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Prospective Clinical Study with New Materials for Tissue Regeneration: A Study in Humans

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El Dr. D. José Eduardo Maté Sánchez de Val como Director de la Tesis Doctoral titulada "Prospective Clinical Study with New Materials for Tissue Regeneration: A Study in Humans" realizada por Dña. Nathalie Jeannette Kollek en el Departamento de Ciencias de la Salud, **autoriza su presentación a trámite** dado que reúne las condiciones necesarias para su defensa.

Lo que firmo, para dar cumplimiento al Real Decreto 99/2011 de 28 de enero, en Murcia a 12 de Septiembre de 2022

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ABSTRACT (English)

Objective This study was performed to evaluate the clinical, radiographic, and histomorphometric outcomes of novel bone grafting materials and dental membranes and to compare the results with current data from literature.

Materials and Methods New synthetic bone substitutes, consisting of biphasic calcium phosphate, in the ratio of 60% hydroxyapatite and 40% β-tricalcium phosphate, were applied in bony defects and covered by either a novel synthetic poly(lactic-co-glycolic) acid (PLGA) or porcine collagen membrane. A sample of 51 biomaterials was placed in a total of 20 patients during different surgical protocols. Implants were simultaneously inserted, and in case of sinus floor elevations 6 months later. Pre- and postoperative cone-beam computed tomographies were taken. Bone biopsies were harvested from augmented sides and processed for histomorphometric evaluation.

Statistical Analysis Averages and ranges were calculated for the percentage of newly formed bone, residual biomaterial, and connective tissue. Data were submitted to analyze the radiological mean differences in length, width, and density. Paired *t*-tests were deployed for the analysis of differences within each group between the baseline (preoperative) and the final (postoperative) measurements.

Results The mean bone gain in length and width were 0.96 ± 3.33 mm (+27.59%) and 1.22 ± 1.87 mm (+30.48%), respectively. The bone density was increased by a factor of 4, reaching an average of 387.47 ± 328.86 HU. Histomorphometric evaluations revealed new bone formation of 41.44 ± 5.37%, residual biomaterial of 24.91 ± 7.31%, and connective tissue of 33.64 ± 4.81%. The mean healing period was 8.32 ± 3.00 months.

Conclusions Data from this study confirmed the suitability of the tested materials in dental surgery. The biomaterials may be recommended for various clinical procedures. A satisfactory level of increase of new bone was reported in augmented sides. No significant

differences were observed between the tested membranes. PLGA might be superior to other membranes for their easier handling.

Keywords: Bone graft substitute; calcium phosphate; biomaterials; PLGA membrane; collagen membrane; osteointegration.

ABSTRACT (Spanish)

Objetivo Este estudio se realizó con el objetivo de evaluar los resultados clínicos, radiográficos e histomorfométricos de nuevos materiales de injerto óseo y membranas dentales así como para comparar los resultados con datos actuales de la literatura.

Materiales y métodos Se aplicaron nuevos sustitutos óseos sintéticos, que consisten en fosfato de calcio bifásico, en una proporción de 60% de hidroxiapatito y 40% de fosfato β-tricálcico, en defectos óseos y se cubrieron con un novedoso ácido poli(láctico-co-glicólico) sintético (PLGA) o membrana de colágeno porcino. Se colocó una muestra de 51 biomateriales en un total de 20 pacientes durante diferentes protocolos quirúrgicos. Los implantes se colocaron simultáneamente, y en caso de elevaciones del piso del seno, 6 meses después. Se tomaron tomografías computarizadas de haz cónico pre- y postoperatorias. Se recolectaron biopsias óseas de los lados aumentados y se procesaron para su evaluación histomorfométrica.

Análisis estadístico Se calcularon los promedios y rangos para el porcentaje de hueso recién formado, biomaterial residual y tejido conectivo. Los datos se enviaron para analizar las diferencias medias radiológicas en longitud, anchura y densidad. Se implementaron pruebas *t* pareadas para el análisis de las diferencias dentro de cada grupo entre las mediciones iniciales (preoperatorias) y finales (postoperatorias).

Resultados La ganancia ósea media en longitud y anchura fue de 0,96 ± 3,33 mm (+27,59%) y 1,22 ± 1,87 mm (+30,48%), respectivamente. La densidad ósea se incrementó por un factor de 4, alcanzando un promedio de 387,47 ± 328,86 HU. Las evaluaciones histomorfométricas revelaron una formación de hueso nuevo de 41,44 ± 5,37%, biomaterial residual de 24,91 ± 7,31% y tejido conectivo de 33,64 ± 4,81%. El período medio de cicatrización fue de 8,32 ± 3,00 meses.

Conclusiones Los datos de este estudio confirmaron la idoneidad de los materiales probados en cirugía dental. Los biomateriales pueden recomendarse para diversos procedimientos clínicos. Se informó un nivel satisfactorio de aumento de hueso nuevo en los lados aumentados. No se observaron diferencias significativas entre las membranas probadas. PLGA puede ser superior a otras membranas por su fácil manejo.

Palabras clave: Bone graft substitute; calcium phosphate; biomaterials; PLGA membrane; collagen membrane; osteointegration.

LIST OF ABBREVIATIONS

BMP = Bone morphogenetic protein

c-fms = receptor for macrophage colony-stimulation factor (on osteoclasts)

FACIT collagens = Fibril-associated collagens with Interrupted triple helices

HA = Hydroxyapatite

MCSF = Macrophage colony-stimulating factor (from osteoblasts)

OPG = Osteoprotegerin

PTH = Parathyroid hormone

PTHrP = PTH-related peptide

Wingless = Wnt

LRP5/6 = LDL Receptor Related Protein 5/6

TGFβ = Transforming growth factor beta

IGF 1/-2 = Insulin-like growth factor-1/-2

PDGF = Platelet-derived growth factor

RANK = Nuclear factor κ B (on osteoclasts)

RANKL= RANK ligand (on osteoblasts)

SCPP = Secretory calcium-binding phospoprotein

Sx = Symptoms

TMJ = Temporomandibular joint

BGS = Bone grafting materials and substitutes

AB = Autogenous bone

AL = Allogenous bone

XE = Xenogeneic bone

AP = Alloplastic bone

BCP = Bisphasic calcium phosphate

CaP = Calcium phosphate

GBR = Guided Bone Regeneration

GTR = Guided Tissue Regeneration

PLGA = poly(lactic-co-glycolic) acid

β-TCP = β-tricalcium phosphate

NB = New bone formation

RB = Residual biomaterial

CT = Connective tissue

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1 - INTRODUCTION

1. Introduction

To attain satisfactory and long-lasting outcomes in the maxillofacial surgery and oral implantology, a detailed knowledge of the possibilities to achieve and preserve a sufficient bone volume, resulting in a successful osseointegration, is essential to integrate in the daily clinical practice. The aim of modern dentistry is to restore the patients' oral health with the most suitable and up-to-date treatments to normal function, comfort, speech and esthetics. The reconstructive surgery is often faced to bony defects in both upper and lower jaw. In this respect, it should be underlined that the molar region in maxilla is more frequent affected by bone loss than the mandible due to its anatomical softer, spongy bone. Generally, the skeleton is a dynamic osseous tissue which undergoes forming and degrading processes, depending if bone is actually needed or not. Each tooth needs an opposing tooth. The occlusal load will be transferred through mastication to the alveolar bone. A missing strain causes atrophy of the alveolar bone and soft tissue changes in the corresponding region. Accordingly, bone defects are caused in a high number by tooth loss as a natural physiologic process or after extractions, and are primary age-related. In Europe, numerous patients have still dentulous jaws at the age of 60 years, but in the years ahead a higher loss of teeth can be detected.[1] A prevalence rate for total edentulism for European people aged 65 to 74 years is determined between 5 to 51%.[2] Risk factors can be dental diseases (e.g., caries, pulpitis, periodontitis, periimplantitis, cysts or factures), lifestyle factors (e.g., smoking, alcohol or drugs) or socio-economic background (e.g., family), whereby the status of the oral health as well as the education and income level are the most decisive.[2,3] Further reasons for bone defects can be trauma, genetic anomalies, cancer, or age-related diseases like osteoporosis. Consequences of not treated complete edentulism are traceable in various areas. From the anatomic point of view, continued bone loss, soft tissue changes (e.g., loss of keratinizing gingiva, soft spots on mucosa), neuromuscular and muscle changes (e.g., ptosis of muscle attachment, more active tongue during mastication) are noticeable. Esthetically, the collapse of the bite results in a loss of the vertical dimension and a rotation of the chin anterior ("chin of a witch"). Increased grooves, in particular

nasolabial, marionette and mental wrinkles are deeper. Besides functional problems (e.g., insufficient masticatory), patients may experience physiological stress.[4]

Summing up, partial or complete edentulism leads to unfavorable conditions for the patients and future prosthetic rehabilitation. Therefore, the reconstruction of alveolar ridge defects is indispensable by means of bone augmentation techniques, commonly assisted by bone substitute materials. Conventional bone grafts are autografts (autologous bone coming from the patient himself), in literature often titled as the "gold standard", allografts (homogenous bone from another human being), and xenografts (heterogenous bone from animals). All bone grafts of biological origin are characterized by specific advantages, but also limitations.[5-8] Conversely, alloplastic bone substitutes, known as alloplasts, are the synthetic non-osseous alternative, enlightening with their beneficial qualities the market of nowadays.[9-12]

In addition, bone graft materials are combined with barrier membranes to sustain the stability of the augmented bone level in the Guided Bone Regeneration (GBR) therapy. Ideally, membranes should guide the slower migrating bone cells to the defect side, while preventing epithelial tissue ingrowth into the graft side.[4]

In this context it is important to add that numerous patients prefer non-autogenous, synthetic bone graft substitutes due to ethical, cultural or religious reasons.[13] Therefore, it is of scientific significance to focus on synthetic biomaterials currently appeared on the dental market in order to verify their benefit for the patient and clinician.

1.1. Bone biology

Bone is an extremely dynamic mineralized tissue which undergoes physiologic turnovers, modelling and remodeling processes, to adapt biomechanically in form, size and density to changing environmental forces a lifetime, whereby maintaining the mineral homeostasis and acid-based balance will be constantly focused. From the functional

standpoint, bone assures structural stability for the human skeleton, allows locomotion and movement, and conserves vital internal organs. Bone acts as a reservoir in the body e.g., for minerals, calcium and phosphate, or growth factors. Further, bone is also responsible for the hematopoietic cell development and production of bone marrow.[14-16] Regarding the different types of bone, the human skeleton consists of short bones (e.g., patellae or sesamoid bones), long bones (e.g., clavicles or femurs), flat bones (e.g., mandible or ribs), and finally, irregular bones (e.g., vertebrae or sacrum).[16]

Taking into consideration the macroscopical structure, bone tissue is composed of cortical (known as compact bone) and trabecular (known as cancellous) bone. Cortical bone is a dense, and solid tissue which surrounds the bone marrow and represents 80% of human bone mass.[14-16] The periosteum (or periosteal surface) attaches the outer cortex to the external surface of the cortical bone through thick collagenous fibers, known as Sharpey's fibers. The endosteum (or endocortical surface) covers the inner surface of the cortical bone which can be also find in trabecular bone. The membranous endosteum contains bone cells and blood vessels; accordingly, it has a greater remodeling activity than the periosteum which is more qualified for the modelling process.[16] Cortical bone is organized by cortical osteons. In the central of each osteon are blood vessels and nerves in the so-called Harversian canal. The cortical osteons (or Haversian systems) consist of concentric lamellae walls and canaliculi which connect the osteocytes with the Harversian canal for communication.[15] Transverse small blood vessels, known as Volkmann's canals, connect the longitudinal orientated Haversian canals with the endosteum for communication.[15] Osteons are separated from each other's by thin cement lines. In cross section, the cortical osteons are cylindrical tightly organized. Cortical bone has a lower metabolic rate.[16] In a human adult skeleton can be found around $21x10^6$ cortical osteons overall and a Harversian area of approximately 3.5 m^2.[16] The porosity of cortical bone is estimated around 5 to 10%, resulting in a maximal resistance to torsion and bending.[15] Both, the high density through the tightly orientated Harversian osteons and the low porosity permit the compressive strength of cortical bone.

Inside the bone is the trabecular bone which is very spongy with a lower density and greater surface area. It is estimated that about 20% of all bones are of trabecular structure.[14-16] Trabecular osteons (or packets) have a semilunar form and are spread in the bone marrow compartment. In the adult skeleton are approximately $14x10^6$ packets with an area of around 7 m^2 in total.[16] Trabecular osteons are connected with the endosteum. Trabecular bone is permeated by sinusoids, resulting in a better vascularization. In addition, a greater metabolic activity (or higher rate of remodeling) due to bone cells on its surface, allow deformation, absorption of loads[15] and a better elasticity.[14] Its porosity is documented around 50 to 90%.[15]

Microscopically, bone tissue can be divided into three types. Firstly, the woven bone (or primary bone) which is produced during the formation in the embryonic skeleton, and seldom locatable in the health adult body. Exceptions are bony defects caused by e.g., fractures, orthognathic surgery, or endosseous implants. In these cases, woven bone triggers the initial and postoperative healing. Woven bone is made of disorganized collagen fibrils which are weak and poorly mineralized. Further, a second type of bone can be found in some literature called composite bone. It has parts of lamellar in woven bone, forming primary osteons. It is relatively quickly produced during postoperative healing. Thirdly, the lamellar bone is the most common one and forms the cortical and trabecular bone. The collagen fibrils are highly organized, strong and well-mineralized, resulting in a significant strength. This strength is dependent on the mineral composition which will be determined during the primary mineralization with hydroxyapatite (HA) and the secondary crystal growth. The adult human skeleton is marked by more than 99% of lamellar bone. Finally, the bundle bone is also lamellar bone and has Sharpey's fibers which permit the attachments of tendons or ligaments.[17]

The bone formation, remodeling and resorption processes are characterized by the following bone cells: osteoblasts, osteocytes and osteoclasts. The osteoblasts are specialized, bone forming cells at the surfaces of bone, originally from pluripotent mesenchymal stem cells that give rise to various cells (e.g., chondrocytes, adipocytes or myocytes). Osteoblasts

can differentiate into osteocytes that support the bone structure in the bone matrix or lining cells, the "inactive osteoblasts" on bone surfaces.[16] The stimulation or inhibition of the osteoblast differentiation is regulated by BMP[15] or the Wnt signaling pathways.[14,16] Wnt molecules are glycoproteins that dock on either a receptor of a Frizzled protein, LRP5 or LRP6 in osteoblasts, thus, after receptor activation, the osteoblast gene expression can be controlled.[14] Furter possibilities are the regulation of osteoclast formation.[18] Proteins that inhibit the Wnt signaling are e.g., the proteins of the Dickkopf family.[14] Mature osteoblasts are small cells with conspicuous large nuclei and organelles-rich (i.e., mitochondrion, endoplasmic reticulum and Golgi complex). They secrete type I collagen for the bone matrix and synthetize proteins such as osteocalcin, a marker for the calcium homeostasis in the bone, or osteonectin for the mineralization of the matrix.[16] The osteocytes are embedded in the lacunae of the osteoid. They lost the ability for cell differentiation. Osteocytes are the main power unit for the metabolism. For maintaining the mineral bone homeostasis,[15] they produce various bone matrix proteins, including osteocalcin and CD44 (or cell adhesion molecule).[16] Microscopically, osteocytes are anatomically different from osteoblasts. They are stellar-shaped with a reduced size of cytoplasm and organelles. Osteocytes are characterized by a large number of cell processes that are connected to each other's by canaliculi. In this way, osteocytes fungate as sensors and are able to communicate directly with themselves.[14,16] The lining cells are osteoblasts on bone surfaces that lost the ability to form bone. They are flat spindle-shaped and poor in cytoplasm. Lining cells regulate the mineral ion balance. They are able to re-differentiate into osteoblasts with the help of PTH.[16] Osteocytes and living cells are the cells whose mechanisms are the less known.[14] The osteoclasts are bone-resorptive cells with large multinucleated cells, lying close to bone surfaces. The osteoclast precursors have the same hematopoietic origin as macrophages. The osteoclastogenesis starts with the production of MCSF by osteoblasts that dock at the c-fms receptor to stimulate the activation of the RANK receptor on osteoclast precursors. RANKL, expressed by osteoblasts, binds to RANK. Thus, the fusion of osteoclast precursors is stimulated, resulting in forming multinucleated immature osteoclasts. Immature osteoclasts become mature through binding to bone matrix via integrin receptors and

forming a ruffled membrane on bone surfaces. Osteoclasts resorb bone in cutting cones.[16,17] There they secrete acid hydrogen ions and proteolytic enzymes like cathepsin K to a resorption lacuna (or sealing zone), where the bone resorption begins by dissolving collagen.[16] Osteoblasts regulate the differentiation of osteoclasts mainly through the production of RANKL and OPG. RANKL is a stimulator of RANK, whereby, OPG inhibits the interaction between RANKL and RANK by binding on RANKL, resulting in the interruption of osteoclast growth.[14] Further regulating factors are PTH, Vitamin-D and calcitonin.[16]

In addition, the bone matrix consists of around 50 to 70% mineral, 20 to 40% organic components, 5 to 10% water, and less than 3% lipids.[14,16] The main elements of bone minerals are calcium, phosphate, along with carbonate and magnesium. Bone comprises HA ($Ca_{10}(PO_4)_6OH_2$) crystals. This crystalline structure is more soluble than geologic HA,[16] thus allows a better metabolic exchange, and makes the bone mechanical stronger, stiffer and resistant to compressive loud.[15] The organic matrix is mainly composed of around 90% of type I collagen, less type III and V collagen, FACIT collagens; the rest 10% are of non-collagenous proteins (e.g., proteoglycans, glycoproteins) or cytokines (e.g., Interleukin-1, Interleukin-6).[16] The organic components are responsible for the residence and flexibility to tensile forces.[15]

The formation of the bone matrix is introduced by chondrocytes and osteoblasts by synthesizing extracellular vesicles, filled with proteins, phospholipids, calcium and inorganic phosphate to start HA crystal formation. The crystals are growing through aggregation of new crystals. Macromolecules will be absorbed by the HA crystal surfaces in the environment and define the final shape or size. The mineralization is promoted by proteins of the SCPP family (e.g., dentin matrix acidic phosphoprotein 1, bone sialoprotein), and regulated by e.g., phosphoprotein kinase and alkaline phosphatase. The process of mineralization will be influenced by systemic hormones and lipids, including PTH, PTHrP and Vitamin D.[16]

In this context, it is of great importance to maintain the molecular bone homeostasis by means of local growth factors or cytokines as well as systematic hormones. Another supporting factor is the dynamic communication between bone cells via gap junctions and cellular processes. The dependence of bones on other organs as kidneys, liver or skin which produce hormones, cannot be underestimated. Women who have low body fat or are in the menopause are predisposed to a lack in bone mass.[17] Mineral imbalance in the skeletal mass leads to well-known diseases like osteoporosis (Sx: low bone mass),[14] osteogenesis imperfecta (osseous mutation) or Morbus Paget (Sx: deformed and thickened bones).[16]

Preventive measures to keep the calcium level in balance are significant. Organs like the kidneys conserve calcium. In particular, patients with kidney or liver diseases or disfunctions suffer under poor bone quality and are risk patients for orthognathic surgery or treatments with implants. The small intestine is responsible for the Vitamin-D-dependent absorption of calcium and phosphate. Calcium-binding proteins absorb 300 mg calcium per day in the intestine.[17] If is not enough calcium available in the body, it has to be taken from bones. The calcium peak in the human skeleton is present between 25 to 30 years, from the 30st year of age, a loss of calcium can be observed.[17] It is recommended for adult humans to examine regularly their endocrine system from physicians. The daily doses of calcium are between 700 to maximal 1500 mg and for Vitamin D around 10 mg during autumn and winter, if diet delivers not enough.[19]

1.1.1. Osteogenesis and bone remodeling

Bone formation is induced endochondral or intramembranous. In both mesenchymal cells become finally osteoblasts. The endochondral ossification is marked by producing a hyaline cartilage as a template that will be replaced by bone. It is significant for the formation of long bones, the longitudinal bone growth in the bones of the skull base and posterior part of the skull,[18] the axial skeleton, the appendicular skeleton[20], and in facture healing.[18] The process starts with the proliferation of chondrocytes to the bone

formation area where they become hypertrophic. They release alkaline phosphatases and mineralize the extracellular matrix. The following step is underlined by the apoptosis of chondrocytes which form the calcification zone. The capillary ingrowth will be supported by vascular endothelial growth factors, the future bone marrow. Osteoprogenitor cells migrate and differentiate to osteoblasts to form new bone. The intramembranous ossification is a direct osteogenesis without cartilage, and occurs in the formation and growth of flat bones, fracture healing and recovery processes after distraction osteogenesis,[18] in the membranous neuro- and viscerocranium, and in parts of the clavicle.[20] Mesenchymal cells form ossification centers in the extracellular matrix and differentiate to specialized bone cells and blood vessels.

