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Chitosan as Bone Scaffold for Craniofacial Bone Regeneration: A Systematic Review

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Abstract

BACKGROUND: The reconstruction of bone defect in the face and head is indispensable yet one of the most challenging procedures to date. Chitosan has emerged as a promising low-cost natural biopolymer for the bone scaffold as an alternative to surgery.

AIM: This study aims to review the effectiveness of chitosan as a bone scaffold for craniofacial bone regeneration.

METHODS: This systematic review used Google Scholar and PubMed as database sources. We included studies regarding chitosan as a bone scaffold for craniofacial bone regeneration. The quality assessment of the study used a checklist from Joanna Briggs Institute for experimental study.

RESULT: We included 18 experimental studies, both *in vivo* and *in vitro* study – the *in vivo* study used animal subjects such as mice, goats, and rabbits. The studies mostly used chitosan combined with other biomaterials such as demineralized bone matrix (DBM), genipin (GP), sodium alginate (SA), resveratrol (Res), polycaprolactone (PCL) and collagen, growth factor and stem cells such as bone morphogenic protein-2 (BMP-2), dental pulp stem cell (DPSC), and human umbilical cord mesenchymal stem cells (hUCMSCs).

CONCLUSION: Chitosan is a natural polymer with promising osteoconductive, osteoinductive, and osteointegrative effects in bone regeneration. Chitosan utilization for bone scaffolds combined with other biomaterials, growth factors, or stem cells gives better bone regeneration results than chitosan alone.

Introduction

Craniofacial bone defect is a common cause of morbidity which contributes to increased health care costs [1]. Craniofacial bone defects are mostly caused by accident or trauma. In the United States, no < 400,000 cases were recorded with facial fractures [2]. In Indonesia, the number of craniofacial bone injuries also increases, concomitant with the rise in traffic accidents [3]. Craniofacial bone defects are also caused by infection, cancer, or congenital abnormalities [1].

Severe craniofacial injury can result in significant soft tissue and bone loss, which require a more complex reconstructive approach such as reconstruction surgery and regeneration for the bone defect [4]. Ideal material for bone regeneration is autologous bone grafts; however, there are still some pitfalls, such as the limitation of graft volume availability, unpredictable bone resorption, and donor site morbidity. Meanwhile, another option is the allograft technique, which carries the risk of disease transmission and adverse host immune reactions. Thus, it is crucial to develop a novel bone scaffold with good biocompatibility and osteoinductivity [4], [5].

Recent research has been studied using natural or synthetic scaffolds as bone scaffold materials for bone regeneration. Several materials have been studied, including the use of natural polymers [6]. Several naturally derived polymers are available for biomedical applications, such as chitosan, collagen, gelatin, alginate, and hyaluronic acid. Chitosan is a natural biopolymer that is very versatile as a biological material to help the healing process of soft and hard connective tissue [7]. Some studies show chitosan or combination of chitosan with other active materials have positive impact for bone regeneration [8], [9], [10]. In this systematic review, we aim to explore the recent literature regarding chitosan as a bone scaffold for craniofacial bone regeneration.

Methods

Search strategy

Google Scholar and PubMed were used to conduct a systematic literature review. We selected the most recent studies about the effectiveness of chitosan as a bone scaffold for craniofacial or other bone regeneration that was published 5 years before (2017–2022). During literature searching, we used the following keywords combined with the Boolean operator: ("chitosan") AND ("bone scaffold" OR "bone engineering") AND ("craniofacial regeneration" OR "bone regeneration") to specify the finding result further. We also searched for the literature or studies listed in article references and chose a study that fulfilled the eligibility criteria.

Study eligibility

We included a study with eligibility criteria using a PRISMA diagram. In the first step, we did literature screening from the online database based on the search strategy keywords. The irrelevant or duplicated study was eliminated. In the second step, the abstract and full-text version of the studies were evaluated and assessed according to the eligibility criteria. The inclusion criteria that we used were studied regarding chitosan as a bone scaffold for craniofacial bone regeneration. The exclusion criteria are non-experimental studies, studies that do not use chitosan as the main material, studies with no full text and not written in English or Bahasa Indonesia.

