Evaluation of swelling ability of scaffold combination of chitosan and hydroxypatite

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ABSTRACT
Background: Tissue engineering is an important treatment strategy and used in current and future regenerative therapies. To achieve the success of tissue engineering, it is necessary to select a scaffold with certain characteristics. The ability to absorb water or swelling in the scaffold material is one of the properties related to architecture and cell activation. Swelling indicates the ability of the scaffold to absorb or retain water from the scaffold. The swelling ability is expected to absorb water from the surrounding tissue and have an impact on the morphology of the scaffold, especially the combination of chitosan and hydroxyapatite on cell growth. **Purpose**: Knowing the ability of water absorption (swelling) on the scaffold combination of chitosan and hydroxyapatite for the purpose of bone tissue engineering. **Method**: Literature review with statistical review method that uses inclusion and exclusion criteria with article design used experimental laboratory in vitro and or experimental laboratory in vivo. **Results**: There are eight articles showing increased swelling ability and five articles showing decreased swelling ability. **Conclusion**: The combination of chitosan and hydroxyapatite biomaterials can increase and decrease the ability of water absorption onthe scaffold so that affects the success of tissue engineering**.**
Keywords: scaffold, chitosan, hydroxyapatite, swelling

INTRODUCTION

Tissue engineering is a strategy used in tissue engineering regenerative therapy with the aim of restoring, regenerating, maintaining or improving the function of damaged tissue or tissue lost due to various diseases.[1] In prosthodontics, tissue engineering is used in prosthodontic therapy for the implantation of prostheses aimed at replacing missing tooth. Missing teeth can usually be caused by tooth fracture or alveolar bone resorption which changes the morphology and quality of the bone after tooth extraction.[2]

For the purposes of tissue engineering, important components are needed, namely cells, matrix, and signals. The matrix required for tissue engineering must be similarly to the extracellular matrix (ECM) because it has functions and effects that affect cell activity. Scaffold is an example of a matrix that can be used to replace ECM because it functions in vitro and in vivo, and has a similar role to ECM.[3] The success of tissue engineering is determined by the characteristics of the scaffold. The ability of water absorption or swelling ability of the scaffold material is one of the properties related to architecture and cell activation. Swelling shows the ability of the scaffold to absorb or retain water from the scaffold so that it has an impact on the morphology of the scaffold and cell growth. The nature of water absorption is influenced by several factors, including the nature of the biomaterial for making scaffolds.[4]

Biomaterials that are often used to make scaffolds include chitosan and hydroxyapatite. Chitosan (CS) is a natural polymer with a linear structure consisting of D-glucosamine linked by glycosidic bonds-(1-4) and a variable number of N-acetyld Glucosamine (NAG) groups. The CS has bioactive, biodegradable, biocompatible, antibacterial proties, and has a hydrophilic surface that is not found in many synthetic polymers. Chitosan plays a role in increasing cell adhesion, proliferation, and differentiation of osteoblasts and mineralization which can support its function as one of the basic ingredients in the design and fabrication of scaffolds to get better results in the improvement of bone tissue engineering.[5]

Hydroxyapatite (HA) is a biomaterial that has biocompatibility and similarity to the mineral composition of hard bone. HA acts as an osteoconductive and osteogenesis that occurs due to cell germination before implantation. Pore size and morphology in HA scaffold are important factors for good osteointegration. The HA pores with sizes ranging 100-150 m are very influential for bone growth and angiogenesis. However, the higher pores size, which is in the range of 200-500 m can be helpful for osteoblast colonization, fibrovascularization, and new bone apposition. The HA scaffold must meet certain criteria, including mechanical mechanical properties similar to those at the bone repair site, biocompatibility, biodegradability, and cell porosity.[6] In order to maintain the integrity of the bio-

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material as an implant against tissue engineering, the mechanical properties must be maintained. A cause of the loss of the mechanical strength is the absorption of water. In addition, blending of synthetic and natural polymers such as CS and HA are used to control not only swelling, but also to improve mechanical performance.[7]

The aim of this review is discussing the ability of water absorption on the scaffold combination of chitosan and hydroxyapatite for the purpose of bone tissue engineering.