Furthermore, the bone is able to skeletal adaption due to the biomechanical environment through modifications in bone mass, geometric structure, matrix organization, and collagen orientation of the lamellae.[17] The changes of bone can have natural and unnatural reasons: maturation of bone, fractures, pathologic processes, aging or functional under- or overload. Bone modelling (or bone growth) are processes to form the bone in shape and size due to genetic constitution, mechanical forces or physiologic circumstances. Bone grows in either longitudinal or appositional direction.[15] Longitudinal growth means that new bone forms until epiphyseal growth plates fuse. Appositional growth describes the process that new bone will be added on the periosteal side while old bone is resorbed on the endosteal side. In contrast, bone remodeling is a lifetime dynamic process of resorption, replacement and modifying of the existing bone to new one. Remodeling effects are bone strength and health, maintenance of calcium homeostasis, and the prevention of an accumulation of microdamage.[16] The remodeling process will be induced by bone through hormones, mineral homeostasis changes or mechanical forces.[18]

The first phase is underlined by the recruitment of monocyte-macrophage osteoclast precursors from circulation and their activation to become osteoclasts in the annular sealing zones on bone matrix (Figure 1). The second, resorptive phase lasts around 2 to 4 weeks.[18] The osteoclasts lower the pH to 4.5 by secreting hydrogen ions in the bone-

resorptive zone,[16] resulting in resorbed cavities on the bone surfaces, the Howship's lacunae.

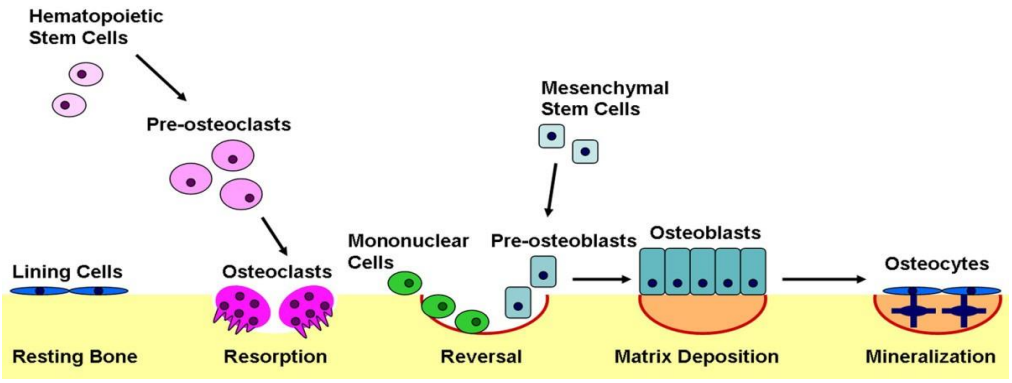


Figure 1. Simple scheme of bone remodelling.[21]

The mineral matrix will be disintegrated by acids proteins (e.g., matrix metalloproteinase or cathepsin K).[19] Thus, Osteoclasts pass away by apoptosis. The next step is the reversal phase which is still not clearly understood. Bone forming cells (e.g., monocytes, osteocytes, preosteoblasts) will be recruited in the Howship's lacunae. Possible coupling signals to start the bone formation may be bone matrix-derived factors (e.g., TGF-β, IGF-1, IGF-2), BMP, PDGF or fibroblast growth factors.[16] Osteoblasts and osteocytes will be recruited. Osteoblasts inhibit the osteoclast resorption through RANKL production. The strain gradient in the Howship's lacunae is decisively: If the strain is increased (or reduced), osteoblasts (or osteoclast) will be activated.[16] The next phase describes the bone formation that lasts around 4 to 6 months.[16,18] The resorbed bone in the lacunae will be refilled with new a collagenous matrix by osteoblasts, the osteoid. The osteoid consists of type I collagen and proteins. After mineralizing the new bone matrix, new bone will be formed by osteoblasts. After the termination of newly formed bone, approximately 50 to 70% of osteoblasts undergo apoptosis,[16] and transform either into osteocytes or lining-cells on the bone surface. Osteocytes build up a canalicular network to connect all bone forming cells, including living osteoblasts and lining cells. Notable is that the bone balance is not the same between the old and new one.[16,18] The new bone is composed of relatively low minerals;

thus, it is easier to change ions with the extracellular fluids. The bone balance (i.e., the volume of the new bone formation minus the old, already resorbed bone) is periosteal slightly positive, whereby endosteal and trabecular slightly negative, accordingly, the cortical and trabecular bone will be thinner by age.[16] After the production of the new osteoid, the mineralization process of cortical and trabecular bone will be finished 130 and 90 days later, respectively.[18] Finally, in the adult skeleton, the cortical bone turnover rate is with around 2 to 3% each year low, whereby the trabecular turnover is much higher to maintain the mineral and metabolic balance.[16]

1.1.2. Alveolar bone

The alveolar bone is divided into the fixed maxilla and movable mandible. The jaws are moving in opposition to each other to enable chewing, biting, and speaking. The upper jaw forms the floor of the nasal cavity for breathing, and the lower jaw holds the tongue in position to support speech and eating. The maxilla is formed though the completed fusion at the intermaxillary suture of two maxillary bones by the sixth week,[22] and is firmly connected to the neuro- (i.e., frontal, ethmoid and sphenoid bones) and viscerocranium (i.e., nasal, lacrimal, zygomatic and palatine paired bones, two inferiors nasales conchae, and vomer). On the lower part of maxilla, the alveolar process, all upper teeth are located. The upper jaw's blood supply is guaranteed by the maxillary artery. The maxilla is supplied by the sensory maxillary nerve (V2) that is the second branch of the trigeminal nerve (V). By the sixth week, the lower jaw is completely fused of the two mandibular processes at the midline, the mandibular symphysis.[22] The center of the arch is thickened and forms a chin, a characteristic in the human skull. The mandibular bone is composed of a horseshoe-shaped body to house the lower teeth, and sharpens in the retromolar triangle, forming two rising rami on left and right side. The rami are shaped by the condylar process, and the coronoid process that is the appendix for the attachment of the temporal muscle. The TMJs shape hinge joints on each side of the head to allow movements of the lower jaw. The

mandible is supplied by the inferior alveolar artery. The mandibular nerve (V3), the third diversion of the trigeminal nerve, branches to the inferior alveolar nerve in the mandibular canal in the body of the mandible and supplies the molar and partly premolar teeth. At the mental foramen under the root tip between the two premolars, the nerves divide into the final branches, the incisive and mental nerves to supply the anterior teeth, lower lip, and chin. Four existing masticatory muscles attached to the mandible permit the jaw occlusion and have the following functions:[23] The masseter muscle causes the forceful closure of the jaw. The temporal muscle has the same function, and additionally it is responsible for the retrusion of the mandible. The medial pterygoid muscle helps to close the jaw and assist the protrusion of the chin. The fourth lateral pterygoid muscle support the protrusion of chin and opening the jaw. Additionally, the mylohyoid and anterior digastric muscles also influence the movement of the mandible and support the chewing and biting, although they do not belong to the masticatory muscles.[23]

The different bone densities inside the alveolar bones are described in Misch's bone density classification scheme (Figure 2),[24] measured radiographically and expressed in Hounsfield unit (HU). Stage *D1* (bone density >1250 HU) outlines the dense cortical bone. Stage *D2* (850-1250 HU) describes the coarse trabecular bone and the outer dense bone. Further, stage *D3* (350-850 HU) delineates the fine trabecular bone and also the thin porous cortical bone. Stage *D4* (0-350 HU) depicts the fine trabecular bone and, finally, the stage *D5* (< 0 HU) is non-mineralized, immature bone. The evaluation of the bone density is important for rehabilitation planning based on the location.

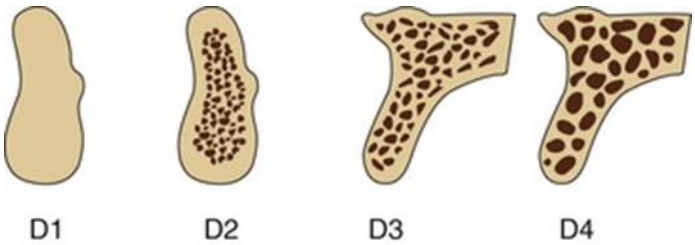
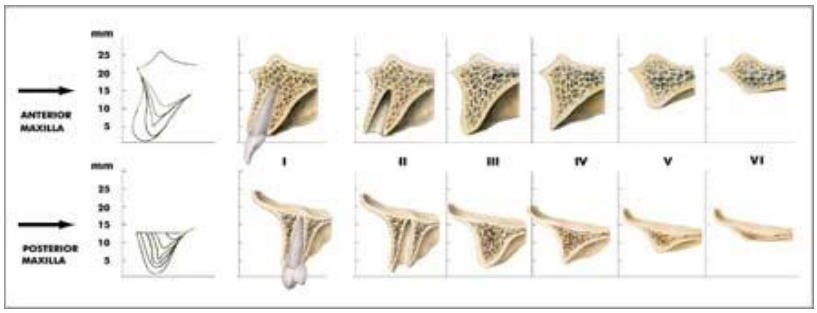


Figure 2. The four bone densities in edentulous patients and their typical anatomic location, described in the stages D1-D4. (D1): dense cortical bone in anterior lower jaw; (D2): porous cortical and coarse trabecular bone in posterior and anterior lower jaw, anterior upper jaw;

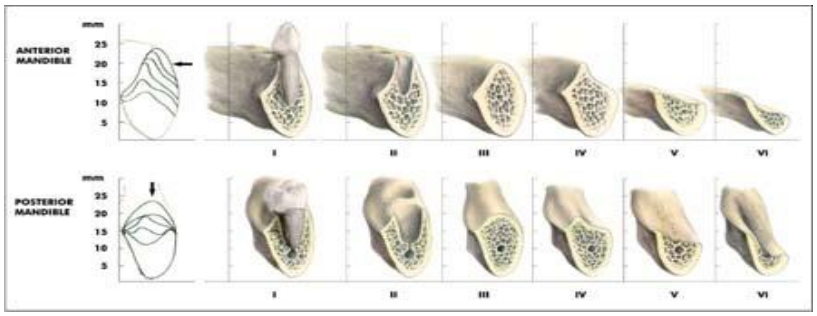
(D3): porous cortical and fine trabecular bone in posterior and anterior upper jaw, posterior lower jaw; (D4): fine trabecular bone in posterior and anterior upper jaw.[24]

The biomechanics describe the stress distribution and strength of bone tissue. The differences of the osseous structure of the jawbones have a great impact on their biomechanics. The maxilla consists primarily of trabecular bone and thin cortices. Stress will be transferred to the entire cranium and is basically loaded in compression.[17] Conversely, the mandible comprises a thick cortical bone and radially oriented trabecular bone, comparable to the structure of long bone that make it stiffer and stronger than the maxilla.[17] The mandible is exposed to forces from different sources including the muscles of massification, teeth and the TMJs, resulting in tensile and compressive loads.[17,25] Wong et al[25] reported that the first strain on the mandible causes deformations in the length, whereby the secondly strain takes influence on the width due to the direction of the mechanical force. Additionally, the author summed up that the maximal stress level can be localized at the contralateral angle and the ipsilateral corpus of the mandible, as well as in the ipsilateral condylar neck area. Summing up, the biomechanical behavior of the jawbone hinge on the material composition, their properties and geometrical form. After the stress terminated, the bone returns to its original structure. Not only the bone possesses the capability to adapt mechanically to the altering environment, but also the condyles of the TMJs are formable for regeneration, recovery and adaption.[17] In the opposite case, if the alveolar bone is not loaded over time, it will be completely resorbed (atrophic).

In the classical study of Cawood and Howell[26], atrophic alveolar processes of jaws of 300 dried skulls were examined to develop a classification of edentulous jaws to describe the stages I-VI of atrophic jaws (Figures 3 A and B). It was concluded that basal bone does not undergo major bone changes in form, whereby changes in the alveolar bone are considerable in both horizontal and vertical directions. Therefore, the alveolar bone changes are foreseeable according to a pattern: the bone loss is vertical and horizontal regarding from the labial view in the anterior mandible and maxilla, and from the buccal view for the posterior maxilla, respectively. A vertical bone loss was observed in the posterior mandible.



(A)



(B)

Figure 3. The modified classifications of edentulous (A) maxilla, and (B) mandible by Cawood and Howell.[27]

With reference to the skeletal changes,[26] the effects in facial soft tissue were concluded.[28] In class I no changes were noticed. In the classes II to IV, the muscles that surround the mouth region collapse, resulting in thinning of the mouth, losing of the lip support and a constricting of the cheeks. In the classes V and VI, a reduction in the vertical height of the lower facial part and the chin prominence are pivotal.

The buccal and palatal/ lingual cortical thickness of edentulous maxilla was measured from 1.04 to 1.69 mm and from 1.36 to 2.06 mm, and for the mandible from 1.36 to 2.06 mm and from 1.66 to 2.39 mm.[29] Accordingly, the thinnest area is the anterior maxilla during the thickest region can be found in the posterior mandible.

Regarding the rehabilitation of edentulous patients with implants and prosthetic reconstructions, the possibilities are limited. The remaining bone is insufficient for conventional implant placements in cases if there is less than a millimeter between oral cavity and nasal or sinus cavities in maxilla or a great bone loss resulting in the exposure of the inferior alveolar nerve in the mandible.[30] Depending on the clinical situation, an augmentation with bone graft substitutes or techniques using only the remaining bone without biomaterials should be considered.

1.1.3. Dental Implants

Since the ancient times, people fabricated implants to replace tooth roots. It all started with implants that were made from materials such as animals' teeth, carved ivory or transplantations of human doners .[31] The semi-success led to further development of material properties, fabrication methods, surgical techniques, and the extensive widening of knowledge. In the years 1980s and 1990s, dental implant systems were widely commercialized through well-known manufacturers and reached their final acceptance.[32]

Over 20 years of clinical and experimental experiences, the Swedish physician and researcher Per-Ingvar Brånemark performed fundamental studies about the improvement of dental implant systems, developed pure titanium implants and became the pioneer in the field of osseointegration. The osseointegration describes the biological and mechanical integration of the implant, or fixtures according to Brånemark' nomenclature, in the bone. Brånemark and his Swedish investigation group presented detailed treatments protocols, including hints for clinical applications:[33] recommendations of specific instruments, drilling methods to avoid thermal and mechanical stress to bone, insertion techniques, and prosthetic loading protocols after an unloaded healing period of 3 to 6 months. In his study, Brånemark emphasized that a successful bone regeneration and osseointegration result if the implant surface is in close contact to the vital host bone without fibrous soft tissue interferences after the healing period.[33]

An implant replaces a single lost tooth or multiple teeth by means of prothesis in edentulous dentitions. A fully functional dental implant consists of a fixture/screw, an abutment, and the final crown. Today, there are various types of implants on the market. *Endosteal* (or endosseous) implants are placed inside the alveolar or basal bone of the jawbones, and have a *blade* (flat shape) or *root* form. Implants with root forms are classified as *cylinder* (or press-fit) implants that are just pushed into the prepared surgical cavity, and *screw-shaped* implants. The screw-shaped implants are screwed with their whole implant body into the bone and are the up-to-date implants nowadays.[32] There is also a differentiation in *one-piece* versus *two-piece* implants. The one-piece implants are composed of one solid, integral unit of an implant body to screw in the bone, and abutment, a connector between the implant and crown. The two-piece implants consist of two separate components: the implant body and a platform that is the connector for e.g., screwed or cemented implant abutments. Further screwed implants are *mini-implants* characterized by small diameters (under 3.0 mm), placed in thin atrophied ridges. In the most cases, the root-formed implants are inserted with the implant collar at bone crest (*bone-level implants*) or occasionally at the soft tissue margin (*tissue-level implants)*. Regarding the implant *macrostructure*, a distinction is made between the first recorded *parallel-sided* implants and the later improved *tapered* screw-shaped design. A further point to consider is the great importance of the *implant threads*, e.g., V-thread, square thread or buttress thread. The different threads result in various degree of strength and in the manner of transmitting the occlusal loads to the surrounding bone.[32] Further characteristic is the implant *microstructure* that describes the surface structure or roughness that can be modified with the aim to increase the surface area, resulting in an improved process of osseointegration. The modification methods can be subtractive (e.g., laser or etching) or additive (e.g., HA coatings) procedures.[32]

In contrast, the less used *subperiosteal* implants are connected with metal posts above the gum line and have a metal framework resting on the jawbone below the gum tissue. An older design are *periosteal* (or transosteal) implants, which are mainly fixed in the

mandible and are fabricated individually due to the width and length of the patient's arch. The implants are held by the metal plate that is at the bottom of the jawbone, fixed by screws in the bone.

