In vitro and in vivo examination of the osteogenic ability of 3D scaffold chitosan-hydroxyapatite: a systematic review

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ABSTRACT
Background: Alveolar bone resorption in dentistry cases can be caused by several factors. Some of which are periodontal disease, post-tooth extraction trauma, post enucleation cyst, and post-tumor surgery. The idea of bone tissue engineering, especially biomaterials will be focused on precise scaffold design in terms of physicochemical in cell adhesion, proliferation, differentiation, and specific organ tissue formation. Chitosan can be combined with hydroxyapatite (HA) in form of scaffold 3D design for bone remodeling procedure. **Purpose**: discover osteogenic ability with scaffold 3D chitosan-HA in vitro and in vivo. **Method**: utilizing literature review by collecting, compiling similarities, and concluding references related to osteogenic ability with scaffold 3D chitosan-HA in vitro and in vivo on bone remodelling process. **Results**: based on journal research with keywords of 3D scaffold, chitosan, HA, bone engineering, in vitro and in vivo, a total of 15 articles were used as references. **Conclusion**: Scaffold integrates bone tissue and provide effective room for new bone formation. Scaffold 3D (combination of chitosan and collagen) plays significant part in bone regeneration and becomes natural polymer containing ion complex as to maximalize characteristics of osteoconductivity contained. Scaffold chitosan/hydroaxipatite possesses osteogenic ability integral to repair bone fracture.
Keywords: 3D scaffold, chitosan, hydroxyapatite, bone engineering, in vivo and in vitro

INTRODUCTION

Alveolar bone fracture in dentistry can be caused by several factors, for instance periodontal disease, post-tooth extraction trauma, post enucleation cyst, and post-tumor surgery. If periodontal disease is not immediately handled, fracture in bone tissue will ensue, leading to tooth loss. With the innovation of bine tissue engineering, biomaterials will be focused on the proper design of *scaffold* in terms of *physicochemical* in cell adhesion, proliferation, differentiation, and specific organ tissue formation.[1]

Scaffold is a media or structure with function to build and help stem cell perform adhesion, proliferation, and differentiation that eventually creates tissue aimed to be replicated.[2] *Scaffold* must be properly formed as to carry correct characteristics and function well. In fact, surface of *Scaffold* should contain suitable morphology for both cell adhesion and cell differentiation. Selection of precise biomaterials to match extracellular matrix of replaced tissue is integral because characteristics of biomaterials will influence growth of stem cell.[3] Requirement of ideal *scaffold* creation involves characteristics of osteoconductive, osteogenic, *biodegradable,* good microstructure as well as precise mechachanic. Also, the most vital requirement is the ability to stimulate cell adhesion and maintain tissue function.[4]

Chitosan is a material commonly used as *graft* material which is less osteoconductive if not combined with other materials. *Chitosan* can be combined with collagen in form of *scaffold 3D* design for bone remodelling. *Chitosan* is an amino polysaccharide (poly-1,4-D-glucosamine) which is widely used as polymer of tissue engineering. *Chitosan* offers numerous advantages in terms of functionality due to its high biocompatibility characteristic, *biodegradable,* and low toxicity. *Chitosan* is chitin derivative obtained through deacetylation process.[5]

Scaffold with singular organic as basic material of *scaffold* is not osteoconductive enough and not quite fulfilling for mechanism characteristic necessary for *scaffold*.[6] Therefore, in creation of *scaffold,* additional materials are necessary for making *scaffold* more efficient in its use.[7] HA has over time focused on the continual development due to its good biocompatibility as well as strong bond with biopolymer and has been proven as biocompatible. Plus, it is well absorbed by tissues in human mouth. In addition, HA also possesses osteoconductive ability and has proven to be able to stimulate osteoblast differentiation growth and bone formation. Positive characteristic of this biomaterials is well-known in dentistry, for instance bone *remodelling,* periodontal defect treatment, tooth implant layering, repair material fills including ceramic resin and glass ionomer powder. *Scaffold 3D Chitosan* functions in the process of bone recovery process because both materials form complex ionic comp-

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ound that can enhance osteoconductivity characteristics.[1] The enhancement of *callus* formation in cracks with *scaffold* containing HA and pyrophosphate compared to *scaffold* control without pyrophosphate and HA or merely pyrophosphate.[9]

HA is a primary inorganic component of a bone, and commonly used as material for bone regeneration process. HA contains good characteristics, for instance non-toxic, non-immunonegic, non-inflammatory, biocompatibility, osteioconduction as well as good bioavailability on bone tissues and cells due to sharing similarities with bone tissue.[10,11] HA possesses the ability in good bond formation on host compared to other bone substitutes. However, due to its slow degradation progress and fragility, it can be modified by *collagen* and *chitosan* as to help repair bone.

