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Original article

Xenografting/Xeno Bone Grafting and Synthesis of Hydroxyapatite from Bovine and Camel Bone

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Abstract

Development of artificial bone grafts has increasingly progressed in the last few years. Significant progress has thereby been made in development, preclinical testing and clinical use of different matrices. In addition to complete synthetically manufactured products, xenografts based on bovine material are in wide spread clinical use. Possible transmission of prions causing bovine spongiform encephalopathies and Creutzfeldt-Jakob disease give the background for discussing the advantages and disadvantages of synthetic versus xenografts. Possible risks using xenografts based on bovine material are discussed. Bone grafts are used as a filler and scaffold to facilitate bone formation and promote wound healing. These grafts are bioresorbable and have no antigen-antibody reaction. These bone grafts act as a mineral reservoir which induces new bone formation. In this review, we will present up to date knowledge about xenografts used in the field of dentistry and I will tray to make this study as firest step for new product of xeno-grafting in future from my nature in Arabian land of Saudi Arabia and produce it from new source (camel bone material comparing to bovine bone material)

Introduction

Bone unions are utilized as a filler and framework to encourage bone development and advance injury mending. These unions are bioresorbable and have no antigen-counter acting agent response. These bone unions go about as a mineral supply which actuates new bone development. Edge absconds create because of medical procedure, injury, disease, or intrinsic contortions. The objectives of rigid substitution are upkeep of form, disposal of dead space, and diminish postoperative contamination; and consequently, improve hard and delicate tissue recuperating. The deficient amount of bone is because of tooth misfortune which brings about quick resorption of alveolar bone because of absence of intraosseous incitement by periodontal tendon (PDL) filaments, for instance, pneumatization of maxillary sinus following tooth misfortune. Bone joining is a surgery that replaces missing bone with material from patient′s own body, a counterfeit, manufactured, or characteristic substitute. Bone uniting is conceivable on the grounds that bone tissue can recover totally whenever gave the space into which it needs to develop. As common bone develops, it for the most part replaces the join material totally, bringing about a completely coordinated district of new bone. Bone deficits of the jaws are usually attributed to accidents, surgical removal of benign lesions or malignant neoplasms, congenital abnormalities, periodontal inflammation, tooth abscess or extraction and finally jaw atrophy due to advanced age or general disease. These bone defects require rehabilitation for a variety of reasons, e.g. maintaining the normal anatomic outline, eliminating empty space, aesthetic restoration and placing dental implants. There are several techniques have been developed to eliminate these bone deformities including bone grafting, guided bone regeneration, distraction osteogenesis, use of growth factors and stem cells. Bones consist of materials of natural or synthetic origin, implanted into the bone defect site, documented to possess bone healing properties. Currently, a variety of bone restorative materials with different characteristics are available, possessing different properties. The aim of this review is to provide a contemporary and comprehensive overview of the grafting materials especially xenografts that can be applied in dentoalveolar reconstruction, discussing their properties, advantages and disadvantages, enlightening the present and the future perspectives in the field of bone regeneration and possibility to produce bone grafting from new source as camel. Xenograft material is viewed as a bone substitute got from another species. The most widely recognized xenograft bone substitute utilized in dentistry today is of cow-like birthplace. Be that as it may, bone substitutes of porcine source are beginning to discover their way into the dental market today. Less regularly utilized xenograft material is gotten from calcifying corals or green growth. Similarly as with allograft, xenografts are handled so all natural segment is disposed of to expel the danger of transmission of infection. A few bone substitutes are remembered for this class, equipped for large scale manufacturing with a generally moderate expense. Among their weaknesses is the way that bone qualities vary contrasted with people while their handling

system may influence their physico–substance properties as on account of allografts just as probability of infection transmission and incitement of immunogenicity. Xenogratfs are bone grafts from a species other than human, such as bovine and are used as a calcified matrix.

Xenogeneic Bone Grafting Materials

These days, an assortment of bone substitutes is accessible for the clinical client. Strikingly, these materials essentially contrast with respect to their crude materials or assembling forms. As an option in contrast to autologous bone tissue (autograft), which is as yet applied as "gold standard" because of its broad regenerative properties, bone substitutes from other normal sources become increasingly more significant in regenerative dentistry. These bone substitute materials are either determined from human (allograft) or creature beginning (xenograft).