METHODS

The source of the article search used the Pubmed, Google Scholar, and Science Direct databases using the keywords *scaffold*, *chitosan*, *hydroxyapatite*, and *swelling*. The search was limited to articles in Indonesian and English, with the year of publication of the article in the last 10 years. Scaffold manufacturing methods, swelling ability observation methods, and scaffold material ratios are not limited in this review. A total of 288 articles were found and as many as 12 articles were selected after the authors read the entire contents of the article based on the relevant topics, inclusion and exclusion criteria.

DISCUSSION

The ability of the scaffold to absorb fluids (swelling) and hydrophilicity is important to create a good interaction between the scaffold and the surrounding tissue so that cell migration and colonization of the scaffold occurs so that swelling can become a standard whether the scaffold is hydrophilic and capable of absorbing large amounts of liquid.[8] In the research of Kartikasari et al.,[9] scaffold containing HA and CS (BHA-G-CS) has an increased swelling ratio which means that the hydrophilicity of these components has a high possibility of cell attachment to absorb nutrient-containing fluids. In the research of Wattanutchariya and Whattanapong,[10] it was also found that the swelling ability increased with the increase in the chitosan-gelatin concentration, but was inversely proportional to the decreased HA concentration. This can occur in order to provide an optimal balance between a favorable surface area for cell attachment and the strength of its structure. Good swelling ability if used in a large area, the higher the better.[11]

Rogina et al.[12] stated that the scaffold with lower organic phase content than inorganic showed a slight increase in swelling capacity because lower HA content could affect the amount of water absorbed so that it could inhibit swelling. This can be an obstacle for HA which plays a role in preventing water seepage into the CS matrix. Meanwhile, in the research of Kar et al,[13] HA plays a role in reducing the hydrophilicity of CS by binding to the hydrophilic -COOH and -NH2. Other organic components in the form of OM play a role in reducing swelling which inhibits the interaction between polymer macromolecules and water molecules, resulting in a decrease in the water content of the CS-OM and CS-OM-HA composite scaffolds. Therefore, the swelling properties of a CS-based composite can be determined based on the appropriate amount of inorganic phase.

The effect of adding nano-HA to Hydrogel ZN-CS/NHAP/β-GP increases swelling in the research of Dhivya et al.,[14] namely the increase in high swelling ability due to fluid retention resulting in relaxation of the mechanical CS chain, which can cause an increase in the surface area of the scaffold. In the study of Shakir et al.[15] showed a significant decrease in the swelling capacity of n-HA/CS and n-HA/CS-ST scaffolds in SBF solution for different time intervals (1, 7, 14, 21, and 28 days). This could be due to the higher intermolecular interaction of n-HA/CS-ST which refers to the possibility of H-bonding between starch OH and CS amino groups. The low swelling rate of scaffold mixture containing starch indicates a higher mechanical strength to support growth into bone tissue.

In research by Pengfei et al,[16] shows a decrea-sed swelling rate because the PVA affects the 3D structure and porosity of the scaffold. Compressive strength of the composite will increase if the PVA content is high and the nHAp content is less than 12.5% has little effect on the spatial structure of this scaffold, namely maintaining stable water absorption ability.[17] The swelling ratio will be stable when the nHAp content in the scaffold is within a certain range. Swelling ratio according to Porrelli, et al,[18] increased by ~1850% after one day. CSL in combination with hDPSC can be used to accelerate bone healing. Then on research Bakopoulou et al,[19] also found an increase in swelling on the scaffold CS/Gel-0.1 showed a value of 980% and was higher than CS/Gel-1 with a value of 590%. Scaffolds with higher swelling ratios are related to the distance between bonds in the hydrogel network. In the research of Iqbal et al,[20] the presence of cross-linkers in varying amounts can ultimately affect the properties of the scaffold whereas the distribution of HA and CS in the matrix to facilitate cellular properties.

