A Mixture of Ceramic Biomaterials (Hydroxyapatite and β-Tricalcium Phosphate) and Chitosan as a Scaffold For Critical Sized Defect Bone

by Dian Agustin Wahjuningrum

Submission date: 26-Sep-2022 01:01PM (UTC+0800)
Submission ID: 1909133303
File name: 93-97_CDJ_11-2-2021_1.pdf (672.58K)
Word count: 3705
Character count: 21223

Conservative Dentistry Journal Vol. 11 No. 2 July-December 2021; 93-97

Literature Review

A Mixture of Ceramic Biomaterials (Hydroxyapatite and β-Tricalcium Phosphate) and Chitosan as a Scaffold For Critical Sized Defect Bone

Dian Agustin Wahjuningrum[1], Fery Setiawan[2], Dwikora Novembri Utomo[3], Setyabudi[1], Ria Chusnita[4], Andi Syahrimayani[4], Arif Rahman Nurdianto[5]

[1]Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga Surabaya-Indonesia
[2]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Indonesia
[3]Orthopaedic and Traumatology Department, Faculty of Medicine, Universitas Airlangga Surabaya-Indonesia
[4]Undergraduate student of Faculty of Dental Medicine, Universitas Airlangga, Surabaya-Indonesia
[5]Lecturer at Universitas Anwar Medika, Sidoarjo, Indonesia

ABSTRACT

Background: *Bone is a living tissue that undergoes a continuous regeneration-remodeling process and the second largest organ implanted after the blood transfusion process. Bones can heal completely, but Critical Size Defects (CSD) require graft materials to support the healing process. There are several graft materials, namely: autologous, allogenous, xenograft, and alloplastic material with their respective advantages and disadvantages through the properties: osteogenesis, osteoconduction, osteoinduction, and others. One of the alloplastic materials is Hydroxypatite/HA and β-Tricalcium Phosphate/β-TCP widely used in the grafting process. HA has the disadvantage of having a low degree of solubility, while β-TCP has a high solubility level when exposed to body fluids.* ***Purpose:*** *To explain the mixture of ceramic biomaterials (Hydroxyapatite and β-Tricalcium Phosphate) and Chitosan as a Scaffold for Critical Sized Defect Bone.* ***Reviews:*** *The CSDs are condition where the bone can not heal by itself. It needs bone graft to bridge the heal of CSDs. One of the transplant materials is ceramic biomaterials contains of HA and β-TCP. Each of material has its strengths and weaknesses so that mixture of these ingredients will increase the positive effects and reduce the negative effects of each ingredient. CSD healing requires the suitable scaffold and biopolymer that can help in healing process.* ***Conclusion:*** *CSD healing requires a scaffold that mimics cancellous bone in the healing process of bone defects played by the mixture of BCP as a bioceramic material and chitosan as a natural biopolymer with low toxicity and high biocompatibility.*

Keywords: *Critical Sized Defects; Hydroxyapatite; β-Tri Calcium Phosphate; Chitosan; Bipashic Calcium Phosphate*

Correspondence: Setyabudi, Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Jl. Mayjen Prof. Dr. Moestopo No 47, Surabaya 60132, Indonesia. E-mail: setyabudi@fkg.unair.ac.id

INTRODUCTION

Bone grafting is a surgical method commonly used to augment bone regeneration in orthopedic treatment procedures. According to statistical data, more than two million bone grafting procedures are performed per year[1,2], making bone the second largest tissue transplant after blood transfusion. Bone grafting materials consist of: autologous bone transplant (bone transplant taken from the individual itself), allogenous bone transplant (bone transplant taken from different individuals but within the same species), xenograft bone transplant (bone transplant taken from different individuals and species), and alloplastic materials. Among the four types of bone grafting materials, autologous bone transplant is called the gold standard bone transplant because it has several properties related to the process of bone regeneration in the defect area (osteoconduction, osteoinduction, and osteogenesis).[2,3]

There are several obstacles that limit the use of autologous bone transplant, which include: limited supply of bone transplant material, complications that occur at the surgical site after the bone graft removal process, and postoperative pain. Allogenous and xenograft bone transplants are the dominant choice after autologous bone graft because they are abundant and available in various forms. However, despite their abundance and availability, both types of bone transplant material have the potential to transmit disease, are agents of infectious disease.[2,4,5]

Physiologically, bone, as the most resilient tissue[3], can heal completely without leaving scar tissue, but if Critical-Sized Defects (CSDs) occur, it requires the help of bone graft material to bridge the bone gap. before bone

regeneration can be achieved. The factors that determine CSDs are shown in Table 1[4,5].