Study selection and data selection

Reviewers screened all related articles in full text that met our eligibility criteria. The abstract is viewed first, followed by the full version. We assessed the selected literature for their evidence before inclusion in the final review (Figure 1). The identified literature is then managed for further analysis. All the selected literature was read thoroughly by the reviewers and captured to extract the principles of the literature.

Quality assessment of the study

The study included in the analysis then undergoes critical appraisal to determine the study quality. We used a checklist from the Joanna Briggs Institute for the experimental study to assess the quality. We give one point for each item on the checklist. A study is classified as good if it has a score equal to or more than half of the maximum total points.

Data synthesis

All relevant studies regarding chitosan as a bone scaffold for craniofacial bone regeneration were included in a narrative synthesis. Because this systematic review is a qualitative report, this study tried to gather information regarding the utilization and effectiveness of chitosan as a bone scaffold for craniofacial bone regeneration. The narrative synthesis was conducted systematically to conclude the role of chitosan as a bone scaffold for craniofacial injury or defect.

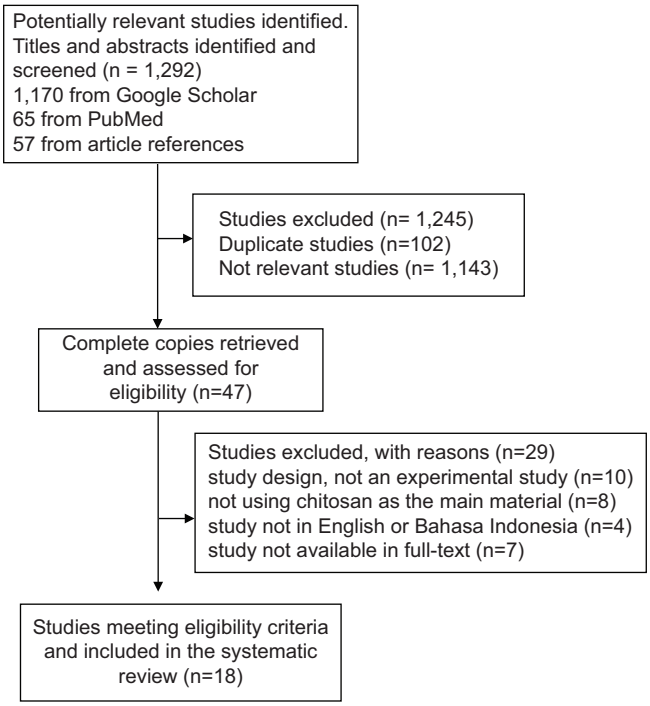

Figure 1: The PRISMA diagram of the study

Results

Study characteristics

A total of 1292 studies were retrieved from the online database. After excluding duplicate studies and irrelevant titles, 47 pieces of literature were assessed for the eligibility criteria. Twenty-nine studies did not meet the inclusion and exclusion criteria; only 18 literature met the eligibility criteria and were included in the qualitative analysis. All of the studies are experimental studies consisting of five *in vivo* studies, nine *in vitro* studies, and four investigating both. Literatures included in this analysis come from several countries such as China, Greece, India, Indonesia, Iran, Poland, and Spain. Most *in vivo* studies used the mouse as a study subject; the others used goats and rabbits. Detail characteristics of the study are shown in Table 1.

