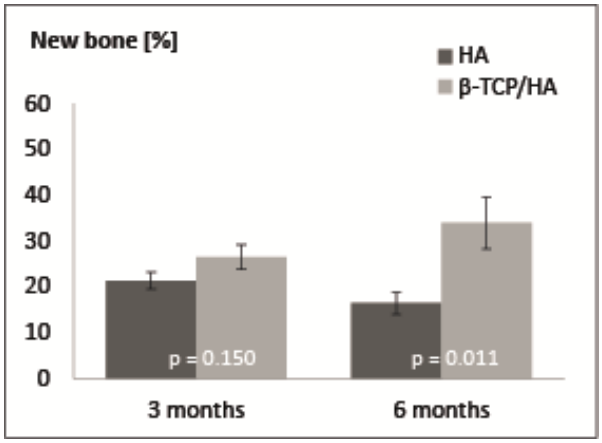

Figure 9: New bone formation after 3 and 6 months (Sokolowski et al., in press)

The surface morphology, size of the particles, and pore density were similar in all groups, with no significant difference between materials. The relative proportion of bone substitute material was nearly the same level in both groups in the first biopsy after 3 months (17.1 % HA, 16.2% β-TCP/HA) (Figure 10).

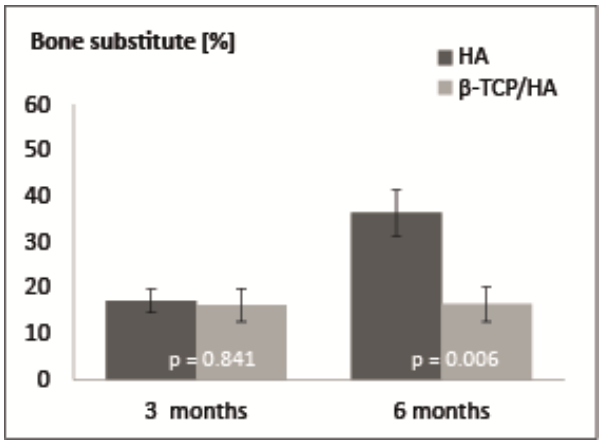

Figure 10: Relative proportion of bone substitute material (Sokolowski et al., in press)

HA showed a higher relative ratio of residual material after 6 months. The difference between the groups was statistically significant after 6 months (p = 0.006). The ratio of new bone area to bone substitute area showed more substitute material and less new bone in the HA group compared to the β-TCP/HA group, whereas more new bone compared to bone substitute could be found after 6 months (Figure 11).

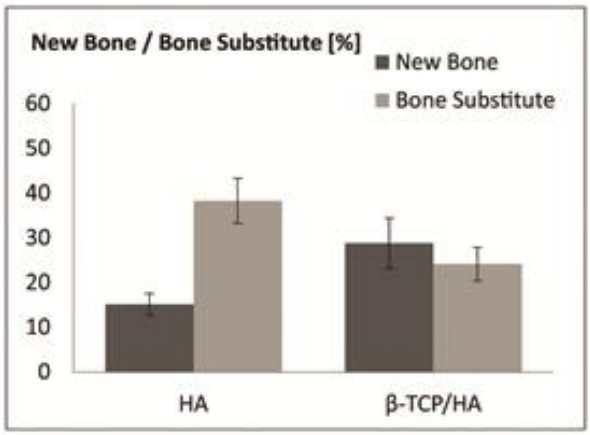

Figure 11: Proportion of new bone to bone substitute (Sokolowski et al., in press)

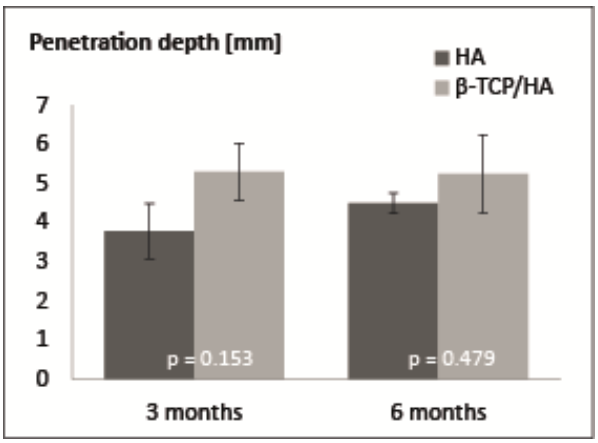

Figure 12: penetration depth of new bone into the substitute material (Sokolowski et al., in press)

The β-TCP/HA group showed a higher bone to substitute material ratio and a higher bone penetration depth into the substitute material (Figure 11 and Figure 12).

In contrast, HA showed a higher contact area with the newly formed bone (Figure 13) with less infiltration into the pores, indicating less resorption compared to β-TCP/HA (Figure 14).

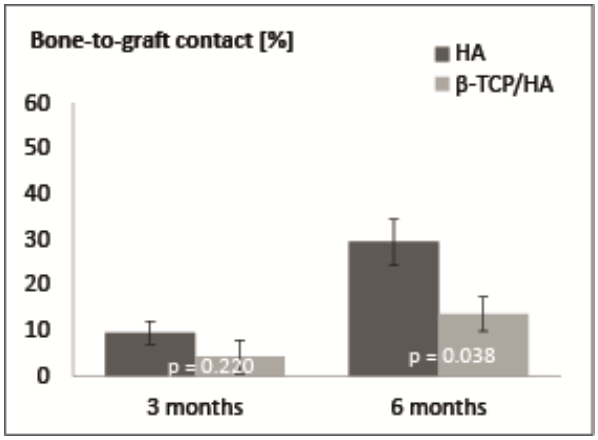

Figure 13: Penetration depth of new bone into the substitute material (Sokolowski et al., in press)

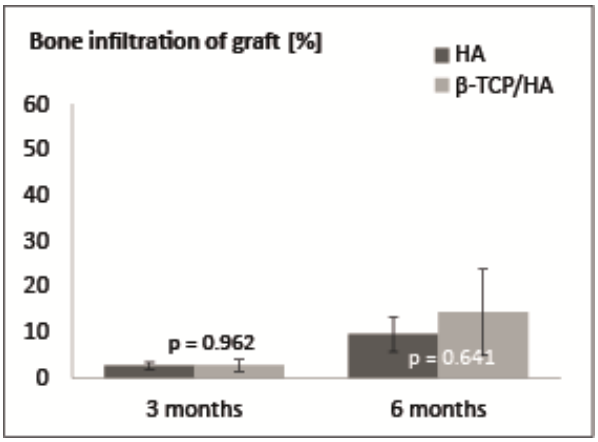

Figure 14**:** Bone infiltration of graft material (Sokolowski et al., in press)

Regarding resorption, both augmentation materials show peculiarities. There seemed to be more pore infiltration and resorption signs in the β-TCP/HA group than in the HA group. Especially after 6 months, tiny remnants of β-TCP/HA granules were visible in newly formed bone and seemed to be undergoing resorption after new bone adherence. Figure 15 shows the general differences between the two graft materials.

While after 3 months small particulate granules of β-TCP/HA surrounded by new bone can be seen, as well as the micropores of bigger particles infiltrated by new bone, HA shows a more stable picture (Figure 15). It is of particular interest that new bone formation primarily occurs at the outer contour of the β-TCP/HA material but quickly infiltrates into the pores. In HA, new bone is extensively attached and in good contact with the substitute material on the edges, indicating a highly biocompatible material. Still, the pores are not as heavily infiltrated as in the compound material. When looking at higher magnification in the 3-month biopsies, one can identify signs of resorption throughout the histologic slides of β-TCP/HA but also still excellent stability of the calcite scaffolds. In contrast, the adherence of new bone to HA is presented prominently, and very few signs of resorption are seen (Figure 16).

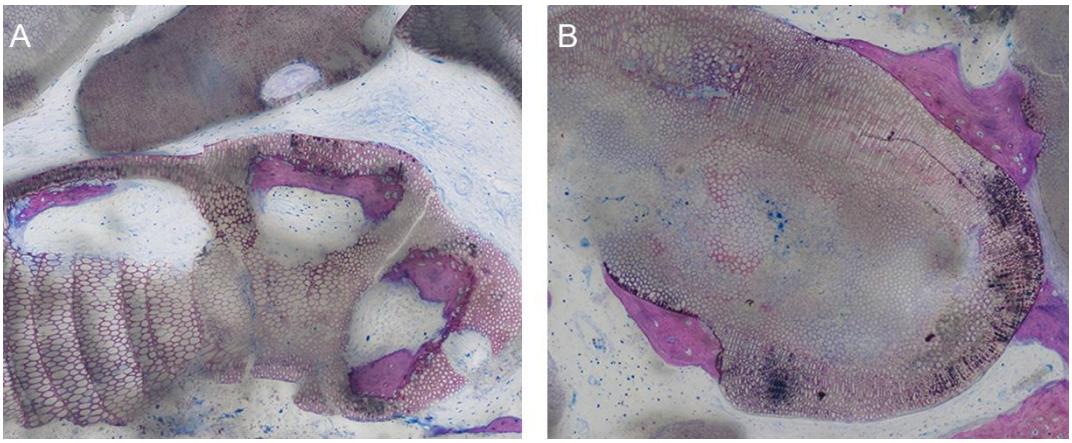

Figure 15: Peculiarities of new bone adherence to β-TCP/HA (A) and HA (B) after 3 months

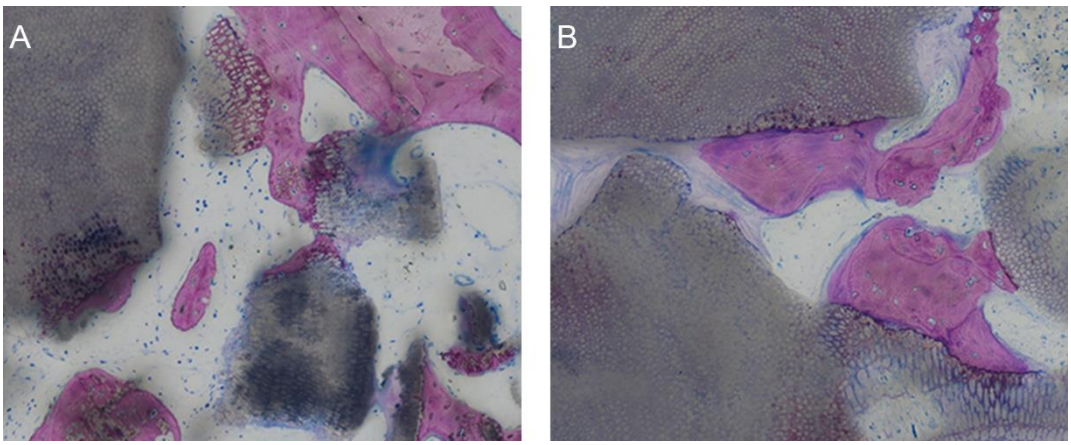

Figure 16: Differences of resorption and attachment of new bone to β-TCP/HA (A) and HA (B) after 3 months

In the 6-month images, the differences become increasingly evident as the new bone resorbs the bone substitute progressively. Still, the HA material mostly stays similar to its appearance in the 3-month pictures with broad contact with new bone and only little infiltration into the micropores (Figure 17).

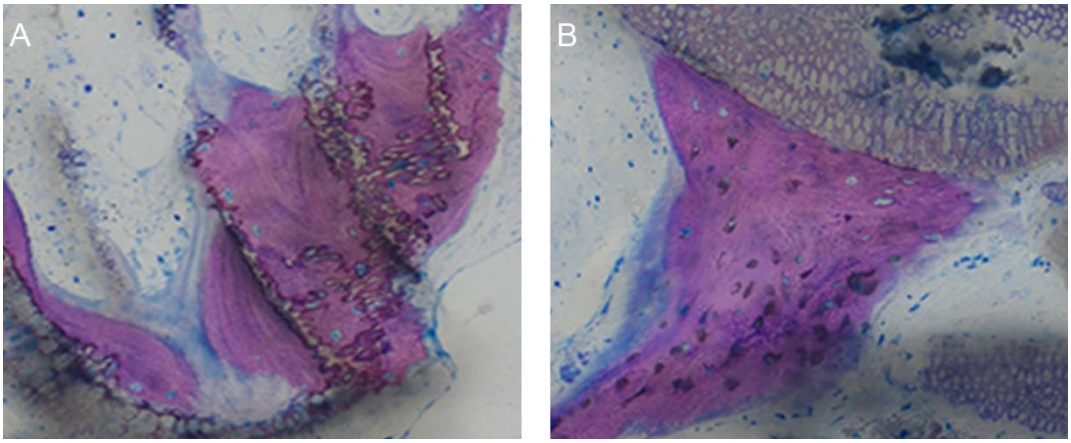

Figure 17: Differences of resorption and attachment of new bone to β-TCP/HA (A) and HA (B) after 6 months

While analyzing the ratio of new bone to bone substitute in the different zones of the 6-month biopsies, it can be seen that HA shows similar values between the crestal and more apical zones. In contrast, in the β-TCP/HA group there is a higher rate of new bone formation and infiltration of the bone substitute in the sinus floor area compared to the more apical zones (Figure 18).

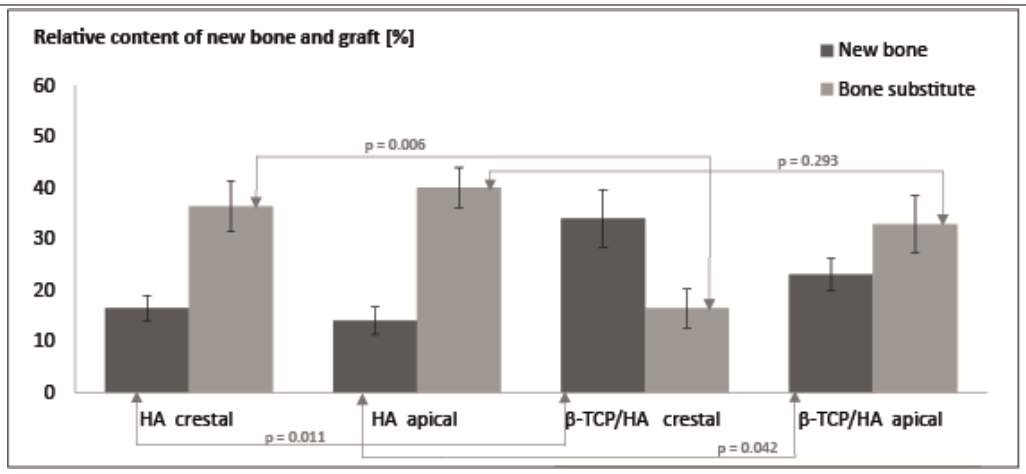

Figure 18: Histomorphometric results obtained with both phycogenic bone substitutes (β-TCP/HA and HA) in biopsies taken 6 months after sinus grafting, comparing the more crestal zones to equally defined zones directly adjacent but more apical. Results are expressed as mean values and standard errors of the mean, and p-values are given. (Sokolowski et al., in press)

This finding is consistent with the overall picture of the histological examination: HA showed a very homogeneous distribution pattern in the respective examined histological regions (new bone, old bone, bone substitute material, connective tissue). This is characteristic of a relatively stable augmentation material with little histological activity regarding absorption or new tissue formation during the first 6 months. In contrast, β-TCP/HA showed a more reactive picture with more resorption, new bone formation, and more degradation and alteration of the bone graft material in the first 6 months.