CSDs are a condition of bone defects that are the most difficult to treat in orthopedic surgery because CSDs will not heal without secondary intervention (surgery)[7]. CSDs occur when the bone defect is larger than 1-2 centimeters in size[8], bone loss is more than 50% of the bone circumference, and the defect is close to 2.5 times the bone diameter due to acute trauma, developmental deformity, non-union cases. chronic, oncological surgery after bone resection, or cases of bone infection/osteomyelitis[2,6,9,10].

To overcome and answers some of the problems and limitations of using autologous, allogenous, and xenograft bone transplants, current attention is paid to bone grafts and substitutes (BGS) and surgical procedures using BGS. One of the BGS materials is biodegradable bioceramic (Hydroxyapatite/HA and -Tricalcium Phosphate (β-TCP)) and natural biodegradable polymer materials. The use of biodegradable BGS is a second generation biomaterial associated with repair of bone defects and is widely used in bone tissue engineering because of its biodegradability. This material will be the main focus of our article.

REVIEW(S)

Healing of bone defects, in general, occurs through two pathways, namely: primary/direct and secondary/indirect.

Tabel 1. Defining Factor Critical-Sized Defects (CSDs)[6]

Defining Factor Critical-Sized Defects (CSDs)
Age
Size of the defect in bone
Anatomical location of the defect,
Defective bone structure and vasculature,
Systemic and metabolic conditions,
Method of healing the defect (primary or secondary),
Adequate supply of nutrients

The basic principle that determines the healing of bone fractures primarily/directly and secondary/indirectly is the immobilization of the fractured bone and the presence or absence of callus formation in the healing process of the bone defect which will be shown in Figure 1. Primary pathway bone healing mainly occurs in gaps. fractures with a size of less than 0.1 mm and fixation occurs completely so that the bone gap is filled directly by the ossification process with no cartilage or callus formation[2]. The Intramembranous Ossification (IMO) stage of healing induces several stages, namely: the process of differentiation of Mesenchymal Stem Cells (MSCs) into osteoblasts associated with the participation of Runx2 or osterix, followed by the formation of an ossification center, osteoid calcification, formation of woven bone around the periosteal tissue. , compact and spongy bone formation, and woven bone replacement[4,11].

Endochondral ossification consists of several stages, namely: condensation and differentiation of Mesenchymal Stem Cells (MSCs) into chondrocytes caused by the participation of Sox9, hypertrophy of chondrocytes, calcification process, degradation by matrix, formation of primary ossification center, and continued secondary ossification center, then maturation. ossification center, and mature bone formation occurs. Bone can undergo the process of ossification through intramembranous ossification, endochronal ossification, and a combination of intramembranous and endochondral ossification. The main difference between the two processes of ossification is in the presence or absence of a cartilaginous phase. Intramembranous ossification occurs when mesenchymal precursor cells proliferate and differentiate directly within osteoblasts, but in the endochondral ossification phase mesenchymal cells in the first stage differentiate into chondrocytes and secrete cartilage matrix. The endochondral ossification process has better biomechanical properties compared to bone formed through the intramembranous ossification process because in the