Quality assessment of the study

Quality assessment of the study was done by using Joanna Briggs Institute checklist for experimental

Table 1: Characteristic of study regarding chitosan as a bone scaffold for craniofacial bone regeneration

Study	Type of study	Study Sample	Scaffold biomaterials	Bone regeneration measurement	Study result
Alidadi *et al.*, 2017, Iran	*In vivo*	Adult male SD rats with radial bone defects.	Chitosan (CS), polymethylmethacrylate (PMMA) and demineralized bone matrix (DBM)	Bone formation and volume, mechanical strength and stiffness, production of inflammatory cells	Chitosan showed better biocompatibility, biodegradability, osteoconductivity and inductivity if combined with DBM and PMMA [8]
Bakopouloua *et al.*, 2019, Greece	*In vitro* and *in vivo*	Male immunocompromised mice	Chitosan/gelatin+dental pulp stem cell 9DPSC)	*In vitro*: Evaluation of viability/ proliferation, osteo/odontogenic gene expression analysis. *In vivo*: implantation	The scaffolds supported cell proliferation and were effective in upregulating osteogenic genes [9]
Bangun *et al.*, 2020, Indonesia	*In vivo*	Alveolar cleft defects of goats	HA/Cs/Gel+BMP-2+hUCMSC	New bone growth, bone density, inflammatory cells recruitment, and neo-angiogenesis	The scaffold showed early bone repopulation and unseen inflammatory cells, and angiogenesis [10]
Cao *et al.*, 2017, China	*In vitro*	Growth medium	Chitosan with Ag-loaded MgSrFe-layered double hydroxide (Ag-MgSrFe/CS)	Cytocompatibility, osteoinductivity and antibacterial activity	The scaffold composite showed remarkable cytocompatibility, osteogenic activity and antibacterial ability [22]
Kowalcyzk *et al.*, 2021, Poland	*In vitro*	Cell culture medium	Chitosan-Human Bone Composite Granulates	Cytotoxicity and Cell Viability, Metabolic Activity and Mineralization	The composite material retained its physicochemical properties after thermal sterilization. Cytotoxicity evaluation proved cell viability within the ISO norm [23]
Gani *et al.*, 2022, Indonesia	*In vivo*	Wistar rat	Combination of Chitosan Gel and Hydroxyapatite from Crabs Shells (Portunus pelagicus) Waste	Expression of inflammatory cytokine gene indicators IL-1 and BMP-2	The combination of chitosan gel and hydroxyapatite inhibited the production of proinflammatory cytokines and increased the production of BMP-2 [13]
Gao *et al.*, 2022, China	*In vivo*	Rabbit model	Chitosan/hydroxyapatite/ minocycline	Physical and chemical property, cytotoxicity, release of minocycline and the bacteriostasis examination	The material showed better effect of promoting periodontal bone formation [14]
Kazimierczak *et al.*, 2019, Poland	*In vitro*	Cell culture medium	Chitosan–agarose reinforced with nanohydroxyapatite	Bioactivity, cytotoxicity, and cell growth	The scaffold is non-toxic to osteoblasts and enhances cell attachment and growth [15]
Kazimierczak *et al.*, 2019, Poland	*In vitro*	Cell culture medium	Chitosan-agarose matrix reinforced with nanohydroxyapatite.	Production of osteogenic markers, osteoblast number	The scaffold has good biocompatibility and osteoconductivity, enhances osteoblasts attachment, growth production of osteogenic markers [16]
Kazimierczak *et al.*, 2021, Poland	*In vitro*	Cell culture medium	Chitosan/Agarose/NanoHA	*In vitro* osteogenic differentiation	The scaffold showed a low risk of inflammatory response and induced osteopromotive properties [17]
Li *et al.*, 2020, China	*In vitro* and *in vivo*	Female SD rats	Chitosan (CS) with nano-hydroxyapatite (n-HA)/ resveratrol (Res)	*In vitro* inflammatory response, cellular biocompatibility, *in vivo* bone regeneration and implantation	The scaffold is promising as a multifunctional filler for the bone defect [18]
Liu *et al.*, 2021, China	*In vitro*	Cell culture medium	Sodium alginate (SA), chitosan (CS), and hydroxyapatite (HA)	Osteogenic properties, cytotoxicity and cell adhesion	The scaffold showed excellent physical, chemical, antibacterial, and osteogenic characteristics [19]
Hu *et al.*, 2022, China	*In vitro*	Cell culture medium	Chitosan/gelatin/ nano-hydroxyapatite multilayer scaffold	Expression of chondrogenic and osteogenic gene	Expression both of the chondrogenic gene and osteogenic gene were increased [11]
Nie at al., 2020, China	*In vitro*	Cell culture medium	Hydroxyethyl Chitosan-Reinforced Polyvinyl Alcohol/Biphasic Calcium Phosphate Hydrogels	Porosity, compressive strength, biomineralization and cytotoxicity	The scaffold effectively reinforced the biomineralization process, improved compressive strength and biocompatibility [24]
Murali *et al.*, 2021, India	*In vivo*	Rat with calvarial defect	Electrospun chitosan membranes (ESCM) modified with short-chain fatty acids	Inflammatory response	No severe inflammatory response was noticed around the ESCMs [20]
Ren *et al.*, 2017, China	*In vivo* and *in vitro*	White rabbits	Nano-hydroxyapatite (n-HA)/ chitosan (CS) and loaded with ciprofloxacin (CIP)	Bone regeneration ability, antibacterial and Physicochemical properties	*In vitro* study: support MSC attachment and proliferation. *In vivo* study showed the scaffold promotes bone tissue formation, blood vessels and better repairability than the control group [21]
Rodríguez-Méndez *et al.*, 2018, Spain	*In vivo* and *in vitro*	Albino Wistar male rats	Strontium (Sr) containing hybrid scaffolds (ionically cross-linked chitosan and microparticles of poly(ε-caprolactone) (PCL)	Physical and chemical characteristics, *in vitro* biological ability and *in vivo* biocompatibility	The scaffold has adequate dimensional stability and osteogenic properties. It also showed *in vivo* biocompatibility and lack of toxicity in rats [25]
Xu *et al.*, 2021, China	*In vitro*	Cell culture medium	Ultra-long tricalcium phosphate nanocrystal-based methacrylate chitosan (UTCP/MAC)	Osteoblast cell viability	The scaffold showed high compatibility and remarkable cell growth [26]