Further research by Zhang, et al,[21] stated that there was a decrease in the swelling ratio of scaf-

RESULTS

Table 1 Results of article characteristics

No	Author and Year of Publication	Research Title and Design	HA:CS Ratio	Scaffold Making Method	Swelling Ability	Research result
1	Kartikasari N, Yuliati A, Listiana A, Setijanto D, Suardita K, Ariani MD, et al, 2016	Characteristic of bovine HA gelatin-chitosan scaffolds as biomaterial candidate for bone tissue engineering (In vitro laboratory experiments)	BHA: CS 70:15	Freeze-drying, fourier transform infrared spectroscopy (FTIR), scouldning electron microscopy coupled with energy dispersive X-ray (SEM-EDX)	Scaffold BHA-GK material has an enlarged swelling ratio of 3.00+0.23 and the percentage of water (WCP) is 74.90 + 1.41% with 30% organic components. The higher the mate-rial composition can increase the swelling ratio and WCP.	Scaffold BHA-GK material has swelling ratio and WCP characteristics above average, and the hydrophilic properties of its components can indicate the possibility of increasing cell adhesion so that it has the ability to absorb liquid containing nutrients.
2	Wattanutchariya W, Changkowchai W, 2014	Characterization of porous scaffold from CS-Gelatin/HA for bone grafting (In vitro lab exp)	CS gel: HA: 1% aceticacid 2.62:2.17: 95.21	Freeze-drying, X-ray diffraction analysis (XRD)	The extent of expansion and swelling ability of the scaffold is good when used over a large area; as soon as possible. Scaffold material with this ratio can reach 95.5% swelling.	The mixture of CS Gelatin and HA scaffold porous has conclusion especially the swelling that increased if there was an increase in CS-gelatin and a decrease in the concentration of HA.
3	Rogina A, Rico P, Gallego-Ferrer G, Ivankovic M, Ivankovic H, 2016	In situ HA content affects the cell differentiation on porous CS/HA scaffolds (In vitro laboratory experiments)	CS:HA 100/0;90/10; 0/20; 70/30;60/40; 0/50; 40/60	FTIR, XRD	Scaffold showed a high swelling ratio (>130%) after 24 hours of immersion in DPBS at 37°C and during swelling, the texture of the scaffold changed from soft sponge to hydrocolloid with increasing water absorption.	Swelling and compressive strength showed a higher value for the composite scaffold with lower HA content for the function of cell and nutrient diffusion, and prevention of body fluid loss.
4	Kar S, Kaur T, Thirugnanam A, 2016	Microwave-assisted synthesis of porous chitosan modified montmorillonite-HA composite scaffolds (In vitro lab exp)	CS:OM:HA 2:10:10	XRD analysis, Attenuated Total Reflectance-FTIR (ATR-FTIR), freeze drying.	HA forms a temporary barrier that prevents water from seeping into the CS and decreases the hydrophilicity of CS by binding to the hydrophilic -COOH and -NH2. Incorporation of CS HA with OM plays a role in reducing swelling.	Improved mechanical properties and bioactivity were observed in the CS-OM-HA composite due to the strengthening of OM-HA. Swelling, degradation, and protein adsorption of the CS-OM-HA scaffold were decreased com-pared to the CS and CS-OM scaffolds. All prepared scaffolds were non-toxic to the MG 63 osteoblast cell line
5	Dhivya S, Saravanan S, Sastry TP, Selvamurugan N, 2015	Nano-HA-reinforced CS composite hydrogel for bone tissue repair in vitro and in vivo (In vitro and in vivo laboratory experiments)	Zn-CS:β-GP:nHAp 8:1:1	SEM, EDX, FTIR, and XRD	The presence of nanoHA (NHAP) in Hydrogel ZN-CS/NHAP/-GP increases swelling, fluid retention causes an increase in surface area (swelling) which can facilitate cell infiltration into the scaffold.	The role of NHAP in thermosensitive CS-based hydrogel to improve its physical & biological characteristics. Increased protein adsorption, swelling, & decreased susceptibility to lysozyme degradation shown by the addition of nano-HA.
6	Shakir M, Jolly R, Khan MS, Iram Ne, Khan HM, 2015	Nano-HA/CS–starch nanocomposite as a novel bone construct: Synthesis and in vitro studies (In vitro lab exp)	CS : HA 85 : 99	FTIR, SEM, transmission electron microscopy (TEM), x-ray diffraction (XRD), thermogravimetric analysis (TGA), and differential thermal analysis (DTA)	Decreased swelling capacity of nHA/CS and nHA/CS-ST scaffolds regularly at intervals of 1,7,14,21 and 28 days. n-HA/CS-ST has a much lower swelling capacity than nHA/CS means nHA/CS-ST has higher molecular interaction enhancement	Scaffold n-HA/CS and n-HA/CS-ST in some spectra showed significant intermolecular interactions between different components in both nanocomposites, improved thermal stability, better bioactivity to facilitate the formation of ingrowths into bone and good osteointegration.