This systematic review is aimed to discuss osteogenic ability with scaffold 3D chitosan-HA in vitro and in vivo.

METHODS

Source of article search utilized *Google Scholar* data base, *Pubmed,* and *Science Direct* by searching keywords, for instance 3D *scaffold*, *chitosan*, *hydroxyapatite*, *bone engineering*, in vitro, in vivo. Search was limited to Indonesian and English articles, with year range of article publication for the last 10 years. *In vitro* and *in vivo* laboratory research involving animal test will be included in the *review.* Only articles discussing about osteogenic ability with *scaffold 3D chitosan*-HA will be included in the *review.* A total of 326 articles were found and a total of 15 articles were selected after the author read throughout the article content based on the most relevant topic in line with inclusion and exclusion criteria.

RESULTS

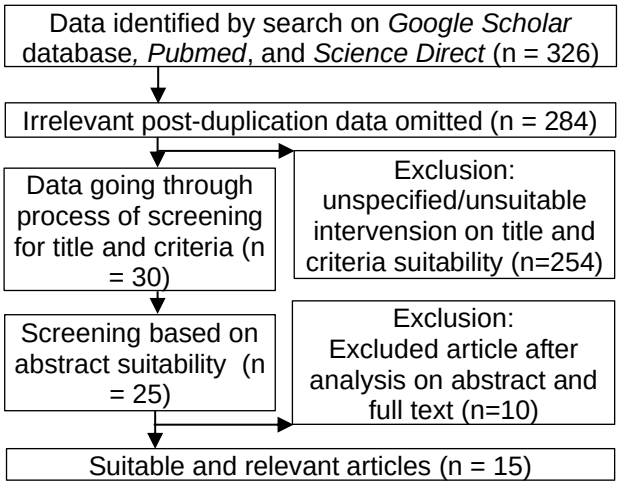

Figure 1 Prisma flow diagram

The PRISMA flowchart (Fig.1) shows the process of searching for articles according to the inclusion and exclusion criteria so that were obtained 15 articles that match the keywords (table 1).

DISCUSSION

The innovation of bone tissue engineering is focused on biomaterials applications that are being developed. One of which is *scaffold. Scaffold* in form of 3D with porous structure suitable for cell adhesion, proliferation, differentiation, and specific tissue formation.[15] Biomaterials that can be created in form of *scaffold* are *chitosan* and HA. *Chitosan* and *collagen* are polymers with characteristics of biodegradable and good biocompatible as well as low toxicity. Therefore, it is suitable to be combined in form of *scaffold* because precise *scaffold* requires those characteristics.[16]

Chitosan is a natural polysaccharide that resembles glycosaminoglycan, especially poly-D-glucosamine in terms of structure. On connective tissue, glycosaminoglycan is the mostly discovered component in extracellular matrix or so-called ECM.[17] *chitosan* is created from deacetylation of chitin by removing acetyl group, leading to create a cationic *chitosan.*[18] The higher the level of deacetylation of chitin is, the greater the cationic characteristic will be. In fact, the higher the level of deacetylation, the lower the biodegradable ability will be.[19] *Chitosan* with positive charge can form ion complex bond by bonding with negative charged materials such as collagen. The cationic characteristic on *chitosan* can stimulate cell adhesion, acting as cell morphology modulator, differentiation, cell movement, synthethic, and cell function.[20] *Chitosan* is generally known to be able to stimulalate growth and differentiation of osteoblast on cell culture. *Chitosan* can be created in any forms and utilized in broad medical aspects for medication, for instance wound healing, gum connective tissue regeneration, and a *scaffold* to regenerate both soft and hard tissue.[21]

On *in vitro research,* obtained effects of *chitosan* on bone formation has achieved cellular level that can affect the enhancement of osteoprogenitor cell differentiation and stimulate new bone formation.[22] Currently, *chitosan* has become one of the most superb materials to research, especially as a medium of bone substitute with multiple characteristics as expected, leading to the creation of precise *scaffold.* However, the idea of *scaffold* as singular material using organic materials is less sustainable to fulfill all criteria necessary for the success of bone tissue regeneration. *Chitosan it-*