BMP-2: Bone morphogenic protein-2, CAH: Chitosan/alginate/hydroxyapatite, Chit/Glu/HA: chit/glu/HA, CIP: Ciprofloxacin, CS: Chitosan, DBM: Demineralized bone matrix, Gel: Gelatin, GP: Genipin, HA: Hydroxyapatite, HE: Hematoxyllin and Eosin, hUCMSC: Human Umbilical Cord Mesenchymal Stem Cells, MSC: Mesenchymal stem cell, n-HA: Nano-hydroxyapatite, nBG: nanobioactive glass, PBS: Phosphate buffered saline, PCL: Poly(ε-caprolactone), PMMA: polymethylmethacrylate, Res: resveratrol, SA: Sodium alginate, SD: Sprague Dawley, Sr: Strontium, UTCP/MAC: Ultra-long tricalcium phosphate nanocrystal-based methacrylate chitosan.

study. Each item from the checklist contributed to one point. A study is considered good quality if it has half or more maximum total points and regarded as low quality if it has less than the half-maximal entire point. The two reviewers evaluated the quality of the study to avoid bias. Of eleven studies involved, all were considered good quality, with a total point range from 7 to 10.

Utilization of chitosan as bone scaffold

Chitosan is a natural copolymer of glucosamine and N-acetylglucosamine derived from crustacea such as shrimps, crabs, and lobster. It has unique characteristics that are biocompatible, stable, biodegradable, has antimicrobial, and immunostimulatory properties [8], [9]. Because

chitosan has a similar structure to the extracellular matrix, glycosaminoglycans (GAGs), it provides a good microenvironment for cell growth. Osteoconductive properties can enhance stem cells or progenitors for osteogenic differentiation and biomineralization. From the analysis of 18 studies included in this systematic review, they all use chitosan combined with other materials as a bone scaffold for the bone regeneration method. A study by Bakopouloua *et al.*, Bangun *et al.*, Hu *et al.*, and Singh *et al.* combined chitosan with gelatin [9], [10], [11], [12], while study by Gani *et al.*, Gao at al., Kazimierczak *et al.*, Li *et al.*, Liu *et al.*, and Ren *et al.* combined chitosan with hydroxyapatite (HA). Hydroxyapatite is an inorganic scaffold biomaterial, while chitosan and gelatin are biodegradable polymers that make good additions to the sturdy HA scaffold [13], [14], [15], [16], [17], [18], [19], [20], [21].

