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Chitosan as a Potential Pulp Capping Material for Dentin-Pulp Regeneration: A Review

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ABSTRACT

Chitosan is a natural biopolymer acquired from chitin, mainly from shell of crustaceans. Its major properties include, nontoxic, biocompatible, antimicrobial, anti-inflammatory, stimulate wound healing and cell proliferation. Due to these properties, chitosan has gained a significant attention due to its wide range of application, including regenerative dentistry. Regenerative dentistry aimed to restore damaged tissues such as dentin and root structures and pulp-dentin complex.

One of the important elements in regeneration of pulp-dentin structure is pulp capping material. Studies found that chitosan exhibit a great potential as a pulp capping material due to its ability to stimulate reparative dentin, maintain pulpal vitality, good antimicrobial activity, adherence properties to restorative material and dentin, and can resist forces during placement of restoration. Nanotechnology in tissue engineering may further enhance the properties and application of chitosan in biomedical field.

This review discussed on the potential of chitosan as a pulp capping material based on the results of current research. This article also briefly discussed on the limitations of chitosan and method to overcome the challenges.

1. Introduction

Chitosan is a natural biopolymer acquired from chitin, mainly from shell of crustaceans. Nacetylglucosamine and glucosamine copolymer units make up the chemical structure of chitosan (Cicciù et

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al., 2019). It is insoluble in water but readily dissolves in acid media. Its major properties include, nontoxic, biocompatible, antimicrobial, anti-inflammatory, stimulate wound healing and cell proliferation (Kmiec et al., 2017). Because of its remarkable biological properties, chitosan is becoming popular in dentistry. One of its intriguing qualities is its ability to develop new biomaterial for various application in dentistry including pulp-dentin regeneration procedure.

One of the approaches to deliver a regenerative material is via pulp capping procedure. It can be defined as a protective base or liner serves as a barrier between restorative material and tooth (American Academy of Pediatric Dentistry, 2023). The goal of pulp capping material is to sustain pulp vitality through either direct or indirect pulp capping (Alex, 2018). Ideal properties of pulp capping material comprise abilities to promote reparative dentin, bactericidal or bacteriostatic, good mechanical properties, and may provide bacterial seal (Cohen & Combe, 1994). The study on pulp capping material is still evolving to improve its quality and properties.

On the other hand, the history of regenerative dentistry dates back to around 1952, when Dr. B. W. Hermann published a case report on the use of Ca (OH)2 in the amputation of important pulp (Hermann, 1952). The regenerative process involves a biologically based procedures aimed to restore damaged tissues such as dentin and root structures and pulp-dentin complex. A review had discussed on the multiple techniques of pulp-dentin regeneration such as via revascularization, stem cell therapy, pulp implant, scaffold, gene therapy and others (Murray et al., 2007).

Therefore, material selection is important to ensure the effectiveness of regenerative process. Calcium hydroxide is a well-known material used for pulp capping up until today (Komabayashi et al., 2016). However, various studies have reported that calcium hydroxide has high solubility rate and poor sealing ability to tooth structure due to its poor adhesive properties. Both characteristics may create marginal leakage underneath the restorations (Li et al., 2015; Zhu et al., 2015; Franzin, N.R. et al., 2021). Due to that, other materials such as calcium phosphate, silicate-based materials, ceramic materials and other synthetic and natural biomaterials including chitosan had currently evolved as a potential pulp capping material (Urgiles et al., 2024). This review aims to present the potential of chitosan as a pulp capping material especially as an aid in dentin-pulp regeneration based on various studies.

2. CRITERIA OF PULP CAPPING MATERIAL

The interactions between dental hard tissues, vital pulp and various restorative materials in a unique environment required great demand on the selection of pulp capping material. Many distinct materials have been employed as a pulp capping materials especially for vital pulp therapy approach. For example, gold foil, calcium hydroxide, corticosteroid/antibiotics mixture, resin-modified glass ionomer cements, and others (Tomson & Duncan, 2021).

The primary goal of pulp capping material is to protect and maintain a viable pulp. In order to enhance the thickness of dentin, the material selected should encourage the pulp's ability to lay down dental hard tissue in the form of reactionary or reparative dentin. Cohen BD in 1994 suggest the ideal properties of pulp-capping materials include:

- i. Stimulate reparative dentin formation.
- ii. Maintain pulpal vitality.
- iii. Antimicrobial activity.
- iv. Adhere to restorative material and dentin.
- v. Resist forces during restoration placement.