Table 1 Characteristics of the articles

Research Title	Author	Subjects & Methods	Variables	Research Results
Osteogenetic properties of electrospun nanofibrous PCL scaffolds equipped with chitosan-based nanoreservoirs of growth factors	Ferrand et al., 2013.	Mouse in vitro - in vivo	Chitosan=bone engineering; regeneration; scaffold; periodontitis	No citotoxicity, biomineralization enhancement, and osteogenetic enhancement. Scaffolds combined with chitosan and BMP-2 shown in vitro and in vivo in calvaria mouse had osteogenetic ability through osteopontin gene expression enhancement and calcium phosphate biomineralization.
Chitosan/HA scaffolds for tissue engineering manufacturing method effect comparison	Sierra et al.,2015	Mouse in vitro - in vivo	chitosan, HA in situ=bone and tissue engineering	Scaffolds showed the best structural integrity and porosity was scaffolds CH//HAis. Scaffolds was recommended material for tissue engineering and bone regeneration.
Graphene oxide enhances chitosan-based 3D scaffold properties for bone tissue engineering	Dinescu et al., 2019	Mouse in vitro - in vivo	Scaffolds chitosan/HA, graphene oxide	Scaffolds chitosan proved to support ostogenic hASC in vitro differenferentiation for 28 days as well as bone repair in vivo on mouse model for 18 weeks. Scaffolds chitosan improved to discover indicator degree on the highest osteogenic both in vitro and in vivo. Therefore, it can be considered the most recommended solution for bone tissue engineering
Evaluation of in vitro and in vivo osteogegenic differentiation of nanoHA/chitosan/poly(lactide-co-glycolide) scaffolds with human umbilical cord mesenchymal stem cells (hUCMSCs)	Wang et al., 2013	Mouse in vitro - in vivo	hUCMSCs, bone marrow mesenchymal stem cells, poly(lactide-co-glycolide) (PLGA), nHA, chitosan = bone tissue engineering	Scaffolds showed mechanism power of scaffolds nHA/chitosan/PLGA can be enhanced. Next, scaffolds nHA/chitosan/PLGA was the most suitable solution for adhesion, proliferation, and differentiation osteogenic hUCMSCs in vitro
Multi-compartment scaffold fabricated via 3D-printing as in vitro co-culture osteogenic model	Giglio et al., 2018	Mouse in vitro	Scaffolds = bone tissue engineering	In vitro 3D model can become insight on bone regeneration to boost experiments in clinic as well as reduce cost to minimalize duration
In vitro and In vivo investigation of osteogenic properties of self-contained phosphate-releasing injectable purine-crosslinked chitosan-HA constructs	Jahan et al., 2020	Mouse in vitro - in vivo	scaffold, osteogenic, chitosan-HA	Results showed enhancement on callus formation in fracture with scaffold bearing both HA and pyrophosphatase or only pyrophosphatase. This result proved that pyrophosphatase-Scaffolds composite HA had the capacity to facilitate fractured bone.
Bone remodeling using a 3D chitosan-HA scaffold seeded with hypoxic conditioned human amnion mesenchymal stem cells	Kamadjaja., 2021	Mouse in vivo	bone tissue engineering; chitosan-HA scaffold; human amniotic mesenchymal stem cells	Toxicity test that combined the use of MTT assay showed that scaffolds CH-HA was 79.42%. also, cells could adhere themselves to surface of scaffolds CH-HA planted in calvaria defect bones.
Study on antibacterial of chitosan/HA doped magnesium composite as a material for bone graft applications	Laksono et al., 2019	in vitro	chitosan, komposit, HA-magnesium, bakteri *E.coli*,	Addition of chitosan influenced chitosan-HA- magnesium composite morphology, showing that composite with added chitosan formed granule with hard surface capable of enhancing biocompatibility.
Enhanced biomineralization and protein adsorption capacity of 3D chitosan/HA biomimetic scaffolds applied for bone-tissue engineering	Nga et al., 2020	Chicken egg shell in vitro	chitosan/HA, scaffolds	scaffolds chitosan-HA showed suitable pores, swelling ratio, pull force, and biodegradation in bone scaffolds. Scaffolds chitosan/HA displayed biomineralization and absorption ability better than in vitro.