Natural-based polymers mimic biological macromolecules and have the advantage of preventing immunological reactions and toxicity, unlike their synthetic counterparts. The other study used other biomaterials such as demineralized bone matrix (DBM), genipin (GP), sodium alginate (SA), resveratrol (Res), polymethylmethacrylate (PMMA), polycaprolactone (PCL), and collagen [8], [13], [18], [19], [22]. Study by Hammouda *et al.* used a combination of chitosan and collagen [27]. Some studies also used chitosan combined with growth factors and stem cells such as bone morphogenic protein-2 (BMP-2), dental pulp stem cell (DPSC), and human umbilical cord mesenchymal stem cells (hUCMSC) [8], [9], [10], [11].

Chitosan effectivity as bone scaffold for bone regeneration

Several parameters are evaluated in the studies, such as morphology, histopathology, cell viability or proliferation, osteogenic differentiation, mineral characterization, and expression of the osteogenic gene, implantation, bioactivity, cytotoxicity, biocompatibility, and inflammatory response. Due to the many parameters used in those 18 studies, we simplified them based on the parameters below:

Scaffold morphology and histopathology

A scaffold is considered good if it has high porosity. Porosity is defined as the permeability, surface area and presence of open pores of the porous structure. A scaffold considered has a high porosity if it has a high surface area or volume ratio. High porosity can help cell adhesion to the scaffold and promote bone regeneration. A study by Alidadi *et al.* compared scaffolds made from chitosan, PMMA, and DBM. The morphology and histopathology examination shows that the chitosan scaffold showed a porous structure with great interconnectivity and a big variation of pores size [8]. Study by Bakopoulou *et al.* using chitosan and gelatin as scaffold showed that the scaffold has pore size distribution between 70–120 μm and interconnecting open pores [9], while scaffold combination of Chitosan and agarose and nanohydroxyapatite by Kazimierczak showed porosity in the range of 50–90% [15], [16], [17].

Osteogenic properties (osteoconductive, osteoinductive, and osteointegrative)

Bone scaffolds must have good osteoinductivity and osteogenic properties to induce *in vivo* bone regeneration and remodeling at the site of bone damage. A study by Alidadi *et al.* studied scaffolds from chitosan, PMMA, and DBM, which found that bone volume percentage in the scaffold group made from a combination of CS, PMMA, and DBM was significantly higher than in the untreated group at 8 weeks after bone injury ($p < 0.01$). Bone defects in the DBM and autograft groups had a significantly higher bone volume than in the chitosan and PMMA groups ($p = 0.009$). Meanwhile, new bone regeneration, osteoinductive, and osteoconductive properties were only seen in the autograft and DBM groups [8]. *In vitro* assessment by Bakupulou *et al.* reveals that a combination of chitosan and gelatin scaffold supported cell viability and proliferation [9].

A study by Bangun *et al.* that compared chitosan scaffold and autologous bone graft revealed that the combination of HA + Chitosan + Gel + BMP-2 + hUCMSCs group showed most superior growth with up to 60% increase in new bone development ($p > 0.05$). HA + Chitosan + Gel + BMP-2 + hUCMSCs scaffold also demonstrated an excellent wound healing process than autologous bone graft, marked with a significant reduction of inflammatory cells and angiogenesis on week 12 follow-up. There is no sign of inflammatory cells and undetected formation of new vascular channels, suggesting faster completion of the bone healing process [10]. Study by Cao *et al.* used chitosan with layered double hydroxide and Ag-MgSrFe [23]. Evaluation of *in vivo* and *in vitro* study of nano-hydroxyapatite/resveratrol/chitosan (n-HA/Res/CS) composite by Li *et al.* showed stimulant BMSCs proliferation, osteo differentiation, enhanced into-chondrostosis, and bone remodeling [18]. The study by Chen *et al.* reported that combination of chitosan, collagen, and calcium phosphate has higher mechanical strength of the scaffolds than chitosan–collagen hydrogels scaffold [24].