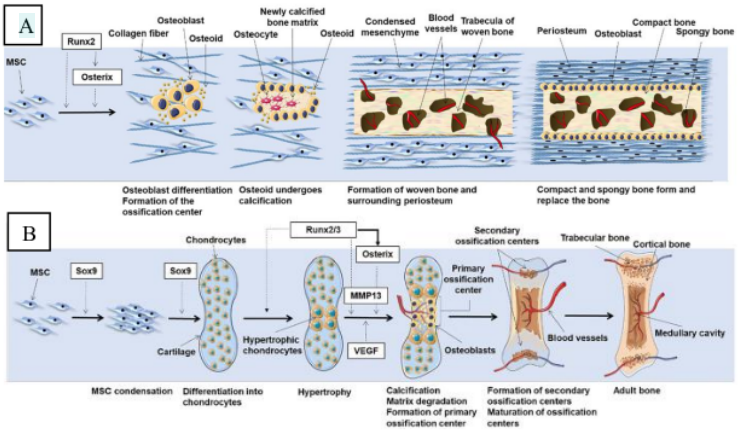

Figure 1. The healing process of bone defects. A. The healing process through the intramembranous pathway (IMO), B. The healing process through the endochondral pathway[10].

endochondral ossification process, there is the manufacture of the cartilage matrix first and then the calcification process begins to occur[2,4,10,11].

Bone grafting materials consist of: autologous bone transplant (bone transplant taken from the individual itself), allogenous bone transplant (bone transplant taken from different individuals but within the same species), xenograft bone transplant (bone transplant taken from different individuals and species), and alloplastic materials. Among the four types of bone grafting materials, autologous bone transplant is called the gold standard bone transplant because it has several properties related to the process of bone regeneration in the defect area (osteoconduction, osteoinduction, and osteogenesis). Although it is the gold standard of bone transplant, there are several obstacles that limit the use of autologous bone transplant, which include: limited supply of bone transplant material, complications that occur at the surgical site after the bone graft removal process, and postoperative pain. Allogenous and xenograft bone transplants are the dominant choice after autologous bone graft because they are abundant and available in various forms. However, despite their abundance and availability, both types of bone transplant material have the potential to transmit disease, are agents of infectious disease (Human Immunodeficiency Virus/HIV in 2 cases since 1989 and an estimated risk prevalence of 1:1.6 million, Hepatitis BC), and are susceptible to infection-rejection process[12,13].

To overcome and answers some of the problems and limitations of using autologous, allogenous, and xenograft bone transplants, current attention is paid to bone grafts and substitutes (BGS) and surgical procedures using BGS. One of the BGS materials is biodegradable bioceramic (Hydroxyapatite/HA and β-Tricalcium Phosphate (β-TCP)) and natural biodegradable polymer materials. The use of biodegradable BGS is a second generation biomaterial associated with repair of bone defects and is widely used in bone tissue engineering because of its biodegradability.

Hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$, is one of the minerals forming calcium apatite, composes 50% of bone weight, and is the main mineral component in teeth and bones so that HA has high biocompatibility and does not trigger the inflammatory system. The properties of HA are osteoconductive, osteointegrative, and have good mechanical properties with compression resistance up to 160MPa. The ratio of Ca/P in HA is 1:1.67[14]. The disadvantage of using HA is related to its low solubility. β-TCP, $Ca_3(PO_4)_2$, has been used as BGS for more than 25 years and is categorized as the "gold standard" material for bone synthesis. The properties of β-TCP are: it is biocompatible, bioresorbable with the same properties as the inorganic phase of bone, and accelerates the bone remodeling process by facilitating colonization of osteogenic cells and has an important effect on angiogenesis[13]. The Ca/P ratio in β-TCP is 1:1.5 which is lower than the Ca/P ratio in HA[2]. However, the weakness of β-TCP lies in its high degree of solubility when in contact with body fluids and is prone to fracture because it has a low compressive strength value of 140 MPa. Therefore, the use of β-TCP is never alone and is always combined with other biodegradable ceramics (HA)[15,16].

In addition to requiring graft materials, the healing process of CSD also requires a scaffold that mimics cancellous bone and the interaction between mineral and organic components of the extracellular matrix. Cancellous bone is a key structural support in receiving structural loads of about 75% of the total body weight. Scaffold with a porous structure in it is associated with supporting processes for tissue interlocking, cell migration, nutrient transport, osseointegration processes which will later be replaced by new host tissues[17]. The extracellular matrix of bone is defined as a dynamic network composed of constituents of organic and inorganic materials (including: collagen, elastin, polysaccharides, and CaP nanocrystals). The extracellular matrix provides a template for the process of cell attachment and proliferation, controlling cell properties by regulating proliferation and differentiation processes through growth factors and cytokines that are soluble and stored in the extracellular matrix of bone[3].