4 | Discussion

4.1 | Influence of the study design

The selection of a suitable study design to establish associations in scientific questions is of the utmost importance when gathering information and medical data (Garattini et al. 2016). Retrospective analyses examine exposure status and compare the incidence of diseases by looking at a cohort back in time. This kind of study design is valuable as a quick, economical, and easy method of answering a question. However, the data is secondary and may be incomplete or biased since researchers do not have any control over the circumstances of how the information was gathered. Before performing a randomized controlled study, retrospective analyses may be a valuable option for making progress in designing the original research and can be helpful in formulating the hypothesis, focusing the main questions, and identifying issues for a prospective study, but such analyses should not be used as a single source for answering complex scientific questions (Jakobsen & Gluud 2013). The study design of a prospective randomized controlled trial randomly assigns participants into an experimental and a control group. During the study, the only expected difference between the two groups is the outcome variable being studied. The groups' homogeneity and balance are of great importance in establishing the validity of information about the scientific topic in question. The randomization process tries to minimize population bias, and the investigators' blinding can be carried out in a controlled approach. To minimalize the risks of systemic errors, design errors, and threads of internal validity, the main pillars of medical science ethics must be fulfilled. Good scientific practice (GSP) represents standards and values that apply throughout an individual's career in healthcare science at any level of practice (AHCS 2012). By performing a study in accord with the Declaration of Helsinki, ICH-GCP, and CONSORT EQUATOR guidelines, the science practitioners have a defined role in delivering and reporting a quality-assured investigation (JAVA 2013). Our study was monitored and supervised by the Coordination Centers for Clinical Trials to ensure compliance with these standards and for verification (KKS, Medical University of Graz). While performing a prospective randomized clinical trial with all its benefits, some disadvantages must be taken into consideration. Due to the character of the study, this approach is expensive in terms of time and money. While patient acquisition and data evaluation are of utmost importance, time schedules may have to be extended to acquire all medical data needed for the assessment.

That being said, randomized controlled trials are generally of a higher level compared to the results of non-randomized cohort studies, case-control studies or case reports, but still there may always be risks of volunteer biases or systemic biases (Deeks et al. 2003; Ioannidis et al. 2001).

The Evidence-Based Medicine Pyramid is a simple diagram described by multiple authors and shows a broad agreement on the relative strength of medical studies and clinical trials (Murad et al. 2016; Paul & Leibovici 2014; Tomlin & Borgetto 2011). While various versions of the evidence pyramid exist, all of them are focused on showing the hierarchy of expert opinions and weaker study designs. Basic science and case series appear at the bottom, followed by case-control and cohort studies in the middle; higher are the randomized controlled trials and, at the very top, systematic reviews and meta-analyses (Golden & Bass 2013; St. John & Mcneal 2017; Tugwell & Knottnerus 2015).

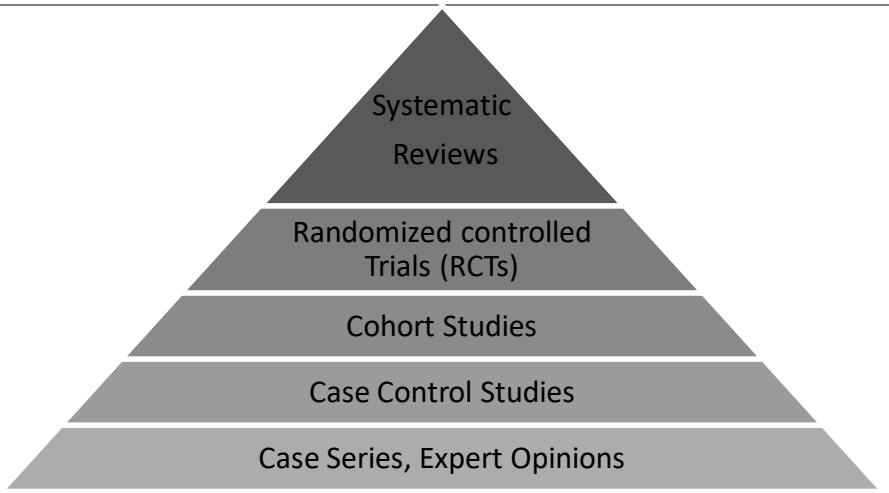

Figure 19: Pyramid of evidence described by various authors (Golden & Bass 2013; St. John & Mcneal 2017; Murad et al. 2016; Paul & Leibovici 2014; Tomlin & Borgetto 2011; Tugwell & Knottnerus 2015).

Based on these considerations and the level of evidence we wanted to provide with our investigation, the choice of study design fell on a prospective clinical randomized controlled trial. Histological studies with specimens retrieved from sinus augmentations comparing phycogenic BCP and HA as bone substitution materials are rare. Therefore, this study will contribute to a broader understanding of plant-based bone graft materials (Ewers et al. 2004; Schopper et al. 2005; Spassova et al. 2007). A power analysis performed prior to the study suggested the number of 20 participants for acquiring significant results.

4.2 | Plant-based bone substitution materials

Calcium phosphate-based biomaterials are used in a continuously increasing number of biomedical applications with the main focus on tissue regeneration within the skeletal system (Albulescu et al. 2019; LeGeros 2008; Tite et al. 2018). It is essential to offer alternatives to xenogeneic or autologous graft materials in a time of climate change, cultural diversity, and with the goal of performing minimally invasive medicine. In a recent study by Offner et al., the acceptance of different bone substitute materials for the management of bone defects among a French population was evaluated. A questionnaire was submitted to 562 participants after explaining different techniques and modalities, resulting in the conclusion that animal-derived xenografts are the least popular modality compared to autografts, allografts, and synthetic bone substitutes. Most refusals were due to the fear of failure, pain, and infection, as well as ethical and religious reasons (Offner et al. 2019). For these patients, plant-derived bone graft materials may offer a promising alternative. The authors admitted that the acceptance of the techniques was not significantly linked to sociodemographics; however, other countries with their own cultural and religious characteristics may have different concerns about the origin of bone substitutes. Thus, bone regenerative medicine is an essential pillar of innovation and a research direction of great potential.

The main limitations in using current bone substitutes are large vertical and horizontal defects (Fernandez de Grado et al. 2018). New technologies, as well as recent research, focus on creating synthetic scaffolds with porosity to promote faster and denser vascularization. Tissue engineering of scaffolds using 3D-printing technology may show promising results for larger defects and defects that need an exact three-dimensional contour. However, the precise design of the micropores still seems to be a challenge. (Touri et al. 2018). In a study by Liu et al., different biphasic calcium phosphate scaffolds were printed using accurately controlled macropore sizes of 0.8, 1.2, and 1.6 mm. Eight New Zealand rabbits were selected to fill 8-mm-diameter calvarial defects with the BCP scaffolds. After 4 and 8 weeks, specimens were harvested to evaluate bone-forming using microcomputed tomographies as well as histological examination. Scaffolds with a 0.8 mm pore size showed superior advantages in initial bone formation and maturation (Liu et al. 2019).

While synthetically derived substitution materials may show a promising way of copying nature, bone graft materials derived from aquatic plants like the calcium-encrusted red marine algae *Corallina officinalis* seem to be a decent, already-available alternative and,

due to their plant-based nature, are created from a renewable resource. Because of their carbonate apatite structure, they show high similarities to the composition of human bone and are not only suitable for patients with ethical or religious concerns but all patients with bone defects needing bone augmentation (de Groot 1983; LeGeros 2008). Auxiliary materials can also be added for better handling and contouring of the granulates. As with other bone substitute materials, combining them with inert carriers can increase their usability. A study by Zhou et al. investigated the effects of Pluronic F-127 (Pluronic, BASF, Mt Olive, NJ) and bone morphogenetic proteins (BMP) in combination with BCP on New Zealand white rabbit calvaria. The amount of new bone formation was not compromised by the addition of Pluronic, which enhanced handling and moldability. In addition, in the compounds with BMP there was a significant increase in new bone (Zhou, Peel & Clokie 2007). A later study by Zhou et al. compared different ratios of β-TCP and HA compound materials with Poloxamer 407 to achieve a stickier and more formable structure. Six weeks after insertion in New Zealand, the white rabbit healing was evaluated qualitatively and histologically. Different ratios of β-TCP and HA did not show any significant outcomes, and Poloxamer 407 did not influence the osteoconductivity (Zhou, Clokie & Peel 2013). These studies show that these biocompatible phycogenic materials can be used either alone or in combination with additives. The manufacturing process of these materials involves thermal treatment of the native algae and hydrothermal transformation of calcium carbonate into hydroxyapatite (Kasperk et al. 1988). The conversion to biphasic calcium phosphate is then performed by the suspension of calcite scaffolds into a slightly acidified aqueous solution of ammonium phosphate and by hydrothermal treatment at a high temperature over an extended period (Ioku & Kamitakahara 2009; Schopper et al. 2005). The granules show rapid and complete hydration during surgery and are a smooth material that is unlikely to injure the Schneiderian membrane. Schopper et al. completed a study with almost pure phycogenic hydroxyapatite and two biphasic calcium phosphate biomaterials with different HA/TCP ratios with identical three-dimensional geometry. The BCPs with 50/50 and 30/70 HA/TCP ratios and almost pure HA were implanted into corticocancellous costal defects of sheep. Overall, the BCP biomaterials had formed significantly more new bone and scaffold resorption was substantially higher than in the HA biomaterial. The different ratios of BCPs did not show significant differences. Still, the study confirmed the assumption that HA/TCP compounding was suitable for improving bone formation and scaffold resorption and, at the same time, maintains the scaffolds' osteoconductive properties (Schopper et al. 2005).

CaP materials are not osteoinductive by themselves. However, osteoinductive characteristics can be induced by designing the graft material with appropriate geometry, topography, and a combination of macroporosity, microporosity, and concavities that allow the entrapment and concentration of circulating growth factors or osteoprogenitor cells responsible for bone formation (LeGeros 2008). In our histologic analysis, we found differences in resorption kinetics and bone growth characteristics between the examined bone substitutes that depended on the porosity of the graft material and the fracturing of the particles. Porosity may vary according to pore geometry, pore size, and the density of a bone graft material (Rateitschak, KH., Wolf 2012; Spassova et al. 2007). Pure ß-TCP materials are known to be completely resorbable and to biodegrade too fast to form vital new bone (Saffar, Colombier & Detienville 1990). Pure HA materials, on the other hand, are known to be almost non-resorbable. However, studies revealed that, although it is an apatite material, phycogenic HA shows higher bioactivity and is slowly replaced entirely by new bone formation (Fujita et al. 2003; Liljensten et al. 2003; Spassova et al. 2007). By combining these two materials, favorable properties of both can be acquired. The higher osteoconductive properties and stability of HA and the higher solubility of ß-TCP lead to significantly more new bone than pure phycogenic HA (Schopper et al. 2005).

By using sinus floor elevation and the lateral window approach, a standardized protocol could be established to perform biopsies with enough bone substitute material for further analysis. Both materials showed different characteristics in resorption speed and progression, which could be explained by different cell behavior surrounding the materials. HA showed signs of more extended stability and good contact with surrounding tissues. In contrast, β-TCP/HA showed a higher infiltrative ingrowth of new bone, especially from the lower sinus floor area compared to the more apical zones, which could result from the higher resorptive activity of β-TCP/HA and, on the other hand, it showed a higher level of new bone formation in the first zone of the sinus floor. Overall, more new bone formation was measured in the β-TCP/HA compound material. While the proportion of new bone in contact with the replacement material increased from 3 to 6 months, it remained at about half the level of HA at both time points (4% after 3 months vs. 14% after 6 months, $p > 0.05$, Fig. 6 c). This circumstance can be explained by β-TCP/HA showing excellent scaffolding characteristics for pore infiltration of the cells during the first months of healing but then being resorbed gradually before a stable contact area could be established around the particles. Bone substitute surrounded and infiltrated by new bone showed a more severe alteration and smaller particle size in the

β-TCP/HA group after 6 months compared to the HA group. β-TCP/HA shows more signs of continuing resorption, and 6 months after augmentation, tiny remnants of β-TCP/HA were visible in the new bone regions, which raises the hypothesis that even after bone infiltration the augmentation material gets resorbed by enzymatic degradation. Almost pure HA shows new bone in broader contact with the graft material particles. After 6 months, the contact area increased to nearly three times the initial value (10% after 3 months vs. 30% after 6 months, Fig. 6 c).

This must be seen as a sign of HA's high osteoconductivity, which was also demonstrated in previous studies like Ewers et al. 2005 (Ewers 2005). The HA group shows a higher relative ratio of residual material after 6 months, which on the one hand indicates a lower resorption rate of HA and, on the other hand, could be explained by a slight compression of the augmentation volume and a subsequent increase in its density, which can be verified by X-ray volume measurements of the sinus graft.

Approaches for harvesting autogenous bone grafts intraorally almost always demand a second surgical site with all its potential negative impacts (Khoury & Hanser 2015; Khoury & Khoury 2006; Sakkas et al. 2016). While significantly increasing bone volume in horizontal defects, autogenous block grafts may need additional sinus floor elevation for vertical augmentation. With the aim of reducing the healing period, sinus grafting using a layering technique has been introduced by various authors (Frenken et al. 2010; Khoury, Keller & Keeve 2017). In this approach, autogenous bone chips are inserted near the basal alveolar crest, while a slowly resorbable substitution material like HA is placed on top cranially (Khoury, Keller & Keeve 2017). Clinical data demonstrate predictable outcomes of this technique. Therefore, cranial coverage of the β-TCP/HA substitute with pure HA as resorption protection might lead to similar advantages without the need for autogenous bone chips.

Nevertheless, our findings show that 6 months after using β-TCP/HA, new bone formation was seen extensively in the basal area of the sinus floor. This might document faster basal substitute resorption and ingrowth of osteogenic cells from the crestal bone boundary. Simultaneously, in the more cranial region, we observed histomorphometric results similar to the more stable HA, which in turn could make a layering technique unnecessary.

When comparing our data with studies about xenogeneic materials, the general picture shows that both materials can be used for sinus floor elevation or general intraoral bone augmentation (Froum et al. 2013). While phycogenic and especially biphasic materials show signs of resorption and remodeling after 6 months and constant volumes,

xenogeneic HA shows a more stable state with vital bone surrounding the particles and with only little change in the graft material (Froum et al. 2013). Analyses suggest that xenogeneic particles do not interfere with the osseous healing process after SFE procedures and promote new bone formation (Orsini et al. 2005).

As these studies are rare, it would also be of particular interest to evaluate phycogenic bone substitution materials for lateral bone augmentation. In a study performed by Kakar et al., the lateral alveolar ridge augmentation procedure using a subperiosteal tunneling technique and alloplastic BCP was evaluated in a pilot study with a population of nine patients with mandibular alveolar ridge deficiencies (Kakar et al. 2018). Lateral ridge augmentation was carried out, and the increase of ridge width was assessed using CBCT evaluation. Histological analysis of new bone formation was performed in one patient by obtaining bone cores at the implant placement re-entry. The histomorphometric assessment 4 months after bone grafting revealed 27.6% new bone and an overall mineralized fraction of 72.3% in the grafted area.

Because of the particulate morphology of the graft material and its lack of exact dimensional stability during the healing period, authors suggest the use of PTFE membranes, resorbable collagen membranes, or more rigid titanium membranes to fix the position of the graft material after augmentation (Watzinger et al. 2000). The quality of the newly formed bone after removal of titanium membranes is described as excellent and more predictable than after using flexible membranes (Lundgren et al. 1995).

4.3 | Limitations

The limitations of the present study naturally include the relatively small sample size and gender ratio of the participants. However, the gender ratio is representative of the patients who presented at our clinic for the procedure. The choice of study design allowed us a prospective, randomized analysis with standardized methods for comparing both materials. Both investigators were blinded to reduce observer bias during evaluation of the histologic images, while performing the colorization of the different tissues on the histologic slides, and during the histomorphometric examination. When the researcher subconsciously projects his/her expectations onto the research, it could unintentionally influence the outcomes and flaw the validity of the data. The fact that both materials, although following different resorption and infiltration patterns, projected in similar shapes and colors also reduced the likelihood of bias.

By using a case report form (CRF), all of the procedures and visits followed a strict protocol which was carried out according to a checklist. To combat possible errors and inaccuracies, the study was monitored by the Coordination Centre for Clinical Trials (KKS), a central service center for research at the Medical University of Graz, and was supported by the center’s expertise in planning, organization, execution, and documentation.