The survival rates of implants are recorded above 90% nowadays,[34-37] dependent on the degree of osseointegration. The degree of osseointegration can be described in obtaining a high quality of bone-to-implant contact (BIC), thus, ultimately in a successful secondary (biologic) stability.[32,34] The primary stability will be reached by mechanical friction of the implant due to its macrostructure and shape in the bone. Directly after the implant insertion, the bone healing process is induced though a cascade of inflammatory, formation and remodeling events, regulated and controlled by growth as well as differentiation factors released by blood vessels.[14-19] The primary stability will be replaced by the secondary stability that is highly relevant for the long-term results.[32] A good integrated implant shows no progressive motion between the implant and the host bone,[38] but has still an adequate space for permitting remodeling events.[39] There are many factors that affect osseointegration positively or negatively. A good general health system, included a healthy host bone bed and a minimal invasive surgery are best conditions for an impeccable osseous implant integration. Further promoting effects are implant-related factors, including implant design, material, shape, length, diameter or chemical composition,[38] adjuvant treatments with bone grafting,[6-10,12] surface treatments with chemical bioactive coatings (e.g., bone morphogenetic proteins),[40] osteogenic biological coatings (e.g., hydroxyapatite),[41] biophysical magnetic stimulation,[42] or finally pharmacological agents (e.g., bisphosphates or simvastatin).[43,44] On the contrary, inhibiting factors include patient-related biological aspects are uncontrolled diabetes,[36,45] high doses of bisphosphonates as a potential risk factor for osteonecrosis of jaw (ONJ) with negative impacts on osseointegration,[36,46,47] the 60 to 79 age group of patients,[45] behavioral factors such as smoking habits, poor oral hygiene, alcohol abuse, bruxism,[45-47] lack of patient compliance, head and neck radiation[45,47] or pharmacological medication e.g., cyclosporine A,[46] postmenopausal estrogen therapy,[45] selective serotonin reuptake inhibitors (SSRIs),[36,46]

proton pump inhibitors (PPIs)[36] or chemotherapeutic agents (e.g., cytostatic).[46] Generally, it is plausible that systematic diseases may have a negative effect on osseointegration, but there is no explicit proof in literature that e.g., osteoporosis or diabetes mellitus pose a proved risk.[36,47-49] Another critical factor is the surgical drilling preparation of the bone, injuries occur between 100 to 500 μm in the mature bone and can be seen as tolerable, but peri-implant gaps exceeding 500 μm decrease the bone quality and the process of osteogenesis.[38,39] The absolute contraindications for implant treatments are recent myocardial infarctions and cerebrovascular accidents, valvular prothesis surgeries, increased bleeding propensities, immunosuppression, active malign cancer treatments, drug consumption, intravenous bisphosphonate use and psychological problems.[50] Moy et al[45] demonstrated that in the most cases the implant rehabilitation is successful by recording considerable low failure rates: 8.16% in the maxilla and 4.93% in the mandible, respectively. Causes for failure of dental implants may be a biomechanical overloading of the implant through unfavorable implant placement or angulation, resulting in e.g., an inadequate fore distribution, loosening, fracture or increased mobility of the implant.[37] Further points are a lack of osseointegration, a loss of surrounding bone supporting, infections or inflammations (e.g., peri-implantitis, peri-mucositis) and persistent pain.[37,47]

Concluding, Albrektsson et al[51] proposed a list of criteria for successful clinical implant treatments: Firstly, the implant is not movable during the clinical examination, and radiolucency between host bone and implant does not exist on a radiographic image. Further, after the first year of implant insertion, the vertical bone is measured less than 0.2 mm annually. Another important point to note is that the patient has to be symptom-free, including no persistent pain, infection or unease. Finally, to evaluate a treatment method as successful, the above criteria should be minimal achieved in a success rate of 85% of the cases after a period of 5 years, and in 80% after 10 years.

1.2. Bone augmentation

In the surgical and prosthetic field, dental implants are widely used along clinicians and are indispensable in treatments of partly or fully edentulous deficiencies. In most cases, dental implants require some sort of bone augmentation due to inadequate bone volume or insufficient bone density. A successful insertion of dental implants requires at least 10 mm bone in vertical height and 3 mm in horizontal width.[52] Recent statistics reveal that globally around 2.2 million bone graft augmentations will be performed each year, and a growth of 13% of surgical procedures for correcting bone defects in dentistry, neurosurgery and orthopedics is noticeable annually.[53] Bone graft materials and substitutes (BGS) constitute an essential part in the reconstruction of articular and osseous defects, not only in the maxillofacial surgery, implantology,[8] periodontology,[54] but also in the orthopedics and traumatology, including the surgery of spine, hip, upper and lower extremities.[6] There are different grafting techniques and materials for use on the market.

The main functions of BGS are to augment the bone to the desired volume and anatomical form, to assist bone healing and remodeling processes, to ensure space for bone regeneration,[54] to serve as a local carrier substance for infused living osteogenic cells or growth factors[8] or other drugs (e.g., antibiotics),[55] and to serve as a frame for tissue engineering.[56]

Broadly, main properties of BGS are osteogenesis, osteoinduction, osteconduction and osteopromotion. *Osteogenic* grafts are of autogenous origin and consist of viable cells that are capable to differentiate and form new bone.[6,10,58] Some examples are the use of rips, chin or cranium.[57] *Osteoinductive* grafts possess growth factors and proteins that stimulate the recruitment of mesenchymal stem and progenitor cells from the surrounding host tissue and their differentiation into osteoblasts.[9,10,58] One example is Demineralized Freeze-Dried Bone Allograft (DFDBA).[58] Further, *osteoconductive* grafts serve as a framework for osteogenesis by enabling the ingrowth of vessels and migration of osteogenic host cells.[9,10,58] Almost all inert materials are osteoconductive.[57] The process is known as "creeping substitution", referring to the motion of the cells from the host bone site into the osseous

defect, and further to the graft for the new bone formation and neovascularization.[57] At least, *osteopromotive* grafts enhance the different bone healing processes by providing stimulating signals such as platelet-rich plasma (PRP).[55]

The requirements for ideal BGS include biocompatibility, osteogenic, osteoconductive and/or osteoinductive properties, a predictable long-term volume and space maintenance, biomechanical stability, bioresorbability, and biodegradability in appropriate time and replacement by newly formed bone, structural similarity to bone to enhance cell adhesion and invading blood vessels, and a low incidence of infection and immunogenicity.[54,57] Many types of BGS of various origins are commercially available for medical applications designed for patients.

Overall, BGS can be divided into the following classifications:[7-9,54,58,59] 1.) *materials of natural origin:* autogen (from the same individual, e.g., donor sites: iliac crest, symphysis, ramus, tuberosity), allogen (from the same species), xenogen (from a different species: bovine and porcine animals), phytogenic (from marine origin: e.g., algae or coral); 2.) *synthetic (alloplastic) materials* (chemically and/or naturally derived nonanimal biomaterials): ceramics (e.g., α-/ β-TCP, biphasic calcium phosphate, HA, bioactive glass, calcium phosphate, calcium sulphate), polymers (e.g., polymethylmetacrylate, poly(lactic-co-glycolic acid) (PLGA), poly(glycolic acid) (PGA), polylactic acid (PLA)), metals (e.g., titanium), cements (e.g., calcium phosphate cement), composites (mixture of various materials, e.g. polymers and bioactive glasses); 3.) *biologics*: growth-factors based (natural and recombinant growth factors, e.g., platelet-derived growth factors (PDGFs), fibroblast growth factors (FGFs) or BMPS), living cell-based (e.g., MSCs, osteogenic cells mixed with cytokines), and gene therapy-based (viral or nonviral). To reach the best possible results of the different materials, structural scaffolds such for allografts and alloplasts can be combined with biological carries.

It is important to consider the graft maturation healing times: autografts (fast), allografts (moderate), xenografts (slow) and alloplasts (slow to unpredictable); and the

further recommendations: If the augmented bone volume is below 4 mm in dimension, 6 to 8 months of recovery time will be necessary, while grafts are greater than 4 mm, 6 to 10 months should be complied.[58] The healing time of the grafting material can be affected by numerous factors such as material-related (e.g., type of material, structure, particle size, porosity, exclusion or inclusion of autogenous bone or chips), the surgery-related (e.g., surgical technique: invasive or not, blood supply at graft site, remaining walls of bone, post-operations) or patient-related (e.g., systematic diseases, habits as poor an oral hygiene, smoking or alcohol).

General complications of all BGS are graft fractures, graft exposure, non-/ or delayed osseointegration and wound infections,[6] additionally specific aspects in the oral surgery include the nerve canal involvement in regeneration sites, exposure of fixation screws during the healing, incision line opening in BGS sites, screw perforations through thin tissue, membrane exposure or high mucosal attachment around the abutment of the implant in the future restorative space.[58]

BGS vary in their composition, structure and fabrication method, resulting in deviations in mechanical actions, and therefore in the scope of application. It is dependent on the size of the defect of the void, a variety of materials in different forms are available, such as bone blocks for more geometry and stability, granules for an easier handling for insertions into performed cavities, and also putties, cements or gels. The design of the scaffold architecture and the mechanical stability of the BGS are determined by morphological properties, including the interconnectivity and porosity of the pores within the graft material. The bone augmentation materials, particularly the manufactured, have different types of porosity in order to imitate the cortical and cancellous structure of autogenous bone,[60] resulting in creating and sustaining the vitality of the graft in the host bone. *Macroporosity* (pore size > 100µm) allows on the one hand the migration and proliferation of cells responsible for osteo- and angiogenesis, and on the other hand, the body fluid circulation and ion exchanges through their interconnectivity.[57] Namely, macropores enhance the bone formation process, but also decrease the mechanical strength

of the grafting material.[61] *Microporosity* (pore size <10μm) facilitates the capillary formation and ingrowth and promotes the fluid distribution, cell transportation and protein distribution.[57] Further *nano-pores* enable the transportation of molecules to forward signals and nutrition and to remove waste; and finally, the *millimetre-pores* enhance the invasion of nerves and blood vessels.[62]

The current literature recommends pore sizes over 300 μm to promote newly formed bone, a better cell ingrowth and bone healing.[63-66] Based on the works on previous and present studies, some authors suggested the optimal pore size between 300-400 μm,[64,65] others reported best results with almost 600 μm.[66] Regarding the vascularization, the highest area of vessel formation was reached with pore sizes >400 μm.[67] In general, the larger the pores, the higher the capillary formation resulting in a greater oxygenation, thus, a faster osteogenesis; conversely, smaller pores provide the osteochondral ossification and reduction of blood vessel ingrowth.[63,67] It is certain that particles smaller than 50 μm are not recommended due to a fast well-documented degradation.[57] In brief, the ideal diameters are still controversial and depend on many factors like the material geometry.

The choice of the adequate BGS, alone, in combination or with additional carriers, is largely dependent on the patient's physical constitution as well as on his expectations, and has to be adapted to the individual needs.

1.2.1. Autograft

Autogenous (or autologous) bone (AB) is a transplant of viable and functional healthy organs or tissues, harvested from one part of the body and implanted to another within the same individual. Moreover, it is strongly entitled as the "gold standard" under the BGS, and is broadly indicated for all deficient areas.[32] AB offers sufficient bone volume with a resorbable and osteoconductive 3-dimensional scaffold including multiple growth factors and living cells that possess all four fundamental biological properties.[6,8,54] In the

dental surgery, the main interest of the AB origin is the mandibular symphysis, tuberosity or mandibular ascending ramus in form of particulate or block graft. Today, various types of BGS are routinely used in dental clinics, whereby AB is significant in procedures like alveolar ridge augmentations.[8]

To the benefits of AB belong their osteogenic potential, biocompatibility and non-immunogenicity, a low patient risk (no disease transmission), predictability and lower costs compared to other BGS.[32,54,68,69] AB has a shorter healing period of 4 months in comparison to other BGS, accordingly, implants can be faster placed.[69] AB deliver the highest possible degree of biological safety.[8] In various osteotomy techniques such as ridge splitting, AB blocks are favored due to their ability to form a three-dimensional volume of lost anatomic ridge contours, therefore demonstrated its effectiveness for the rehabilitation of larger bone defects.[69] However, the limits of AB are a relatively quick, but predictable resorption rate, a limited supply, and the possible necessity for a second stage surgery and/or general anesthesia. Final disadvantages are the needed second surgical side, including possible operative or post-operative complications e.g., increased blood loss or operative time, surgical risks, pain, infections, inflammations, donor site morbidity or potential of scarring.[6,8,32,68] At this point it has to be emphasized that the efficacy of bone graft alternatives is often compared to the biological properties of AB.

In general, AB can be classified into cancellous, cortical or cortico-cancellous bone that is either vascularized nor avascular,[6,68] and available both fresh and frozen.[70] Porous cancellous AB is often obtained from the iliac crest, femur, fibula or radius, consisting of a porous scaffold with a cellular matrix that is highly biological active.[68] Initially, cancellous bone graft provides poor support, but later on it gives structural support during bone formation .[68] Cancellous AB are rarely used in the dentistry. Instead, denser cortical AB has a mainly osteoconductive character. Their low porosity effects in a smaller surface area.[68] Therefore, cortical AB is less biological active and also less resorbable than cancellous bone.[68,69] In all, cortical AB gives strong mechanical and structural support marked by a minimal resorption, but requires a longer time to become vascularized and finally

integrated. Intraoral bone grafts are primary cortical and can be harvested from the symphysis that offers a great bone volume, but includes also a higher incidence of postoperative complications like neurosensory changes.[69] However, the posterior mandible is a safer and rather uneventful option chosen as donor site[8] for harvesting particulate AB or AB blocks in the dimensions at a maximum of 40 mm in length and greater than 10 mm in height.[69] Misch et al[69] underlined that cortical bone is the "ideal" option for onlay augmentations and the volume loss was less than 25% in his study. Lastly, the softer cortico-cancellous bone consists of both, a trabecular and dense bone structure, and is very effective due to maximize the remodeling, revascularization and bone healing process.[8] For larger intraoral bone defects, cortico-cancellous block graft is preferred from the iliac crest.[69] With regard to the studies of Misch et al[69], the healing time of implants was around 2 months in cortical grafts and 4 months in cortico-cancellous grafts. Furthermore, an avascular bone graft is a cortical span bone, whereby vascularized are won by free tissue transfers with connected vessels for the blood supply. They are harvested from e.g., ribs, iliac crest or fibula,[6] and are better incorporated in the surrounding bone tissue.[68] Revascularization causes mesenchymal differentiation into osteogenic, chondrogenic or further cell types.[54]

In order to amplify the osteoinductive properties and structural integrity, AB or allografts can be mixed with platelet rich plasma (PRP).[6] PRP is composed of a low concentration of human platelets in plasma, including growth factors as well as cell adhesion molecules like e.g., fibrin or fibronectin.[6]

Furthermore, for treatments in esthetic zones, it is common to use autologous free gingival grafts, especially harvested from the palate to magnify the keratinized tissue to improve the mucosal thickness as well as the graft coverage. Bone grafting should be conducted at least 6 to 8 weeks after extraction in order to permit the epithelialization processes and the maintenance of the soft tissue architecture.[69]

Pogrel et al[71] set up criteria obtained by the findings of his study for the use of vascularized and non-vascularized graft materials in the dentistry. Vascularized grafts recorded a high success rate in the treatment of primary reconstructions, after previously irradiations, during the simultaneous replacement of soft tissue or mandibles > 9 mm in length. However, avascular bone grafts are considered as a first choice for treatment to form the bone volume and contour for facial esthetics and implant insertions, as well as for the secondary reconstructions of osseous defects < 9 mm in length. The recent systematic review conducted by Moura et[72] showed a success rate of 87.6% for reconstructions with avascular bone grafts in the mandible which were caused primary by benign tumors in 58.8% of the cases.

The 5-year-study of Altiparmak et al[73] presented a similar success rate of implants inserted in AB blocks for ridge augmentations (92.45%) and of implants placed in natural host bone (85%). Furthermore, the average marginal bone loss was marked by 1.47 mm by AB and 1.58 mm by host bone group, respectively.