If there should arise an occurrence of these materials, the got hard extracellular framework dependent on calcium phosphates ought to at last fill in as bone substitute In view of the physicochemical closeness of this class of bone substitutes to the autologous bone tissue, it can be expected that these materials are the perfect decision for rigid recovery. Especially, cow-like bone is utilized as source tissue in the day by day dental practice, as if there should arise an occurrence of the two principally applied bone substitute materials Bio-Oss™ and cerabone.

Safety aspects and purification processes:

For the clinical use of bone substitutes from normal sources, it is natural to filter the giver tissue from immunogens to ensure a recovery process without confusions, for example, dismissals or illness transmissions. To guarantee the sheltered use of such bone substitute materials, distinctive decontamination steps of the contributor tissue are applied. The initial step is the appropriate determination of giver creatures before the commencement of the cleaning process. Hence, for the creation of Bio-Oss™ and carbon ® cow-like femoral heads from enrolled providers situated in Australia and New Zealand are handled as the two nations are perceived to have an insignificant BSE chance as indicated by the World Organization for Animal Health (OIE). Thereafter, complex cleansing advances including both substance and physical techniques are applied for a total sanitization. Be that as it may, those strategies are incidentally talked about in view of conceivable dismissal responses or an exchange of microorganisms while applying bone joining materials. In this unique situation, the temperature treatment for the cleansing assumes a significant job. Bio-Oss™ is prepared at temperatures of roughly 300 °C, while the bone substitute material cera bone® is decontaminated by remarkably higher temperatures of up to 1,250 °C.1, 2 This distinction in temperature is by all accounts critical for the protected use of xenogeneic bone substitute The filtration procedure of ox-like bone tissue was assessed in an ongoing survey by Kim et al.3 Interestingly, the creators reasoned that the inactivation of prions in Bio-Oss™ is preferably founded on the applied temperature over a consequence of the treatment with profoundly focused sodium hydroxide (NaOH). While this concoction procedure was portrayed as proficient by Wenz et al.,4 the unwavering quality and affectability of the pre-owned tests were addressed by Kim et al.3 In this audit, the creators depict that prions may be adequately demolished by warming up to 1,000 °C for five minutes. Besides, the agreeing EU-rules for clinical gadgets using creature tissues and their subordinates (Part 1: Application of hazard the board, EN ISO 22442-1), call attention to that a treatment at temperatures over 800 °C is lessening the danger of the transmission of Transmissible Spongiform Encephalopathies (TSEs) to an adequate least.

Inflammation and bone regeneration:

Information from preclinical and clinical investigations show practically identical qualities for new bone arrangement, remaining bone joining material and connective tissue for both xenogeneic bone substitutes referenced above, these results allude to comparable organic air conditioning tivities of Bio-Oss' and cerabone®. Nonetheless, if there should arise an occurrence of cerabone® higher quantities of multinucleated gi-subterranean insect cells (MNGCs) were found inside the main days after its implantation' Furthermore, the correlation with various different investigations shows that the underlying number of MNGCs if there should arise an occurrence of cerabone® is essentially lower as found in the embed bed of quick de-gradable manufactured materials dependent on tricalcium phosphates. These outcomes affirm a few different examinations asserting the drawn-out steadiness of xeno-generic bone substitutes as it was indicated that MNGCs are associated with the biodegradation of bone-joining materials by phagocytosis.,' Strikingly, the MNGCs were distinguished as remote body monster cells (FBGCs) in light of their atom articulation. Be that as it may, more data is as yet expected to get further ends in regards to their separation." Interestingly, the corruption procedure of bone substitutes the procedure of bone tissue recovery is firmly associated by means of the significant cell types, for example, macrophages and MNGCs In this specific situation. Altogether, it can be concluded that the xenogeneic bone substitute material cerabone® is able to ensure the highest possible safety from disease transmission due to the high temperature treatment. Furthermore, it is assumable that the relatively high numbers of multinucleated giant cells express high amounts of anti-inflammatory molecules and support a fast and high implant bed vascularization and therefore, might favor the bone regeneration process.