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Table 1 ……continuing

In vitro evaluation of HA, chitosan, and carbon nanotube composite biomaterial to support bone healing	Paretris et al., 2021	Mouse in vitro	HA, chitosan, carbon nanotube composite biomaterial, vero cells, MSCs	This biomaterials composite can be used with MSC. biomaterials composite HA, chitosan, and carbon nanotube are not cytotoxic. Therefore, they are applicable for in vivo test
Chitosan and HA scaffolds with amoxicillin for bone repair	Ponciano et al., 2021	Mouse in vitro	Chitosan, HA, scaffolds, amoxicillin	Scaffolds resulted from this research not only has physical characterteristics, but also chemical and biological. Scaffolds with HA 30% exhibiting the best result for cells in vitro survival. Test of viability proved that fibroblast could grow and proliferate on scaffolds. This also proved that biomaterials were non-toxic and biologically acceptable as scaffolds.
In vivo bone regeneration induced by a scaffold of chitosan/dicarboxylic acid seeded with human periodontal ligament cells	Sukpaita et al., 2019	Mouse in vivo	chitosan, scaffold, periodontal ligament cells	scaffolds chitosan/DA stimulates bone formation in vivo. Scaffolds with or without top primary hPDLC could enhance bone tissue repair on mouse calvarial defect. Scaffolds chitosan/dicarboxylic acid could function as host or itself to repair bone defect, and it is suitable for bone tissue engineering.
Indirect 3D printing technology for the fabrication of customised β-TCP/chitosan scaffold with the shape of rabbit radial head—an in vitro study	Wang et al., 2019	Rabbit in vitro	β-TCP, Chitosan, scaffolds	Scaffold β-TCP/chitosan had good function and degradation level, and in vitro cell test also confirmed that scaffold had sustainable cytocompatibility and bioactivity
Chitosan/HA composite coatings on porous Ti6Al4V titanium implants: in vitro and in vivo studies	Zhang., 2020	Mouse in vitro - in vivo	Chitosan/HA, titanium implants	Experiment in vitro showed that porous Ti-implant had no biological toxicity. In vivo test result also showed that porous tissue was beneficial because bone tissue could grow into porous. So, this exhibited good osseointegration. Porous Ti with chitosan/HA layer could enhance cell proliferation and differentiation MC3T3-E1 and osseointegration in vivo
Comparative study of porous HA/chitosan and whitlockite/chitosan scaffolds for bone regeneration in calvarial defects	Zhao et al., 2017	Mouse in vitro	HA, whitlockite, chitosan	Membrane composite whitlockite/chitosan had better biocompability, enhance human MSCs osteogenic proliferation and differentiation ability compared to HA/Chitosan. In fact, porous scaffold whitlockite/chitosan could significantly boost bone regeneration on calvarial defect.

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itself is nearly not osteoconductive enough. Therefore, the ability to form new bone is not yet optimal. An approach to handle to answer such hindrance is to design a composite by combining forces of different materials so that weaknesses of two different materials can be minimalized.[6] One of organic materials that also plays a great deal in tissue engineering is collagen.[23] Collagen possesses anionic characteristics or negative charged. A combination of *chitosan* (cationic) and collagen (anionic) can form ion complex that can maximalize osteoconductive characteristic of a material on bone fracture. Optimal osteoconduction can support cell adhesion of bone formation integral to the new bone formation.[4]Osteogenic ability of *scaffold chitosan* can stimulate new bone growth.[24] *Scaffold* is created out of biomaterials and factor of osteogenic signaling as well as bone tissue engineering that appears to be the most effective method to regenerate any bones, including either weight-sustaining bones or non-weight-sustaining bones. The use of natural or synthethic *scaffold* for bone regeneration has been considered a promising alternative for natural bone graft. Factors of bioactive osteogenic growth also provide suitable condition for stem cell adhesion and capture or so-called osteoblast, that leads to proper osteogenic differentiation both *in vitro* and *in vivo.* Bone morphogenetic protein 2 (BMP2) is a protein-based growth factor with integral role in stem cell osteogenic differentiation.[24] Potential of osteogenic from *scaffold* covered with layers of CHI and BMP-2 can reduce a number of bioactive components, reducing economical cost.