In the 10-year prospective case series study, Chappuis et al[74] obtained the results of lateral ridge augmentations using AB blocks, covered by DBBM and collagen membrane in Guided Bone Regeneration (GBR). The 10-year implant success rate with AB blocks was 98.1%, whereby a minimal graft resorption of 7.7% was observed,[74] thus, similar to commonly survival rates of implants in pristine bone.[34-37] The chin graft obtained significant better results than the retromolar graft and women had a higher bone loss after 10 years than men.[74] The studies revealed that the quality of incorporated AB blocks is similar to the density of native bone,[73,74] even surpass the natural bone.[69]

Summing up, AB improves the bone quantity and quality in a satisfactory way, but regarding the implant success rates, there are no significant differences between all BGS, with the addition that patients prefer non-autogenous bone due to its drawbacks.[75]

1.2.2. Allograft

Allogenous (=homogenous) bone (AL) is a transplant of living organs and tissue or bone of cadaveric sources from a genetically nonidentical donor of the equal species.[6-8,57] Analogically, isografts are grafts of two different individuals, but from the same genetic origin.[57] AL can be obtained from either an adult nor embryonic bone of neutral crest or mesoderm, whereby the fetal bone has a higher degree of osteoinductivity.[76]

In general, the procedures of harvesting, processing, storage, and the final distribution of the AL are controlled by the American Association of Tissue Banks (AATB).[77] The AATB established clear rules and dictate defined parameters.[57,77,78] Only these licensed tissue banks are allowed to supply AL after ensuring the safe by removing the contamination and antigenic potential and maintaining the ethical use of donated human tissues. The first tissue bank US Navy was founded by Dr. George Hyatt in 1949 at the Naval Medical Center located in Bethesda (Maryland, United States).[79] It was the first program worldwide that established standards such as donor criteria, processing methods, graph registrations and documentations, principles of tissue transplantation, and clinical evaluations. Various methods as cryopreservation, freeze-drying or irradiation sterilization of tissue were developed to process, preserve and sterilize AL. Tissue processors are responsible for the donor suitability, valuation, transport, storage, processing and distribution.[57] The donor suitability is strongly monitored and standardized. Immediately upon arrival, the processing of AL starts with the cleaning and decontamination through antimicrobial, antimycotic and antifungal solutions, followed by freezing at -80 °C and dehydration to reduce antigenicity.[78] The graft size will be standardly reduced between 250 to 1000 μm.[57] To produce demineralized AL, the mineral components of the bone will be eliminated with hydrochloric acid baths and buffered with solutions to remove residual acid.[78] AL is sterilized by means of several methods such as heat, chemicals, ethylene oxide gas, supercritical CO_2, gamma or electron beam radiation.[57,78] After the termination of all postprocessing tests, the tissues are processed into usable grafts for the global market. Consequently, after all these processing methods, the biological and mechanical properties

of AL are weakened.[77] For the industrial regulation, the Food and Drug Administration (FDA) is the ultimate authority.[78] The FDA established a registration and listening system for human cellular and tissue-based products (HCT/Ps). All human AL has to undergo procurement steps. Hospitals have to report all patient deaths to tissue banks that send a team for donor screening.[78] The potential donors are examined with microbiological and serological tests to detect bacteria, fungi, antibodies of human immunodeficiency virus, hepatitis and syphilis, respectively.[78] In the USA, the donor ages are between 12 to 80 years old, and about 86.5% of the donors are younger than 70 years and three of four donors are men.[57]

Notably, AL are subdivided into fresh, frozen, and processed freeze-dried grafts (mineralized, demineralized or mixed) subgroups,[77] commonly used in the dentistry in treatments like GBR, sinus grafts and periodontal procedures for maxillary and mandibular defects.[8,32,77,80] AL is beneficial in ready availability and predictability. There is no need of a second surgical side, thus the possibility of avoiding morbidity.[7] Mineralized AL is osteoconductive, whereby demineralized, fresh and frozen AL have osteoinductive and osteoconductive capabilities.[32,57,77,81] Disadvantageously, AL in mineralized form has a potential for immunogenicity, though demineralized AL is at least immunogenic.[77] AL is slightly capable to transmit a disease and infections,[6-8] may conflict in cultural issues, and is not suitable for larger graft sites.[32] Furter drawbacks are the procurement costs and the inconsistent graft incorporation.[6,8] The resorption time is for demineralized AL fast, and for mineralized AL from medium to slow.[32] Fresh and frozen AL is rarely used due to limited shelf life and its high risk of immunogenicity and infections.[7] Mineralized AL is available as cortical (e.g., *MinerOss Cortical*™) or cancellous (e.g., *MinerOss Cancellous*™) particulate. It is a freeze-dried bone allograft (FDBA) which still comprises all natural bone components including BMPs after commercial processing.[81] The graft source of mineralized cortical AL is mainly from extremity bone such as femur, tibia or fibula.[57] Cortical FDBA is mechanically strong and a popular graft in the implantology.[57] However, mineralized cancellous AL has its origin from trabecular bones, mainly from the metaphyseal region of

long bones.[57] It is softer and more porous, resulting in a better process of angiogenesis. Besides, cortical FDBA is covered by endosteum leading to a possible benefited bone incorporation. A further advantageous point is that cancellous bone integrates well in its surroundings, thus a reduction of micromotion of the graft can be observed.[57] It was established that FDBA should have a residual moisture around 6% or less.[78] In addition, FDBA is also offered as a cortical and cancellous mix and combine the benefits of both. A further division of AL is the demineralized freeze-dried bone AL (DFDBA) or demineralized bone matrix (DBM). DBM is proprietary produced in a few processes under patent protection, whereby DFDBA is nonproprietary and may be made of several processes.[57] Vehicles such as glycerol, hyaluronic or collagen are usually added to DBM to improve handling and adaptability.[8] Both forms are removed by its mineral and cellular components due to reduce the infection rate and host immune response, resulting in a less mechanical strength and worse scaffold for space maintenance compared to fresh, frozen AL and FDBA.[8,81] The demineralization process is supportive for the release of soluble factors such as BMPs while storing enough calcium for the hydroxyapatite crystal formation.[81] The maximum permissible amount of residual calcium in the DFDBA graft should be 8% by weight or less in order to fulfil entirely its osteoinductive properties.[57,78,81] In contrast, FDBA has to be demineralized by the recipient bone which will in turn cause a prolongation in function because of a longer osteoclastic breakdown.[81] In addition, new achievements in graft materials are mineralized and demineralized cortical mixtures (70:30 volume per volume) that contain all beneficial qualities of DFDBA, including maintaining space and a fast incorporation.[57] A further product is the collagen-based AL. Collagen promotes the deposition of minerals, ingrowth of vessels, and the binding of growth factors, and is often combined with BMPs or even better with carriers such as HA.[77] Nowadays, the most commonly used AL are FDBA and DFDBA.[81] However, there is an inconsistency between the question if FDBA grafts are better for dental applications[57,77] or either DFDBA.[80,81]

AL is commercially available in form of blocks, segments, chips, cubes for larger defects, or gel, pastes, and putties.[8,57]

Interesting to note is that Holtzclaw et al[78] stated in his review that some international scandals regarding AL were caused in the past. Human body corpuses were stolen, and human tissues were marketed without authorized consent and appropriate testing for diseases according to the FDA regulations. After that, the regulations became even more strict.

Another important point to consider is the bioburden of AL provoking infections of the operated areas in the human oral cavities. In a recent report of 509 bone grafts collected from around 110 multiorgan donors, Ilays et al[82] figured out the bacterial contamination rates for surface swabs and bone cultures for all grafts of 16.6 and 6.1%, respectively.

Another current systematic review of Baseri et al[83] showed that bacterial contamination rates of the bone were greater in cadaver donors (19.9%) and samples and swab tests (13.2%) in comparison to living donors (7.5%) and bone fragment cultures (6.3%). In 63.2% of the cases of all selected bones, the predominated isolated bacteria was Staphylococcus spp.

Ultimately, the recent review of Donkiewicz et al[84] confirmed no significant difference between the clinical results of AB and AL as pre-implant bone grafting materials.

1.2.3. Xenograft

Xenogenous (=heterogenous) bone (XB) is a transplant of tissue or organs from a donor of a different species than the host.[57] In the beginning of the 19th century, organic XB was mainly harvested from os purum or os novum,[57] and subsequently, inorganic XE

became commercially available in the 1960s, produced by Scopp.[85] Scopp et al[85] called the first bovine bone *Boplant* that was deprived from calf bone.

Generally, the advantages of XE are the ready availability and the lack of necessity of a second surgical site.[32] Downsides are the following:[8,32] relative slow or variable resorption rates, only osteoconductive properties, an increased inflammatory response, immunogenicity, a potential for disease transmission, and cultural concerns. Due to the absence of living cells and the biological part, an additional tissue treatment is beneficial to facilitate the retention of the cells with osteoinductive capabilties.[8] XE can be applied in procedures of bone grafting, maxillary sinus elevations, and ridge augmentations,[70] but are not recommendable for too large graft sites.[32]

Today, XE is offered in demineralized, freeze-dried, and/or deproteinized form.[57] In the dentistry, XE are originating from animals like bovine, porcine, equine, chitosan, silk or phytogenic species like coral-based, Gusuibu or algae-based.[8,54,57] Bovine and porcine XE are usually made of cancellous bone, thus, show similar biochemical and -mechanical characteristics to the human bone in porosity, density and calcium content.[57] In general, the processing and production of XE is FDA-controlled and similar to those of AL.[57] Notably, the most used XE is the deproteinized bovine bone.[77] In the deproteinized form all organic components (i.e., immunogenic factors, around 40% by weight) are eliminated whereby a calcified matrix with pure HA is left.[57] The macro- and microstructure of the XE is not changed. XE is a pure mineral with a medium to slow resorption rate.[32]

XE can be obtained as solid or porous block, pure HA, bovine or porcine pericardium, collagen-based products (resorbable collagen membranes from tendon and skin) or as coralline, chitosan or spongious grafts.[54,57]

A broadly popular deproteinized bone is *Bio-Oss*™. This bovine bone is treated chemically and thermically by special methods to remove all organic components and to gain pure HA. The particle size of *Bio-Oss*™ is between 0.25 to 1 mm, and especially these pore dimensions revealed the capability to enhance new bone formations.[77] *Bio-Oss*™ is

marked by 75% porosity, thus it is beneficial for osteoconductivity, stimulating angiogenesis and bone ingrowth,[8] but worse for initial stability due to an increased surface area.[77] In the human study of maxillary sinus augmentation with 9 biomaterials and 94 patients, Scarano et al[86] showed new bone formation in AB and *Bio-Oss*™ of 40.1 and 39%, and residual graft of 18 and 31%, respectively. These results demonstrate the biological similarity of *Bio-Oss*™ with natural bone, whereby the high amount of residual graft indicates a low resorbability. Another study also confirmed that around 10 to 13% more residual graft of bovine bone was found compared to AB.[87] Schlegel et al[88] describes *Bio-Oss*™ as a "permanent implant" due to its non-resorptive properties. A further study revealed also comparable results between AB and *Bio-Oss*™, however, the authors claimed to see histologically more connective tissue surrounded by the *Bio-Oss*™ particles than newly formed bone after a year.[89] The microvascular density in alveolar ridge regenerations of both materials is not significantly different, but bovine bone has a longer healing period than AB.[90] Other famous bovine products on the market are *OsteoGraf*™ and *Cerabone*™ that both also imitate the biomechanical characteristics of natural bone.[8]

Another promising XE is Chitosan, a natural biopolymer deprived from the exoskeleton of crustaceans, comprising of glucosamine and N-acetylglucosamine that support the bone regeneration by stimulating mesenchymal stem cells for the differentiation into osteoblasts, providing structural scaffold, and forming mineralized bone matrix.[8,77] Chitosan is biocompatible, biodegradable, and has an antibacterial, and antifungal activity.[91] Its dental application areas are for bio-dental materials and engineering, periodontal and dentin-pulp treatments,[91] as well as in local drug delivery systems.[92] Furthermore, chitosan is offered in numerous forms e.g., films, beads, hydrogels or included in complex scaffolds, but because of its poor mechanical properties, chitosan is often mixed with other materials like e.g., bioactive glasses, gelatin, calcium phosphates, or growth factors.[8,92]

A further XE is silk, gained from the silkworm Bombyx mori that is a natural biopolymer of proteins, sericin, and fibroin.[8] By removing sericin, the remained silk fibroin

is the useful component for bone scaffolds in e.g., sponge, fibers or hydrogel forms. Silk fibroin is biocompatible, degradable, good tissue integrable, oxygen and water permeable.[8] It has poor mechanical properties and therefore used mainly in membranes for GBR.

Coral-based materials are from marine coral, and are known since the 1970s.[77] Some examples are *ProOsteon*™, *BioCoral*™ and *InterPore*™.[8,9] They consist commonly of calcium carbonate which is industrially transformed through high heat treatments with ammonium phosphate into crystalline HA[8] in order to adapt to the natural bone composition of HA, besides a small portion of calcium carbonate and phosphate.[57] HA is naturally brittle, poor resorbable, has a good compressive, but low tensile strength, and is used as a structural scaffold that is similar to trabecular bone.[8] Therefore, HA is often combined with AB and/or acts alone as a carrier material for osteoinductive growth factors to promote bone healing in defects.[8] The study of Giuliani et al[93] in human maxillary defects demonstrated on x-ray microtomography that coralline-derived scaffold grafts formed new vessels, homogenous and well-connected bone and had a good biomaterial resorption. In a 5-year-follow-up clinical trial, Yukna et al[94] showed a decrease in periodontal probing depths as well as in gingival recessions.

In a study, Wang et al[95] prepared a hydrogel and mixed coralline HA, silk fibroin, glycol chitosan with umbilical cord mesenchymal stem cells from humans and inserted it in femoral condyle defects in rats. Hence, the outcomes showed a good promoting bone repair, thus, the combination of many XE products can be very beneficial and in future a promising graft.[95]

In addition, a further XE of phytogenic origin is the medical herb Gusuibu, a dried rhizome of perennial pteridophyte Drynaria fortunei that has osteoinductive abilities, an enhanced alkaline phosphatase activity, effecting positively on the calcification and bone remodeling process.[8,77] In the Chinese medicine, Gusuibu is successfully used for bone fractures and osteoarthritis.[8]

The final XE to mention is the marine red algae that will be chemically converted, like the coralline materials, to HA.[77] Algae are clinically used since 1988.[8] A famous phytogenic example is *AlgiPore*™, a bone-analog calcium phosphate.[54] It is available in three grain sizes from 0.3 to 2 mm relating to the bone defect, and integrates in a period of around 15 months. The seaweed material is able to reconstruct and contour at least a triple wall alveolar bony defect,[54] has a great surface area for the adhesion of proteins, and is resorbable.[8] It is a space filler and also often combined with others materials to enhance its properties. The reconstructive study of Ewers[96] demonstrated an implant survival rate of 95.6% with *AlgiPore*™ after 14 years in sinus lift procedures. Another study showed newly formed bone around and within the pores of *AlgiPore*™ and described it as appropriate treatment in atrophic maxilla.[97]

In the literature, clinical studies of a sufficient number are still lacking regarding algae- and coral-based materials.

In conclusion, XE can be used either alone, or mixed with other bone substitutes. Ideally, they should unite the osteoregenerative properties of AB and AL, while eliminating its limits.

1.2.4. Alloplast

Alloplastic (=synthetic) bone substitute (AP) is an inorganic implant of nonosseous material, available in a non-biologically or biologically derived form.[57] In 1892, Dressman implanted the first AP, consisting of calcium sulfate, in humans.[98] Nowadays, APs are produced under the guidelines of the pharmaceutical industry, fulfilling the GMP (Good Manufacturing Practice) requirements, supervised and regulated by FDA and the International Organization for Standardization (ISO).[57]

Advantages of APs are: no disease transmission, no immunogenicity, unlimited supply, greater acceptance, no necessity of a second surgical site, lower morbidity, volume

maintenance for cell infiltration and remodeling processes, biocompatibility, safety and efficacy in preclinical and clinical studies, predictability.[12,32] APs are bioactive: they have the capability to form bone apatite- and carbonate-like crystals of HA on their surfaces in order to allow attachment with the cells and to connect directly with the surrounding bone tissue for a strong biomaterial-bone interface.[9] AP can be modified on its composition by manufacturing processes by reaching a good biological stability. Its structural properties are similar to those of natural bones.[9,12] There is a big variability along the different types of AP due to the resorption time from fast to slow,[12,32] therefore it is of great significance that clinicians know the characteristics of each AP. Depending on the application, different resorption rates of APs are required for e.g., providing long-term or short-term space maintenance while bone formation, adjusted by the compound of calcium phosphate concentrations. The solubility/ degradation rate of the AP is the extent of calcium phosphate dissolution and depends on factors like the technique of material formation, composition, calcium/phosphate ratios, morphology, or porosity.[5,9,57] The aim is that finally the AP will be completely resorbed. Bone remodeling procedures need around 3 to 6 months; therefore, this interval is appropriate for a complete AP resorption.[12] Additionally, AP with slow resorption rates is preferred in GBR and sinus elevation procedures. In general, APs have osteoconductive capabilities,[5,9] whereby some authors claimed that through their appropriate three-dimensional structure APs may be osteoinductive because they are able to blind endogenous bone morphogenetic proteins in circulation and are possible effective carriers of bone cells.[9] In general, the biological performance of AP depends on many factors such as chemical composition, morphology (e.g., granule size, porosity, pore size, crystallinity), or mechanical stability.[5,10,11] These properties can be modified by thermal (sintering methods) or chemical treatments.[11,99] Through the manipulation of the properties of synthetic materials, mechanisms like bone formation or regeneration can be directly and positively influenced.[11,12,100] In the experimental study, Maté Sánchez de Val et al[11] showed that the grain size of AP influences the process of integration and regeneration of biomaterials. The crystallinity of the material increased with raising temperature of sintering, whereby no significant differences in density were detected. It was noted that the

highest grain size (2000-4000 μm) had the best results compared with the others groups with smaller grain sizes. It was reached by the highest sintering temperature (1000°C), resulting positively in a smoother surface of the crystals, less resorption, better stability and a high release of calcium/phosphate molar ratios that stimulate osteogenesis. High concentrations in the environment of the bone changes the pH, provokes a slight inflammatory response that forms fibrous tissue, and finally stimulates new bone formation. In return, the resorption is raising with the decline of crystallinity, but an increase of surface area. Tadic et al[10] added that a very small crystallite size of biomaterials, included carbonate and/or collagen matrix, increases the solubility.

The different composition of APs covers the wide range of clinical applications and underlines their importance in the dental field of today: for coatings to stabilize implants, filling defects in oral and maxilla reconstructions, ridge augmentations and preservations, sinus elevations, GBR, immediate tooth root replacements, classical reconstructions of periodontal, periapical or endodontic surgery and treatments of periimplantitis.[9,11,12,99,101,102] AP is offered mainly in particulate form, but also as putty, paste, gel or plaster.[12] Moreover, AP can be mixed with cell transplantations or growth factors to enhance the promotion of bone regeneration.[12]

The most used APs are made of calcium phosphate (CaP), furthers are bioactive glasses, calcium sulfate, synthetic polymers, metals, and composites.[10,12,99] CaP materials are represented by HA, α- and β-tricalcium phosphates (α-TCP and β-TCP), biphasic calcium phosphates (BCP), and finally unsintered CaPs or calcium deficient apatites.[9] According to LeGeros[9], the bone and CaP interface is very dynamic, thus, CaP is cooperating directly with the newly forming bone. Regarding its composition, CaP is the most similar AP to natural bone mineral. Synthetic CaP grafts are chosen especially for large defects.[5] The most used APs were BCP (33%), β-TCP (22%) and HA (13%) approved by the FDA between 2010 and 2020 in the USA.[12] Bone graft substitutes should resemble by means of macro-and microporosity the structure of natural human bone. Porosity allows biological and biochemical processes like bone cell ingrowth, protein adsorption, and vascularization,

hence, interactions of the biomaterial surfaces with the surrounding tissue.[9,61,103] Coral and bovine-derived HA have fixed macroporosity (mean diameter of 190 to 230 μm). For all other synthetic AP, the microporosity and macroporosity is variable. Commercially are offered pore sizes of 100 to 400 μm. Various studies confirmed that APs are able to form a very strong bone-biomaterial surface and integrate immediately in the host bone.[9] AP can be mixed with other bone grafting materials, mainly AB, to enhance its osteogenic behavior.[5] Synthetic AP is prepared by precipitation of calcium deficient apatite with calcium to phosphate ratios of 1.67 (pure HA), 1.5 (β-TCP) or less than 1.67 (BCP or unsintered CaP) and differing sintering temperatures.[9,12] The optimal sintering temperature was experimentally confirmed by 1250°C for a three-layer CaP structure.[61] Additionally, pore graded CaPs showed 40% higher flexural strength than homogenous CaPs with one single pore size. Consequently, mechanical properties depend strongly on the degree of porosity.