Ideal properties of grafts

- Non-toxic-Non-antigenic with patient acceptance
- Resisting to infection
- No root resorption or ankyloses
- Strong and resistant
- Stimulates osteoinduction & framework for osteocooduclion
- Easily adaptable

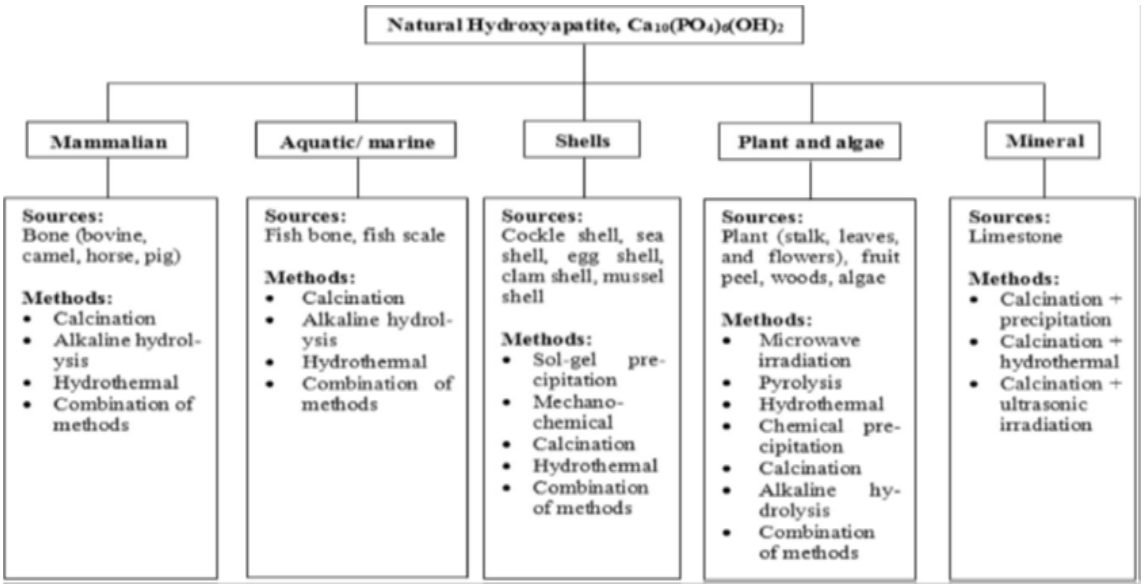
Hydroxyapatite: Hydroxyapatite, a naturally occurring form of calcium phosphate, is the main mineral component of bones and teeth. Natural hydroxyapatite and bone have similar physical and chemical characteristics make it biocompatible. Its porous structure resembles native bone. The biocompatibility, biodegradability and bioactivity make it extensively useful in interdisciplinary fields of sciences like chemistry, biology, and medicine. Calcium phosphate-based ceramics are of great interest as substitutes of synthetic bone graft due to their similarities in composition to bone mineral and bioactivity as well as osteoconductivity. Hydroxyapatites (HAP) is a naturally occurring mineral form of calcium apatite comprising of about 50% of the weight of the bone, which accounts for its excellent osteoconductive and osteointegrative properties It is a main component of bone mineral

but in some cases carbonate-apatite is a main hard tissue component, as in dental enamel. One of the most common apatites used as bioceramic in medicine and dentistry is hydroxyapatite (HAP) due to its bioactivity and osteoconductive properties in vivo. The advantage of using HAP as a bioceramic or biomaterial compared to other bioceramics, such as Bioglass or A-W glass-ceramic, is its chemical similarity to the inorganic component of bone and tooth.

Chemically hydroxyapatite Ca5 (PO4)3 OH but often written as Ca10 (PO4)6 (OH)2.

Natural hydroxyapatite

Natural hydroxyapatite is usually extracted from biological sources or wastes such as mammalian bone (e.g. bovine, camel, and horse), marine or aquatic sources (e.g. fish bone and fish scale), shell sources (e.g.cockle, clam, eggshell, and seashell), and plants and algae and also from mineral sources (e.g. limestone).