Therefore, recently studied materials for pulp capping should aim to possess those characteristics to provide a desirable quality for pulp therapy.

3. POTENTIAL OF CHITOSAN AS PULP CAPPING MATERIAL

3.1 Stimulate reparative dentin formation

The objective of regenerative dentistry is to develop biomaterials that can facilitate dentin-pulp complex's regeneration. An in-vivo study observed the effect of chitosan on the formation of odontoblast-like cells using chitosan-coated red snapper fish scales as a direct pulp capping medicament on healthy rats and with reversible pulpitis. Based on the study, odontoblast-like cell formation was observed in group of rats treated with chitosan compared to without any treatment. It is suggested that chitosan-coated red snapper fish scales help to stimulate the progenitor cells and promotes formation of transforming growth factor-beta 1 (TGF-β1) as an initial stage for the production of odontoblast-like cells (Tifani et al., 2023).

In a study that investigate the pulp capping ability and reparative dentin formation in dog model via chitosan bilayer membrane containing TGF- β 1 loaded microspheres claim that the formulation provides a better growth factor delivery for dental regenerative applications. The results plotted in the study shows that there are proliferation of odontoblast-like cells and increase in thickness of reparative dentin for the chitosan membrane group. However, the plot demonstrates improved results upon incorporation of growth factor or other active material into the chitosan membrane (Yang et al., 2012). It is agreed by recent study that shows higher deposition of mineralized matrix upon infusion of calcium hydroxide β -glycerophosphate (β GP) with porous bioactive chitosan scaffold (Bordini et al., 2022).

Earlier research in 2014 aimed to study the biomimetic of remineralization and demineralization of dentin using scaffold containing chitosan. The materials used in this study is carboxymethyl chitosan/amorphous calcium phosphate (CMC/ACP). They mentioned that the formulation is able to mimic the stabilizing effect of dentine matrix protein 1 (DMP1) on ACP in the biomineralization of dentine. Although ACP nanoparticles are the active elements to achieve intrafibrillar mineralization of collagen, chitosan is also crucial to provide stabilization of the scaffold. Hence, CMC/ACP nanocomplexes could be a viable indirect pulp capping material for the treatment of deep caries since they demonstrate a promising effect of remineralisation on demineralised dentine (Chen et al., 2015). As a result, dentinal tissue can be maintained to the greatest extent possible.

3.2 Maintain pulpal vitality

The intentions of pulp therapy are to preserve the vital pulp tissue and encourages its repair. Thus, pulp capping material stimulate the repair process by enhance the recruitment, migration, proliferation, and differentiation of human dental pulp stem cells (DPSCs) (Sangwan et al., 2012). A study mentioned that vascular endothelial growth factor (VEGF) plays a crucial role in dentin-pulp regeneration. However, its administration is always challenging. Chitosan hydrogels were used as a bioactive material and delivery method in this study. Their findings found that chitosan hydrogel has a potential function and possible carrier of bioactive molecules in pulp capping therapy. It is found that it may steadily release the VEGF and support the odontogenic differentiation of DPSCs (Wu et al., 2019).

Besides placement of VEGF into the chitosan hydrogel, another study provides an idea to combine chitosan and hydroxyapatite. Their potency to promote angiogenesis and fibroblast cell proliferation were studied in-vivo on molars of *Rattus norvegicus* rat (Sularsih et al., 2024). The samples were grouped into five groups in which glass ionomer cement (GIC), calcium hydroxide (Ca (OH)₂), chitosan (CH),

hydroxyapatite (HA), and chitosan and hydroxyapatite (CH-HA) were placed respectively. Histopathological examination of the blood vessels and and fibroblast cells were studied after Day 3, 7, and 14. The results found that the CH-HA group showed the greatest blood vessels and fibroblast cell proliferation upon healing of direct pulp capping. In comparison, GIC group presented with the lowest healing process. On the other hand, the CH and HA only group express better cell proliferation compared to Ca (OH)₂ group. Immunohistochemistry examination of VEGF expression on Day 3 of the experiment also demonstrates a consistent result. Highest expression of VEGF was observed in CH-HA group. The study concluded that formulation of chitosan and hydroxyapatite could aid in pulp healing and maintaining pulp vitality.