DISCUSSION

Figure 2 shows about the main discussion of this article. It begins from the defects that defects can occur in bone in the form of defects that are Critical-Sized Defects (CSDs) and not CSDs. In non-CSD defects, the healing process will proceed well without scarring, but if CSDs occur, bone grafting materials and scaffolds are needed to play a role in the CSDs healing process. There are four types of bone graft materials used for the healing process of CSDs, namely: autograft, allograft, xenograft, and alloplastic materials, with each material having advantages and disadvantages. Autograft material is the goal standard as bone graft material because it is taken from the same person and species so that the rejection process is very minimal as a side effect of bone grafting. However, autograft poses several problems, namely: the availability-abundance of bone graft material taken and causes several post-operative conditions which can be: postoperative pain and death at the donor site of bone graft material.

To overcome the shortcomings due to the use of autografts, a grafting procedure can be carried out using allograft and xenograft-based bone graft materials, with different allograft bone graft materials taken from individuals of the same species but from different people, while for xenograft bone graft materials taken from different species. Allograft and xenograft materials have limited use due to the potential for disease transmission and are prone to rejection by the activation of the major histocompatibility complex (MHC). The initial osteoinduction process by allograft and xenograft can trigger inflammatory cells so that inflammatory cells quickly surround the allograft and xenograft area causing necrosis of osteoprogenitor cells.

Another effort used to overcome the shortage of grafting materials is autograft, allograft, and xenograft by using alloplastic material (Bone Graft Synthetic/BGS). One of the

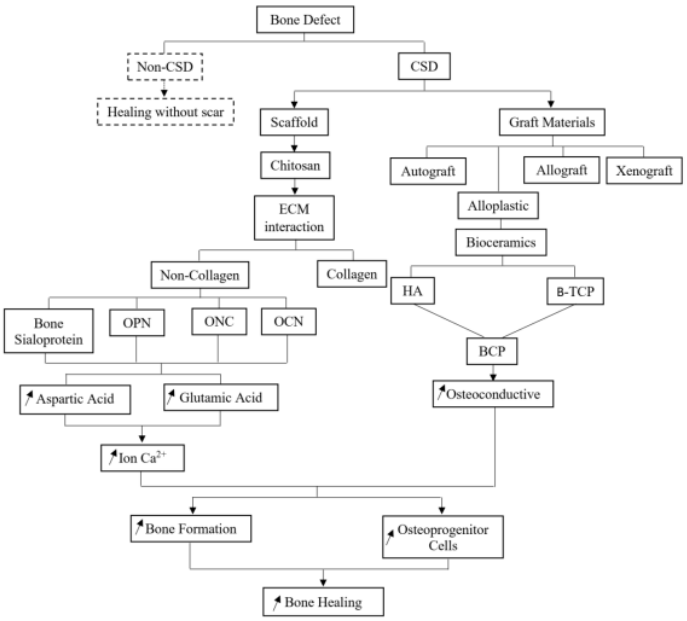

Figure 2. Concept Mapping script discussion.

materials that is the focus of writing this review article is BGS which is derived from degradable bioceramics, namely: Hydroxyapatite (HA) and β-Tricalcium Phosphate (β-TCP). HA is a calcium apatite-forming material and composes about 50% by weight of bone with good osteoinductive and osteointegrative properties. The ratio of Ca/P ratio in HA = 1:1.67 so that HA has the same mechanical properties as cancellous bone. The weakness of HA is the very low level of solubility when implanted as bone graft material on bone with a solubility percentage of 5.4% after being implanted in bone for a period of six months. β-TCP is another biodegradable class of bioceramics which has a Ca/P ratio of 1:1.5 which is below the Ca/P ratio of HA so that the mechanical property value of β-TCP is lower than that of HA. In contrast to HA which has a low degree of solubility, β-TCP has a high degree of solubility after implantation in bone with a percentage of 85.4% solubility when in contact with body fluids. β-TCP accelerates the process of bone remodeling by facilitating colonization of osteogenic cells. nutrition through increased capillary blood vessels and the potential for angiogenesis. However, due to its brittle nature, β-TCP cannot be used alone and must be combined with HA[13]. The combination of HA and β-TCP (Biphasic Calcium Phosphate (BCP)) has a high value of osteoconductivity to stimulate bone formation and osteoprogenitor cells.