Expression of osteogenic gene and formation of mineralized tissue

A study by Bakupulou *et al.* that used a combination of chitosan, gelatin, and DPSC showed that incubation of DPSCs inside the chitosan and gelatin scaffolds effectively induced upregulation that statistically significant of all osteogenic markers. Their

study also found that the regenerated mineralized tissues were analyzed after 6 weeks of implantation, which showed osteoid formation in a significantly higher amount and complete bone mineralization [9]. Study by Cao *et al.* used a composite scaffold consisting of chitosan with layered double hydroxide and Ag-MgSrFe element to induce the extracellular matrix mineralization and increase the osteogenic related gene expression such as alkaline phosphatase (ALP), runt-related transcription factor (RUNX2), and BMP-2 [23]. ALP is an osteogenic marker for osteoblastic differentiation that expressed early, RUNX2 is vital for formation of bone and cartilage, and BMP-2 is essential in the construction of fibrocartilage and development of tendon-bone junction [9]. The other study by Kazimierczak using CAH scaffold found the scaffold combination supports the production of osteogenic markers (collagen, bALP, and osteocalcin) by MC3T3-E1 and hFOB 1.19 cells [16].

Antibacterial and anti-inflammatory properties

Cao *et al.* found that scaffold composite from chitosan with LDH and Ag-MgSrFe showed antibacterial properties. Ag nanoparticles in the composite scaffold effectively prevent Staphylococcus aureus biofilm formation [23]. Study by Liu *et al.* using scaffold from sodium alginate (SA), chitosan (CS), and hydroxyapatite (HA) also showed antibacterial properties against *S. aureus* [19]. Study by Ren *et al.* using scaffold composite using chitosan and nano-hydroxyapatite and loaded with ciprofloxacin (n-HA/CS-CIP) showed excellent antibacterial activity. It supported the proliferation and attachment of MSC. *In vivo* study also showed that n-HA/CS-CIP enhanced bone tissue and blood vessel formation and good capability for bone defect repair [21].

Sometimes, the graft development is followed by an increasing level of pro-inflammatory cytokines that can impair bone development and formation. A study by Li *et al.* used the n-HA/Res/CS scaffold composite showed an anti-inflammatory effect by reducing the expression of inflammatory cytokines such as TNF-α, IL1β and iNOS [18].

Cytocompatibility

The ideal bone scaffold material should have excellent cytocompatibility characterized by no harm to the host and promoting remarkable osteointegration between the scaffolds and bone tissue. A study by Cao *et al.* found that a scaffold composite of chitosan with layered double hydroxide and Ag-MgSrFe promotes cell proliferation on their surfaces. It also did not show toxicity to the hBMSCs [23]. The other study by Kazimierczak *et al.* used a combination of Chitosan and agarose and nanohydroxyapatite, which showed a non-toxic effect on osteoblasts and favored cell attachment and growth [15], [16], [17]. Study by Nie *et al.* using a scaffold comprised hydroxyethyl chitosan with polyvinyl alcohol/biphasic calcium phosphate hydrogels showed sufficient *in vitro* biomineralization to facilitate the mechanical characteristics of the scaffold and good cytocompatibility [25].

A study by Rodríguez-Méndez *et al.* using Sr(II)/Chitosan/PCL scaffolds showed sufficient dimensional stability, osteogenic activity, and biocompatibility in rats through *in vivo* evaluation and minimal toxicity. This scaffold is a potential scaffold material for craniofacial bone regeneration [25]. Study by Xu *et al.*, using nanocomposite made of UTCP/MAC, showed high cell viability and proliferation good biocompatibility for the growth of osteoblast cells [26].