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7	Ma P, Wu W, Wei Y, Ren L, Lin S, Wu J, 2021	Biomimetic gelatin/CS/polyvinyl alcohol/nano-HA scaffolds for bone tissue engineering (In vitro lab exp)	CS : HA 5: 12.5	Electromicron (EM), micro-CT, mechanical tests, degradation experiments, pH & swelling tests, FTIR, XR	The swelling capacity of the scaffold decreased with increasing PVA. swelling capacity GCP2, GCP3 and GCP4 were significantly lower than GCP1. nHAp 12.5% has effect on GCPH scaffold to maintain water absorption ability	The mixed Gel, CS and PVA matrix showed adjustable pore size, porosity, swelling, degradation and mechanical strength. Then nHAp was introduced as scaffolds fabrication for bone tissue engineering.
8	Porrelli D, Gruppuso M, Vecchies F, Marsich E, Turco G, 2021	Alginate bone scaffolds coated with a bioactive lactose modified CS for human dental pulp stem cells proliferation and differentiation (In vitro lab exp)	CS : HA 0.2 : 3	SEM, X-ray microcomputed tomography analysis, ATR-FTIR,	The CSL (Chitosan Lactosed) structure swelled rapidly in the first minutes of the experiment; reached maximum swelling (~1850%) after 1 day.	CSL was used as a coating for the porous scaffold, cell adhesion and osteogenic activity increased synergistically when differentiation stimuli were added. CSL induces hDPSC towards bone phenotype so that CSL-coated scaffold in combination with hDPSC can be used to accelerate bone healing.
9	Bakopoulou A, Georgopoulou A, Grivas I, Bekiari C, Prymak O, Loza , et 2018[19]	Dental pulp stem cells in CS/gelatin scaffolds for enhanced orofacial bone regeneration (In vitro and in vivo lab exp)	CS : HA 2 : 9	SEM–EDX analysis, XRD & Rietveld refinement analysis, X-ray powder diffraction, XRD;thermo-gravimetry,TG	The degree of swelling of the scaffold CS/Gel-0.1 showed a value of 980% and was higher than that of CS/Gel-1, which was measured by a value of 590%.	In vitro studies revealed that the CS/Gel type of scaffold supports viability, cell proliferation, and demonstrated extensive formation of a HA-rich nanocrystalline calcium phosphate phase.
10	Iqbal H, Ali M, Zeeshan R, Mutahir Z, Iqbal F, Nawaz MAH, et al, 2017	CS/HA/hydroxypropylmethyl cellulose (HPMC) spongy scaffolds-synthesis and evaluation as potential alveolar bone substitutes (In vitro lab exp)	CS:HA 1:1.25	SEM, freeze drying	high HA caused by CS/HA/HPMC interaction resulted in a scaffold with better pore size, low porosity and low swelling ratio. Whereas swelling increased with increasing HPMC concentration.	The presence of cross-linkers in varying amounts can affect the properties of the scaffold, where the even distribution of HA and CS in the matrix can facilitate cellular properties. Scaffold composition can be adjusted for mineralized tissue formation.
11	Zhang XY, Chen YP, Han J, Mo J, Dong PF, Zhuo YH, et al, 2019	Biocompatiable silk fibroin/car-boxymethyl CS/strontium substituted HA/cellulose nanocrystal composite scaffolds for bone tissue engineering (In vitro laboratory experiments)	CS : HA 4 : 1	Freeze drying, Cross linking	The swelling ratio of SF/CMCS/Sr-HAp scaffolds decreased significantly compared to SF/CMCS because addition of Sr-HAp reduced the hydrophilicity of carboxymethyl CS and silk fibroin	Scaffold SF/CMCS-based incorporating Sr-HAp and/or CNC to improve mechanical properties and osteoinductivity. The interconected porous structure, improved mechanical properties, and protein adsorption prove the superiority of SF/CMCS/Sr-HAp/CNC.
12	Salim SA, Loutfy SA, El-Fakharany EM, Taha TH, Hussien Y, Kamoun EA, 2021.[22]	Influence of CS & HA incorporation on properties of electrospun PVA/HA nanofibrous mats for bone tissue regeneration: nanofibers optimization and in-vitro assessment (In vitro laboratory experiments)	CS:HA 1.5 : 2	electrospinning, SEM, FT-IR and, Mechanical Tensile	PVA/HA/CH achieved a high swelling ratio of ~325%, compared to PVA/HA of ~300%, PVA/HA/HAP showed a swelling ratio of ~170% after 2 days of swelling; however, the addition of CS into the nanofibers (PVA/HA/CH/HAP nanofibers) reached a swelling ratio of ~240%, after 4 days.	The incorporation of CS into NF significantly increased swelling, protein adsorption, hemocompatibility, and antimicrobial activity of NF mats. However, incorporation of HA into NF reduced swelling, increased mechanical/thermal stability, and increased the adhesion and proliferation behavior of WI38 cells.