The sources and examples of techniques used for synthesising natural HAp. Stoichiometric HAp is basically composed of calcium and phosphorus with molar ratio of Ca/P equal to 1.67. This ratio has been proven to be the most effective in promoting bone regeneration. Natural HAp is non-stoichiometric and is either deficient in calcium or phosphorus. Calcium positions are the most common vacancy in HAp where cations such as Na, Mg2, and Al3 are substituted in the calcium positions, while carbonate ions can substitute for either phosphate or hydroxyl ions while fluoride ions substitute for hydroxyl ions. The presence of trace elements in some natural HAp mimics the apatite produced from human bone. The trace elements are essential in th regeneration of the bone and accelerates the process of bone formation [9]. Research by Balamurugan et al. proved that 3–5 mol% silicon added to synthetic hydroxyapatite (Si-HAp) increased cell growth density which enhanced osteoblast growth. Research by *Capuccini et al*. showed that the presence of 1–10% of strontium ions in synthetic Hap enhanced osteoblast activity and differentiation and also inhibited osteoclast proliferation and production. Carbonate-substituted hydroxyapatite was also proven to enhance osteogenesis by enhancing bio resorption. The usage of HAp extracted from natural sources can be considered to be an environmentally friendly, sustainable, and economical process to fabricate these materials since these materials are available in large quantities. This can result in positive contributions to the economy, environment, and to general health.

Extraction of hydroxyapatite (HAp) from natural sources Mammalian sources in this review I focused in (bovine and camel) as comparing between this tow sources for HAP. The extraction of HAp from bovine bone was frequently reported in literature compared to other sources such as camel, horse and, porcine. The cortical part of the femoral bone is usually used because they are morphologically and structurally similar to human bone. Reviewing the literature shows that the properties of the extracted HAp, such as the Ca/P ratio, size, shapes and crystalline phases of Ca-P have been discussed. These properties differ with the applied extraction methods and thus the parameters such as calcination temperatures and pH. Generally, most literature have reported that pretreatment of the bone is usually done before proceeding with the extraction method. The pretreatment involves washing and removing the dirt, fats, protein, and other components such as bone marrows and soft tissues. Some literature reported the usage of boiling water to remove organic components from the bone by boiling for times of 8 h or more. A combination of boiling and washing with solvents such as acetone and chloroform have been employed for the pretreatment of bone. Another pretreatment method that has been widely used is washing the bone alternatively with surfactant and alkali solutions to remove the soft tissues and de cellularise it. The bone was also cut into smaller pieces before or after removing the organic constituents. Most majority of literature reported that the bone was cut first into smaller pieces before boiling or treated with the solvent to remove the unwanted components such as bone marrow located inside the bone. In this review, most of the methods for extraction of HAp from mammalian bones used the calcination method which is either the sole process or a combination of calcination with other methods. The calcination process involves heating the bone in a furnace at different temperatures of up to 1400 C in order to completely remove the organic matter and kill the pathogens which may be present [9]. Barakat et al. employed the alkaline hydrothermal hydrolysis treatment to extract HAp from bovine bone. The extracted HAp was shows that the calcination method was the most popular method for fabrication compared to others. The calcination process removes the organic constituent in the bone by the thermal process. The organic matter is converted to carbon dioxide and ash (calcium phosphate compounds) as per Eq. (1). This calcium

phosphate usually does not decompose at temperature below 1200_C and will be present as different calcium phosphate (CaP) phases. Increasing temperatures increases the crystallinity of the HAp particles along with other calcium phosphate phases such as β-TCP. On the other side, use of high calcination temperatures will remove all pathogens and prevent the possibility of transmission of diseases such as bovine spongiform encephalopathy and Creutzfeldt Jakob disease. Researches have concluded that pathogens cannot survive at temperature above 800 _C. Mammalian bone Organic matter HAp water Calcination CaP phases CO2. Other methods such as alkaline heat treatment have been used to extract HAp from mammalian bone. In this method, the alkaline solution usually NaOH is used to remove the organic matter from the bone. The NaOH solution hydrolyzes the organic component in the bone and the remaining calcium phosphate is rinsed and separated using filtration. However, alkaline heat treatment produces low crystallinity Hap compared to calcination. Methods used for extraction of HAp from mammalian sources.