In addition to bone graft material, the healing process of CSD also requires scaffold material for the interaction process with the extracellular matrix. One of the materials used as scaffold is chitosan. The bioactive activity of chitosan is characterized by the presence of osteoconductive, osteoproductive, and osteoinductive properties which are characterized by increased adhesion, proliferation, differentiation and mineralization of osteoblasts. There is a secretion of several growth factors, namely: Bone Morphogenetic Proteins-2 and 7 (BMP-2 and BMP-7) which play an important role in the bone healing process where BMP is produced by osteoblasts so that it is involved in the process of skeletonization and ectopic bone formation. BMP plays a role in the recruitment of osteoprogenitor cells in areas where bone formation occurs. Composite Chitosan with Hydroxyapatite is also able to induce the differentiation of osteoblast cells because it is associated with the induction of Mesenchymal Stem Cells (MSCs) which are able to support the bone regeneration process. In addition, chitosan also has antibacterial activity related to the cationic property of chitosan[17-21].

The extracellular matrix provides a template for the process of cell attachment and proliferation, controlling cell properties by regulating proliferation and differentiation processes through growth factors and cytokines that are soluble and stored in the extracellular matrix of bone. The extracellular matrix contains two main types of proteins, namely: collagenous proteins and non-collagenous proteins (NCPs). Collagen proteins, especially type I collagen, are the most abundant proteins in bone tissue that play an important role in interactions with NCPs. Type I collagen can initiate the growth process of carbonated apatite minerals and affect the structural characteristics and control the three-dimensional distribution of apatite[3,22].

NCP consists of: Bone sialoprotein (BSP), osteonectin (ONC), osteopontin (OPN), and osteocalcin (OCN) which

are the main structures of NCP found in the extracellular matrix of bone. The majority of NSP contains aspartic acid (Asp) and glutamic acid (Glu) which have high affinity for calcium ions (Ca^{2+}). BSP is a phosphoprotein containing several segments of poly-glutamic acid so that it is associated with binding to apatite. Osteonectin is a glycoprotein rich in cysteine with high levels in bone tissue. Osteopontin is an amino acid protein that is negative and able to bind to HA. In addition, osteopontin is also an important regulator of osteoclast activity. Osteocalcin is an NCP protein in the extracellular matrix synthesized by osteoblasts so that it has a high affinity for HA. Osteocalcin controls the process of bone formation. Increased differentiation of osteoblasts will be associated with increased formation and healing of bone defects because osteoblasts are the main matrix for bone formation[3]. In conclusion, CSD healing requires a scaffold that mimics cancellous bone in the healing process of bone defects played by the mixture of BCP as a bioceramic material and chitosan as a natural biopolymer with low toxicity and high biocompatibility.

ACKNOWLEDGEMENT

The authors thank the Ministry of Research, Technology, and Higher Eduation, Indonesia, for financing this study.