The histologic analysis allows qualitative and bone histomorphometry, a quantitative evaluation of bone microarchitecture, and bone remodeling by providing insight into cellular changes (Malhan et al. 2018). In histologic analyses, there is always the limitation of the quality of the biopsies as well as the quality of the processing and photographing of the slides (Hayat 1993). Surgical peculiarities like the insertion depth of the trephine bur, the cooling and rotational speed of the bur, and the surgeon's experience may also influence the quality of the biopsy. Because of individual specifications for each biopsy like residual bone height, the shape of the crestal bone, the volume of augmentation, the compression of the material, and, of course, individual healing patterns, distinct measurement methods had to be implemented to secure valid outcomes. After the slices were scanned using a light microscope with a high-resolution digital camera, all images were converted to TIFF files, an abbreviation for "tagged image file format," a computer file format for storing raster graphics images using lossless compression (Ostebee 1997). Each image was divided into zones according to precise parameters. On identifying the most crestal point of bone substitute, a zone was defined for analysis that extended one burr in width and 1 mm in length apically. In the 6-month group, another such zone apical

to the first one was specified. Based on automated counting and computing of the percentage distributions of the differently colored pixels, relevant measurements were derived for new bone, old bone, soft tissue, graft, bone infiltration of graft, bone-to-graft contact, and penetration depth. This histomorphometric analysis method is a valid option comparable to a range of programs that can perform fully automated or semi-automated morphometry of histologic slices (van ’t Hof et al. 2017; Chavassieux, Arlot & Meunier 1985). The range of software and tools includes open-source freeware programs as well as expensive commercial programs that all have the same goal: to color and mark the sections of a histologic image to differentiate between the tissues, such as osteoid, osteoclasts, new bone, old bone, soft tissue, and fibroblasts.

While processing the biopsies and preparing them for microscopical analysis, the staining, cutting angle, and selection of the region of interest is of great importance for valid outcomes (Donath & Breuner 1982). Even while following a predefined protocol, exact measurements can sometimes be imperfect because of different shades of stains for the same tissues, overlapping of tissues during the sawing process, or excessive air space between the tissues due to the biopsy technique. In addition, the exact method of dehydrating the slices with increasing concentrations of alcohol, followed by infiltration with increasing amounts of resin for embedding, is crucial. These parameters must be secured by controlled circumstances. For this purpose, the process of dehydration and infiltration had to be documented, the temperature of the environment had to be tracked according to the process protocol, and an exact timetable had to be followed for the specimens to show high-quality images. The undecalcified ground sections were then sawed into 50 µm thick slices and stained with azure II and methylene blue in alkaline solution and counterstained with basic fuchsin in water. Even while documenting the whole procedure and trying hard to maintain the study environment, there is always the possibility of obtaining flawed specimens, which occurred in four samples. During the study, these four slides had to be excluded due to tissue inconsistencies which would have resulted in the misleading evaluation and misinterpretation of the results.

Statistical values have to be seen in the context of the clinical situation and within the mean variation (Thiese, Arnold & Walker 2015). There are tools and guidance for researchers to consider when determining an analysis plan and clinical trial as well as a series of study-specific checklists to prevent incorrect or flawed outcomes. To avoid these kinds of errors, we strictly followed the EQUATOR network CONSORT checklist (Schulz, Altman & Moher 2010). This statement is used worldwide to improve and assist in the reporting of randomized controlled trials by implementing a 25-item checklist for

methodical research.

Several factors can also influence the statistical outcomes, and direct conclusions cannot always be drawn and interpreted without considering cofactors (Krousel-Wood, Chambers & Muntner 2006). Nevertheless, statistical analysis and evaluation is a crucial pillar of evidence-based medicine, and randomized controlled studies of high quality can contribute to a broader understanding of connections between different interventions and outcomes (Shelton 2014).

5 | Conclusion

In the present study, bone formation and biomaterial degradation of monophasic and biphasic, both phycogenic, bone graft materials were investigated histomorphometrically at 3 and 6 months following sinus floor augmentation. The results of the study show that HA and β-TCP/HA are both highly biocompatible bone substitution materials with specific physiochemical and histologic characteristics. No inflammations, allergic reactions, or losses of augmentation material could be observed. Within the limitations of this study, the present results support the literature findings that almost pure phycogenic hydroxyapatite and phycogenic biphasic calcium phosphate bone graft materials show promising results in the broad field of bone tissue regeneration. While autologous bone is the most biocompatible, secure and vital option for bone augmentation, synthetic, plant-based and animal-based options play a big role in bone augmentation surgeries due to the lack of a second surgical site with benefits for the patients regarding healing and morbidity. The broad field of bone substitutes is a fast paced and innovative research area with multiple indications in different medical professions. By clinical investigations the outcomes and effectiveness can be evaluated and lead to more certainty among the dental surgeons and practitioners. A further study evaluating the clinical outcome and volume differences between the two groups in up to 24 months is currently being carried out at our department. Longer-term histological and histomorphometric studies will be necessary to fully understand the resorption times of both biomaterials. The methods described were effective for the objectives in question and can be reproduced for investigating other bone substitute materials.

6 | References

van ’t Hof, R.J., Rose, L., Bassonga, E. & Daroszewska, A. 2017, ‘Open source software for semi-automated histomorphometry of bone resorption and formation parameters.’, *Bone*, vol. 99, pp. 69–79.

Aarden, E.M., Burger, E.H. & Nijweide, P.J. 1994, ‘Function of osteocytes in bone’, *Journal of Cellular Biochemistry*, pp. 287–99.

Aghaloo, T.L. & Moy, P.K. 2007, ‘Which hard tissue augmentation techniques are the most successful in furnishing bony support for implant placement?’, *The International journal of oral & maxillofacial implants*, vol. 22 Suppl, pp. 49–70.

AHCS 2012, ‘Good Scientific Practice’, *Academy for Healthcare Science*.

Albulescu, R., Popa, A., Enciu, A., Albulescu, L., Dudau, M., Popescu, I.D., Mihai, S., Codrici, E., Pop, S., Lupu, A., Stan, G.E., Manda, G. & Tanase, C. 2019, *Comprehensive In Vitro Testing of Calcium Phosphate-Based Bioceramics with Orthopedic and Dentistry Applications*, vol. 10, pp. 1–41.

Allegrini, S.J., Koening, B.J., Allegrini, M.R.F., Yoshimoto, M., Gedrange, T., Fanghaenel, J. & Lipski, M. 2008, ‘Alveolar ridge sockets preservation with bone grafting--review.’, *Annales Academiae Medicae Stetinensis*, vol. 54, no. 1, pp. 70–81.

Alvernia, J.E., Hidalgo, J., Sindou, M.P., Washington, C., Luzardo, G., Perkins, E., Nader, R. & Mertens, P. 2017, ‘The maxillary artery and its variants: an anatomical study with neurosurgical applications.’, *Acta neurochirurgica*, vol. 159, no. 4, pp. 655–64.

Aoki, N., Maeda, M., Kurata, M., Hirose, M., Ojima, Y., Wada, K. & Shibuya, Y. 2018, ‘Sinus floor elevation with platelet-rich fibrin alone: A Clinical retrospective study of 1-7 years’, *Journal of Clinical and Experimental Dentistry*, vol. 10, no. 10, pp. e984–91.

Artzi, Z., Nemcovsky, C.E., Tal, H. & Dayan, D. 2001, ‘Histopathological Morphometric Evaluation of 2 Different Hydroxyapatite-Bone Derivatives in Sinus Augmentation Procedures: A Comparative Study in Humans’, *Journal of Periodontology*.

Von Arx, T., Fodich, I. & Bornstein, M.M. 2014, ‘Proximity of premolar roots to maxillary sinus: A radiographic survey using cone-beam computed tomography’, *Journal of Endodontics*.

Ash, P., Loutit, J.F. & Townsend, K.M. 1980, ‘Osteoclasts derived from haematopoietic stem cells.’, *Nature*, vol. 283, pp. 669–70.

Atwood, D.A. 1963, ‘Postextraction changes in the adult mandible as illustrated by microradiographs of midsagittal sections and serial cephalometric roentgenograms’, *The Journal of Prosthetic Dentistry*, vol. 13, pp. 810–24.

Avila-Ortiz, G., Gubler, M., Romero-Bustillos, M., Nicholas, C.L., Zimmerman, M.B. & Barwacz, C.A. 2020, ‘Efficacy of Alveolar Ridge Preservation: A Randomized Controlled Trial.’, *Journal of dental research*, vol. 99, no. 4, pp. 402–9.

Bansal, R., Patil, S., Chaubey, K.K., Thakur, R.K. & Goyel, P. 2014, ‘Clinical evaluation of hydroxyapatite and beta-tricalcium phosphate composite graft in the treatment of intrabony periodontal defect: A clinico-radiographic study.’, *Journal of Indian Society of Periodontology*, vol. 18, no. 5, pp. 610–7.

Bansal, S., Chauhan, V., Sharma, S., Maheshwari, R., Juyal, A. & Raghuvanshi, S. 2009, ‘Evaluation of hydroxyapatite and beta-tricalcium phosphate mixed with bone marrow aspirate as a bone graft substitute for posterolateral spinal fusion.’, *Indian journal of orthopaedics*, vol. 43, no. 3, pp. 234–9.

Bassetti, R., Bassetti, M., Kremer, U. & Mericske-Stern, R. 2010, ‘Existiert das Kombinationssyndrom?’, *Schweiz Monatsschr Zahnmed*, vol. 120 9, pp. 771–8.

Baxter, D.J.G. & Shroff, M.M. 2011, 'Developmental maxillofacial anomalies.', *Seminars in ultrasound, CT, and MR*, vol. 32, no. 6, pp. 555–68.

Beaumont, C., Schmidt, R.J., Tatakis, D.N. & Zafiropoulos, G.-G. 2008, 'Use of Engineered Bone for Sinus Augmentation', *Journal of Periodontology*.

Bechara, S., Kubilius, R., Veronesi, G., Pires, J.T., Shibli, J.A. & Mangano, F.G. 2017, 'Short (6-mm) dental implants versus sinus floor elevation and placement of longer (≥10-mm) dental implants: a randomized controlled trial with a 3-year follow-up', *Clinical Oral Implants Research*.

Di Benedetto, A., Gigante, I., Colucci, S. & Grano, M. 2013, 'Periodontal Disease: Linking the Primary Inflammation to Bone Loss', *Clinical and Developmental Immunology*.

Bijur, P.E., Silver, W. & Gallagher, E.J. 2001, 'Reliability of the visual analog scale for measurement of acute pain', *Academic Emergency Medicine*.

Block, M.S. & Kent, J.N. 1997, 'Sinus augmentation for dental implants: the use of autogenous bone.', *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons*, vol. 55, no. 11, pp. 1281–6.

Block, M.S. & Kent, J.N. 1997, 'Sinus augmentation for dental implants: The use of autogenous bone', *Journal of Oral and Maxillofacial Surgery*, vol. 55, no. 11, pp. 1281–6.

Bołtacz-Rzepkowska, E. 1987, 'Zagadnienia korelacji między higieną jamy ustnej i stanem uz ę bien ia.', *Wrocławska Stomatologia*, p. 315.

Boyne, P.J. & James, R.A. 1980, 'Grafting of the maxillary sinus floor with autogenous marrow and bone.', *Journal of oral surgery (American Dental Association : 1965)*, vol. 38, no. 8, pp. 613–6.

Brugnami, F., Caiazzo, A. & Leone, C. 2009, 'Local intraoral autologous bone harvesting for dental implant treatment: Alternative sources and criteria of choice', *Keio Journal of Medicine*, pp. 24–8.

Brugnami, F., Caiazzo, A. & Leone, C. 2010, 'Review of Intraoral Harvesting for Bone Augmentation: Selection Criteria, Alternative Sites, and Case Report', *Compendium*, vol. 31, no. 7.

Bucholz, R.W., Carlton, A. & Holmes, R. 1989, 'Interporous hydroxyapatite as a bone graft substitute in tibial plateau fractures.', *Clinical orthopaedics and related research*, no. 240, pp. 53–62.

Budach, W., Hehr, T., Budach, V., Belka, C. & Dietz, K. 2006, 'A meta-analysis of hyperfractionated and accelerated radiotherapy and combined chemotherapy and radiotherapy regimens in unresected locally advanced squamous cell carcinoma of the head and neck', *BMC Cancer*.

Burt, B. & Ismail, A. 1986, 'Diet, nutrition, and food cario- genicity', *J Dent Res*, no. 65, pp. 1475–1484.

Campana, V., Milano, G., Pagano, E., Barba, M., Cicione, C., Salonna, G., Lattanzi, W. & Logroscino, G. 2014, 'Bone substitutes in orthopaedic surgery: from basic science to clinical practice.', *Journal of materials science. Materials in medicine*, vol. 25, no. 10, pp. 2445–61.

Carlsson, G.E. 2004, 'Responses of jawbone to pressure.', *Gerodontology*, vol. 21, pp. 65–70.

Cawood, J.I. & Howell, R.A. 1988, 'A classification of the edentulous jaws.', *International journal of oral and maxillofacial surgery*, vol. 17, pp. 232–6.

Chai, F., Raoul, G., Wiss, A., Ferri, J. & Hildebrand, H.F. 2011, '[Bone substitutes: Classification and concerns].', *Revue de stomatologie et de chirurgie maxillo-faciale*, vol. 112, no. 4, pp. 212–21.

Chanavaz, M. 1990, ‘Maxillary sinus: anatomy, physiology, surgery, and bone grafting related to implantology--eleven years of surgical experience (1979-1990).’, *The Journal of oral implantology*, vol. 16, no. 3, pp. 199–209.

Chavassieux, P.M., Arlot, M.E. & Meunier, P.J. 1985, ‘Intermethod variation in bone histomorphometry: comparison between manual and computerized methods applied to iliac bone biopsies.’, *Bone*, vol. 6, no. 4, pp. 221–9.

Chen, T.-W., Chang, H.-S., Leung, K.-W., Lai, Y.-L. & Kao, S.-Y. 2007, ‘Implant placement immediately after the lateral approach of the trap door window procedure to create a maxillary sinus lift without bone grafting: a 2-year retrospective evaluation of 47 implants in 33 patients.’, *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons*, vol. 65, no. 11, pp. 2324–8.

Chiapasco, M., Casentini, P. & Zaniboni, M. 2009, ‘Bone augmentation procedures in implant dentistry.’, *The International journal of oral & maxillofacial implants*, vol. 24 Suppl, pp. 237–59.

Chiroff, R.T., White, E.W., Weber, K.N. & Roy, D.M. 1975, ‘Tissue ingrowth of Replamineform implants.’, *Journal of biomedical materials research*, vol. 9, no. 4, pp. 29–45.

Choukroun, J., Diss, A., Simonpieri, A., Girard, M.-O., Schoeffler, C., Dohan, S.L., Dohan, A.J.J., Mouhyi, J. & Dohan, D.M. 2006, ‘Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part IV: clinical effects on tissue healing.’, *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics*, vol. 101, no. 3, pp. e56-60.

Clermonts, E.C. & Birkenhager-Frenkel, D.H. 1985, ‘Software for bone histomorphometry by means of a digitizer.’, *Computer methods and programs in biomedicine*, vol. 21, no. 3, pp. 185–94.

Cordaro, L., Bosshardt, D.D., Palattella, P., Rao, W., Serino, G. & Chiapasco, M. 2008, ‘Maxillary sinus grafting with Bio-Oss or Straumann Bone Ceramic: histomorphometric results from a randomized controlled multicenter clinical trial.’, *Clinical oral implants research*, vol. 19, no. 8, pp. 796–803.

Covani, U., Ricci, M., Bozzolo, G., Mangano, F., Zini, A. & Barone, A. 2011, ‘Analysis of the pattern of the alveolar ridge remodelling following single tooth extraction’, *Clinical Oral Implants Research*, vol. 22, pp. 820–5.

Cullinan, M.P., Ford, P.J. & Seymour, G.J. 2009, ‘Periodontal disease and systemic health: Current status’, *Australian Dental Journal*, vol. 54, pp. S62–9.