Discussion

Malformed or fractured bones due to a congenital defect or a traumatic injury require craniofacial reconstruction to repair the defect [4], [5]. A defect is considered critical when it does not spontaneously heal and requires intervention. Bone grafting is a conventional practice for reconstructing critical size bone defects, using autologous bone harvested from the patient. Autologous bone graft is considered as the gold standard in bone reconstruction procedure. However, there are several limitations to this method. Grafting initiate secondary operative site associated with morbidities such as infection and chronic pain. In the case of a large graft, it raises a possibility of instability and risk of fracture at the donor site, and therefore, there is only limited amount of autograft availability. An alternative regenerative treatment approach involves using novel biomaterials that can be used as scaffolds and implanted at the lesion site to encourage bone growth and repair [1], [6]. There are several scaffold materials from biopolymers available, both natural and synthetic. Natural-derived biopolymers such as chitosan, collagen, and gelatin can be used as scaffold material due to their similar structure, chemical conformation, and biochemical characteristics to the natural bone organic matrix. They have minimal immunogenic reactions and enhance good cell response and function while contributing to tissue remodeling [4]. Ideally, these biomaterials should be osteoconductive, osteoinductive, and osseo-integrative. Osteoconductive is defined as ability to promote recruitment of bone cells (progenitor) from the host, while osteoinductive is described as the ability to promote the bone precursor cells transformation into osteoblasts, and osseo-integrative capability of supporting appropriate host or graft interaction with minimal immune response [5].

This systematic review aims to explore the utilization and effectivity of chitosan as a bone scaffold for bone regeneration. From the analysis and review

results, we found that chitosan can be combined with other biomaterials, growth factors, or stem cells and give a better result if combined with other materials rather than chitosan alone. Chitosan has biodegradable, biocompatible, and biological renewable characteristics. It is also bacteriostatic and non-toxic. Chitosan is also a promising biomaterial that is multipurpose, derived from the crustaceans consisting of glucosamine and N-acetylglucosamine [7], [8], [9]. However, these biomaterials show weak mechanical characteristics. Synthetic polymers have poor potentiality in providing cell adhesion or migration and proliferation. Still, they offer good mechanical properties, and their mechanical strength and degradation rate can be adjusted to reach the best performance. Other biomaterials such as hydroxyapatite (HA), polymethylmethacrylate (PMMA), nano-bioactive glasses, gelatin, and collagen can ensure excellent osteoconductivity if combined with chitosan [8], [13], [18], [19], [26]. Moreover, the addition of growth factors such as BMP-2 and stem cells to the chitosan-based scaffolds may improve the biological properties of chitosan, providing a better regenerative effectivity for bone regeneration [8], [9], [10], [11].

Chitosan can be processed in various forms, including hydrogels, films, and scaffolds, because chitosan hydrogels can be formed through a chemical process in the formation of covalent cross-links. Making chitosan scaffold is mostly done using the freeze-drying method. From our analysis, most of the studies processed chitosan scaffolds through the freeze-drying method and formed them into porous scaffolds [8], [9], [10]. From the overall study, chitosan has shown promising results as a bone scaffold for craniofacial or other bone regeneration [8], [27]. However, there is a limitation in our study; from 18 studies included in this systematic review, only four studies use an animal model with craniofacial or calvaria defect as the study subject [10], [13], [14], [28]. Due to limited studies available, we still included a study that evaluated chitosan for bone regeneration for craniofacial bone defects and bone defects in general. With the continuous development of research in scaffolding, further experimental study of chitosan and its combination with stem cells, growth factors, and other biomaterials is likely to become a promising therapy in the future.

Conclusion

Chitosan is a natural polymer with promising osteoconductive, osteoinductive, and osteointegrative effects in bone regeneration. Chitosan can be combined with other biomaterials such as DBM, genipin, sodium alginate, resveratrol, polycaprolactone and collagen, growth factors such as BMP-2 and stem cells such as dental pulp stem cell, and human umbilical cord mesenchymal stem cells. Chitosan utilization for bone scaffolds combined with other biomaterials, growth factors, or stem cells gives better bone regeneration results than chitosan alone.

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