REFERENCES

1. Campana V, Milano G, Pagano E, Barba M, Cicione C, Salonna G, Lattanzi W, Logroscino G. Bone substitutes in orthopaedic surgery: from basic science to clinical practice. J Mater Sci: Mater Med. 2014;25: 2445-61; DOI: 10.1007/s10856-014-5240-2
2. Wang W, Yeung KWK. Bone grafts and biomaterials substitutes for bone defect repair: A review. Bioactive Materials. 2017;2:224-47; http://dx.doi.org/10.1016/j.bioacmate.2017.05.007
3. Farbod K, Nejadnik R, Jansen JA, Leeuweburgh SCG. Interactions Between Inorganic and Organic Phases in Bone Tissue as a Source of Inspiration for Design of Novel Nanocomposites. Tissue Engineering Part B. 2014; 20(2):173-88
4. Kalfas IH. Principles of bone healing. Neurosurg Focus. 2001; 10(4): 1-4
5. Oryan A, Monazzah S, Sadegh AB. Bone injury and Fracture Healing Biology. Biomed Environ Sci. 2015; 28(1): 57-71, doi: 10.3967/bes2015.006
6. Reichert JC. Tissue Engineering Bone – Reconstruction of Critical Sized Segmental Bone Defects in a Large Animal Model. Thesis: Queensland University of Technology. 2010
7. Bezstarosti H, Metsemakers WJ, van Lieshout EMM, Voskamp LW, Kortram K, McNally MA, Marais LC, Verhofstad MHJ. Management of critical-sized bone defects in the treatment of fracture-related infection: a systematic review and pooled analysis. Archives of Orthopaedic and Trauma Surgery. 2020:1-16; https://doi.org/10.1007/s00402-020-03525-0
8. Nauth A, Schemitsch E, Norris B, Nollin Z, Watson JTW. Critical-Size Bone Defects: Is There a Consensus for Diagnosis and Treatment? J Orthop Trauma. 2018; 32(3): S7-11
9. Tarchala M, Engel V, Barralet J, Harvey EJ. A pilot study: Alternative biomaterials in critical sized bone defect treatment. Injury Int J Care Injured. 2017; 2017:1-9; https://doi.org/10.1016/j.injury.2017.11.007
10. Wei S, Ma JX, Xu L, Gu XS, Ma XL. Biodegradable materials for bone defect repair. Military Medical Research. 2020; 7(54):1-25; https://doi.org/10.1186/s40779-020-00280-6
11. Kenkre JS, Bassett JHD. The bone demodelling cycle. Annasl of Clin Biochem. 2018; 55(3): 308-27, DOI: 10.1177/000456.3218759371
12. Venkataraman N, Bansal S, Bansal P, Narayan S. Dynamics of bone graft healing around implants. J Int Clin Dent Res Organ. 2015; 7: 40-7
13. De Grado GF, Keller L, Gillet YI, Wagner Q, Musset AM, Jessel NB, Bornert F, Offner D. Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management. Journal of Tissue Engineering. 2018; 9: 1-18; DOI: 10.1177/2041731418776819
14. Kuffner BHB, Facci AD, Sachs D, Silva G. Study of the microstructure and mechanical properties of beta tricalcium phosphate-based composites with alumina addition produced by powder metallurgy. REM. 2017; 70(4):459-64; http://dx.org/10.1590/0370-44672017700082
15. Chicot D, Tricoteaux A, Lesage J, Leriche A, Descamps M, Constantin ER. Mechanical Properties of Porosity-Free Beta Tricalcium Phosphate (β-TCP) Ceramic by Sharp and Spherical Indentations. New J of Glass and Ceramics. 2013; 3:16-28
16. Aguilar CR, Pinto UO, Reyes EAA, Juarez RL, Alfonso I. Characterization of β-tricalcium phosphate powders synthetized by sol-gel and mechanosynthesis. Boletin De La Sociedad Espanola De Ceramica Y Vidrio. 2018; 2018:213-20; https://doi.org/10.1016/j.bsecv.2018.04.004
17. Saad MPM, Prakoso AT, Sulong MA, Basri H, Wahjuningrum DA. Impacts of dynamic degradation on the morphological and mechanical characterisation of porous magnesium scaffold. Biomechanic and Modeling in Mechanobiology. 2019; 18:797-811; https://doi.org/10.1007/s10237-018-01115-z
18. Mooduto L, Wahjuningrum DA, Agatha PA, Lunardhi GJ. Antibacterial effect of chitosan from squid pens against Porphyromonas gingivalis bacteria. IJM. 2019; 11(2): 177-80
19. Wahjuningrum DA, Subijanto A, Taqiya AK, Anggini FD, Hasib A, Mooduto L, Puteri FH. Cytotoxicity of Chitosan Derived from Shrimp for Bone Scaffold on Adipose Tissue-Derived Mesenchymal Stem Cells. APJMT. 2019; 8(4): 115-7
20. Basri H, Prakoso AT, Sulong MA, Saad AMM, Ramlee MH, Wahjuningrum DA, Sipaun S, Ochsner A, Syahrom A. Mechanical degradation model of porous magnesium scaffolds under dynamic immersion. J Materials: Design and Application. 2019; 0(0):1-11 DOI: 10.1177/1464420719881736
21. Islam MM, Shahruzzaman M, Biswas S, Sakib MN, Rashid TU. Chitosan based bioactive materials in tissue engineering applications-A review. Bioactive Materials. 2020; 5:164-83
22. Hasib A, Wahjuningrum DA, Ibrahim MHR, Kurniawan HJ, Ernawati R, Hadinoto MEK, Mooduto L. ALP (Alkaline Phosphatase) Expression in Simple Fracture Incident in Rat (*Rattus Norvegicus*) Femur Bone Supplemented by *Apis Mellifera* Honey. JIDMR. 2020; 13(3):887-91