Daculsi, G., LeGeros, R.Z., Heughebaert, M. & Barbieux, I. 1990, ‘Formation of carbonate-apatite crystals after implantation of calcium phosphate ceramics.’, *Calcified tissue international*, vol. 46, no. 1, pp. 20–7.

Dahlin, C., Sennerby, L., Lekholm, U., Linde, A. & Nyman, S. 1989, ‘Generation of new bone around titanium implants using a membrane technique: an experimental study in rabbits.’, *The International journal of oral & maxillofacial implants*, vol. 4, no. 1, pp. 19–25.

Danesh-Sani, S.A., Loomer, P.M. & Wallace, S.S. 2016, ‘A comprehensive clinical review of maxillary sinus floor elevation: anatomy, techniques, biomaterials and complications.’, *The British journal of oral & maxillofacial surgery*, vol. 54, no. 7, pp. 724–30.

Deeks, J.J., Dinnes, J., D’Amico, R., Sowden, A.J., Sakarovitch, C., Song, F., Petticrew, M. & Altman, D.G. 2003, ‘Evaluating non-randomised intervention studies’, *Health Technology Assessment*.

Deguchi, T., Takano-Yamamoto, T., Yabuuchi, T., Ando, R., Roberts, W.E. & Garetto, L.P. 2008, 'Histomorphometric evaluation of alveolar bone turnover between the maxilla and the mandible during experimental tooth movement in dogs.', *American journal of orthodontics and dentofacial orthopedics : official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics*, vol. 133, no. 6, pp. 889–97.

Delloye, C., van Cauter, M., Dufrane, D., Francq, B.G., Docquier, P.-L. & Cornu, O. 2014, 'Local complications of massive bone allografts: an appraisal of their prevalence in 128 patients.', *Acta orthopaedica Belgica*, vol. 80, no. 2, pp. 196–204.

DMSIV 2006, *Vierte Deutsche Mundgesundheitsstudie (DMS IV)*, no. Dms Iv.

Docherty, N. 2012, 'Netter's head and neck anatomy for dentistry, 2nd edition', *British Dental Journal*.

Donath, K. 1988, 'Die Trenn-Dünnschliff-Technik zur Herstellung histologischer Präparate von nicht schneidbaren Geweben und Materialien', *Der Präparator*, Bochum, pp. 197–206.

Donath, K. & Breuner, G. 1982, 'A method for the study of undecalcified bones and teeth with attached soft tissues: The Säge-Schliff (sawing and grinding) Technique', *Journal of Oral Pathology & Medicine*.

Dudic, A. & Mericske-Stern, R. 2002, 'Retention mechanisms and prosthetic complications of implant-supported mandibular overdentures: long-term results.', *Clinical implant dentistry and related research*, vol. 4, pp. 212–9.

Dumitrescu, A.L. n.d., *Bone Grafts and Bone Graft Substitutes in Periodontal Therapy*.

Elgali, I., Omar, O., Dahlin, C. & Thomsen, P. 2017, 'Guided bone regeneration: materials and biological mechanisms revisited.', *European journal of oral sciences*, vol. 125, no. 5, pp. 315–37.

Engels, H. 2003, 'Misserfolge in der oralen Implantologie. Ein kleines Brevier zur Vermeidung von Misserfolgen.', *Handbuch zum BDIZ / EDI Implantat-Register*, Basic.Dent-Verlag, Berlin, pp. 273–93.

Ewers, R. 2005, 'Maxilla sinus grafting with marine algae derived bone forming material: A clinical report of long-term results', *Journal of Oral and Maxillofacial Surgery*, vol. 63, no. 12, pp. 1712–23.

Ewers, R., Goriwoda, W., Schopper, C., Moser, D. & Spassova, E. 2004, 'Histologic findings at augmented bone areas supplied with two different bone substitute materials combined with sinus floor lifting: Report of one case', *Clinical Oral Implants Research*.

Faour, O., Dimitriou, R., Cousins, C.A. & Giannoudis, P. V 2011, 'The use of bone graft substitutes in large cancellous voids: any specific needs?', *Injury*, vol. 42 Suppl 2, pp. S87-90.

Farzad, M. & Mohammadi, M. 2012, 'Guided bone regeneration: A literature review', *Journal of Oral Health and Oral Epidemiology*, vol. 1.

Felice, P., Pistilli, R., Piattelli, M., Soardi, E., Pellegrino, G., Corvino, V. & Esposito, M. 2013, '1-stage versus 2-stage lateral maxillary sinus lift procedures: 4-month post-loading results of a multicenter randomised controlled trial.', *European journal of oral implantology*, vol. 6, no. 2, pp. 153–65.

Fernandez de Grado, G., Keller, L., Idoux-Gillet, Y., Wagner, Q., Musset, A.-M., Benkirane-Jessel, N., Bornert, F. & Offner, D. 2018, 'Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management', *Journal of tissue engineering*, vol. 9, pp. 2041731418776819–2041731418776819.

Frayssinet, P., Mathon, D., Lerch, A., Autefage, A., Collard, P. & Rouquet, N. 2000, 'Osseointegration of composite calcium phosphate bioceramics.', *Journal of biomedical materials research*, vol. 50, no. 2, pp. 125–30.

Frenken, J.W.F.H., Bouwman, W.F., Bravenboer, N., Zijderveld, S.A., Schulten, E.A.J.M. & ten Bruggenkate, C.M. 2010, 'The use of Straumann Bone Ceramic in a maxillary sinus floor elevation procedure: a clinical, radiological, histological and histomorphometric evaluation with a 6-month healing period.', *Clinical oral implants research*, vol. 21, no. 2, pp. 201–8.

Fritsch, H. 1989, 'Staining of different tissues in thick epoxy resin-impregnated sections of human fetuses.', *Stain technology*, vol. 64, no. 2, pp. 75–9.

Froum, S.J., Wallace, S., Cho, S.-C., Rosenburg, E., Froum, S., Schoor, R., Mascarenhas, P., Tarnow, D.P., Corby, P., Elian, N., Fickl, S., Ricci, J., Hu, B., Bromage, T. & Khouly, I. 2013, 'A histomorphometric comparison of Bio-Oss alone versus Bio-Oss and platelet-derived growth factor for sinus augmentation: a postsurgical assessment.', *The International journal of periodontics & restorative dentistry*, vol. 33, no. 3, pp. 269–79.

Fry, R., Patidar, D., Goyal, S. & Malhotra, A. 2016, ' Proximity of maxillary posterior teeth roots to maxillary sinus and adjacent structures using Denta scan ® ', *Indian Journal of Dentistry*.

Fujita, R., Yokoyama, A., Kawasaki, T. & Kohgo, T. 2003, 'Bone augmentation osteogenesis using hydroxyapatite and β-tricalcium phosphate blocks', *Journal of Oral and Maxillofacial Surgery*.

Gaasbeek, R.D.A., Toonen, H.G., van Heerwaarden, R.J. & Buma, P. 2005, 'Mechanism of bone incorporation of beta-TCP bone substitute in open wedge tibial osteotomy in patients.', *Biomaterials*, vol. 26, no. 33, pp. 6713–9.

Galois, L. & Mainard, D. 2004, 'Bone ingrowth into two porous ceramics with different pore sizes: an experimental study.', *Acta orthopaedica Belgica*, vol. 70, no. 6, pp. 598–603.

Galois, L., Mainard, D. & Delagoutte, J.P. 2002, 'Beta-tricalcium phosphate ceramic as a bone substitute in orthopaedic surgery.', *International orthopaedics*, vol. 26, no. 2, pp. 109–15.

Garattini, S., Jakobsen, J.C., Wetterslev, J., Bertelé, V., Banzi, R., Rath, A., Neugebauer, E.A.M., Laville, M., Masson, Y., Hivert, V., Eikermann, M., Aydin, B., Ngwabyt, S., Martinho, C., Gerardi, C., Szmigielski, C.A., Demotes-Mainard, J. & Gluud, C. 2016, 'Evidence-based clinical practice: Overview of threats to the validity of evidence and how to minimise them', *European Journal of Internal Medicine*.

Ghosh, S.K., Nandi, S.K., Kundu, B., Datta, S., De, D.K., Roy, S.K. & Basu, D. 2008, 'In vivo response of porous hydroxyapatite and beta-tricalcium phosphate prepared by aqueous solution combustion method and comparison with bioglass scaffolds.', *Journal of biomedical materials research. Part B, Applied biomaterials*, vol. 86, no. 1, pp. 217–27.

Golden, S. & Bass, E. 2013, 'Validity of Meta-analysis in Diabetes: Meta-analysis Is an Indispensable Tool in Evidence Synthesis', *Diabetes care*, vol. 36, pp. 3368–73.

Gosau, M., Rink, D., Driemel, O. & Draenert, F.G. 2009, 'Maxillary sinus anatomy: a cadaveric study with clinical implications.', *Anatomical record (Hoboken, N.J. : 2007)*, vol. 292, no. 3, pp. 352–4.

Gouin, F., Yaouanc, F., Waast, D., Melchior, B., Delecrin, J. & Passuti, N. 2010, 'Open wedge high tibial osteotomies: Calcium-phosphate ceramic spacer versus autologous bonegraft.', *Orthopaedics & traumatology, surgery & research : OTSR*, vol. 96, no. 6, pp. 637–45.

de Groot, K. 1983, 'Ceramics based on calciumphosphates', *Ceramics in surgery*, pp. 79–90.

Gross, U.M. & Strunz, V. 1977, 'Surface staining of sawed sections of undecalcified bone containing alloplastic implants', *Biotechnic and Histochemistry*.

Gunzburg, R., Szpalski, M., Passuti, N. & Aebi, M. 2002, *The use of bone substitutes in spine surgery: a state of the art review*, vol. 10, Springer Science & Business Media.

Hallman, M., Sennerby, L. & Lundgren, S. 2002, 'A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture.', *The International journal of oral & maxillofacial implants*, vol. 17, no. 5, pp. 635–43.

Hallman, M. & Thor, A. 2008, 'Bone substitutes and growth factors as an alternative/complement to autogenous bone for grafting in implant dentistry.', *Periodontology 2000*, vol. 47, pp. 172–92.

Handschel, J., Simonowska, M., Naujoks, C., Depprich, R.A., Ommerborn, M.A., Meyer, U. & Kubler, N.R. 2009, 'A histomorphometric meta-analysis of sinus elevation with various grafting materials.', *Head & face medicine*, vol. 5, p. 12.

Hatano, N., Sennerby, L. & Lundgren, S. 2007, 'Maxillary sinus augmentation using sinus membrane elevation and peripheral venous blood for implant-supported rehabilitation of the atrophic posterior maxilla: case series.', *Clinical implant dentistry and related research*, vol. 9, no. 3, pp. 150–5.

Hayat, M.A. 1993, *Stains and cytochemical methods*, Springer Science & Business Media.

Heinrich-Weltzien, R. 1997, *Edentulism and Dental Status in Older Populations of Eight European Countries*, no. 10, pp. 189–99.

Henderson, B. & Kaiser, F. 2018, 'Bacterial modulators of bone remodeling in the periodontal pocket', *Periodontology 2000*.

Hildebolt, C., Molnar, S., Elvin-Lewis, M. & McKee, J. 1988, 'The effect of geochemical factors on prevalences of dental diseases for prehistoric inhabitants of the state of Missouri', *American Journal of physical anthropology*.

Imai, Y., Sato, T., Mori, S. & Okamoto, M. 2002, 'A histomorphometric analysis on bone dynamics in denture supporting tissue under continuous pressure', *Journal of Oral Rehabilitation*, vol. 29, pp. 72–9.

Ioannidis, J.P.A., Contopoulos-Ioannidis, D.G., Haidich, A.B., Pappa, M., Pantazis, N., Kokori, S.I., Tektonidou, M.G., Contopoulos-Ioannidis, D.G., Ioannidis, J.P.A. & Lau, J. 2001, 'Comparison of evidence of treatment effects in randomized and nonrandomized studies', *Journal of the American Medical Association*.

Ioku, K. & Kamitakahara, M. 2009, 'Hydroxyapatite Ceramics for medical application prepared by hydrothermal method', *Phosphorus Research Bulletin*, vol. 23, pp. 25–30.

Irmen, A. & Litina, A. 2016, *Population Aging and Inventive Activity*.

Jakobsen, J.C. & Gluud, C. 2013, 'The necessity of randomized clinical trials', *Journal of Advances in Medicine and Medical Research*, pp. 1453–68.

Jakse, N., Eskici, A., Pertl, C., Seibert, F.-J. & Lorenzoni, M. 2001, 'A modified technique of harvesting tibial cancellous bone and its use for sinus grafting', *Clinical Oral Implants Research*, vol. 12, no. 5, pp. 488–94.

Jarcho, M. 1981, 'Calcium phosphate ceramics as hard tissue prosthetics.', *Clinical orthopaedics and related research*, no. 157, pp. 259–78.

JAVA 2013, 'Declaration of Helsinki World Medical Association Declaration of Helsinki', *Bulletin of the world health organization.*

Jensen, S.S., Eriksen, J. & Schiodt, M. 2012, 'Severe bleeding after sinus floor elevation using the transcrestal technique: a case report.', *European journal of oral implantology*, vol. 5, no. 3, pp. 287–91.

Jensen, S.S. & Terheyden, H. 2009, 'Bone augmentation procedures in localized defects in the alveolar ridge: clinical results with different bone grafts and bone-substitute materials.', *The International journal of oral & maxillofacial implants*, vol. 24 Suppl, pp. 218–36.

Ji-Ye, H., Xin-Feng, Z. & Lei-Sheng, J. 2013, 'Autonomic control of bone formation: Its clinical relevance', *Handbook of Clinical Neurology*, vol. 117, Elsevier, pp. 161–71, viewed 25 February 2019, <https://www.sciencedirect.com/science/article/pii/B9780444534910000146>.

Jin, H.-Y., Teng, M.-H., Wang, D., Li, X., Liang, J.-Y., Wang, W.-X., Jiang, S. & Zhao, B.-D. 2019, 'Modified Disk-Up Sinus Reamer for Sinus Floor Elevation and Simultaneous Implant Placement: An Animal Study with Miniature Pigs', *Journal of Investigative Surgery*, vol. 0, no. 0, pp. 1–10.

John, H.-D. & Wenz, B. 2004, 'Histomorphometric analysis of natural bone mineral for maxillary sinus augmentation', *The International journal of oral & maxillofacial implants*, vol. 19, pp. 199–207.

St. John, K. & Mcneal, K. 2017, 'The Strength of Evidence Pyramid: One Approach for Characterizing the Strength of Evidence of Geoscience Education Research (GER) Community Claims', *Journal of Geoscience Education*, vol. 65, pp. 363–72.

Jordi, C., Mukaddam, K., Lambrecht, J.T. & Kühl, S. 2018, 'Membrane perforation rate in lateral maxillary sinus floor augmentation using conventional rotating instruments and piezoelectric device-a meta-analysis', *International journal of implant dentistry*, vol. 4, no. 1, p. 3.

Kakar, A., Kakar, K., Sripathi Rao, B.H., Lindner, A., Nagursky, H., Jain, G. & Patney, A. 2018, 'Lateral alveolar ridge augmentation procedure using subperiosteal tunneling technique: a pilot study', *Maxillofacial plastic and reconstructive surgery*, 1Yenepoya University Dental College, University Road, Mangalore, 575018 India., p. 3.

Kalyvas, D., Kapsalas, A., Paikou, S. & Tsiklakis, K. 2018, 'Thickness of the Schneiderian membrane and its correlation with anatomical structures and demographic parameters using CBCT tomography: a retrospective study', *International journal of implant dentistry*, vol. 4, no. 1, p. 32.

Kandelman, D., Bordeur, J.M., Simard, P. & Lepage, Y. 1986, 'Dental needs of the elderly: a comparison between some European and North American surveys.', *Community dental health*.