Research conducted by Wang by implanting *scaffold* on mouse with defect of skull then evaluating bone formation by using micro-computed tomography (micro-CT) and histology examination. The researcher successfully modified OGP on *scaffold* and proved that *scaffold* posed an osteogenic effect significant through kinase/extracellular protein path that is arranged by kinase protein (MAPK/ERK) activated by mitogen both *in vitro* and *in vivo.*[26]

Research conducted by Sukpaita showed that *scaffold chitosan/dicarboxylic acid* proved to be able to enhance bone regeneration on calvaria mouse defect.[24] New bone formation can be seen in the 6th week and 12th week on the defect impleplemented with *scaffold chitosan/dicarboxylic acid.* solvent of *chitosan* can enhance synthethic of type I collagen and hPDLC differentiation into osteoblast. This is proven to have potential to induce new bone formation on defect mouse. *Scaffold chitosan* was also developed so that it could release growth factors which included BMP-2 and insulin-like growth factor-1 (IGF-1). This *scaffold* could enhance restoration and regeneration of rabbit bone. This showed that *chitosan* was the finest *scaffold* material for bone formation.[24]

Research performed by Nga showed that scaffold 3D chitosan and HA exhibited completely porous tissue with porous size approximately 265 mm, and porosity with average 75,01%; pull force of scaffolding was 2.45 MPa, completely matching trabekular.[13] Addition of HA into *chitosan* matrix can efficiently reduce swelling. The percentage of *scaffold chitosan*-HA and maintained level of composite degradation matching the *scaffold*. The percentage of *scaffold chitosan*-HA was 46.37% after 28 days soaked in physiology solvent. *Scaffold chitosan*-HA showed biomineralization ability higher than *scaffold chitosan,* releasing apatite layer after 15 days of incubation of body fluid simulation. The presence of HA imitating biological apatite in *scaffold* composite facilitated higher protein absorption ability, compared to *scaffold chitosan.* The result showed that *scaffold chitosan*-HA had great potential as biocompatible material for bone tissue engineering. [14]

Scaffold shows integral role as biomaterial in bone repair due to its role in neoformation, neovascularisation, adhesion facilitation, and cell activity enhancement. Porous *scaffold* tissue enhances surface as to provide space for cell fixation and support chemical bond on close tissues. Moreover, the high level of porosity responsible for arranging bioactivity that directly affects structural permeability due to degraded *scaffold.* Biomaterial degradation in biological condition is one of the most relevant variables because it is directly related to long-term durability after being implanted in patient.[12] Research conducted by Sultana proved that *scaffold* HCG (HA*, chitosan, gelatin*) showed fine result with interconnected porous tissue. The size of porous from this *scaffold* allows *scaffold* to perform cell adhesion, proliferation, and nutrition supply enhancement that enable bone tissue growth quickly and precisely.[27]

In previous research, it is evident that concentration of HA exceeded 80%, creating a fragile *scaffold. Chitosan* and HA were homogen combined *in situ* synthethic HA using coprecipitation method and porous tissue generated from lyophilisation showed fine porosity and some cells could grow in 3D *scaffold* porous.[7] Porous had diameter about 50-100 m. Porous about 40-100 m enabled growth into blood vessel to facilitate vascularisation and

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bone mineralization. In this matter, *scaffold* has this dimension porous. Therefore, it can be used for bone remodelling. The smallest porous less than 20 mm was also essential for protein absorption, ionic leaching, and osteoblast adhesion on *scaffold.* According to Manjubala, *scaffold* applied for tissue engineering should contain interconnected porous tissues and high porosity to provide space for cell adhesion, proliferation, and migration. The ability of swelling is pivotal for *scaffold in vitro* and *in vivo* in tissue formation.[25]

Based on literature review conducted, *scaffold* 3D was a combination of *chitosan* and collagen with role in bone regeneration because a combination of both materials became natural polymer containing ion complex as to maximalize osteoconductivity contained. *Scaffold 3D chitosan*-HA possesses osteogenic ability vital to bone fracture repair.

Based on research outcome, it is evident that there are suggestions available for researchers starting from conducting in-depth research and literature review in regards to *scaffold* materials with osteogenic ability. In addition, it is necessary for a research and a literature review to be conducted further as to discover advantages of *scaffold 3D chitosan*-HA on bone fracture.

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