HA [$Ca_{10}(PO_4)_6(OH)_2$] (e.g., Endobon™, Ostim™) can be derived naturally from coralline or bovine bone or synthetically (=HA ceramic),[5,9] and is commercially offered in forms of porous nonresorbable, solid nonresorbable, and porous, nonceramic resorbable.[57] Both bovine and synthetic HA shows similar efficacy as graft material in osseous regeneration processes in human bony defects, however, it should be mentioned that bovine HA is more economical.[104] Natural HA possesses some minor components of natrium, magnesium, kalium or strontium and an interconnecting macroporosity from the original bone source conserved, whereby synthetic HA is pure.[8,9] HA is an inorganic part of the natural bone and is therefore very similar to the bone apatites.[8,105] After the implantation, HA directly bonds to bone through carbonated calcium-deficient apatite layer at the bone and implant surface.[105] Furthermore, HA enhances the adhesion and differentiation of osteoblastic cells. In addition, its scaffold serves as delivery and controlled release system of vehicles for e.g., cytokines that attract bioactive molecules like BMPs.[105] Further beneficial properties are no toxicity, an outstanding hydrophilicity for vessel uptake; but limitations are a lack of appropriate microporosity, a delayed resorption, low

mechanical strength, accordingly, not recommendable for high load-bearing sites.[8] HA is produced in a dense or macroporous form, available as granules or blocks. It can be used in all kind of bone substitutes with low loading-stress, especially for coatings on metallic implants for the dentistry and orthopedics, as scaffolds for tissue and material engineering, and carrier for drugs.[8,9,105] According to the mechanical strength, the tensile strength for dense and porous HA is ranging from 79 to 106 MPa and 42 MPa, respectively. To compare, cortical bone has a mechanical strength of 69 to 110 MPa.[9] Generally, pure HA is at least soluble of all CaP components,[12] and shows a slow resorption time.[5] In the nanotechnology, new nano-structed HA with lengths of 1 to 100 μm were designed. The benefits of nanocrystalline HA are a higher dissolution and bioactivity degree than coarser ceramic crystals, a greater surface area for an improved fracture toughness, a higher promotion of osteoblast adhesion, proliferation and osseointegration.[8,105] However, it should be emphasized that there is not enough evidence for a broadly application in dentistry.[8,105] In the *in vivo* study with critical-sized defects in the femur, Pearson et al[106] demonstrated that HA scaffolds had greater mineralization to total volume and bone density compared with pulverized AB, concluding that porous HA is a good alternative.

Beta-tricalcium phosphate (β-TCP) [β-$Ca_3(PO_4)_2$] (e.g., Vitoss™, Cerasorb™) is one of the two polymorphs of TCP (α- and β-TCP). β-TCP is produced by high or lower sintering temperatures, in water-free or solid-state acid-based chemical mediums.[12] It is widely used, offering good biocompatibility and resorbability. β-TCP has a bone regenerative potential comparable to that of AB and AL (FDBA and DFDBA).[57,107] Comparing β-TCP to AB, similar outcomes in bone formation and healing were achieved,[108] and even less pain and fewer side effects with β-TCP.[109] Disadvantageously, β-TCP has poor mechanical properties in compressive strength,[8] and a medium resorption time,[5] but faster than those of HA.[8]

Biphasic calcium phosphate (BCP) is available in products like e.g., Triosit™, MBCP™ or Osteosynt™. BCP is a mixture with varying weight ratios of HA and β-TCP, resulting in different dissolution properties: the higher the ratios, the lower the extent of

dissolution.[9] The HA/β-TCP ratios, as well as the dual porosity and the subsequent interconnectivity are decisive properties for BCP.[103] BCP fulfills the benefits of both β-TCP and HA: biocompatibility, resorbability and a good mechanical strength in comparison to HA or β-TCP, whereby the compressive strength is much lower than those of cortical bone.[8] According to Bouler et al[103], BCP optimizes the bone regeneration due to the carrying-capacity of its appropriate matrix for bone colonization, the dosage and release of bioactive molecules, and their progressive resorbability. Miron et al[110,111] demonstrated in two *in vivo* models that BCPs are able to promote ectopic bone formation, therefore, they have a confirmed osteoinductive potential. Depending on the modifications and altering the ratio of HA/TCP, the resorption time can be controlled from slow to fast possible. BCP is the promising substitute of today and may be seen as a very good alternative to AB with future applications in tissue engineering.[102,103]

Unsintered CaP [$(CaNa)_{10}(PO_4\ HPO_4)_6(OH)_2$] (e.g., Osteogen™) or calium-deficient apatites are resorbable products. Unsintered CaP is produced in two ways: firstly, precipitation with temperatures between 25° to 100°C or secondly, by hydrolysis of amorphous CaP, dicalcium phosphate dihydrate, alpha-tricalcium phosphate or octacalcium phosphate.[5]

Further, Calcium sulfate (CS) [$CaSO_4$] (e.g., OsteoSet™) is made of a hemihydrate powder, hydrated to form CS dihydrate, followed by an exothermic reaction to a solid form.[12] The compressive strength of CS is higher than that those of cancellous bone.[57] CS is mostly used as an adjunct to other BGS in periodontal regeneration therapies.[57] CS is relatively economic, highly moldable and biocompatible, but it is also marked by characteristics including a faster resorption than human bone and a relatively risk of infections and inflammation.[8]

In addition, noncommercial CaP materials (e.g., whitlockite or octacalcium phosphate) are substitutions in β-TCP or apatite structures to influence the crystal structure and the dissolution properties of CaP.[9] Some examples are magnesium-substituted or zinc-

substituted TCP. In addition, the sprayed CaP coatings on dental and orthopedic implants are CaP cements, made of solid (CaP with or without other Ca compounds), and liquid (inorganic or organic acids or natrum phosphate solutions) components (e.g., Norian™, Hydroset™.[9] CaP cements have a good mouldability and biocompatibility, but a slow speed of cell adhesion, and are brittle.[8]

Bioactive glass (BG) (e.g., Perioglas™, Biogran™) is a noncrystalline amorphous silicate-based material, combined with other additives like acid oxides (e.g., phosphorous pentoxide) and alkaline oxides (e.g., magnesium oxide), available in compact and porous form.[12] Their application ranges from augmentations of unilateral cleft alveolar bone, management of periodontal bony defects to preservations of alveolar ridge bone after tooth removals in orthodontics.[8,112] It is important to note that BG is quick resorbed.[5] The first regeneration of bioactive glasses is represented by the well-known *Bioglass 45S5*, made of siliciumdioxid, and discovered by Larry Hench in 1962.[57] *Bioglass 45S5* is characterized by very strong interfacial bonds with the surrounding tissue and has one of the highest *in vivo* bioactivities among all bioceramics.[112] BGs are similarly composed to the bone mineral resulting in good outcomes in biomedical applications.[57] Advantageous are the possibility to control the rate of degradation, a very good osteoconductivity, bioactivity, antimicrobial, antibacterial and angiogenic properties, and a complete resorbability.[8,57,112] Limitations are some mechanical properties: low strength, toughness, reliability, brittles and a poor fractur resistance.[8,57,112] BG bonds chemically with host tissues by forming a bonelike apatite layer. Due to the brittleness, BGs are limited as load-bearing scaffold, accordingly, to improve their mechanical and biological performance, a new generation of BGs were introduced: the BG-based composites with polymer matrices (e.g., BioGraft®). The novel BGs contain additionally growth factors, nanoparticles or composites. For the applications in hard tissues (i.e., bones and teeth), the composites are enhanced by e.g., bio-metals, carbon nanomaterials, titanium silver or magnesium alloys; and in soft tissues, porous polymers are mixed with BG-based composites.[112] BGs can be produced by melting-quenching method at temperatures over 1300°C or the chemical-based sol-gel method, adding today

nanoparticles to improve surface area.[57] In a recent *in vitro* study of Anil et al[113] five different BGS, including, two XEs and three APs were tested, resulting in superior outcomes in physical and crystalline properties for the APs. The product with the best physiochemical properties in this study was the AP with components of HA and BG. BG contributed to the excellent bone regeneration.

Synthetic polymers (e.g., Bioplant HTR, Synthetic Bone™) are biocompatible, radioopaque and very porous, available as a resorbable or non-resorbable form. The biggest concern is its degradation to acidic products.[8]

Another group are metals (e.g., OSS Builder™) marked by characteristics of outstanding mechanical strength and biocompatibility.[8] Metals are acting as a membrane barrier, are not resorbable, thus a second surgical treatment is necessary. A possibility of soft tissue dehiscence and exposures are reported.[8]

In general, non-bioactive materials like metals or synthetic polymers are not able to build up a strong bone-material interface.[9]

Finally, the last group to mention are composites (e.g., NanoBone™, Fortoss Vital™) that have osteoconductive and osteoinductive capabilities, a good cell adhesion, and are resorbable.[8] In the literature, there are insufficient studies relating to composites as bone graft substitutes.

1.2.5. Guided Bone Regeneration and Membranes

The Guided Bone Regeneration (GBR) is a method using barrier membranes and BGS for the reconstruction of bony defects. The membranes perform the function of biological and mechanical barriers to prevents a contour collapse from mucogingival compression, protect bony defects and wounds from the invasion of undesired non-osteogenic cell populations (e.g., epithelial cells, fibrous tissue and granulation tissue)

which interfere negatively in the process of osteogenesis.[114] Membranes are occlusive and selective permeable for particular cells; therefore, they allow osteogenic cell populations (e.g., osteoprogenitors, osteoblasts, cells for neovascularization) to enter, and the diffusion of growth factors, signaling molecules or bioactive substances are provided.[114,115] The barrier membranes select the adequate cell repopulation. Additionally, the membranes guide the migration of the slower-migrating osteogenic cells into the defect site and support the early angiogenesis. After the surgical intervention, an initial thrombus is formed, vessel ingrowth, further the replacement of woven bone, and later the transformation in lamellar bone.[58] The GBR technique is successfully used for alveolar bone regenerations, including treatments of peri-implant osseous defects, the preservation of alveolar sockets after tooth extractions, lateral and vertical bone augmentations with or without implant placements.[114] Besides, the Guided Tissue Regeneration (GTR) method is approved for periodontal procedures, for instance, periodontal furcation defects, intrabony pockets and socket preservation.[115]

The ideal barrier membrane is biocompatible with a good integration in the host tissue, creates and maintains the regenerative space, prevents the loss of the reconstructed shape and position of the bone, stabilizes the blood clot, supports the cell-migration with the appropriate porosity, possess mechanical strength, high durability, and a predictable resorption time similar to those of the bone regeneration rate.[58,114,115] Further, an easy handling, manipulations due to shape forming and easy placement are important factors for the clinicians.[115] Smooth membranes are beneficial for an overlying flap that can be easily adapted for closure.[58] Studies confirmed that GBR is able to regenerate critical sized maxillofacial and calvaria defects, and additionally, neo-osteogenesis could be observed.[114]

Moreover, GBR uses non-resorbable or resorbable membranes. Non-resorbable membranes are bio-inert materials, consisting of titanium foils, including expanded polytetrafluoroethylene (e-PTFE), dense polytetrafluoroethylene (d-PTFE), both with or without titanium reinforcement, and titanium meshes.[58,116] Generally, they are recommended for use in load-bearing regions e.g., vertical ridge augmentations.[115] An

advantage is their excellent biocompatibility, mechanical strength, rigidity, stability for bone formation and graft, and a more favorable space maintenance.[58,116] Drawbacks of this type of membranes are more frequent wound dehiscence, an increased risk of exposure and tissue ingrowth, and the need of a second stage surgery for removal, resulting in a possible higher risk of morbidity and higher costs.[58,116] In the dentistry, the first membranes were e-PTFE (e.g., GORE-TEX®). They have two different sites: one layer is around 1 mm thick and possesses a porosity of 90% with approximate pore sizes of 5 to 20 μm for the ingrowth of epithelia cells, whereby the other layer has a thickness of 0.15 mm and a porosity of 30% for new bone ingrowth and excluding fibrous tissue.[58] The e-PTFE membranes are exposed to risks such as a high incidence of premature exposure of around 30 to 40% ,[115] followed by possible infections, difficulty of removal because of the high porosity, including an increasing risk of exposing newly formed bone to bacteria.[58] It is important to add that a too early removal may result in a resorption of fresh regenerated bone, in turn, a too late removal, may affect in possible bacterial contamination.[115]

A further development are the d-PTFE membranes (e.g., Cytoplast™) that are characterized by a higher density material with less than 0.3 μm pores in order to avoid the complications of e-PTFE membranes. Beneficial factors are a lower risk of bacterial contaminations, consequently, less associated infections.[58,115] The smaller pore sizes allow a better oxygenation of the tissue and the passage of small molecules and prevent the fibrous tissue ingrowth, resulting in an easier removal.[58]

The titanium-reinforced PTFE membranes are very stable through the included titanium strut and show good outcomes of treatments of large osseous defects, irregular thicknesses of bone, volume and contour stability. Many studies of horizontal and vertical ridge augmentations revealed good outcomes regarding excluding fibrous tissue ingrowth, support in graft mobility, and space maintaining.[58,117-119]

The titanium mesh membranes (e.g., Ti-Micromesh) are very effective through its strength and toughness in space maintenance, easy to manipulate according to the desired

shape, biocompatible, support the blood supply from periosteum through the holes, and prevent a contour collapse from mucogingival compression.[58,115,116] Disadvantageously, there is an increased incidence of wound dehiscence, risk of soft-tissue coverage during the healing procedures, and the removing is challenging.[115]

The next group of membranes are the resorbable membranes that are recommended for non-load-bearing regions e.g., in maxillary sinus elevations.[115] Resorbable membranes are divided into natural, tissue-derived from allogenic or xenogeneic collagen, pericardium, human amnion and chorion tissue, and human acellular freeze-dried dermal matrix (ADM), or synthetic membranes, comprising of polyesters e.g., polylactic acid (PLA), polyglycolic acid (PGA), PLGA (poly-D,L(lactic-co-glycolic)acid).[58,115,116]

Collagen membranes, consisting of type I or III, are commonly from porcine or bovine sources and are the most popular membranes in dentistry.[58,116] They are biodegradable, and have a reduced risk of infections and tissue damage. A second stage surgery is not necessary, in consequence, a decreased patient morbidity. To the beneficial factors belong an easy handling, support of the healing by attraction of fibroblasts and the blood clot stabilization through the platelet aggregation, low-antigenicity, and high tensile strength.[58,120] They are available in different forms, including tape/plugs, regular membranes, extended membranes, pericardium, and acellular dermal matrix.[58] Collagen membranes are degraded through enzymatic reactions that are similar to those in the human body.[58] They have variable resorption rates ranging from 0.5 to 10 months[115] that can be modified by manufacturing processes due to the number of cross-linking components.[58] A non-cross-linked collagen membrane has a short half-life time of degradation around 7 to 28 days, whereby the cross-linked membrane possesses a prolonged biodurability up to 6 months.[116] There are various methods (e.g., ultraviolet light treatments), chemical cross-linking techniques (e.g., with glutaraldehyde) or natural agents (e.g., ribose) to enhance the cross-links of the membrane; summing up, the higher the cross-linking structure, the slower rate of degradation.[116] It has been shown that both membranes

provided osteogenic functions, whereby the cross-linked collagen membrane was superior in a larger quantity of attached cells, total calcium deposition, alkaline phosphatases and vascular endothelial growth factors A .[120] Long term results of implants inserted simultaneously in GBR technique with an e-PTFE and collagen membranes or without membranes as control group with a median of 12.5 years .[121] All membranes reached a high implant survival rate ranging from 92 to 93%. No statistically significant radiographic outcomes were recorded in determined marginal bone level.[121]

Synthetic resorbable membranes are biocompatible without antigenicity and immunogenicity. Resorption rates are varying between 1.5 to 24 months and are dependent on the type of material.[115] To avoid the micromovements of the membranes, tenting screws, bone and BGS are often used to support them.[58,116]

PGA and PLA membranes are biodegradable with resorption rates between 5 and 12 months.[116] PGA is very hydrophilic and possesses a highly crystalline structure, marked by a fast degradation rate; PLA has an additional methyl group on alpha carbon, resulting in different chemical, mechanical and physical properties, but is very similar to PGA.[122] PLGA is a co-polymer that is preferred than PGA/PLA for bone substitutes due to the degradation rates that can be better controlled by varying the ratio between its monomers:[122] the higher the content of hydrophilic glycolic acid unit, the faster the degradation rates.[123] The hydrolytic degradation of PLA/PGA and PLGA works through de-esterification and the fragmented monomers of each polymer are removed completely by natural pathways. In an animal model of critical-sized calvaria and mandible defects, PLGA membranes were compared with PLA and cross-linked collagen membranes.[123] The study showed that PLGA membranes are safer, more biocompatible and have a better controlled resorption rate. They have also a good bone regeneration capacity, and according to the study, PLGA can be recommended for pre-implantology and pre-odontology surgery. In general, tissue and alveolar bone healing last around 2 and 16 weeks, respectively, so an ideal membrane has to fulfil at least 16 weeks the barrier function. In the experimental study, the PLGA membranes lost its structural integrity after 16 weeks and

were completely resorbed after 26 weeks *in vitro*. In the study were no statistically significant differences referring new bone formations between the membranes observed.[123]

The choice of the type of membrane is one of the final elements of the GBR regeneration protocol and important for the particular surgical treatment. Some authors[115] proposed criteria for the selection of adequate membranes due to the clinical treatment (Table 1).

Table 1. Criteria for the selection of appropriate membranes.[115]

Surgical procedures	**Resorbable Membrane**	**Non-resorbable Membrane**
Sinus floor elevations	Yes.	No.
GTR	With BGS.	• No. • Exceptional cases: Yes, but without BGS.
Alveolar Ridge preservations	Yes	Yes
Monocortical blocks	With AB.	• In great osseous defects, • Vertical augmentations, • With AL.

GBR	• Lateral ridge augmentations, • With XE, • With AL and XE.	• Lateral ridge augmentations with AB or AL, • Vertical ridge augmentations.
Ridge crest splitting	Yes.	No.
Biologics	With BGS.	Without BGS.