Kang, D.-W., Yun, P.-Y., Choi, Y.-H. & Kim, Y.-K. 2019, 'Sinus bone graft and simultaneous vertical ridge augmentation: case series study.', *Maxillofacial plastic and reconstructive surgery*, vol. 41, no. 1, p. 36.

Kasperk, C., Ewers, R., Simons, B. & Kasperk, R. 1988, 'Algae-derived (phycogene) hydroxylapatite. A comparative histological study', *International Journal of Oral and Maxillofacial Surgery*.

Kelly, E. 1972, 'Changes caused by a mandibular removable partial denture opposing a maxillary complete denture.', *The Journal of prosthetic dentistry*, vol. 27, pp. 140–50.

Khan, S.N., Cammisa, F.P.J., Sandhu, H.S., Diwan, A.D., Girardi, F.P. & Lane, J.M. 2005, 'The biology of bone grafting.', *The Journal of the American Academy of Orthopaedic Surgeons*, vol. 13, no. 1, pp. 77–86.

Khoury, F. & Hanser, T. 2015, 'Mandibular bone block harvesting from the retromolar region: a 10-year prospective clinical study.', *The International journal of oral & maxillofacial implants*, vol. 30, no. 3, pp. 688–97.

Khoury, F. & Hanser, T. 2019, 'Three-Dimensional Vertical Alveolar Ridge Augmentation in the Posterior Maxilla: A 10-year Clinical Study.', *The International journal of oral & maxillofacial implants*, vol. 34, no. 2, pp. 471–80.

Khoury, F., Keller, P. & Keeve, P.L. 2017, 'Stability of Grafted Implant Placement Sites After Sinus Floor Elevation Using a Layering Technique: 10-Year Clinical and Radiographic Results', *The International journal of oral & maxillofacial implants*, vol. 32, no. 5, p. 1086—1096.

Khoury, F. & Khoury, C. 2006, 'Mandibular bone block grafts: Diagnosis, instrumentation, harvesting techniques and surgical procedures.', in F. Khoury, H. Antoun & P. Missika (eds),*Bone Augmentation in Oral Implantology.*, Quintessenz, Berlin, London, p. 115–212.

Kim, S.-H., Kim, K.-H., Seo, B.-M., Koo, K.-T., Kim, T.-I., Seol, Y.-J., Ku, Y., Rhyu, I.-C., Chung, C.-P. & Lee, Y.-M. 2009, 'Alveolar bone regeneration by transplantation of periodontal ligament stem cells and bone marrow stem cells in a canine peri-implant defect model: a pilot study.', *Journal of periodontology*, vol. 80, no. 11, pp. 1815–23.

Kim, S.-Y., Choi, Y.-H. & Kim, Y.-K. 2018, 'Postoperative malocclusion after maxillofacial fracture management: a retrospective case study', *Maxillofacial Plastic and Reconstructive Surgery*.

Kim, S.-Y., Kim, Y.-K., Kim, H.-S., Yun, P.-Y., Kim, S.-G. & Choi, Y.-H. 2017, 'Extraction socket sealing using palatal gingival grafts and resorbable collagen membranes.', *Maxillofacial plastic and reconstructive surgery*, vol. 39, no. 1, p. 39.

Kirmeier, R., Payer, M., Wehrschuetz, M., Jakse, N., Platzer, S. & Lorenzoni, M. 2008, 'Evaluation of three-dimensional changes after sinus floor augmentation with different grafting materials.', *Clinical oral implants research*, vol. 19, no. 4, pp. 366–72.

Koshino, T., Murase, T., Takagi, T. & Saito, T. 2001, 'New bone formation around porous hydroxyapatite wedge implanted in opening wedge high tibial osteotomy in patients with osteoarthritis.', *Biomaterials*, vol. 22, no. 12, pp. 1579–82.

Krousel-Wood, M.A., Chambers, R.B. & Muntner, P. 2006, 'Clinicians' guide to statistics for medical practice and research: part I', *The Ochsner journal*, vol. 6, no. 2, pp. 68–83.

Küçükkurt, S., Alpaslan, G. & Kurt, A. 2017, 'Biomechanical comparison of sinus floor elevation and alternative treatment methods for dental implant placement', *Computer Methods in Biomechanics and Biomedical Engineering*.

Kühl, S., Gotz, H., Brochhausen, C., Jakse, N., Filippi, A., D'Hoedt, B. & Kreisler, M. 2012, 'The influence of substitute materials on bone density after maxillary sinus augmentation: a microcomputed tomography study.', *The International journal of oral & maxillofacial implants*, vol. 27, no. 6, pp. 1541–6.

Kühl, S., Payer, M., Kirmeier, R., Wildburger, A., Acham, S. & Jakse, N. 2015, 'The influence of particulated autogenous bone on the early volume stability of maxillary sinus grafts with biphasic calcium phosphate: a randomized clinical trial.', *Clinical implant dentistry and related research*, vol. 17, no. 1, pp. 173–8.

Kühl, S., Payer, M., Kirmeier, R., Wildburger, A., Wegscheider, W. & Jakse, N. 2014, 'The influence of bone marrow aspirates and concentrates on the early volume stability of maxillary sinus grafts with deproteinized bovine bone mineral - first results of a RCT.', *Clinical oral implants research*, vol. 25, no. 2, pp. 221–5.

Kühnel, T.S. & Reichert, T.E. 2015, 'Trauma of the midface', *Laryngo- rhino- otologie*, vol. 94, pp. S206–47.

Künzel, W. 1989, *Gerontostomatologie*, Berlin.

Kurlej, W., Łagowska, K., Szulgan, A., Gredes, M. & Gworys, B. 2006, 'The application of Human Loss Permanent Dentition Indes (TLI) and Decay changes index (DEF) to make appraisal of chosen students`dentitions', *Movement and Health*, Opole, pp. 505–9.

Laczko, J. & Levai, G. 1975, 'A simple differential staining method for semi thin sections of ossifying cartilage and bone tissues embedded in epoxy resin', *Mikroskopie*, vol. Wien 31(1), pp. 1–4.

Lake, S., Iwanaga, J., Kikuta, S., Oskouian, R.J., Loukas, M. & Tubbs, R.S. 2018, 'The Incisive Canal: A Comprehensive Review.', *Cureus*, vol. 10, no. 7, p. e3069.

Lalabonova, H. & Daskalov, H. 2013, 'JAW CYSTS AND GUIDED BONE REGENERATION (a late complication after enucleation)', *Journal of IMAB - Annual Proceeding (Scientific Papers)*.

de Las Casas, E.B., de Almeida, A.F., Cimini Junior, C.A., Gomes, P. de T.V., Cornacchia, T.P.M. & Saffar, J.M.E. 2007, 'Determination of tangential and normal components of oral forces.', *Journal of applied oral science : revista FOB*, vol. 15, pp. 70–6.

Laurencin, C.T. & El-Amin, S.F. 2008, 'Xenotransplantation in orthopaedic surgery.', *The Journal of the American Academy of Orthopaedic Surgeons*, vol. 16, no. 1, pp. 4–8.

LeGeros, R.Z. 2008, 'Calcium phosphate-based osteoinductive materials', *Chemical Reviews*.

LeGeros, R.Z., Parsons, J.R., Daculsi, G., Driessens, F., Lee, D., Liu, S.T., Metsger, S., Peterson, D. & Walker, M. 1988, 'Significance of the porosity and physical chemistry of calcium phosphate ceramics. Biodegradation-bioresorption.', *Annals of the New York Academy of Sciences*, vol. 523, pp. 268–71.

Lekholm, U. & Zarb, G. 1985, 'Patient selection and preparation.', *Tissue integrated prostheses. Osseointegration in Clinical Dentistry*, pp. 109–209.

Liljensten, E., Adolfsson, E., Strid, K.G. & Thomsen, P. 2003, 'Resorbable and nonresorbable hydroxyapatite granules as bone graft substitutes in rabbit cortical defects', *Clinical Implant Dentistry and Related Research*.

Linde, A., Thoren, C., Dahlin, C. & Sandberg, E. 1993, 'Creation of new bone by an osteopromotive membrane technique: an experimental study in rats.', *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons*, vol. 51, no. 8, pp. 892–7.

Links between oral health and general health - the case for action 2011, Dental Health Services Victoria.

Liu, F., Liu, Y., Li, X., Wang, X., Li, D., Chung, S., Chen, C. & Lee, I.-S. 2019, 'Osteogenesis of 3D printed macro-pore size biphasic calcium phosphate scaffold in rabbit calvaria.', *Journal of biomaterials applications*, vol. 33, no. 9, pp. 1168–77.

Liu, J. & Kerns, D.G. 2014, 'Mechanisms of guided bone regeneration: a review.', *The open dentistry journal*, vol. 8, pp. 56–65.

Ludwig, P. & Niedermeier, W. 2002, 'Teilprothese', *Checklisten der Zahnmedizin Prothetik*, Thieme, Stuttgart, pp. 65–103.

Lundgren, D., Lundgren, A.K., Sennerby, L. & Nyman, S. 1995, 'Augmentation of intramembraneous bone beyond the skeletal envelope using an occlusive titanium barrier. An experimental study in the rabbit.', *Clinical oral implants research*, vol. 6, no. 2, pp. 67–72.

Malhan, D., Muelke, M., Rosch, S., Schaefer, A.B., Merboth, F., Weisweiler, D., Heiss, C., Arganda-Carreras, I. & El Khassawna, T. 2018, 'An Optimized Approach to Perform Bone Histomorphometry ', *Frontiers in Endocrinology* , p. 666.

Malhotra, A. & Habibovic, P. 2016, 'Calcium Phosphates and Angiogenesis: Implications and Advances for Bone Regeneration.', *Trends in biotechnology*, vol. 34, no. 12, pp. 983–92.

Marks, S.C. & Seifert, M.F. 1985, 'The lifespan of osteoclasts: experimental studies using the giant granule cytoplasmic marker characteristic of beige mice.', *Bone*, vol. 6, pp. 451–5.

Marx, R.E., Sawatari, Y., Fortin, M. & Broumand, V. 2005, 'Bisphosphonate-induced exposed bone (osteonecrosis/osteopetrosis) of the jaws: Risk factors, recognition, prevention, and treatment', *Journal of Oral and Maxillofacial Surgery*, pp. 1567–75.

McNeill, C. 1997, 'Management of temporomandibular disorders: Concepts and controversies', *Journal of Prosthetic Dentistry*, vol. 77, pp. 510–22.

Micheelis, W. & Reich, E. 1997, *Dritte Deutsche Mundgesundheitsstudie*, Köln.

Mikulasek, A., Fuchs, R. & Wisbauer, A. 2018, *Demographisches Jahrbuch 2018*, Vienna.

Misch, C.E. 1990, 'Density of bone: effect on treatment plans, surgical approach, healing, and progressive boen loading.', *The International journal of oral implantology : implantologist*.

La Monaca, G., Iezzi, G., Cristalli, M.P., Pranno, N., Sfasciotti, G.L. & Vozza, I. 2018, 'Comparative Histological and Histomorphometric Results of Six Biomaterials Used in Two-Stage Maxillary Sinus Augmentation Model after 6-Month Healing', G. Sammartino (ed.),*BioMed Research International*, vol. 2018, p. 9430989.

Monje, A., Diaz, K.T., Aranda, L., Insua, A., Garcia-Nogales, A. & Wang, H.-L. 2016, 'Schneiderian Membrane Thickness and Clinical Implications for Sinus Augmentation: A Systematic Review and Meta-Regression Analyses.', *Journal of periodontology*, vol. 87, no. 8, pp. 888–99.

Mossey, P.A., Little, J., Munger, R.G., Dixon, M.J. & Shaw, W.C. 2009, 'Cleft lip and palate.', *Lancet (London, England)*, vol. 374, no. 9703, pp. 1773–85.

Mourad, M., Jetmore, T., Jategaonkar, A.A., Moubayed, S., Moshier, E. & Urken, M.L. 2017, 'Epidemiological Trends of Head and Neck Cancer in the United States: A SEER Population Study', *Journal of Oral and Maxillofacial Surgery*.

Moy, P.K., Lundgren, S. & Holmes, R.E. 1993, 'Maxillary sinus augmentation: histomorphometric analysis of graft materials for maxillary sinus floor augmentation', *Journal of oral and maxillofacial surgery*, vol. 51, no. 8, pp. 857–62.

Mroz, T.E., Joyce, M.J., Steinmetz, M.P., Lieberman, I.H. & Wang, J.C. 2008, 'Musculoskeletal allograft risks and recalls in the United States.', *The Journal of the American Academy of Orthopaedic Surgeons*, vol. 16, no. 10, pp. 559–65.

Murad, M.H., Asi, N., Alsawas, M. & Alahdab, F. 2016, 'New evidence pyramid', *Evidence-based medicine*, vol. 21, no. 4, 2016/06/23., pp. 125–7.

Nandi, S.K., Kundu, B., Ghosh, S.K., De, D.K. & Basu, D. 2008, 'Efficacy of nano-hydroxyapatite prepared by an aqueous solution combustion technique in healing bone defects of goat.', *Journal of veterinary science*, vol. 9, no. 2, pp. 183–91.

Nishimoto, M., Kan, J., Rungcharassaeng, K., Roe, P., Prasad, H. & Lozada, J. 2019, 'Histomorphometric Analysis of Maxillary Sinus Grafts: A Pilot Study', *The International Journal of Oral & Maxillofacial Implants*.

Offner, D., de Grado, G.F., Meisels, I., Pijnenburg, L., Fioretti, F., Benkirane-Jessel, N. & Musset, A.-M. 2019, 'Bone Grafts, Bone Substitutes and Regenerative Medicine Acceptance for the Management of Bone Defects Among French Population: Issues about Ethics, Religion or Fear?', *Cell Medicine*.

Ofner, P; Errath, M. 2004, 'Randomizer: A Web-based Trial Management and Patient Randomization System for Clinical Trials', *Jahrestagung der Deutschen Gesellschaft für Medizinische Informatik, Biometrie und Epidemiologie (gmds)*, Innsbruck, Austria.

Okazaki, A., Koshino, T., Saito, T. & Takagi, T. 2000, 'Osseous tissue reaction around hydroxyapatite block implanted into proximal metaphysis of tibia of rat with collagen-induced arthritis.', *Biomaterials*, vol. 21, no. 5, pp. 483–7.

Orsini, G., Traini, T., Scarano, A., Degidi, M., Perrotti, V., Piccirilli, M. & Piattelli, A. 2005, 'Maxillary sinus augmentation with Bio-Oss particles: a light, scanning, and transmission electron microscopy study in man.', *Journal of biomedical materials research. Part B, Applied biomaterials*, vol. 74, no. 1, pp. 448–57.

Orwoll, E.S. & Adler, R.A. 2018, 'Osteoporosis in men', *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*.

Osburn, R.C. 1974, 'Preservation of the alveolar ridge: a simplified technique for retaining teeth beneath removable appliances.', *The Journal of the Indiana State Dental Association*, vol. 53, no. 1, pp. 8–11.

Ostebee, A. 1997, 'Encyclopedia of Graphics File Formats, Second Edition', *The American Mathematical Monthly*.

Otake, I., Kageyama, I. & Mataga, I. 2011, 'Clinical anatomy of the maxillary artery.', *Okajimas folia anatomica Japonica*, vol. 87, no. 4, pp. 155–64.

Paul, M. & Leibovici, L. 2014, 'Systematic review or meta-analysis? Their place in the evidence hierarchy.', *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, England, pp. 97–100.

Pieske, O., Wittmann, A., Zaspel, J., Loffler, T., Rubenbauer, B., Trentzsch, H. & Piltz, S. 2009, 'Autologous bone graft versus demineralized bone matrix in internal fixation of ununited long bones.', *Journal of trauma management & outcomes*, vol. 3, p. 11.

Pjetursson, B.E. & Lang, N.P. 2014, 'Sinus floor elevation utilizing the transalveolar approach.', *Periodontology 2000*, vol. 66, no. 1, pp. 59–71.