A Mixture of Ceramic Biomaterials (Hydroxyapatite and β-Tricalcium Phosphate) and Chitosan as a Scaffold For Critical Sized Defect Bone

ORIGINALITY REPORT

15%	7%	13%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	mmrjournal.biomedcentral.com Internet Source	3%
2	Gabriel Fernandez de Grado, Laetitia Keller, Ysia Idoux-Gillet, Quentin Wagner et al. "Bone substitutes: a review of their characteristics, clinical use, and perspectives for large bone defects management", Journal of Tissue Engineering, 2018 Publication	2%
3	"Handbook of Bioceramics and Biocomposites", Springer Science + Business Media, 2016 Publication	2%
4	mafiadoc.com Internet Source	2%
5	Dawei Zhang, Xiaowei Wu, Jingdi Chen, Kaili Lin. "The development of collagen based composite scaffolds for bone regeneration", Bioactive Materials, 2018	1%

	Publication	
6	G T Mulyati, M Maksum, B Purwantana, M Ainuri. "The workload of rice harvester in Java Indonesia", IOP Conference Series: Earth and Environmental Science, 2020 Publication	1 %
7	Wenhao Wang, Kelvin W.K. Yeung. "Bone grafts and biomaterials substitutes for bone defect repair: Areview", Bioactive Materials, 2017 Publication	1 %
8	"Tissue Engineering and Regenerative Medicine", Journal of Tissue Engineering and Regenerative Medicine, 2012. Publication	1 %
9	mts.intechopen.com Internet Source	1 %
10	www.pubfacts.com Internet Source	1 %
11	rjme.ro Internet Source	1 %
12	wrap.warwick.ac.uk Internet Source	1 %
13	patentimages.storage.googleapis.com Internet Source	<1 %

14	P. De Falco, E. Barbieri, N. Pugno, H. S. Gupta. "Staggered Fibrils and Damageable Interfaces Lead Concurrently and Independently to Hysteretic Energy Absorption and Inhomogeneous Strain Fields in Cyclically Loaded Antler Bone", ACS Biomaterials Science & Engineering, 2017 Publication	<1 %
15	Wagner, Wilfried, JÃ¶rg Wiltfang, Hans Pistner, Murat Yildirim, Bettina Ploder, Miranda Chapman, Nina Schiestl, and Edith Hantak. "Bone formation with a biphasic calcium phosphate combined with fibrin sealant in maxillary sinus floor elevation for delayed dental implant", Clinical Oral Implants Research, 2012. Publication	<1 %
16	www.mdpi.com Internet Source	<1 %

Exclude quotes	Off	Exclude matches	< 10 words
Exclude bibliography	On		

A Mixture of Ceramic Biomaterials (Hydroxyapatite and β-Tricalcium Phosphate) and Chitosan as a Scaffold For Critical Sized Defect Bone

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5