Source	Method of extraction	Ca/P ratio	Crystalline phases	Particle size	Shape
Bovine bone	Alkaline hydrothermal hydrolysis	1.86	HAp	Nano	Nanoflakes
	Subcritical water process	1.56			
	Calcination	1.67	HAp (750 _C)	<420 μm	
		1.5	HAp, β-TCP (850 _C)	420–500 μm	
		>1.67	HAp, β-TCP	20–100 μm	Irregular
	Alkaline heat treatment	>1.67	HAp	20–100 μm	Irregular
	Calcination vibro milling	1.66	HAp	(800 _C) <100 nm (<2 h)	Needle like
	Hydrothermal calcination		HAp, Hap dehydroxylate		Irregular (700 _C) semispherical(800 _C)
Camel bone	Calcination	1.66 2.036	HAp (1000 _C) HAp (700 _C)	79 nm- 0.9 μm 97 nm	Irregular

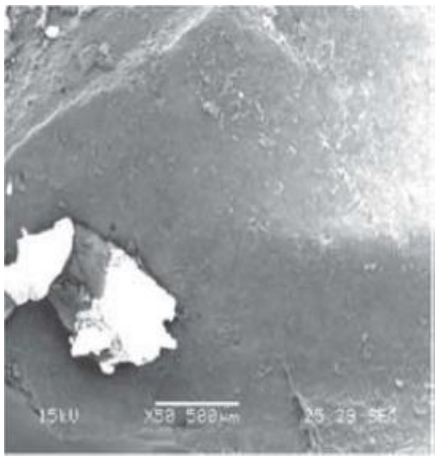
The lake studies and in few literature about method to extraction HAP from camel bone I found one experimental study do it in KSA for Structural characteristics of camel-bone gelatin by demineralization and extraction it was helpful in my review this study was focus on how extracted bone gelatin. Bone gelatin is primarily used for pharmaceutical purposes because of its high level of purity. Prior to extraction of gelatin from bone samples, the bone must be demineralized using dilute acid solution to remove inherent calcium composition that laces the bone matrix. Structurally, the function of calcium salt deposited in the organic matrix of bone is to maintain the bone integrity by holding the scaffolds and crosslinks that improves bone strength and rigidity. Removal of calcium salt from bone through acidulation with dilute acid is an essential step toward successful gelatin production since result of acid treatment produces bone protein(collagen) which contained water-soluble gelatin. Extraction temperature, pH, and extraction period are common operating parameters influencing gelatin production. The effect of processing conditions on the properties of gelatin from fish bone with pretreatment of hydrochloric acid has been reported.[9] Adoption of varied level of processing parameters and raw material sources often dictates the consistency in functionality of gelatin or collagen.

Recently, there has been increased interest in effective use of underutilized resources and industrial wastes to reduce production cost and environmental hazards. Utilization of animal bone for gelatin production offers opportunities including waste to wealth and waste minimization.

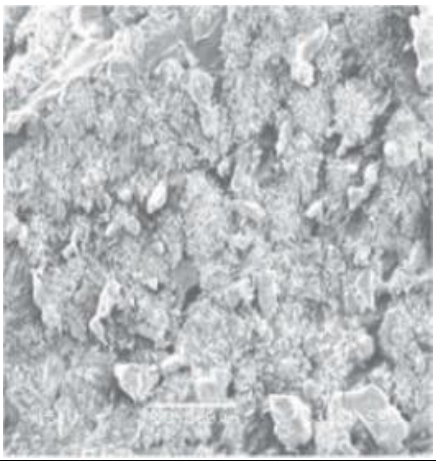
Even developed demineralization processing conditions for cattle bone are kept as company secret, and conflicting values are reported by other practitioners. Therefore, the focus of the present investigation covered the effect of processing conditions on characteristics of ossein and gelatin from camel bone.

Morphology of camel bone and ossein Scanning electron microscope was used to obtain morphological characteristic of camel bone and ossein morphological changes of camel bone due to demineralization. The initial smooth and intact surface of camel bone was lost during to demineralization. After 2 days, the resulting matter surface becomes rough which increases after 5 days of demineralization. Ca salt, hydroxyapatite, inherent in bone structures is responsible for strengthening of the matrix and crosslinks.