Pjetursson, B.E., Tan, W.C., Zwahlen, M. & Lang, N.P. 2008, 'A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation: Part I: Lateral approach', *Journal of Clinical Periodontology*.

Puri, K., Kumar, A., Khatri, M., Bansal, M., Rehan, M. & Siddeshappa, S.T. 2019, '44-year journey of palatal connective tissue graft harvest: A narrative review.', *Journal of Indian Society of Periodontology*, vol. 23, no. 5, pp. 395–408.

Rancitelli, D., Borgonovo, A.E., Cicciu, M., Re, D., Rizza, F., Frigo, A.C. & Maiorana, C. 2015, 'Maxillary Sinus Septa and Anatomic Correlation With the Schneiderian Membrane.', *The Journal of craniofacial surgery*, vol. 26, no. 4, pp. 1394–8.

Rateitschak, KH., Wolf, H. 2012, *Farbatlanten der Zahnmedizin, Band 1: Parodontologie*, *Georg Thieme Verlag Stuttgart*, vol. 3, Georg Thieme Verlag Stuttgart.

Rauch, F. 2014, *Chapter 25 - Bone Histomorphometry*, in J.R. Shapiro, P.H. Byers, F.H. Glorieux & P.D.B.T.-O.I. Sponseller (eds), Academic Press, San Diego, pp. 237–42.

Revell, P.A. 1983, 'Histomorphometry of bone', *Journal of clinical pathology*, vol. 36, no. 12, pp. 1323–31.

Riben, C. & Thor, A. 2012, 'The maxillary sinus membrane elevation procedure: Augmentation of bone around dental implants without grafts - A review of a surgical technique', *International Journal of Dentistry*.

Roberts, T.T. & Rosenbaum, A.J. 2012, 'Bone grafts, bone substitutes and orthobiologics: the bridge between basic science and clinical advancements in fracture healing.', *Organogenesis*, vol. 8, no. 4, pp. 114–24.

Rohen, J.W. & Lütjen-Drecoll, E. 2006, *Funktionelle Anatomie des Menschen, Lehrbuch der makroskopischen Anatomie nach funktionellen Gesichtspunkten*, Stuttgart.

Rolski, D., Kostrzewa-Janicka, J., Zawadzki, P., Życińska, K. & Mierzwińska-Nastalska, E. 2016, 'The Management of Patients after Surgical Treatment of Maxillofacial Tumors', *BioMed research international*, vol. 2016, 2016/09/26., p. 4045329.

Rozas, N.S., Sadowsky, J.M. & Jeter, C.B. 2017, 'Strategies to improve dental health in elderly patients with cognitive impairment: A systematic review', *Journal of the American Dental Association*, vol. 148, no. 4, p. 236–245.e3.

Sabitzer, S., Sokolowski, A., Sokolowski, A. & Wegscheider, W. 2018, 'Veränderungen der dentalen Situation in zunehmendem Alter Doktor der Zahnheilkunde', Graz Medical University.

Saccardin, F. & Kühl, S. 2018, 'Maxillary sinus floor elevation: Lateral window technique', *Implantologie*, vol. 26, pp. 27–37.

Saffar, J.L., Colombier, M.L. & Detienville, R. 1990, 'Bone Formation in Tricalcium Phosphate-Filled Periodontal Intrabony Lesions. Histological Observations in Humans', *Journal of Periodontology*.

Sai, S. & Fujii, K. 2005, *Beta-Tricalcium Phosphate as a Bone Graft Substitute*.

Saikia, K.C., Bhattacharya, T.D., Bhuyan, S.K., Talukdar, D.J., Saikia, S.P. & Jitesh, P. 2008, 'Calcium phosphate ceramics as bone graft substitutes in filling bone tumor defects.', *Indian journal of orthopaedics*, vol. 42, no. 2, pp. 169–72.

Sakkas, A., Ioannis, K., Winter, K., Schramm, A. & Wilde, F. 2016, 'Clinical results of autologous bone augmentation harvested from the mandibular ramus prior to implant placement. An analysis of 104 cases.', *GMS Interdisciplinary plastic and reconstructive surgery DGPW*, vol. 5, p. Doc21.

Sam, G. & Pillai, B.R.M. 2014, 'Evolution of Barrier Membranes in Periodontal Regeneration-"Are the third Generation Membranes really here?".', *Journal of clinical and diagnostic research : JCDR*, vol. 8, no. 12, pp. ZE14-7.

Sbordone, C., Toti, P., Guidetti, F., Califano, L., Bufo, P. & Sbordone, L. 2013, 'Volume changes of autogenous bone after sinus lifting and grafting procedures: a 6-year computerized tomographic follow-up.', *Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery*, vol. 41, no. 3, pp. 235–41.

Schaller, D.O. 2009, *Alveolarkamm-und Sinusbodenaugmentation mit autogenem Knochen–eine retrospektive Studie über 13 Jahre*.

Schenk, R.K., Buser, D., Hardwick, W.R. & Dahlin, C. 1994, 'Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible.', *The International journal of oral & maxillofacial implants*, vol. 9, no. 1, pp. 13–29.

Schlegel, K.A., Fichtner, G., Schultze-Mosgau, S. & Wiltfang, J. 2003, 'Histologic findings in sinus augmentation with autogenous bone chips versus a bovine bone substitute', *The International journal of oral & maxillofacial implants*, vol. 18, no. 1, pp. 53–8.

Schmid-Schwap, M. 2006, 'Totalprothetik', in E. Piehslinger (ed.),*Grundlagen der zahnärztlichen prothetik*, 2. Auflage., Universimed, Wien.

Schneider, C.V. 1660, *Liber de catarrhis*, Wittenberg.

Schopper, C., Moser, D., Sabbas, A., Lagogiannis, G., Spassova, E., König, F., Donath, K. & Ewers, R. 2003, 'The fluorohydroxyapatite (FHA) FRIOS® Algipore® is a suitable biomaterial for the reconstruction of severely atrophic human maxillae', *Clinical Oral Implants Research*.

Schopper, C., Ziya-Ghazvini, F., Goriwoda, W., Moser, D., Wanschitz, F., Spassova, E., Lagogiannis, G., Auterith, A. & Ewers, R. 2005, 'HA/TCP compounding of a porous CaP biomaterial improves bone formation and scaffold degradation - A long-term histological study', *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, vol. 74, no. 1, pp. 458–67.

Schroeder, H.E. 2000, *Orale Strukturbiologie*, Thieme, Stuttgart.

Schulz, K.F., Altman, D.G. & Moher, D. 2010, 'CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials.', *Trials*, vol. 11, p. 32.

Schumacher, G.H. 1994, ‘Zähne und Zahnhalteapparat’, in E. Olbrichs, K. Sames & A. Schramm (eds),*Kompendium der Gerontologie*, Ecomed, Landsberg/Lech.

Setz, J. & Körber, E. 2007, ‘Totalprothetik’, in W. Gernet, R. Biffar, N. Schwenzer & M. Ehrenfeld (eds),*Zahnärztliche Prothetik*, Thieme, Stuttgart, pp. 134–6.

Shelton, J.D. 2014, ‘Evidence-based public health: not only whether it works, but how it can be made to work practicably at scale’, *Global health, science and practice*, vol. 2, no. 3, pp. 253–8.

Silva, L. deF, de Lima, V.N., Faverani, L.P., de Mendonca, M.R., Okamoto, R. & Pellizzer, E.P. 2016, ‘Maxillary sinus lift surgery-with or without graft material? A systematic review.’, *International journal of oral and maxillofacial surgery*, vol. 45, no. 12, pp. 1570–6.

Simunek, A., Cierny, M., Kopecka, D., Kohout, A., Bukac, J. & Vahalova, D. 2005, ‘The sinus lift with phycogenic bone substitute: A histomorphometric study’, *Clinical Oral Implants Research*.

Slade, G.D. 1997, ‘Concepts of Oral Health, Disease and the Quality of Life’, *Measuring Oral Health and Quality of Life*, p. 172.

Smiler, D.G., Johnson, P.W., Lozada, J.L., Misch, C., Rosenlicht, J.L., Tatum, O.H.J. & Wagner, J.R. 1992, ‘Sinus lift grafts and endosseous implants. Treatment of the atrophic posterior maxilla.’, *Dental clinics of North America*, vol. 36, no. 1, pp. 151–8.

Sohn, D.S., Moon, J.W., Moon, K.N., Cho, S.C. & Kang, P.S. 2010, ‘New Bone Formation in the Maxillary Sinus Using Only Absorbable Gelatin Sponge’, *Journal of Oral and Maxillofacial Surgery*.

Sokolowski, A., Sokolowski, A. & Wegscheider, W. 2015, ‘Kieferkammatrophie im Seitenzahnbereich der Mandibula in Abhängigkeit von der prothetischen Versorgung’, Graz Medical University.

Sokolowski, A.A., Sokolowski, A.A., Schwarze, U.Y., Theisen, K., Payer, M., Lorenzoni, M. & Wegscheider, W. n.d., ‘Phycogenic bone substitutes for sinus floor augmentation: Histomorphometric comparison of hydroxyapatite vs. biphasic calcium phosphate in a randomized clinical pilot study.’, *International Journal of Oral Implantology (In press)*.

Solar, P., Geyerhofer, U., Traxler, H., Windisch, A., Ulm, C. & Watzek, G. 1999, ‘Blood supply to the maxillary sinus relevant to sinus floor elevation procedures.’, *Clinical oral implants research*, vol. 10, no. 1, pp. 34–44.

Soriano, R.M. & M Das, J. 2020, *Anatomy, Head and Neck, Maxilla*, Treasure Island (FL).

Spassova, E., Gintenreiter, S., Halwax, E., Moser, D., Schopper, C. & Ewers, R. 2007, ‘Chemistry, ultrastructure and porosity of monophasic and biphasic bone forming materials derived from marine algae’, *Materialwissenschaft und Werkstofftechnik*.

Spivak, J.M. & Hasharoni, A. 2001, ‘Use of hydroxyapatite in spine surgery.’, *European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society*, vol. 10 Suppl 2, pp. S197-204.

Stimmelmayr, M., Beuer, F., Schlee, M., Edelhoff, D. & Guth, J.-F. 2014, ‘Vertical ridge augmentation using the modified shell technique--a case series.’, *The British journal of oral & maxillofacial surgery*, vol. 52, no. 10, pp. 945–50.

Strub, J.R., Kern, M., Türp, J.C., Witkowski, S., Heydecke, G. & Wolfart, S. 2011, *Curriculum Prothetik*, 4., Quintessenz Verlag.

Summers, R.B. 1994, ‘A new concept in maxillary implant surgery: the osteotome technique.’, *Compendium (Newtown, Pa.)*, vol. 15, no. 2, p. 152, 154–6, 158 passim; quiz 162.

Szulgan, A., Kurlej, W. & Łagowska, K. 2005, 'The research over loosing human permanent dentition', *Międzynarodowa Konferencja Naukowo-Szkoleniowa 'Holistic View on Elderly Patients'*, Wroclaw, pp. 17–8.

Tallgren, A. 2003, 'The continuing reduction of the residual alveolar ridges in complete denture wearers: A mixed-longitudinal study covering 25 years', *Journal of Prosthetic Dentistry*, vol. 89, pp. 427–35.

Tan, W.C., Lang, N.P., Zwahlen, M. & Pjetursson, B.E. 2008, 'A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation. Part II: transalveolar technique.', *Journal of clinical periodontology*, vol. 35, no. 8 Suppl, pp. 241–54.

Tanaka, K., Sailer, I., Iwama, R., Yamauchi, K., Nogami, S., Yoda, N. & Takahashi, T. 2018, *Relationship between cortical bone thickness and implant stability at the time of surgery and secondary stability after osseointegration measured using resonance frequency analysis*, vol. 48, no. 6, pp. 360–72.

Tanoue, S., Kiyosue, H., Mori, H., Hori, Y., Okahara, M. & Sagara, Y. 2013, 'Maxillary artery: functional and imaging anatomy for safe and effective transcatheter treatment.', *Radiographics : a review publication of the Radiological Society of North America, Inc*, vol. 33, no. 7, pp. e209-24.

Tatum, H.J. 1986, 'Maxillary and sinus implant reconstructions.', *Dent Clin North Am*.

Testori, T., Fabbro, M., Weinstein, R. & Wallace, S. 2010, *SinusbodenaugmentationChirurgische Techniken und alternative Konzepte*, Quintessenz Verlag.

Tetsch, J., Tetsch, P. & Lysek, D.A. 2010, 'Long-term results after lateral and osteotome technique sinus floor elevation: a retrospective analysis of 2190 implants over a time period of 15 years.', *Clinical oral implants research*, vol. 21, no. 5, pp. 497–503.

Thiese, M.S., Arnold, Z.C. & Walker, S.D. 2015, 'The misuse and abuse of statistics in biomedical research', *Biochemia medica*, vol. 25, no. 1, pp. 5–11.

Thorwarth, M., Wehrhan, F., Srour, S., Schultze-Mosgau, S., Felszeghy, E., Bader, R.D. & Schlegel, K.A. 2007, 'Evaluation of substitutes for bone: Comparison of microradiographic and histological assessments', *British Journal of Oral and Maxillofacial Surgery*.

Tilkeridis, K., Touzopoulos, P., Ververidis, A., Christodoulou, S., Kazakos, K. & Drosos, G.I. 2014, 'Use of demineralized bone matrix in spinal fusion.', *World journal of orthopedics*, vol. 5, no. 1, pp. 30–7.

Tite, T., Popa, A.C., Balescu, L.M., Bogdan, I.M., Pasuk, I., Ferreira, J.M.F. & Stan, G.E. 2018, 'Cationic substitutions in hydroxyapatite: Current status of the derived biofunctional effects and their in vitro interrogation methods', *Materials*.

Tiwari, T., Scarbro, S., Bryant, L.L. & Puma, J. 2016, 'Factors Associated with Tooth Loss in Older Adults in Rural Colorado', *Journal of Community Health*.

Tolstunov, L. 2014, 'Classification of the alveolar ridge width: implant-driven treatment considerations for the horizontally deficient alveolar ridges.', *The Journal of oral implantology*, vol. 40 Spec No, pp. 365–70.

Tomlin, G. & Borgetto, B. 2011, 'Research Pyramid: a new evidence-based practice model for occupational therapy.', *The American journal of occupational therapy : official publication of the American Occupational Therapy Association*, vol. 65, no. 2, pp. 189–96.

Tonelli, P., Duvina, M., Barbato, L., Biondi, E., Nuti, N., Brancato, L. & Delle Rose, G. 2011, 'Bone regeneration in dentistry', *Clinical Cases in Mineral and Bone Metabolism*.

Touri, M., Moztarzadeh, F., Osman, N.A.A., Dehghan, M.M. & Mozafari, M. 2018, '3D-printed biphasic calcium phosphate scaffolds coated with an oxygen generating system for enhancing engineered tissue survival.', *Materials science & engineering. C, Materials for biological applications*, vol. 84, pp. 236–42.

Tugwell, P. & Knottnerus, J.A. 2015, 'Is the "Evidence-Pyramid" now dead?', *Journal of Clinical Epidemiology*.

Ulm, C., Tepper, G., Blahout, R., Rausch-Fan, X., Hienz, S. & Matejka, M. 2009, 'Characteristic features of trabecular bone in edentulous mandibles', *Clinical Oral Implants Research*, vol. 20, pp. 594–600.

Underwood, A.S. 1910, 'An Inquiry into the Anatomy and Pathology of the Maxillary Sinus.', *Journal of anatomy and physiology*, vol. 44, no. Pt 4, pp. 354–69.

de Vries, R.B.M., Oerlemans, A., Trommelmans, L., Dierickx, K. & Gordijn, B. 2008, 'Ethical aspects of tissue engineering: a review.', *Tissue engineering. Part B, Reviews*, vol. 14, no. 4, pp. 367–75.

Wang, H.L. & Carroll, M.J. 2001, 'Guided bone regeneration using bone grafts and collagen membranes.', *Quintessence international (Berlin, Germany : 1985)*, vol. 32, no. 7, pp. 504–15.