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folds containing HA (SF/CMCS/Sr-HAp) compared to scaffolds containing CS (SF/CMCS) due to the addition of Sr-HAp which reduced the hydrophilicity of carboxymethyl CS and silk fibroin. In addition, by providing more physical crosslinks to the carboxymethyl CS chains and silk fibers, the addition of Sr-HAp and CNC made the scaffold network structure more stable. In the study of Salim et al, [22] the PVA/HA/CH combination scaffold achieved a high swelling ratio compared to PVA/HA. The addition of CS which has a hydrophilic group that allows the penetration of water molecules in the scaffold chain. As a result, PVA/HA/CH showed the highest hydrophilicity and swellability. This was inversely proportional to the addition of salinized HA nanoparticles into the NF. The incorporation of HA into the nanofibers significantly reduced the swelling rate due to the interaction between the HA nanoparticles and the –OH group. On the other hand, the presence of CS can increase the swellability and penetration rate of nanofibers, due to the high hydrophilicity of CS.

From the evaluation of the 12 articles used, there are eight articles that show increased swelling of the scaffold and lead to biomaterial properties that can meet tissue engineering requirements such as an increase in the surface area of the scaffold, increased cell adhesion, overall distribution of cell nutrients, good mechanical strength, and in combination with other stem cells, for example human dental pulp stem cell (hDPSC) can support the repair of defective bone tissue. There are four articles which show that the decrease in swelling can occur due to several factors such as; combination of other organic components that can inhibit the hydrophilicity of the scaffold. The presence of other components can reduce the swelling ratio, but in some cases can also help the mechanical strength of the scaffold itself.

According to the authors' understanding, the high amount of CS in the scaffold can affect the cell properties such as increasing porosity and also making the scaffold enlarging or have an increased swelling ratio. However, the decreased HA when combining materials on the scaffold can also improve the mechanical properties of the scaffold with too large a porosity so that the mechanical, physical, and thermal properties of the scaffold can be balanced with the swelling ability which is not as great as when CS is added. Several studies have shown that swelling is initially beneficial for adhesion or cell growth in 3D scaffold mode because it causes an increase in pore size, but if swelling increases continuously it will cause loss of mechanical integrity and compressive strength of the surrounding tissue, as stated by Chen et al.[23] Therefore, the amount of HA within a certain range can still maintain the optimal swelling ratio.

Scaffold with combination of CS and HA biomaterials has the ability to increase the potential for successful bone tissue engineering by increasing the swelling ratio. The increased swelling ratio can help in cell migration, cell adhesion, and is able to absorb and distribute nutrients that are important for cells. Meanwhile, slightly decreased swelling can benefit several aspects such as increasing compressive strength and cell porosity.

So, further research is needed on the optimal swelling ability and ratio, component ratio and particle size of HA/CS which can produce the most ideal swelling ability in determining the modification of scaffold manufacture.

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