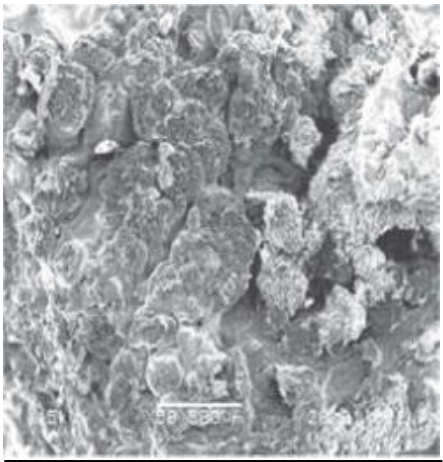
Morphological analysis of camel bone and ossein



Camel bone



Ossein (after 2 days soaking in 6 % HCl)



Ossein (after 5days soaking in 6 % HCl)

Other study done in Iran subjected in comparative study of impact of animal source on physical, structural, and biological properties of bone xeno graft. In this study, methods was in three bone substitute materials intended to serve as bone xenografts were derived from the cow, camel, and ostrich using the thermochemical processing procedure. The physicochemical properties, in vitro cytocompatibility and in vivo bone regeneration capability of the prepared deproteinized bone grafts, were assessed and compared with OCS-B as an approved product in the global market. The physical tests confirmed the hydroxyapatite nature of the final products. SEM and BET analysis showed morphological and structural differences between the products due to differences in the animal sources. In vitro studies showed the prepared deproteinized bone was free of processing chemicals and was biocompatible with mouse fibroblast and myoblast cell lines. In vivo studies revealed that the bone formation capability of the DBB, DCB, and DOB has no significant difference with one another and with OCS-B despite their structural differences. The DCB showed the highest graft residue after two month. No signs of immunogenicity were observed in the study groups compared to the blank group DBB, DCB, and DOB may offer a favorable cell response and bone regeneration similar to those of commercial bovine bone material.

Results

There are various ceramic processing methods for HA synthesis such as precipitation, sol-gel, hydrothermal processing, etc., HA was precipitated from phosphoric acid and calcium phosphate and the stoichiometric calcium to phosphorus (Ca/P) ratio for HA synthesis is 1.67. Higher Ca/P ratio results in the formation of calcium hydroxide in addition to HA. The effect of different pH of the precipitation solution on the properties of HA was investigated. Properties of synthesized powders were characterized for use as dental composites.

Calcium hydroxide is formed in the structure of hydoxyapatite precipitated from calcium hydroxide and phosphoric acid with Ca/P ratio more than the stoichiometric one (1.67). The presence of calcium hydroxide in the structure leads to an increase in the amount of free calcium hydroxide and its dissolution in the environment which increases pH and reduces bacteria growth. Calcium hydroxide has antibacterial characteristics, enhances enzymes and growth factors release, and increases the rate of drug release Basrah Journal of Veterinary Research, Vol.15, No.3,20

Conclusion

Hydroxyapatite is shown to be a significant material for biomedical applications due to its biodegradability, biocompatibility and bioactivity. HAP is a beneficial biomaterial for dental and medical applications. Natural hydroxyapatite can easily be extracted from raw bovine bone by the thermal decomposition method.

Extracted hydroxyapatite is useful for dental implants due to its biocompatibility properties, osseo-integration, and similar mechanical properties when compared with the human tooth. The results showed that calcinating at 650°Cfor 3 h produces semi-decomposed powder, while heating at 750°C for 6 h synthesizes hydroxyapatite from the raw bovine bone powder. It can be inferred that treatment temperature and time are key parameters in determining the composition of the extracted product. Through experimental study to extract gelatin bone from camel it help to produce xeno-bone grafts from new source using Calcination methods as we do in extract from bovine bone in future.

Ethics approval and consent to participate

This review study still theoretical until now depend on previous study about subjects

References

[1] Nasr HF, Reidy AME, Yukna RA. Bone and bone substitutes. Periodontology 2000, 1999;19: 74-86

[2] Carranf a FA, Takei HH, Cochran DL. Chapter-67, Reconstructive Periodontal Surgery. Corranzo's Clinical Periodontology, 10. edition: 968-969.