Watzinger, F., Luksch, J., Millesi, W., Schopper, C., Neugebauer, J., Moser, D. & Ewers, R. 2000, 'Guided bone regeneration with titanium membranes: a clinical study.', *The British journal of oral & maxillofacial surgery*, vol. 38, no. 4, pp. 312–5.

Wildburger, A., Payer, M., Jakse, N., Strunk, D., Etchard-Liechtenstein, N. & Sauerbier, S. 2014, 'Impact of autogenous concentrated bone marrow aspirate on bone regeneration after sinus floor augmentation with a bovine bone substitute - a split-mouth pilot study', *Clinical Oral Implants Research*.

Wilson, D.B. 1979, 'Embryonic development of the head and neck: Part 3, The face.', *Head & neck surgery*, vol. 2, no. 2, pp. 145–53.

Yang, H.-M., Bae, H.E.K., Won, S.-Y., Hu, K.-S., Song, W.-C., Paik, D.-J. & Kim, H.-J. 2009, 'The buccofacial wall of maxillary sinus: an anatomical consideration for sinus augmentation.', *Clinical implant dentistry and related research*, vol. 11 Suppl 1, pp. e2-6.

Yang, M., Nam, G.E., Salamati, A., Baldwin, M., Deng, M. & Liu, Z.-J. 2018, 'Alveolar bone loss and mineralization in the pig with experimental periodontal disease.', *Heliyon*, vol. 4, no. 3, p. e00589.

Yildirim, M., Spiekermann, H., Handt, S. & Edelhoff, D. 2001, 'Maxillary sinus augmentation with the xenograft Bio-Oss and autogenous intraoral bone for qualitative improvement of the implant site: a histologic and histomorphometric clinical study in humans.', *The International journal of oral & maxillofacial implants*, vol. 16, no. 1, pp. 23–33.

Zackrisson, B., Mercke, C., Strander, H., Wennerberg, J. & Cavallin-Ståhl, E. 2003, 'A systematic overview of radiation therapy effects in head and neck cancer', *Acta Oncologica*.

Zhou, A.J.-J., Clokie, C.M.L. & Peel, S.A.F. 2013, 'Bone formation in algae-derived and synthetic calcium phosphates with or without poloxamer.', *The Journal of craniofacial surgery*, vol. 24, no. 2, pp. 354–9.

Zhou, A.J.J., Peel, S.A.F. & Clokie, C.M.L. 2007, 'An evaluation of hydroxyapatite and biphasic calcium phosphate in combination with Pluronic F127 and BMP on bone repair', *Journal of Craniofacial Surgery*.

Zimmermann, G. & Moghaddam, A. 2011, 'Allograft bone matrix versus synthetic bone graft substitutes.', *Injury*, vol. 42 Suppl 2, pp. S16-21.

7 | Appendix

7.1 | Material Data sheet – Symbios® Algipore®

Gebrauchsanweisung **3001 DE**

FRIOS® System
FRIOS® ALGIPORE®

Indikationen

FRIOS® ALGIPORE® ist bestimmt für die Rekonstruktion von Knochendefekten in der Kiefer- und Gesichtschirurgie sowie in der augmentativen Implantatchirurgie.
- Implantologie: Sinusbodenelevation, Augmentation des unzureichenden Implantatlagers, Augmentation nach Perforation des Knochens bei der Implantation/laterale Defekte
- Zysten: Defekte nach Exstirpation von Knochenzysten
  - Defekte nach Wurzelspitzenresektion
  - Defekte nach operativer Entfernung retinierter Zähne
  - Andere mehrwandige Knochendefekte der Alveolarfortsätze

Kontraindikationen

Bei der Patientenauswahl sind die allgemeinen Kontraindikationen für zahnärztliche/chirurgische Eingriffe zu beachten. Dazu zählen unter anderem:
Gefäßerkrankungen, nicht eingestellter Diabetes mellitus und andere Stoffwechselkrankheiten, die die Knochenregeneration beeinträchtigen, Störungen der Blutgerinnung, Antikoagulantien-Therapie.
Temporäre Kontraindikationen sind akute und chronische Entzündungsprozesse am Ort der Implantation, unzureichende Weichgewebsabdeckung sowie Zustand bei oder nach Chemo- oder Strahlentherapie.
Orale Kontraindikationen sind alle Kontraindikationen für einen chirurgischen Eingriff in der Mundhöhle.
Bei einer Augmentation in Zusammenhang mit einer zeitgleichen oder späteren Implantation sind auch alle Kontraindikationen für zahnärztliche Implantate zu beachten:
- Mangelnde Bereitschaft zur oralen Gesamtrehabilitation
- Mangelhafte Mundhygiene
- Nicht behandelte Parafunktionen wie z.B. Bruxismus
- Zu geringer Interokklusalabstand
- Mangelhafte Okklusion und/oder Artikulation, die auch durch geeignete Therapiemaßnahmen nicht korrigiert werden können
- Absolute Kieferkammaugmentation

Bei Tabak- und Alkoholabusus gelten die Einschränkungen für zahnärztlich-chirurgische Maßnahmen.
FRIOS® ALGIPORE® darf nicht verwendet werden, wenn eine ausreichende Weichgewebsabdeckung nicht möglich ist oder lokale Infektionen im Knochen und/oder Weichgewebe festgestellt werden.
FRIOS® ALGIPORE® darf nicht zur Augmentation solcher anatomischer Strukturen verwendet werden, bei denen eine unmittelbare Belastung des Knochendefekts oder die Wanderung der Granulate nicht zuverlässig verhindert werden kann.
FRIOS® ALGIPORE® darf nicht in der Nähe von Nerven oder Nervenaustrittspunkten appliziert werden.

Warnhinweise

Diese Gebrauchsanweisung ist vor der Anwendung von FRIOS® ALGIPORE® zu lesen. Bei Unklarheiten bezüglich der Indikation oder der Art der Anwendung ist der Einsatz von FRIOS® ALGIPORE® zu unterlassen, bis alle Punkte geklärt sind.
FRIOS® ALGIPORE® darf nur seiner Indikation entsprechend nach den allgemeinen Regeln für medizinisches/chirurgisches Handeln sowie unter Beachtung der Arbeitsschutz- und Unfallverhütungsvorschriften angewendet werden. Das Material darf nach dem Öffnen der Blisterverpackung nicht zu einer späteren Verwendung gelagert werden, da die Sterilität des Produktes nicht mehr gewährleistet werden kann.
Die nachfolgenden Beschreibungen reichen bei in augmentativen Verfahren unerfahrenen Behandlern nicht aus, um eine fachgerechte Anwendung sicherzustellen. Daher empfehlen wir die Einweisung in die Handhabung durch einen darin erfahrenen Anwender. FRIOS® ALGIPORE® darf nur von Zahnärzten und Ärzten verwendet werden, die mit der zahnärztlichen Chirurgie, einschließlich Diagnose und präoperativer Planung vertraut sind.
Für eine Verarbeitung und Anwendung des Produkts außerhalb seines bestimmungsgemäßen Gebrauchs ist jegliche Haftung für hierbei verursachte Schäden ausgeschlossen.

Als Komplikationen können u. a. auftreten:
- Unzureichende Knochenregeneration
- Dislokation oder Freilegung von FRIOS® ALGIPORE® Partikeln

Chirurgische Komplikationen können u. a. sein:
- Postoperative Blutungen
- Infektionen
- Dehiszenzen
- Fistelbildung

Vorsichtsmaßnahmen

Folgende Vorsichtsmaßnahmen sind vor bzw. während einer Behandlung zu treffen:
- Vor jedem Eingriff sicherstellen, dass alle benötigten Teile, Instrumente und Hilfsmittel vollständig, funktionsfähig und in der erforderlichen Menge vorhanden sind.
- Zur eigenen Sicherheit immer geeignete Schutzkleidung tragen.
- Patienten so lagern, dass die Gefahr der Aspiration von Teilen minimiert wird. Alle im Mund des Patienten verwendeten Teile gegen Aspiration und Verschlucken sichern.
- Alle Produkte, die zum einmaligen Gebrauch bestimmt sind, dürfen nicht wiederverwendet werden. Bei Nichtbeachtung besteht die Gefahr von Infektionen!

Nebenwirkungen

Als Begleiterscheinung chirurgischer Eingriffe können auftreten:
- Temporär lokale Schwellungen, Ödeme, Hämatome
- Temporär Einschränkungen des Empfindungsvermögens/der Kaufunktion

Produktbeschreibung

FRIOS® ALGIPORE® ist ein pflanzliches Knochenaufbaumaterial, das aus Rotalgen gewonnen wird. Die knochenähnlichen Eigenschaften und die interkonnektierende Porosität von FRIOS® ALGIPORE® bewirken, dass eine Vaskularisierung und Revitalisierung des Defektbereiches sowie die Bildung neuer Knochensubstanz stattfinden. In Abhänggkeit von der Qualität des Knochenlagers erfolgen die Neubildung von Knochen und die Resorption von FRIOS® ALGIPORE® unterschiedlich schnell. Die Herstellung erfolgt unter validierten GMP-Bedingungen. Die Übertragung von Keimen, Viren oder Prionen ist ausgeschlossen. FRIOS® ALGIPORE® ist biokompatibel und pH-neutral. Immunreaktionen sind keine bekannt.

Empfohlene Patientenvorbereitung

Vor der Implantation sollte eine klinische und radiologische Untersuchung des Patienten durchgeführt werden, um eventuelle Knochenkrankheiten auszuschließen und um die Platzierung des Augmentats zu planen. Beachten Sie bei der Auswertung von Röntgenbildern, dass FRIOS® ALGIPORE® eine etwas höhere Röntgendichte als Knochen aufweist. Dies kann eventuell zugrunde liegende pathologische Zustände des lokalen Knochens kaschieren oder eine Knochenveränderung vortäuschen. Das die Rekonstruktionen überlagernde Gewebe muss frei von Entzündungen und Ulzerationen sein. Falls notwendig, sollte der Patient mindestens eine Woche vor der Operation keine Prothese tragen, damit gereiztes Gewebe heilen kann.

Anwendung

Die Auswahl der Korngröße ist in Abhängigkeit von der Defektgröße zu treffen.
Empfohlene Korngrößen:
Korngröße 0,3 mm bis 0,5 mm z.B. Auffüllen von kleinen Defekten
Korngröße 0,5 mm bis 1,0 mm z.B. Augmentation lateraler Defekte
Korngröße 1,0 mm bis 2,0 mm z.B. Augmentation bei Sinusbodenelevation

WICHTIG!
Die nachfolgenden klinischen Angaben stellen nur eine mögliche Empfehlung dar und sind im Einzelfall durch den Anwender kritisch zu überprüfen.
FRIOS® ALGIPORE® **muss bis zur Sättigung** mit Patientenblut aus der Wunde oder mit Venenblut gemischt werden.
FRIOS® ALGIPORE® darf nicht mit Kochsalzlösung gemischt werden. Bei großer Korngröße ist es vorteilhaft, die Körner ein wenig zu zerdrücken, um die innere Porenstruktur offen zu legen und die Aufnahme von Blut zu verbessern.
Vor Platzierung des mit Blut gemischten Granulats müssen Weichgewebsreste aus dem zu augmentierenden Bereich entfernt sein. Eine gering durchblutete knöcherne Kompakta kann mechanisch angefrischt werden. Auch kleine Perforationen der Kompakta mit z.B. einem Rosenbohrer beschleunigen die spätere Knochenregeneration.
Je nach Größe des Knochendefekts kann FRIOS® ALGIPORE® direkt in den gesäuberten, blutenden Knochendefekt eingebracht werden. Wir empfehlen jedoch das vorherige Vermischen mit frischem Patientenblut unter sterilen Bedingungen, dadurch werden Serumproteine und Wachstumsfaktoren absorbiert. Falls möglich, sollten autologe Knochenspäne beigemischt werden, da diese den Einheilungsprozess zusätzlich verbessern.
Die Mischung sollte manuell in einem sterilen Gefäß mit einem sterilen Spatel oder einem anderen geeigneten sterilen Instrument vorgenommen werden. Für den Fall, dass mehr als die geplante Menge für die Durchführung benötigt wird, ist es ratsam, zusätzliches Material bereitzuhalten.
Die Mischung in kleinen Mengen mit sterilem Instrument (z.B. chirurgischer Löffel) applizieren, möglichst große Kontaktfläche zwischen ortsständigem Knochen und FRIOS® ALGIPORE® Granulat nutzen.
FRIOS® ALGIPORE® nicht zu fest in den Defekt stopfen, da ein Überfüllen des Defekts eine hohe gingivale Schleimhautspannung verursachen könnte, was Wund- oder Gewebsdehiszenzen zur Folge haben kann.
FRIOS® ALGIPORE® Granulate sollten im Defekt stabil liegen. Zur Unterstützung der Stabilität empfehlen wir die Verwendung einer Membran mit ausreichender Barrierefunktion. Bei Wundverschluss FRIOS® ALGIPORE® vollständig durch Membran (z.B. FRIOS® BoneShield) und Mukoperiostlappen abdecken. Der augmentierte Bereich darf während der Heilung nicht mechanisch belastet werden.

HINWEIS: In Einzelfällen wurde beim Einsatz von resorbierbaren Membranen auf Polylactidbasis aufgrund der besonderen Abbauart dieser Membranmaterialien eine forcierte Resorption von Augmentat und Knochen mit unzureichender Knochenregeneration beobachtet. Wir empfehlen daher den Einsatz von nicht resorbierbaren Membranen, z.B. FRIOS® BoneShield aus Titan.

Verwendung von FRIOS© ALGIPORE© in Verbindung mit Dentalimplantaten
Das Knochenaufbaumaterial FRIOS® ALGIPORE® ist osseokonduktiv. Die Geschwindigkeit und das Ausmaß der Knochenneubildung hängen von der Qualität des ortsständigen Knochenlagers und von den Verarbeitungsbedingungen von FRIOS® ALGIPORE® ab.
- Bei einzeitigem Vorgehen kann die prothetische Versorgung des Implantats nach 6 Monaten erfolgen.
- Bei zweizeitigem Vorgehen sollte die Implantation in den augmentierten Bereich bei noch ausreichend vorhandener Restknochenhöhe nach 6 Monaten, die prothetische Versorgung nach weiteren 6 Monaten erfolgen.
- Bei zweizeitigem Vorgehen und einem gering ausgeprägten lokalen Knochenlager, z.B. bei Sinusbodenaugmentation mit einer Restknochenhöhe von unter 5 mm, sollte die Implantation nach 6-9 Monaten erfolgen. Die prothetische Versorgung sollte bei FRIOS® ALGIPORE® frühestens nach 6 Monaten erfolgen.

Postoperative Versorgung
Weisen Sie den Patienten auf die Notwendigkeit einer regelmäßigen Mundhygiene hin. In den ersten 7–10 Tagen nach der Operation kann die Mundhygiene durch Spülungen mit einer geeigneten Mundspüllösung ergänzt werden. Postoperativ sind mechanische Belastungen des Implantatonsorts zu vermeiden. Eine provisorische, weich unterfütterte Prothese kann aus ästhetischen Gründen getragen werden.