Regarding the clinical procedure, after the insertion of BGS in the defect site, the membrane should be placed over the graft material. The membrane has to be stretched widely enough so that the BGS particles are entirely covered and by the way protected against bacterial contamination and soft tissue ingrowth. It is important to close tension-free with buccal, periosteal and mylohyoid releases.[116] To avoid membrane failure, the d-PTFE and titanium meshes need a minimum of 2 mm between the margin of the membrane and the site of the bordering root of the tooth, however, the resorbable membranes and ADM can be placed directly over roots without expected complications.[58] Wang and Boyapati[124] issued the PASS principles to obtain a predictable bone regeneration: starting with the primary wound closure to enable healing, then the blood supply with the attraction of mesenchymal stem cells for angiogenesis, space creation and maintaining, and finally, clot stability of wound and implant. In the GBR technique, it should be focused to create and maintain space between the membrane and the graft by means of e.g., tenting screws or pins, titanium-reinforced membranes or titanium meshes, resulting in an unmoving support of the BGS.[58] Comparing the two types of membranes, it was reported in two studies of Cucchi et al[118,119] that the both types of membranes showed no differences in vertical bone gain and complications rate of implant stability, whereby the non-resorbable

d-PTFE membrane emerged in a greater bone density, a thinner pseudo-periosteum layer above the new bone formation and a smaller rate of surgical and healing complications than titanium meshes covered by resorbable cross-linked collagen membranes. In a recently published systematic review[117], five non-resorbable membranes (e-PTFE, d-PTFE, titanium mesh, titanium-reinforced e-PTFE and d-PTFE) were compared to five resorbable membranes (cross-linked and non-cross-linked collagen membranes, PLA, PEG (polyethylene glycol), PLA910 (polylactic acid 910)) and two combined (titanium mesh plus cross-linked and non-cross-linked collagen membranes), reaching the highest vertical bone gain with titanium-reinforced d-PTFE membranes. All membranes showed similar outcomes regarding bone regeneration and common complications like soft tissue injuries and membrane exposure. Regarding cytocompatibility and cell adhesion, collagen membranes reached the best results in comparison to e-PTFE and PLA membranes.[125]

In general, barrier membranes demonstrated good results in their clinical application,[114-123,125] but there are also authors that claim limited evidence of their effectiveness.[126]

1.3. Objective of the thesis

The general objective of the present thesis is to evaluate the usefulness, reliability, and efficacy of the two novel synthetic bone substitute materials and the two membranes of synthetic and porcine origin in an experimental model, with focus on their suitability for the clinical daily work. The biomaterials should augment the bone volume in defect sites to improve the stability for implant placements and future prosthetic rehabilitation. For the investigation of their impact on living bone tissue, the materials are implanted in human subjects, and after a minimum of six months healing time, the results are verified. To achieve the general objective, further secondary targets are set.

Firstly, the clinical evaluations start with preoperative examinations of the bone and their documentation. Further, the biomaterials are tested and combined with implant placements in the following surgical procedures: sinus lift elevations, bone regeneration in 3rd molar region, alveolar ridge augmentations, socket preventions, and alveolar crest-splitting. During these surgeries, the handling of the materials is exactly examined.

Secondly, the radiographic evaluation is focused on the integration of the biomaterials in the host bone, the identification of infections or inflammations, and of quantity (vertical and horizontal volume) as well as quality (density) of the bone.

Thirdly, for the histomorphometric analysis, bone biopsies are harvested and these samples are examined with the light microscope. It will be determined the quantity of newly formed bone, residual biomaterial, and the surrounded connective tissue. On this way, it is possible to analyze the cellular reactions of the host bone tissue to the augments.

At the end, the obtained results will be compared with those of the current literature to conclude the efficiency of the tested bone graft substitute materials and barrier membranes.

1.4. Hypothesis of the study

The novel biomaterials, combined with the overlying barrier membranes to provide occlusive properties, will act effectively in the experimental model in humans.

1.5. Justification of the study

In the current literature, it is rarely to find data about these novel biomaterials in human studies, but instead numerous *in vitro* and *in vivo* models are present. The most studies are focused on either sinus floor elevations or ridge augmentations. In the present

study, the biomaterials were implanted in five different surgical procedures. No studies could be found that published pre- and postoperative measurements due to the bone increase in length, width, and density. Accordingly, to close this gap, the present study was designed. Evaluating this data specifically focused on clinical, radiographic and histomorphomethric analysis will provide valuable insight of these novel biomaterials and their behavior and cellular reactions in biological bone tissue. Regarding the application of synthetic biomaterials, there is no cause for concerns in ethical, cultural or moral issues. For these reasons, verifying the materials recently appeared on the dental market, is of great significance.

On this way, clinicians get more possibilities to choose the appropriate biomaterial and may be able to improve the assessment of their treatments.

2 - MATERIALS AND METHODS

2. Materials and methods

2.1. Study design und ethics

All patients involved had provided their informed consent prior to inclusion in the study. The experiments were performed under the guideline established by the Declaration of Helsinki as revised in 2008 for medical research involving humans. Possible side effects or complications would be immediately treated in accordance with current medical university knowledge. The study and all associated documents were approved by the ethics committee of the Catholic University of Murcia (UCAM). Patients were recruited at the department of Master's Degree in Implant Dentistry at the dental clinic of UCAM, and were treated from March 2018 to December 2020 within this project. The present study was an analysis of the records of 20 systemically healthy patients (7 females and 13 males), partially or completely edentulous, and finally restored with implants for prosthetic rehabilitation. The mean age of the patients was 54.09 ± 11.26 years (ranging from 38 to 75) at the time of surgery. The follow-up period was set as a minimum of a half year. In total, 35 clinical acts have been implemented, and 51 samples of the study material were used.

2.2. Inclusion and exclusion criteria

Healthy patients, without uncontrolled systematic pathology, were included in the study. The exclusion criteria were the following:

- uncontrolled diabetes,
- a history of renal failure or radiation treatment in the head or neck region,
- current chemotherapy,

- treatments with bisphosphonates,
- metabolic bone disorders,
- hemophilia,
- pregnancy,
- drug or alcohol abuse,
- poor oral hygiene,
- non-collaborating patients, and
- patients who do not accept the consent or treatment report.

2.3. Study materials

This study included the following four tested materials:

• TIXXU® GRAFT (Synthetic Bone Substitute Granules, Bredent Medical GmbH & Co. KG, Germany), produced by Biomatlante, Vigneux-de-Bretagne, France, REF: TX0302G01, n= 23),

• TIXXU® GRAFT (Injectable Synthetic Bone Substitute Putty, Bredent Medical GmbH & Co. KG, Germany), produced by Biomatlante, Vigneux-de-Bretagne, France, REF: TX1002PU50DE, n= 11),

• TIXXU® CONTROL (Synthetic PLGA Membrane, Bredent Medical GmbH & Co. KG, Germany), produced by Biomedical Tissues, SAS, Nantes, France, REF: TICO2030, 20x30 mm, n= 10), and

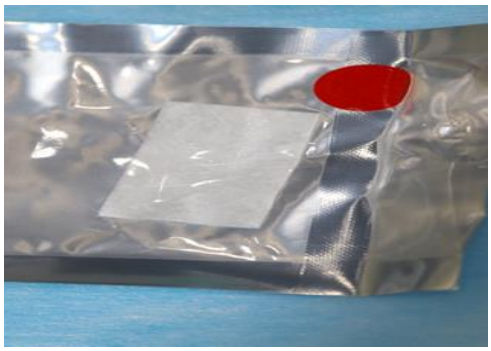
• EZ Cure™ (Porcine Collagen Membrane, produced by Biomatlante, Vigneux-de-Bretagne, France, 20x30 mm, REF: 0702EZC2030, n= 7).

Abbreviation: n= number of samples.

All biomaterials were resorbable. A second-stage surgery for removal was not necessary. They were obtained directly from the manufacturer in sealed packaging, and used without further treatments (Figure 4).

(A)

(B)



(C)

Figure 4. (A) TIXXU® GRAFT granules, (B) TIXXU® GRAFT putty, (C) TIXXU® CONTROL PLGA membrane.

2.3.1. TIXXU® GRAFT granules and putty

The biomaterials were produced with the MBCP technology with bioactive CaP, categorized as biphasic material (BCP) with ratios of 60% HA [Ca10(PO4)6(OH)2] and 40% β-TCP [Ca3(PO4)2] in the granule form. The putty version contained additionally a

hydrogel (hydroxypropylmethylcellulose) for the malleability. BCP was obtained by sintering precipitation.[9] The main elements of this material were calcium and phosphate in a ratio of approximately 1.5 to 2.2. The material had a total and intraparticle porosity of 74 and 32%, respectively. The pore size and distribution were around 65 to 72%, and the particle density was approximately 3.2%. The biomaterials had a micro- (< 10 μm) and macroporous (100-600 μm) structure with documented grain sizes between 0.5-1 and 0.8-1.5 mm in diameter. The resorption rate was approximately 6 months. To avoid osmotic pressure, the granules had to be prehydrated with patient's own blood or saline solution before their implantation in osseous defects. The materials should be in direct contact with the living bone to support the vascularization. An overfilling with the materials was not permissible.

2.3.2. TIXXU® CONTROL and EZ Cure™ membranes

The following tested materials were the barrier membranes. The fully synthetic membrane was made of poly(lactic-co-glycolic acid) (PLGA). The membrane had a double-layered structure, fabricated in two processes of freezing and lyophilization with a PLGA solution.[122] The outer side of the membrane was covered by a smooth fascia of a dense glossy layer to prevent the ingrowth of gingival fibroblast cells, and connective tissue invasion. The inner layer of the membrane had a porous matt fascia structure with nonwoven microfibres for the promotion of osteogenic cells, and a controlled bone regeneration. The bilayer transmembrane structure allowed angiogenesis. According to the manufacturer's specifications, the resorption rate was around 6 months. The second membrane had a cross-linked structure, originally from porcine. It was a commonly recommended collagen type III membrane for GBR with a documented resorption rate of around 3 months. The used size of both membranes was 20 mm x 30mm. Th membranes had to be positioned over the complete bone defect, including the graft substitutes. Attachments, pins or screws could be used if necessary. After cutting the PLGA membrane

to appropriate size under dry conditions, the membrane had to be moistened before applied on the augmented side. Pre-wetting for the collagen membrane was not necessary.

2.4. Preoperative examinations

Potential patients were informed about study conditions. The clinical evaluation report included a standard extra- and intraoral check-up. All patients received careful periodontal examinations, including the assessment of supra- and subgingival plaque, gingivitis, probing depth, followed by oral hygiene instructions and, if indicated, periodontal therapy. The clinical cases were documented preoperatively, during the surgery, and postoperatively with full-HD pictures (camera: Canon EOS 200D, Japan; macro ring flash lite system: Meike MK-14EXT; macro lens 105 mm: Sigma F2.8 EX DG OS HSM, Japan).

2.5. Radiological analysis

Standardized three-dimensional radiographs were obtained by means of paralleling cone-beam computed tomography (CBCT) device (Orthophos SL 3D, Dentsply Sirona, United States/Germany), using a digital imaging software system (SIDEXIS 4, Dentsply Sirona). CBCT scans were taken before and at a minimum of 6 months after the surgery. For comparing the bone volume changes, parameters such as the length, width, and density of the recipient graft site were analyzed, first without, and finally with the applied material by using a dental planning software (Blue Sky Plan 4, United States). The alveolar bone of maxilla and mandible was measured in their entire length (basal-occlusal distance) and width (bucco-palatal/lingual distance) at three points. Density was measured in four random points. Always the same pre- and postoperative cross-sectional views were used for analysis in CBCT scans (Figure 5).

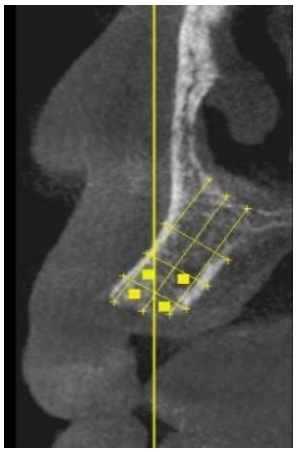


Figure 5. Example of a CBCT scan in a cross-sectional view in maxilla: measurements of the length, width, and density (squares).

2.6. Surgical protocol

All surgeries were performed under local anesthesia, according to the manufacturer's protocol, in the maxilla (n=16) and mandible (n=5). Depending on the locus of the bony defect, five different clinical treatments were performed: sinus floor elevation (n=6), bone regeneration in third molar region (n=1), socket prevention (n=15), ridge augmentation (n=11), and crest splitting (n=2). The materials were applied in the bone defects to augment and increase the bone volume; cases in combination with membranes, n=14, and without, n=6. The needed quantity of the materials was previously calculated in a patient's protocol, including the adjusted diameter, length, and amounts of the implants (Bioner® Implant Systems, Barcelona, Spain), and a further prosthetic rehabilitation plan. In all clinical situations, implants were immediately inserted at the bone crest level, expected during the sinus lift augmentation. After the elevation of the sinus membrane, the implants were placed in the healed bone at least in 6 months' time. In total, 57 implants were inserted during the study period in either the augmented sites or areas which were in close contact with biomaterials in the same jaw. After the surgeries, each patient received

an anti-inflammatory treatment: 400 mg of ibuprofen every 8 hours for 3 days, and 0.12% chlorhexidine gel every 12 hours for 2 days. Patients were asked to follow the general guidelines after surgical procedures. Temporary prosthetic restauration was done. Sutures were removed after 8 to 10 days postoperative.

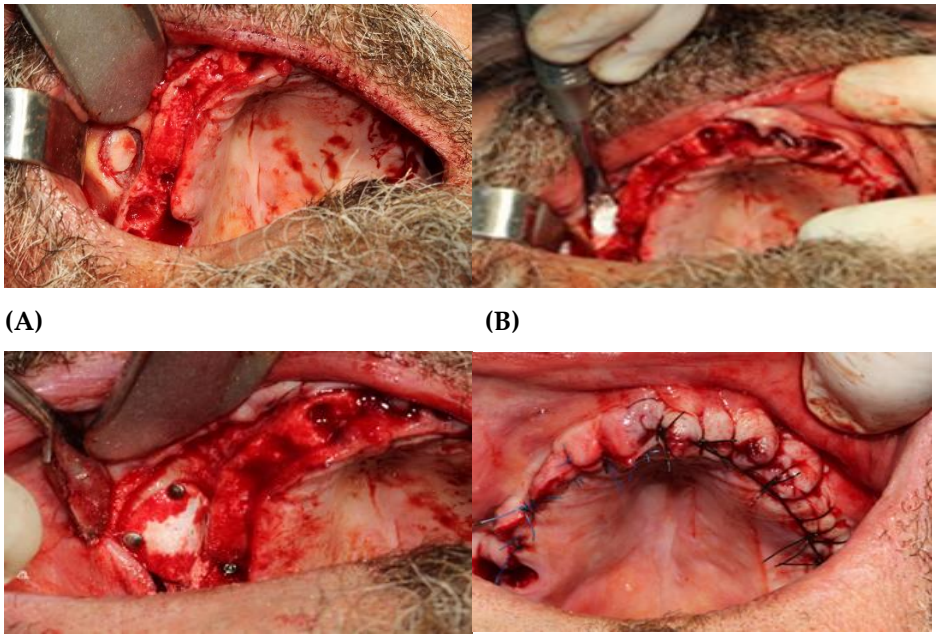
2.7. Surgical procedures

All procedures performed in patients were related to a relative risk. While working with BGS, it was essential to ensure an appropriate blood supply to the graft. A proper modelling and fixation of the grafts, without over-contouring with the graft materials, and covering by a suitable barrier membrane were the basic steps. A tension-free flap was created by releasing incisions. The reconstructed augment should not be exposed to high load or compression, therefore it was better to avoid removable prothesis or the use of low-stress prothesis, if possible. A sufficient healing period was essential for a successful graft integration. In the study, all tested materials and dental implants were implanted simultaneously, in case of sinus elevations 6 months later. In general, to augment bone volume, there are exiting various methods. Three techniques were included in this study: the onlay and inlay grafting, and the ridge expansion. The onlay grafting is used to apply the BGS over the bony defect area to increase the width or height for implant insertions. The inlay grafting is a sandwich method to apply BGS between two separated parts of the jaw. The ridge expansion technique describes the longitudinal splitting of the alveolar ridge crest to create space for BGS.

2.7.1. Maxillary Sinus augmentation

The posterior maxilla is often challenging for implant placements. In the maxilla, the sinus is the critical structure to protect. Therefore, numerous surgical approaches were developed to manage deficient maxillary alveolar ridges, including direct and indirect

approaches for sinus elevations. For instance, the Le Fort I osteotomy is a method to divide the upper jaw from the skull base to achieve an unhindered access to the maxilla. In this technique larger grafting volumes can be inserted. Another method is through the lateral nasal wall (an intraoral antrostomy) to manage sinus pathologies or via the tooth socket directly to the maxilla.[127] In this present study, the basic lateral window procedure of Caldwell-Luc was used with a delayed of at least 6 months' implant placement. In this technique (Figure 6), it would be possible to place an implant simultaneously, if more than 4 mm of bone height could be ensured,[127] but it was not done in this study. The incision was made from the lateral incisor to the second molar tooth at the height of crestal bone. The muco-periosteal flap was created tension-free. The surgical exposure of the lateral bone window was made by means of osteotomy to get access to the maxillary sinus. Using a cutting burr, the bone was removed carefully in circle form creating a window that outlined the Schneiderian membrane. This membrane had been divided from bone. The BGS were placed into the new created space, and covered by either the PLGA or collagen membrane. Finally, the flap was closed and sutured.



(A) **(B)**

(C) **(D)**

Figure 6. Sinus maxillary augmentation. (A) Opening of the sinus, (B) implantation of TIXXU® GRAFT putty, (C) TIXXU® CONTROL PLGA membrane, and (D) tension-free closure.

2.7.2. Socket prevention

After tooth extractions and cleaning of the wound by removing inflammatory tissue, BGS and dental implants were simultaneously placed in the alveolus, covered by one of the tested barrier membranes, to prevent volume bone loss or gingival collapse (Figure 7). Suturing was performed. Primary wound closure was not necessary. It was important to keep the whole procedure as minimal invasive as possible, so incisions and creating traumatic flaps should be avoided. Generally, socket prevention is not required after all extractions, but highly recommended in the esthetic zone, in cases of delayed implant placements or bone defects after extractions.[128] In cases of greater socket defects, it is recommended to place the implant at least 4 months later, but there does not exist a standardized surgical protocol.[128] The socket grafting technique can be oriented to the quantity of remaining bone walls after the extraction:[129] The defect of the socket is differentiated between the classes 1 to 5 bony walls. A thick bony socket with 5 walls (>1.5 mm) can be treated with an immediate implant placement, a delayed treatment with implant placement and socket grafting is not necessary. For a thin 5-bony-wall socket (<1.5 mm), it is recommended to use graft material. In 4-wall sockets, the buccal wall is often missing, and they should be grafted with BGS, covered by a longer-acting membrane. A 3-, 2-, or 1-wall socket needs a larger amount of BGS to reconstruct the defect. In these cases, AB in form of only blocks may be useful. In 3-walled bony sockets, membrane tent screws are often used to maintain space for GBR.