[3] Reynolds MA, Reidy AME, Branch-May GL, Gunsolley IC. The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. Ann Periodontal 2003; 8(1): 227-265.

[4] Den.' & Medical Device. Product information on Osteo-Bill •, 2008.

[5] Dentsply-Friadent. Product information on PepGen P-15•, 2008.

[6] Rita Singh, Lanka Mahesh. Infections Resulting from Bone Grafting Biomaterials. International Journal of Oral Implantology and Oinkal Research, May-August 2013,4(2h68-71

[7] AL Durnitrescu Chemicals in Surgical Periodontal Therapy, Bone Grafts and Bone Graft Substitutes in Periodontal Therapy.

[8] Emrnings to al. Chemically modified osseous material for restoration of bone defects./ Periodontal 1974; 45:385.

[9] Boyne at al. Transplantation, implantation and grafts. Dent Clio NAm 1971; 15: 434.

[10] Krejci et al. Osseous grafting in periodontal therapy. Part I - Osseous graft material. Comp. font Edu. Dent VII11987, No.10.

[11] Barone A, Ricci M, Covani U, Nannmark U, Azarmehr I, Calvo-Guirado J. Maxillary sinus augmentation using pre-hydrated cortico-cancellous porcine bone: histomorphometric evaluation after 6 months. Clin Implant Dent Relat Res, 14 (June (3)) (2012), pp. 373-379

[12] Turhani D, Cvikl B, Watzinger E, Weißenböck M, Yerit K, ThurnherD, et al. In vitro growth and differentiation of osteoblast-like cells on hydroxyapatite ceramic granule calcified from red algae. J Oral MaxillofacSurg, 63 (June (6)) (2005), pp. 793-799

[13] Gille J, Dorn B, Kekow J, Bruns J, Behrens B. Bone substitutes as carriers for transforming growth factor-β1 (TGF-β1). IntOrthop, 26 (4) (2002), pp. 203-206

[14] Christian S, Doris M, Alexis S, Georgios L, Else S, Franz K, et al. The fluorohydroxyapatite (FHA) FRIOS® Algipore® is a suitable biomaterial for the reconstruction of severely atrophic human maxillae. Clin Oral Implants Res, 14 (2003), pp. 743-749

[15] Scarano A, Degidi M, Perrotti V, Piattelli A, Iezzi G. Sinus augmentation with phycogene hydroxyapatite: histological and histomorphometrical results after 6 months in humans. A case series. Oral MaxillofacSurg, 16 (March (1)) (2012), pp. 41-45

[16] Demers C, Hamdy CR, Corsi K, Chellat F, Tabrizian M, Yahia L. Natural coral exoskeleton as a bone graft substitute: a review. Biomed Mater Eng, 12 (1) (2002), pp. 15

[17] Comparative study of impact of animal source on physical, structural, and biological properties of bone xenograft Fatemeh Gashtasbi 1, Shahriar Hasannia 1, Sadegh Hasannia 1 2, Mohammad Mahdi Dehghan 3, Farzin Sarkarat 4, Abbas Shali 1 Xenotransplantation. 2020 Jul 12;e12628 doi: 10.1111/xen.12628

[18] Structural characteristics of camel-bone gelatin by demineralization and extraction Hassan A. AL-Kahtani, Irwandi Jaswir, Elsayed A. Ismail, Mohammed Asif Ahmed, Ademola Monsur Hammed, Saeed Olorunnisola & Fitri Octavianti International Journal of Food Properties Saudi Arabia 2017

[19] Xenogeneic bone grafting materialsAuthors: Dr Mike Barbeck, Dr Ronald Unger, Prof. Dr Frank Witte, Prof. Dr Sabine Wenisch & Prof. Dr Dr Reiner Schnettler, Germany 2017

[20] Synthesis and characterization of hydroxyapatite from bovine bone for production of dental implants Jamiu K Odusote1, Y Danyuo2, Abdulazeez D Baruwa1 and Akeem A Azeez Journal of Applied Biomaterials &Functional Materials 2019