Lieferformen - Lagerungshinweise - Sterilisation

FRIOS® ALGIPORE® ist zum **einmaligen Gebrauch** bestimmt und **darf nicht** erneut sterilisiert werden. Reste einer angebrochenen Packung entsorgen.
FRIOS® ALGIPORE® ist gammasteril in versiegelten Glasflaschen, die sich in einem sterilen Blister befinden, verpackt.
Bei beschädigter Blister-Verpackung, beschädigtem Siegel, geöffneter Glasflasche oder nach Ablauf der auf dem Etikett angegebenen Sterilitätsgarantie FRIOS® ALGIPORE® nicht mehr verwenden. FRIOS® ALGIPORE® bei Raumtemperatur in der Originalverpackung lagern. Lagerung unter in Zahnarztpraxen üblichen Bedingungen. Keiner direkten Sonneneinstrahlung aussetzen.
Folgende Packungsgrößen sind lieferbar:
Korngröße 0,3 mm bis 0,5 mm - 0,5 ml
Korngröße 0,5 mm bis 1,0 mm - 1,0 ml
Korngröße 0,5 mm bis 1,0 mm - 2,0 ml
Korngröße 1,0 mm bis 2,0 mm - 1,0 ml
Korngröße 1,0 mm bis 2,0 mm - 2,0 ml

Copyright und Warenzeichen

CE / CE 0123

CE: Medizinprodukte der Klasse I
CE0123: Medizinprodukte der Klassen IIa, IIb, III gemäß Richtlinie 93/42/EWG

Hersteller:
DENTSPLY Implants Manufacturing GmbH Steinzeugstraße 50 68229 Mannheim Germany
Tel. (0621) 4302-010 Fax (0621) 4302-011 www.dentsplyimplants.com

Stand der Information: 14.12.2012 Rev. 011

Änderungen vorbehalten.
Nicht alle Produkte sind in allen Ländern erhältlich.

DENTSPLY IMPLANTS

7.2 | Material Data sheet – Symbios® Biphasic Bone Graft Material

Gebrauchsanweisung

SYMBIOS® biphasisches Knochenaufbaumaterial

Indikationen

SYMBIOS® biphasisches Knochenaufbaumaterial ist für folgende klinische Anwendungsbereiche bestimmt:
- Augmentation oder Rekonstruktion des Alveolarkammes
- Augmentation bei der maxillaren Sinusbodenelevation
- Behandlung knöcherner Defekte, z.B.:
  - nach Exstirpation von Zysten
  - Wurzelspitzenresektion oder
  - parodontaler Knochendefekte
- Auffüllung von Extraktionsalveolen zum Erhalt des Alveolarkammes
- Auffüllen von Knochendefekten (parodontal oder peri-implantär) in Verbindung mit Membranen im Rahmen der gesteuerten Geweberegeneration (GTR)

Kontraindikationen

Allgemeine Kontraindikationen für oralchirurgische Eingriffe sind zu beachten. SYMBIOS® biphasisches Knochenaufbaumaterial sollte bei Patienten mit folgenden Erkrankungen nicht angewendet werden:
- Akute und chronische Infektionen im Operationsbereich
- Gefäßerkrankungen
- Infektions- oder Stoffwechselerkrankungen, die die Knochenregeneration oder Wundheilung beeinträchtigen, z.B. schlecht eingestellter Diabetes mellitus
- Systemische Erkrankungen wie z.B. Blutgerinnungsstörungen
- Osteoporose
- Antikoagulantien-Therapie
- Immunsuppressive Therapie
- Unzureichendes Weichgewebe

Alle Erkrankungen, bei denen der behandelnde Arzt einen chirurgischen Eingriff für kontraindiziert erachtet. Vorsicht ist geboten im Falle von schlechter Knochenqualität und bei starken Rauchgewohnheiten.
SYMBIOS® biphasisches Knochenaufbaumaterial darf nicht in unmittelbarer Nähe von Nerven appliziert werden.
SYMBIOS® biphasisches Knochenaufbaumaterial ist nicht zur Augmentation lasttragender oder instabiler Indikationen zu verwenden.
Wird SYMBIOS® biphasisches Knochenaufbaumaterial in Verbindung mit einer zeitgleichen oder späteren Implantation verwendet, müssen auch alle Kontraindikationen für Dentalimplantate in Betracht gezogen werden.

Warnhinweise

Die Gebrauchsanweisung muss vor der Anwendung von SYMBIOS® biphasischem Knochenaufbaumaterial aufmerksam gelesen werden. Der Anwender trägt die ausschließliche Verantwortung für die korrekte Anwendung der Materialien sowie für die sachgemäße Durchführung des chirurgischen Eingriffs. Die Gebrauchsanweisung alleine befähigt nicht, in augmentativen Techniken unerfahrene Anwender, eine fachgerechte Behandlung sicherzustellen. Daher wird dringend empfohlen, zuvor eine Einweisung in die Handhabung des Produkts durch einen erfahrenen Anwender und eine Schulung der chirurgischen Techniken der Knochenaugmentation in Anspruch zu nehmen.
SYMBIOS® biphasisches Knochenaufbaumaterial darf nur von Chirurgen oder Zahnärzten verwendet werden, die mit der Knochen- und Weichteilanatomie vertraut sind und Erfahrung in der medizinischen Diagnostik, der präoperativen Planung sowie in den entsprechenden chirurgischen Eingriffstechniken besitzen.
SYMBIOS® biphasisches Knochenaufbaumaterial darf nur in den angeführten Indikationsbereichen angewendet werden, entsprechend der allgemeinen Richtlinien für chirurgische und oralchirurgische Versorgung von Knochendefekten. Die Vermeidung bzw. Reduzierung allgemeiner Risiken bei chirurgischen Eingriffen liegt ebenfalls im Verantwortungsbereich des Anwenders. Aseptische Operationsbedingungen müssen eingehalten werden.
AlgOss Biotechnologies ist nicht verantwortlich für Komplikationen durch falsche Indikation, falsche Kombination von Material und Operationstechnik sowie Grenzen der Behandlungsmethode. Jegliche Haftung für hierbei verursachte Schäden ist ausgeschlossen.

Vorsichtsmaßnahmen

Folgende Vorsichtsmaßnahmen sind vor bzw. während einer Behandlung zu treffen:
- Vor jedem Eingriff sicherstellen, dass alle benötigten Teile, Instrumente und Hilfsmittel vollständig, funktionsfähig und in der erforderlichen Menge vorhanden sind.
- Zur eigenen Sicherheit immer geeignete Schutzkleidung tragen.
- Patienten so lagern, dass die Gefahr der Aspiration von Teilen minimiert wird.
- Alle im Mund des Patienten verwendeten Teile gegen Aspiration und Verschlucken sichern.
- Alle Produkte, die zum einmaligen Gebrauch bestimmt sind, dürfen nicht wiederverwendet werden. Bei Nichtbeachtung besteht die Gefahr von Infektionen!

Nebenwirkungen

Materialbedingte Nebenwirkungen sind nicht bekannt.
Als Folge chirurgischer Eingriffe können temporär Schwellungen und Hämatome auftreten.
Zu möglichen Komplikationen zählen:
- Unzureichende Knochenbildung
- Verschiebung oder Freilegung von biphasischem Knochenaufbaumaterial
- Verzögerte Knochen- oder Wundheilung
- Unzureichende Vaskularisierung

Bei jedem chirurgischen Eingriff im Kiefer sind allgemeine OP-Komplikationen möglich, die nicht unbedingt auf das Knochenaufbaumaterial zurückzuführen sind:
- Intra- bzw. postoperativ: Blutungen, Nerv- oder Gefäßverletzungen, Perforation von Weichgewebe, Perforation der Sinusmembran, Beschädigung von Zähnen.
- Frühkomplikationen: Blutungen, Hämatome, Ödeme, Infektionen, Nahtdehiszenzen, gingivale Schwellung / Hyperplasie, Schmerzen, Emphysem, Nervenschäden durch chirurgisches Trauma.
- Spätkomplikationen: Weichgewebsdehiszenz, Membranexposition, Abszessbildung, Schleimhautirritation, Fistelbildungen, Sinusitis, Schwellung, Sequesterbildung von teilweise schon osseointegriertem Augmentationsmaterial, Verlust des inserierten Materials, Sensibilitätsstörungen (Hyp-, Dys-, Parästhesie), sekundäre Nervschädigung.

Komplikationen können durch sorgfältige Patientenauswahl, atraumatische Operationstechniken und den bestimmungsgemäßen Einsatz von SYMBIOS® biphasischem Knochenaufbaumaterial weitestgehend vermieden werden.

Produktbeschreibung

SYMBIOS® biphasisches Knochenaufbaumaterial ist ein resorbierbares anorganisches Knochenaufbaumaterial, pflanzlichen Ursprungs, das aus Rotalgen gewonnen wird. Die chemische Zusammensetzung dieses interkonnektierend (durchgängig) porösen biologischen Produkts ist mit der des menschlichen Knochens vergleichbar. SYMBIOS® biphasisches Knochenaufbaumaterial ist ein biphasisches Komposit mit einem Phasenverhältnis von 20% Hydroxylapatit und 80% β-Trikalziumphosphat. Je höher der Trikalziumphosphat-Gehalt des Produkts ist, desto schneller erfolgt die Resorption und damit die Möglichkeit von schnellerer Knochenregeneration. Das Produkt ist biokompatibel, osteokonduktiv und pH-neutral. Die Geschwindigkeit des Biomaterialabbaus und der Ersatz durch autogenen Knochen sind abhängig von der Qualität des Knochenlagers. Die Herstellung von SYMBIOS® biphasischem Knochenaufbaumaterial erfolgt unter validierten GMP-Bedingungen. Die Übertragung von Bakterien, Viren oder Prionen ist ausgeschlossen. Fremdkörperreaktionen sind keine bekannt.

Empfohlene Patientenvorbereitung

Vor der Anwendung von SYMBIOS® biphasischem Knochenaufbaumaterial ist eine eingehende klinische und röntgenologische Untersuchung des Patienten durchzuführen, um eventuelle Erkrankungen des Knochens auszuschließen und den Zustand der Hart- und Weichgewebe vor der Augmentation zu beurteilen.

Anwendung

Die Auswahl der Korngröße ist in Abhängigkeit von der Defektgröße zu treffen.
Empfohlene Korngrößen:
Korngröße 0,2 mm bis 1,0 mm, z.B. Auffüllen von kleinen Defekten
Korngröße 1,0 mm bis 2,0 mm, z.B. Augmentation lateraler Defekte und bei Sinusbodenelevation
WICHTIG!
Die angeführten klinischen Anweisungen stellen allgemeine Empfehlungen dar und sollten im Einzelfall durch den behandelnden Arzt kritisch geprüft werden.
SYMBIOS® biphasisches Knochenaufbaumaterial **muss bis zur Sättigung** mit frischem Eigenblut des Patienten angemischt werden. Das Eigenblut kann entweder intraoperativ aus der Wunde oder durch venöse Entnahme gewonnen werden. Durch die Sättigung mit Blut adsorbiert das Knochenaufbaumaterial Serumproteine und Wachstumsfaktoren.
Vor dem Einbringen des Augmentats muss Granulationsgewebe im Augmentationsbereich vollständig eliminiert werden.
Eine möglichst große direkte Kontaktfläche zwischen SYMBIOS® biphasischem Knochenaufbaumaterial und dem versorgenden Knochen sowie eine gute Vaskularisation des umgebenden Knochenlagers ist notwendig, um die Bildung von neuem Knochen zu gewährleisten. Die Durchblutung gering durchbluteter Kontaktstellen kann durch Anrauen (Anfrischen) des Knochens mit einem Rosenbohrer erhöht werden. Eine Beimischung von autologen Knochenspänen (Knochenchips) führt zu einer Verbesserung des Einheilprozesses und zu einer Beschleunigung der Knochenneubildung.
Das mit Blut gesättigte SYMBIOS® biphasische Knochenaufbaumaterial wird mit Hilfe von sterilen Instrumenten in kleinen Portionen in den Defekt eingebracht, wobei auf einen möglichst guten Kontakt zum ortsständigen Knochen zu achten ist. Eine Überfüllung des Defekts sollte vermieden werden, da eine starke Schleimhautspannung zu einer Verschiebung oder Abwanderung von Partikeln führen kann. Für eine optimale Geweberegeneration muss der Defekt vollständig mit einer Membran abgedeckt werden. Wir empfehlen daher den Einsatz von langsam resorbierbaren Membranen oder nicht resorbierbaren Membranen. Die Verwendung einer schnell resorbierbaren Membran wird nicht empfohlen. Die Wunde muss spannungsfrei verschlossen werden und während der Heilungsphase darf der augmentierte Bereich nicht mechanisch belastet werden.

Verwendung von SYMBIOS© biphasischem Knochenaufbaumaterial in Verbindung mit Dentalimplantaten
Das Ausmaß und die Geschwindigkeit der Knochenneubildung sind vor allem abhängig vom Kontakt zum ortsständigen Knochen und von dessen Qualität.
- Bei einzeitigem Vorgehen kann die prothetische Implantatversorgung je nach Region und Defekt nach 4 bis 6 Monaten erfolgen.
- Bei zweizeitigem Vorgehen und ausreichender Restknochenhöhe (mindestens 5 mm) kann die Implantation in den augmentierten Bereich je nach Region und Defekt nach 4 bis 6 Monaten und die prothetische Versorgung nach weiteren 4 bis 6 Monaten erfolgen.
- Bei zweizeitigem Vorgehen und einer Restknochenhöhe unter 5 mm kann die Implantation je nach Region und Defekt erst nach 6 bis 9 Monaten und die prothetische Versorgung nach weiteren 6 Monaten erfolgen.

Parodontologie
Eine wichtige Voraussetzung für eine erfolgreiche Parodontalbehandlung ist die Beherrschung bakterieller Infektionen und eine adäquate Mundhygiene. Es wird empfohlen die Patienten diesbezüglich sorgfältig aufzuklären und präoperativ vorzubereiten. Vor der Anwendung von SYMBIOS® biphasischem Knochenaufbaumaterial zur Füllung parodontaler Defekte ist, neben der Plaquekontrolle, eine präzise Behandlung der parodontalen Läsion (Wurzelglättung, Debridement) erforderlich. Um eine optimale Geweberegeneration zu erreichen, ist eine Membran zur Abdecken des Defektes erforderlich.

Postoperative Versorgung
Es ist sehr wichtig den Patienten auf die Notwendigkeit einer guten Mundhygiene hinzuweisen. In den ersten 7-10 Tagen postoperativ sollten Spülungen mit einer antibakteriellen Mundspüllösung unterstützend erfolgen. Nach dem operativen Eingriff ist eine mechanische Belastung am Implantationsort unbedingt zu vermeiden.

Lieferformen - Lagerungshinweise - Sterilisation

SYMBIOS® biphasisches Knochenaufbaumaterial ist gammasteril und ist für den **einmaligen Gebrauch** an einem Patienten bestimmt. SYMBIOS® biphasisches Knochenaufbaumaterial **darf nicht** erneut sterilisiert werden. Unverbrauchte Reste einer geöffneten Verpackung sind zu verwerfen. SYMBIOS® biphasisches Knochenaufbaumaterial ist in einem Glasfläschchen verpackt, das in ein Tyvek-Säckchen, als Sterilverpackung, eingeschweißt ist. Sowohl das Glasfläschchen als auch der Inhalt sind steril.
SYMBIOS® biphasisches Knochenaufbaumaterial darf bei beschädigter Sterilverpackung, nach Ablauf des auf der Verpackung angegebenen Haltbarkeitsdatums oder bei Öffnung der Verpackung unter unsterilen Bedingungen vor dem Gebrauch nicht mehr verwendet werden.
SYMBIOS® biphasisches Knochenaufbaumaterial ist in der Originalverpackung bei Raumtemperatur zu lagern. Keiner direkten Sonneneinstrahlung aussetzen.

Folgende Packungsgrößen sind lieferbar:
Korngröße 0,2 mm bis 1,0 mm - 0,5 ml
Korngröße 0,2 mm bis 1,0 mm - 1,0 ml
Korngröße 1,0 mm bis 2,0 mm - 1,0 ml
Korngröße 1,0 mm bis 2,0 mm - 2,0 ml

Erklärung der Symbole

STERILE R — Steril durch Bestrahlung

CE 0123

CE0123: Medizinprodukte der Klassen IIa, IIb, III gemäß Richtlinie 93/42/EWG

Hersteller: AlgOss Biotechnologies GmbH, Schumanngasse 15, 1180 Wien, Österreich
Tel.: +43 (1) 4075820 Fax: +43 (1) 407582-10 E-Mail: office@algoss.at www.algoss.at

3101 Rev.000 Stand der Information: 2014 - 04

DENTSPLY IMPLANTS