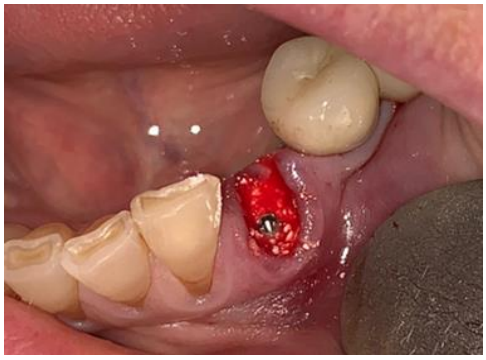
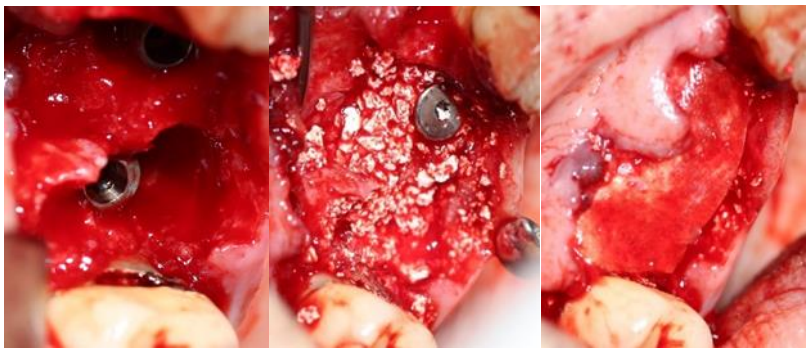


Figure 7. Socket prevention in of tooth 34 in mandible, and simultaneously implant placement. TIXXU® GRAFT granules; Bioner implant.

2.7.3. Ridge augmentation

The vertical and horizontal (or lateral) ridge augmentations are techniques to treat patients with insufficient bone volume in the height and width in the anterior maxilla and the whole mandible by following the GBR protocol. In the posterior mandible, it should be paid attention to protect the inferior alveolar nerve. Both techniques are often combined (Figure 8). The vertical ridge augmentation is related to a higher complications rate than the horizontal augmentation due to coronal pressure through the provisional prothesis or masticatory muscle functions, resulting in wound dehiscence and a possible loss of graft material.[130] To avoid these complications, other options were developed such as the sandwich osteotomy or alveolar bone distractions. During the surgeries with the vertical and horizontal augmentation techniques, the bone height and width were increased with onlays in average 3 to 4 mm on the alveolar ridges. The horizontal ridge augmentation is a more stable, predictable and not invasive procedure, and often combined with ridge splitting and expansion.[131] In general, the complication rate is relatively high and associated with e.g., wound dehiscence, exposure of the grafts, infections, inflammations, failure of biomaterial integration or delayed bone resorption.[130] The primary wound closure was very important in ridge augmentations.



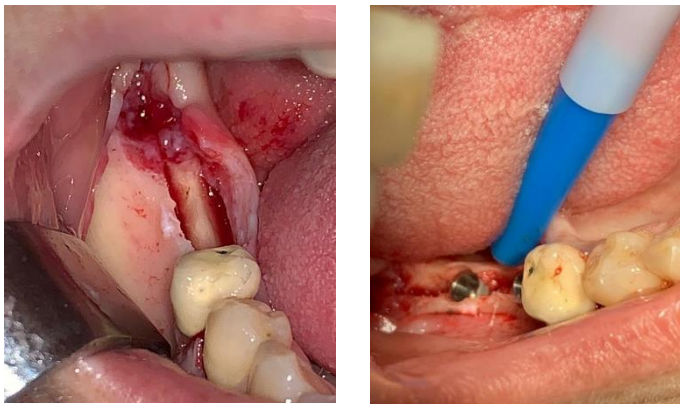
(A) **(B)** **(C)**

Figure 8. Ridge augmentation. (A) Extractions of teeth 15, 16 and immediate implant insertions in the maxilla, (B) applied TIXXU® GRAFT granules and putty; (C) Covered by TIXXU® CONTROL PLGA membrane.

2.7.4. Alveolar ridge-split expansion

This technique, also known as the *crest-splitting* or *expansion method*, is used to treat horizontal alveolar ridge deficiencies. An indication for this procedure is a ridge of 3 to 5 mm thickness.[131] In short, the existing ridge will be splitted, expanded to create intra-alveolar space for particulate BGS and the implant placed. This procedure is an intrapositional grafting. In the study were two patients treated with alveolar ridge-split expansions in the mandibular (Figure 9), where the procedure is normally done in two stages; in the maxilla is normally one stage recommended.[131] In the first stage, after the extractions, incisions on the middle of the crest line and vertical were made. On the buccal site, a full-thickness flap was created. Then the bone was splitted along the four corticotomie lines in the area where the future implants were planned. The split corticotomies were crestal, apical, and two verticals to create a "window". Around 3 to 4 weeks later, the alveolar splitting was performed along the windows osteotomies to widen the split and expand the bone. The buccal bone plate, including soft tissue, formed the

osteoperiosteal flap which were slightly repositioned laterally. In the new created intraalveolar gap, implants were placed, surrounded by BGS and covered by membrane. The primary wound closure was done and the repositioned buccal flap tension-free sutured. Common complications of alveolar ridge expansions may be early membrane exposure or infections due to GBR failure.[131]



(A) **(B)**

Figure 9. (A) Crest-splitting in the mandible regio 46-48, and creation of vertical window along the corticotomies, including alveolar ridge expansion, (B) Three implant placements, applied TIXXU® GRAFT granules and putty, covered by collagen membrane (not visible on the image).

2.8. Histologic processing and bone biopsies

Bone biopsies of augmented areas were taken with trephine needles after 6 months from the placement of the biomaterials, processed for ground sectioning. In total, 11 biopsies were harvested. The sample preparation consisted of the non-decalcified sections and selective staining. The protocol started with the fixation process by means of 10% buffered formalin to preserve the tissues. The specimens were dehydrated with raising

concentrations of ethanol from 70 up to 100% over a period of several days, until reaching water replacement by alcohol in the tissues. In the next process the samples were cleared from ethanol and lipids for a good infiltration. The embedding of the non-decalcified samples was done in methyl methacrylate, then polymerized, and sectioned using a diamond saw (EXAKT Apparatebau, Norderstedt, Germany). Always two segments were cut from each biopsy sample. After the sectioning, the cut surface was polished. The complete specimen blocks were stained with haematoxylin and eosin. The cut surface of the blocks was bonded to a glass slide. A further cut was performed creating a stained sample slide. The exposed sample surface was polished and finally covered by a glass. Sections in the edges of the specimens were avoided to provide always the same quality.

2.9. Histomorphometry

The digital quantitative analysis was performed by using calibrated digital images, ranging from 4x to 40x magnifications (Leica microscope Q500Mc, Leica DFC320s, 3088x2550 pixels, Barcelona, Spain). The most central section of the biomaterials was elected for the histomorphometric analysis. Further tools were the MIP 4.5 software (Microms Image Processing Software, Consulting Image Digital, Barcelona, Spain) and a video camera (Sony DXC-151s 2/3-CCD RGB Color, Japan). The areas of interest were marked, and their values were calculated digitally for the total percentage with the ImageJ software (W. Rasband, National Institutes of Health, Maryland, United States). The evaluation consisted the measurements of new bone formation (NB), residual biomaterials (RB), and connective tissue (CT) in relation to the total measurement area. Values were expressed in percentage.

2.10. Statistical analysis

The obtained results were transferred into the Excel program (Microsoft Corporation, Redmond, Washington, United States). The data analysis was performed with the statistical software SPSS 20.0 (International Business Machines, New York, United States). The descriptive method was used to analyze the radiological mean differences in length, width, and density. Quantitative variables in the form of mean, median, standard deviation (SD), maximum, and minimum were calculated for radiologic and histomorphometric evaluations. The paired *t*-test was deployed for the analysis of the differences within each group between baseline (preoperative) and final (postoperative) measurements. A *p*-value of <0.05 was established to be statistically significant.

3- RESULTS

3. Results

The values followed a normal pattern of dispersion with a 95 percent confidence interval. Table 2 summarized the statistically significant differences (p<0.05) between the measurements of the parameters pre- and postoperative. The univariate analysis showed a bone gain in length, and width, of 27.59%, and 30.48%, respectively. The bone density was increased by a factor of 4. The mean healing period was 8.32 ± 3.00 months.

Table 2. Radiographic measurements of total alveolar bone in cross-sectional view in CBCT and evaluation.

	Preoperative, mean ± SD	**Postoperative, mean ± SD**	***p*-Value**
Length	15.59 ± 7.39 mm	16.55 ± 6.15 mm	0.00396*
Width	9.67 ± 2.87 mm	10.89 ± 2.58 mm	$1.112e^{-09}$*
Density	381.89 ± 384.47 HU	769.35 ± 378.40 HU	$<2.2e^{-16}$*

Abbreviations: CBCT, cone beam computed tomography; SD, standard deviation.

*Statistical significance: $p < 0.05$.

3.1. Clinical evaluation

Preclinical situation

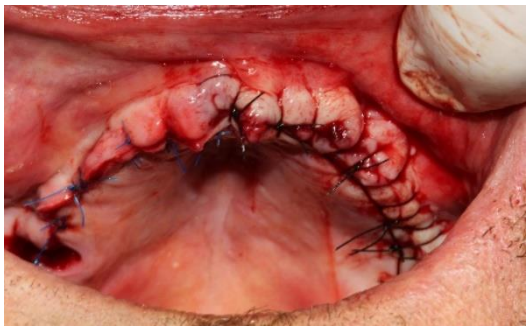
40% of the patients had no denture before operation, and the rest were priorly prosthetic pre-treated.

Operation Day

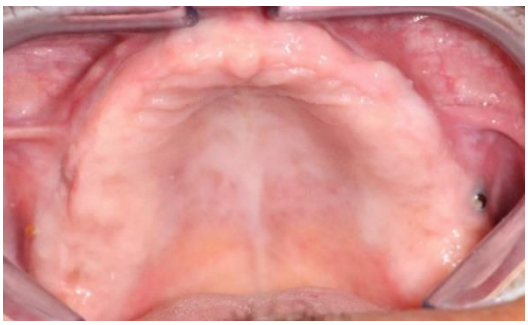
In 76% of the cases, BGS were applied in the maxilla, and in 24% in the mandible, whereby their distribution was incidental. Membranes were used in 14 of 20 cases. The planned prosthetic rehabilitations after substitute grafting and implant insertion were either a fixed hybrid acrylic prothesis or crowns and/or bridges. All crowns and bridges were of metal-ceramic reconstruction.

Postoperative situation

Seven of the 57 implants were lost in nonaugmented areas. In all other cases, uneventful healing was observed. Neither regional bony infections nor rejection of the BGS could be identified in clinical and radiographic examinations. Regarding the membranes from a clinical point of view, PLGA membranes were rigid under dry conditions, easier to cut into the appropriate shape to cover the bony defects. Attention should be paid to place the porous side of the PLGA membrane correctly on the bone surface without confusions of the sides. After moistening, the mechanical flexibility increased, and a simple placement was possible. In comparison, the collagen membrane was softer, and stuck slightly at the instruments. The permeable cross-linked collagen structure allowed cell attachment from all sides. While suturing with soft tissue, the mechanical strength of both membranes seemed to be enough. The tested membranes did not disturb tissue healing or bone healing as demonstrated in Figure 10.



(A)



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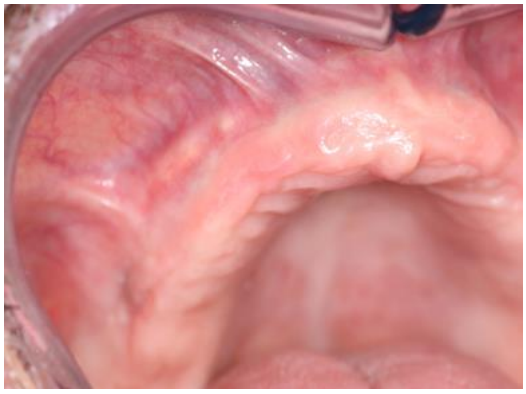

(C)

Figure 10. (A) After alveolar ridge augmentations and sinus floor elevations bilateral. (B-C) postoperative after the healing period. No mucositis or inflammations observed. Hard alveolar bone palpable without soft fibrous tissue interferences. Ideal basis for implant and prosthetic rehabilitation. (B) palatal, and (C) lateral view.

3.2. Radiographic evaluation

Immediately after the surgery, the CBCT images showed a greater radiopacity of the augmented sites in comparison to the host bone. A radiolucent line marked the not yet ossified space between the bone substitutes and the surrounded alveolar bone. Postoperatively, a decrease of material radiopacity and an increase of radiolucency at the graft-alveolar bone interface were observed. No signs of osteolysis could be found. The biomaterials were well included in the host bone, marked by increased radiodensity around it. The mean bone gain in length, width, and density was 0.96 ± 3.33 mm, 1.22 ± 1.87 mm, and 387.47 ± 328.86 HU, respectively (Table 3).

Table 3. Mean bone gain in length, width, and density postoperative.

	Length [mm]	**Width [mm]**	**Density [HU]**
n*	315	315	420
Range	14.81	10.12	1,524.75
Mean	0.96	1.22	387.47
SD	3.33	1.87	328.86
Minimum	-4.71	-1.44	-281
Maximum	10.10	8.68	1,243.75
Median	0.17	0.74	403.75

*Number of measurements.

3.3. Morphometric evaluation

New bone regeneration and osteoclastic activity, detected by the absorbed lacunae of the inserted biomaterials, were observed (Figures 11 and 12). In all samples, the newly formed bone was in close contact with the graft material. The substitutes were surrounded by CT (Figures 11 and 13).

The NBs were well vascularized. Some osteocytes were found (Figure 13). In the samples, bone remodeling and absorption processes (Figure 11, 12), and the ingrowth of NB into the biomaterials (Figure 12, 13) were observed. Some macropores seems to be

invaded with bone, and others remained empty (Figure 12). Percentage values of NB, RB, and CT are listed in Table 4.

Table 4. Percentages of NB, RB, and CT after a healing period of 6 months.

%	**Mean**	**Median**	**SD**
New bone formation	41.44	42.61	5.37
Residual biomaterial	24.91	27.37	7.31
Connective tissue	33.64	33.52	4.81
Total	99.99		

Abbreviation: SD, standard deviation.

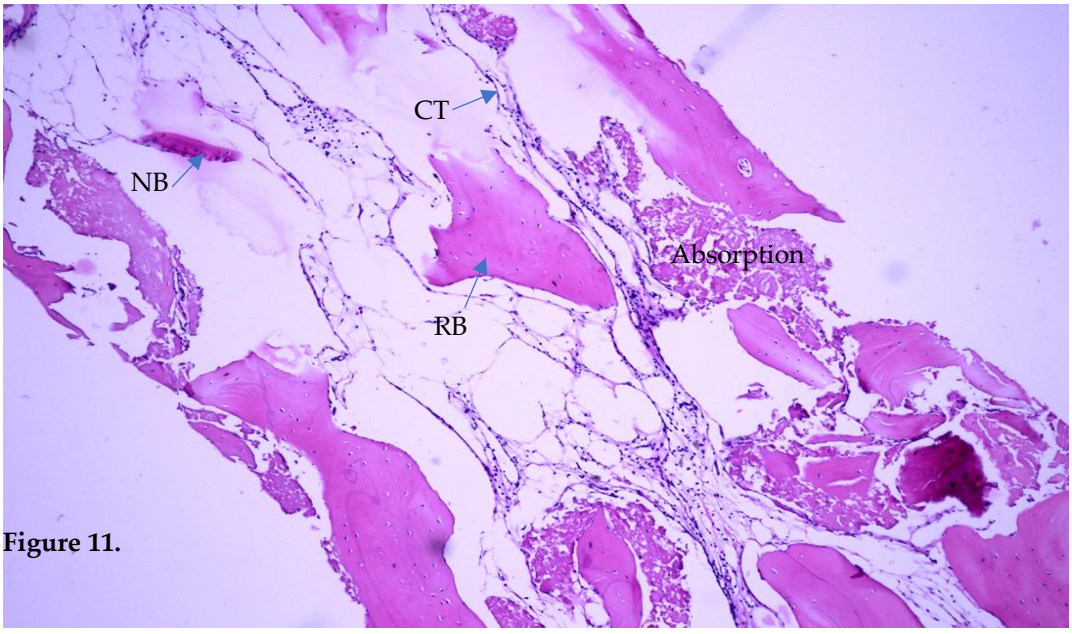

Figure 11.

Figure 11. Macroscopic image postoperative, stain Hematoxylin and eosin (H&E), magnification *10x*. Residual biomaterial (RB), absorption, connective tissue (CT), new bone formation (NB); Arrows indicate the affiliation.

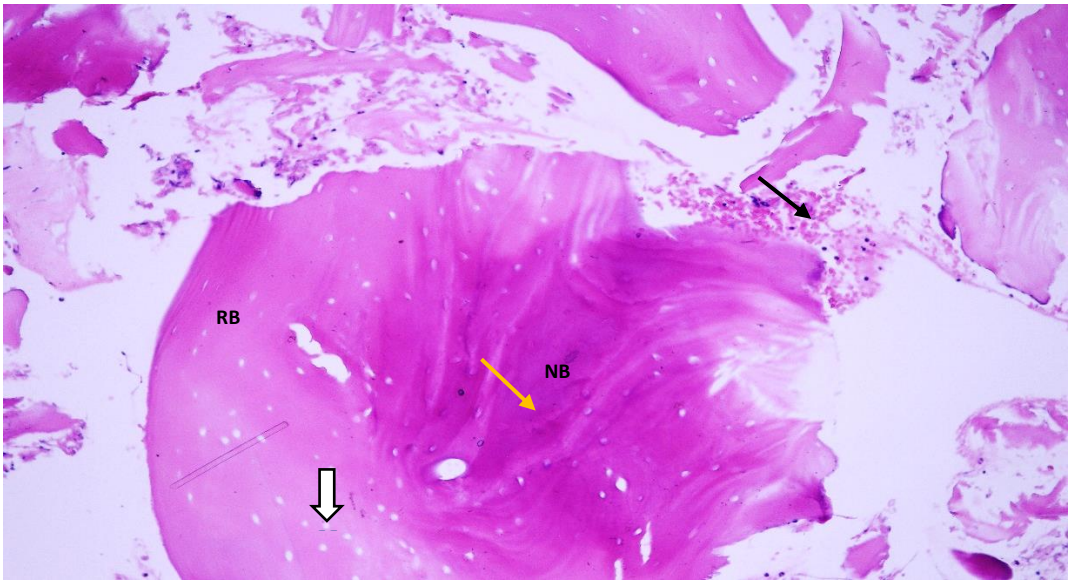

Figure 12. Macroscopic image postoperative, stain Hematoxylin and eosin (H&E), magnification *20x*. Bone ingrowth inside the biomaterials (orange arrow), remodeling process (Absorption, black arrow), newly formed bone (NB), residual biomaterial (RB), macropores (white arrow).

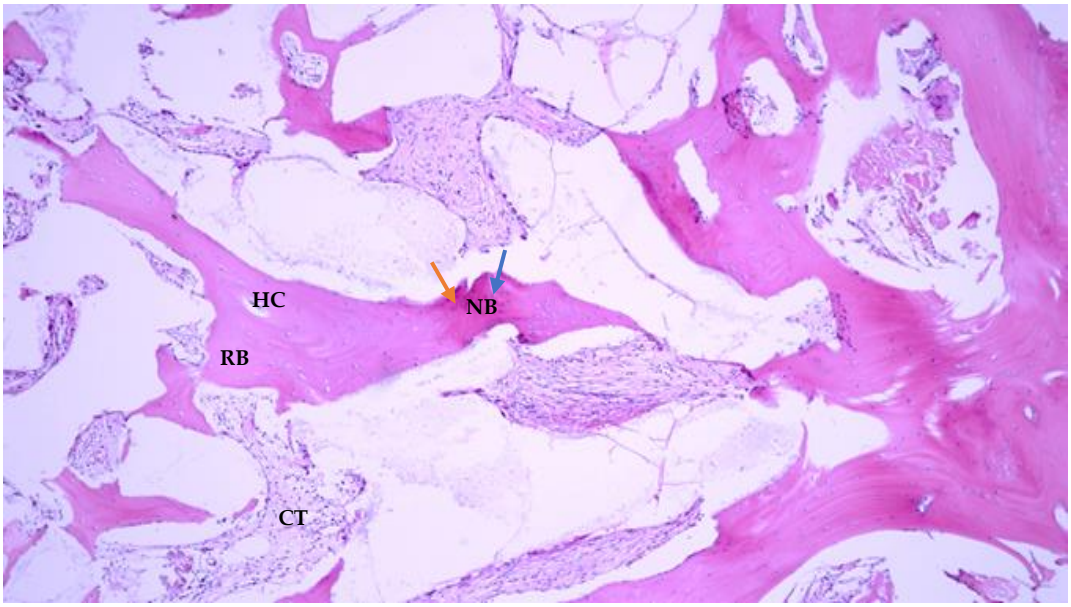

Figure 13. Macroscopic image postoperative, stain Hematoxylin and eosin (H&E), magnification *10x*. Bone ingrowth inside the biomaterials (orange arrow), residual biomaterial (RB), new bone formation (NB), osteocyte in NB (blue arrow), Harversian canal (HC) surrounded by osteons, connective tissue (CT).

4- DISCUSSION AND FUTURE PERSPECTIVE

4. Discussion

The disciplines of synthetic materials and their biomechanics proved to be a very decisive factor for the understanding of device-based function to make the effort of the best decision for the patient. The chemical, mechanical and physical properties of the biomaterial components have always to be evaluated for any dental application.

First of all, one of the first extensive studies by Daculsi et al[132] demonstrated the efficiency of BCP as grafting material of MBCP technology covering bone defects approximately 30 years ago. The authors performed the studies in patients with tumoral resections in spine and experimentally in an animal model. Histologically, the biodegradation through the partial dissolution of the MBCP crystals, especially the β-TCP content, by multinucleated cells were observed at least 2 weeks after graft implantation. During the first month, the degree of biodegradation was greater than after when the new bone formation increased. Initially, bone formation occurred in the cortical part of the MBCP graft, then in the ceramic macropores and spaces between the implant blocks in the intramedullary area, and finally, remodeling processes were observed in the inside of the bone substitute between 8 and 18 weeks. After 18 weeks, it remained a cortical bone deficient and the bone formation have not been completely finished yet. Instead, the clinical results demonstrated bone ingrowth into the MBCP blocks and new cortical bone formation by filling the surgical defects after 16 months.

The biodegradation mechanism started immediately after the AP implantation beneath the ceramic surface.[133] The optimal pH for osteoclastic cells and resorptive activity was ranging between 6.8 to 7.2 or even more acidic.[134] Accordingly, the osteoclast-induced resorption of the material was activated, resulting in a release of calcium and phosphate, and an increase of these elements in the surrounded host bone tissue that was close to the substitute.[11,133] Osteoclasts had the same behaviour at the implantation site as at the natural bone by forming resorption lacunae with ruffled borders. In the lacunae, osteoclasts introduced the phagocytosis of the CaP crystals on the biomaterial surface. The crystals lost

their electron density by breaking their structure.[133] Microscopic analysis revealed dissolution zones predominate on BCP materials.[134] The formed endophagosomes and pseudopodia-like plasmaprotrusions of the ruffled borders incorporated the fractured particles into vacuoles that were released to cytoplasm. There followed the phagocytosis of the fractured particles by monocytes and macrophages.[133,134]

After the degradation, the higher levels of calcium and phosphate ions in the microenvironment stimulated osteogenesis in the tissue by attracting osteoblasts.[11] In the following, changes of pH in the tissue evoked a mild inflammatory that caused fibrous tissue formation. Thusly, high extracellular calcium levels effected simultaneously an inhibition of osteoclastic activity and the attraction of osteoblasts. Experimentally, it was proven that modifications of size, porosity or crystallinity of the AP influenced the degradation, integration of the material and new bone formation significantly. The corresponding methods have been already discussed in chapter "Alloplast". A successful osteointegration included the complete replacement by autologous tissue after biomaterial degradation.[133] In this context, it should be focussed on the solubility of AP that was defined as the extent of dissolution of calcium and phosphate depending on HA and β-TCP molar ratios.[61]

Comparing BCPs with different ratios of HA/β-TCP (100/0, 79/21 and 57/43), the recently performed study by Ortiz-Puigpelat et al[135] concluded to reach high amounts of NB with the ratio of 57/43. After 6 months, a bone density of 880.55 HU was measured by means of microcomputed technology in an *in vivo* model, accordingly, minimal higher than in this present study amounted.

The *in vivo* studies on rabbit bone cells by Yamada et al[136] confirmed that BCP (60% HA/ 40% β-TCP) might be seen as the satisfactory mixture for the clinical application. HA operated for a longer time as a mechanical strong scaffold with long term stability regarding its slow 1 to 2 years resorption rate,[9,132,136] whereas β-TCP was approximately 10 to 20 times faster resorbed due to its higher calcium concentration during the first 3 months.[137] The

properties of the combination of both materials were comparable to the biological degradation rate of natural human bone,[138] and may be the best compromise for the desired bioactivity.[132]

Moreover, comparing the histomorphometric results of the present study with data from the literature while respecting similar healing periods, Friedman et al[139] obtained 38.8% NB in ridge augmentations and maxillary sinus grafting. Mangano et al[140] used BCP produced by the same MBCP technology in a clinical study for sinus augmentation and obtained 28.3% NB. The systematic review and meta-analysis of Danesh-Sani et al[141] compared histomorphometric results after sinus floor augmentation of all types of grafting materials in different healing times from 136 studies. Regarding the healing period of 4.5 to 9 months, the AB had the highest amount of NB (43.15%) compared with other graft materials, followed by AP (29.27% NB), and AL with the lowest amount of NB (25.02%). Notably, AP was not clearly differentiated. In the randomized controlled trail by Schmitt et al[142], four different grafting materials were compared in 30 patients after 5 months in sinus augmentations: Straumann® Bone Ceramic (BCP), Bio-Oss® (anorganic bovine bone), Puros® (mineralized cancellous bone allograft MCBA), and AB. The AB reported the highest amount of NB (42.74%), followed by MCBA with 35.41% and BCP 30.28 %. The bovine bone revealed the lowest amount of NB (24.90%). The randomized controlled clinical study by Lindgren et al[143] obtained with BCP an average NB of 41.1% that is comparable to those of the present study, and deproteinized bovine bone (DBB) NB of 41.6% after 8 months of healing time in maxillary sinus floor augmentations. The DBB particles had significantly more contact with NB compared to BCP particles (87.9 and 53.9%, respectively), but without clinical relevance underlined by the authors. Further studies compared the efficacy of BCP against AB and concluded higher results in NB with AB: 38.63 to 41%,[144] or 36.8 %[145] versus NB in BCP of 26.68 to 33.70%,[144] or 28.2 %.[145]

Taking all results into consideration, it is remarkable that the present study demonstrated the capacity of the novel biomaterials to promote higher amounts of NB compared with conventional BCPs, XEs, and ALs.[139-143] The tested BCP reached similar high

amounts of NB to ABs and demonstrated the resemblance in behavior to AB.[142-145] According to Fellah et al[146], BCP in ratio of 60/40 (HA/β-TCP) had even a better stability and osteogenic features than AB in critical-sized bone defects. AB was too quickly resorbed from 19.4 initially to 1.7.% in the 12th week.

One disadvantage of AB was the higher rate of bone formation initially, but in turn also a faster resorption rate.[147] In contrast to the rougher surface of AB, BCP had initially a smooth surface which had to be prepared by macrophages for improving the bone cell apposition. Lee et al[137] observed the first bone formation at around 12 weeks after implantation. BCP inhibited a too early osteoclastic resorption;[137] therefore, BCP seems to be more stable and predictable in behavior. In a systematic review, Troeltzsch et al[148] stated the clinical efficacy of grafting materials in alveolar ridge augmentations. After an investigation of a total of 184 papers, a horizontal mean bone gain of 2.2 ± 1.2 mm was described for APs. The highest vertical gain of 4.5 ± 1mm was reached by AB mixed with AL or XE, and the overall mean of all grafts was 3.7 ± 1.2mm. The results are comparable to those of this study.

Regarding to the radiographic results of the tested materials, it has to be emphasized that the bone gain of width was greater than those of the length because mechanically occurs a greater occlusal pressure through the provisional prothesis, during biting processes and masticatory functions. Probably, these factors influence negatively the graft substitute integration. Accordingly, based on the present study, one might conclude that horizontal augmentation with APs, in the form of granules and/or putty, is recommendable with a confirmed satisfactory outcome up to a height of 3 to 4 mm. For augmentations with synthetic particulate bone graft higher than 4 mm, further clinical studies are necessary.

With reference to the histomorphometric outcomes, the tested materials seem to be very biocompatible and osteoconductive since high amounts of NB were reported with

uneventful healing. It is usual for synthetic BGS that the amount of RB is still high after only 8 months postoperative because its resorption rate is approximately 6 months.

Relating to the membranes, some studies underlined better outcomes for GBR using barrier membranes,[149] and others showed no significant differences.[150] In the present study, the results of radiographic and histomorphometric evaluations according to the PLGA and collagen membrane did not differ substantially, and both membranes had good outcomes as physical and biological barriers. In general, no systemic toxicity has been observed yet for PLGA membranes,[151] and collagen membranes are generally cytocompatible because of their natural origin.[125] Hoornaert et al[123] figured out that PLGA membranes had a higher bone regeneration rate of 30% NB compared with cross-linked porcine collagen membranes (24.6%). PLGA was hydrolyzed after 6.5 months, whereby collagen membranes were already completely resorbed in around 8 weeks. Collagen membranes have faster degradation rates depending on different factors, including the tissue of origin or mechanical properties. Alveolar bone healing was marked at around 4 months;[123] therefore, barrier membranes should sustain their biological function during this period. Consequently, PLGA membranes might be safer, and have a better predictable resorption rate. The *in vitro* and *in vivo* studies of Won et al[152] reported a similar level of biocompatibility and bone regeneration potential of PLGA and collagen membranes (NB 24.26 and 13.84%, respectively). The study asserted that PLGA membranes were more reliable in retaining form in the oral cavity than collagen membranes, which lost a tad of stability under wet conditions. Finally, the percentages of rate of rehabilitation of dehiscence defects for PLGA and collagen membranes were reported in the literature to be 70.20 and over 80%, respectively, whereby the complication rates were the highest for PLGA (37.4%) and lowest for the collagen membrane (10.4%).[148]

Summing up all data from the present and previous studies,[148-152] apart from small differences, both types of membranes are biocompatible and suitable for GBR.

Overall, BCPs have a great potential in dental treatments. Miron et al[110] demonstrated, firstly, that only AB and BCP were capable to differentiate bone marrow stromal cells towards osteoblasts, and secondly, BCP supported ectopic bone formation. According to Park et al[153], BCP produced with MBCP technology had an almost 100% high stem cell-carrying potential, and a better osteogenic differentiation if loaded with stem cells compared with Bio-Oss in an ectopic transplantation model.

Numerous studies stated that BCP in the ratios of 60/40 of HA and β-TCP may be a good and promising alternative to AB,[146,153] and even entitled as the "gold standard of nowadays".[103] These statements can be confirmed by the outcomes of the present study.

In future perspectives, more attention should be focused to hone the skills of synthetic BCPs, especially in their osteoinductivity performances, in combination with tissue engineering, including stem cells, bioactive bone agents, growth factors, cell culture technology or printed three-dimensional scaffolds. To optimize the regenerative capabilities of bone and membranes in GBR, some studies led to promising results by adding bioactive agents, including silver nanoparticles,[154] advanced platelet-rich fibrin,[155] or mesenchymal stem cells.[156] It was shown that leucocyte- and platelet-rich fibrin membranes modified with silver nanoparticles had enhanced mechanical properties relating to strength and stiffness as well as a higher antibacterial activity and residence against bacterial contamination compared to membranes alone.[154] Silver nanoparticles are used in the maxilla facial surgery and implantology in treatments like wound or bone healing, GTR and GBR, dental implant modifications or oral cancer. A further treatment option is to use products with leucocyte-platelet-rich fibrin (L-PRF) or the novel advanced platelet-rich fibrin (A-PRF) for tissue regenerations, both further developments of platelet-rich plasma (PRP).[155] L- and A-PRFs are consisting of higher concentrations of leukocytes and a flexible, tridimensional fibrin mesh for cellular adhesion to enhance angiogenic, antimicrobial and osteogenic abilities, and to enable the release of signalling molecules over a longer time in tissues. The A-PRFs have the advantage to resemble the physiology of wound healing due to a greater concentration of growth factors, for instance, vascular

endothelial growth factors, platelet-derived growth factors or ant-inflammatory cytokines.[155] The application field of L- and A-PRFs are the periodontology, implantology and maxillofacial surgery. In addition, to increase the bone density, human umbilical cord mesenchymal stem-cells were injected in osteoporotic mandibular defects in an animal model.[156] The study results showed higher levels of alkaline phosphatase, osteocalcin, and type 1 collagen which influence the bone metabolism, and an enormous increase of trabecular bone area and bone density. Further new findings were made by Roldan et al[157]. The authors have been discovered that the bone morphogenetic protein-7 (BMP-7) enhanced the degradation and bone formation of BCPs in a ratio of 60/40 (HA/β-TCP) through the osteoclast-induced resorption. In contrast, TCP degraded through dissolution and no osteoclasts could be found on the ceramic surface. Both BCP and TCP were explicitly osteoinductive in this model. In the recent years, it was focused to design highly bioactive BCP ceramics by means of, for example, partial substitution of calcium ions by strontium ions, resulting in an enhanced osteoinductivity, including a higher protein absorption ability, greater attachment of bone marrow stromal cells, and proliferation of protein gene expression; further, promotion of new bone and an increased degradation.[158,159] Commercially available BCP is irregular, porous and has large grain sizes, whereby natural bone has more nanocrystalline incorporated. To mimic these properties, BCP ceramics with nanocrystalline were developed experimentally.[160] As a result, a stronger osteoinductivity and bone regenerative and osteogenic abilities were obtained. Due to its very good tested biological and physical efficacy, it is recommended by the authors to use by in GBR treatments. The last novel technique to mention is the nanotechnology. BCP was coated with nano-HA.[161] Accordingly, the *in vitro* investigations showed that nano-HA did not influence the mechanical properties i.e., density, flexural or compressive strength or porosity, of BCP ceramics. In contrast, the nano-HA coatings as scaffolds of BCPs increased the osteoinductive potential, the viability of mesenchymal stem cells on the ceramic surfaces that produced higher amounts of alkaline phosphatase, collagen type 1, and osteocalcin than on non-modified BCPs scaffolds.

Taking everything into conclusion, BCPs are the subject of much attention for dental applications as the biomaterial of nowadays, accordingly, they are very auspicious for tissue engineering in future.

5- CONCLUSIONS

5. Conclusions

In the present study, the novel biomaterials were successfully used for bone substitution in various clinical situations without notable risks or complication rates, and may be an adequate and promising alternative to autogenous bone.

From the clinical point of view, the tested synthetic materials can be recommended for the following dental surgical procedures: sinus lift elevations, regeneration in 3rd molar regions, ridge augmentations, socket preventions and alveolar crest-splitting. The novel biomaterials were easy to handle and characterized by sufficient long as well as predictable resorption rates.

Regarding the bone substitute in form of granules, it is recommended to crush the granules with an appropriate flat surgical instrument to reduce the particle sizes, make them fine-grained and to expand the surface area of the ceramics that will be in direct contact with the surrounding bone. In contrast, the putty material is malleable and sticks well at bony walls. Both the granules and putty form can be used for smaller as well for larger defects in GBR protocols.

Furthermore, the PLGA and collagen membrane exhibited good biocompatibility, and fulfilled all biological and mechanical properties as barrier membranes for the daily clinical work. The PLGA membrane might be superior in easier handling, sizing, and positioning.

According to the radiographic evaluations, it might be concluded that deficient bone volume and bony voids could be uncomplicated augmented with the novel particulate material with MBCP technology up to heights of 3 to 4 mm. Outstanding clinical outcomes were reported compared with those of current literature: a good integration of the biomaterials in the recipient tissue, no infections nor inflammations, an increased degree of vertical and horizontal bone augmentation and volume stability, and a great bone density after 8 months.

Histomorphometrically, the high amount of new bone formations indicated the biocompatibility and resorbability of these materials.

The hypothesis is confirmed that these novel biomaterials had similar clinical outcomes compared with previous studies in humans, even better than expected.

With the limitations of a clinical study in humans, the number of patients was restricted. All radiographic evaluations were done on the most precise manner as possible. Although all images were obtained by standardized three-dimensional radiographs and by means of the most modern paralleling cone-beam computed tomography (CBCT) devices, nevertheless, it cannot be excluded that all images were always made in the same position because patients can move during the imaging, resulting in small differences of received CBCT scans. Further, the clinical outcomes are also dependent on the cooperation of the patient. Particularly in the implantology and the future prosthetic rehabilitation, it is significant to instruct the patient to maintain a very good oral hygiene pre- and postoperative to avoid further complications like periimplantitis, infections or inflammations, resulting in possible graft integration and healing complications.

Further studies are necessary to verify the clinical efficiency of these biomaterials for long-term results as well as for patients with medical history or risks.

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