Furthermore, to prevent progression to irreversible pulpitis or pulp necrosis, hydrogels or scaffolds should not induce or induce only a low inflammatory or immune response (Colombo et al., 2014). Many dental hydrogels are engineered to be non-toxic and free from harmful chemicals. According to a study on regenerative material, the immune response from the hydrogels can be modulate by its physical and chemical properties. This process called immunomodulation allow a controlled and low inflammatory response (Bu et al., 2022). Ducret et al. (2019) have designed an innovative cellularized fibrin hydrogel supplemented with chitosan. Although the percentage of viable dental pulp-mesenchymal stem/stromal cell (DP-MSC) was seen similar in fibrin-alone and fibrin-chitosan hydrogels at both Day 0 and Day 7, the cell proliferation by the expression of nuclear marker MKI67 both at gene and protein level with RT-qPCR and immunohistochemistry shows otherwise. The fibrin-chitosan hydrogels express significant level of expression between Day 2 and Day 4 indicates marked cell proliferation. These combinations are currently preferred by the researchers due to its positive impact on the tissue engineering. To support, a recent *invivo* study on the effect of nanotchitosan derived from red snapper fish scales on pain and pulp inflammation found that nanochitosan reduced the levels of inflammatory mediators such as TNF-α and leukocyte cells (Widyastuti et al., 2024).

3.3 Antimicrobial activity

Remaining bacteria post-cavity preparation may lead to pulpal injury. Antibacterial properties of pulp-capping material during permanent restoration are crucial to prevent further pulpal damage. Pulpal inflammation can also be avoided if the surrounding bacteria been arrested, thus maintaining the pulp vitality. Among various oral microorganisms, study shown that *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Enterococcus faecalis* are the main culprit in dental caries formation and progression into the root canal system (Siqueira & Rôças, 2008). Because of that, the effectiveness of pulp therapy might depend on the good antimicrobial properties exerted by pulp capping materials.

One of the favorable properties of chitosan is its antimicrobial properties towards bacteria, viruses, fungi, and even algae (Erpaçal et al., 2019). Chitosan inhibits bacterial growth mainly via bacteriostatic action. Research that developed an antibacterial adhesive using carboxymethyl-chitosan (CMC) aimed to to study its antibacterial activity against *S. mutans*. Etch-and-rinse adhesive were applied without CMC application in the negative control group. There was a considerable suppression of *S. mutans* growth in CMC-containing adhesive group compared to the negative control. More efficient suppression was seen upon CMC concentration of 5 to 10 mg/mL and no significant finding was noted beyond the concentration (Yao et al., 2022).

A paper from Dental Materials Journal in 2020 presented on the antibacterial property of chitosan against *E. faecalis* especially in endodontic treatment. 6 different types of chitosan with different molecular weight were used in this study. The study reported that the 1,700 and 2,100 kDa have the lowest MBC at 2 mg/mL, suggesting that they have the highest antibacterial activity. They also studied on the time required for bactericidal activity of chitosan towards *E. faecalis*. The findings imply that chitosan might be an

effective antibacterial agent against *E. faecalis* with a contact time more than 10 minutes (Supotngarmkul et al., 2020). Another recent study that incorporates chitosan hydrogels in extracellular matrix for dentinpulp complex regeneration shows an inhibitory effect on *E. faecalis* biofilms and the formulations demonstrated a remarkable ability to regulate the growth of the bacteria (Osman et al., 2025).

Various factors affecting the antimicrobial activity of chitosan. One of the factors mentioned in a study is that a combination of bacterial cell binding and DNA binding mechanisms could provide the antibacterial action of chitosan (Chen & Chung, 2012). Moreover, antioxidant activity of chitosan cause neutralisation of free radicals and binding to metal ions thus, converting various constituents into stable compounds. This antioxidant activity is mostly caused by the basic group's hydroxyl (-OH) and amino (-NH) (Yildirim et al., 2016). Chitosan inclusion is therefore beneficial for improving dental materials' biocompatibility, particularly with regard to their antibacterial properties.

3.3.1 Mechanism of actions of chitosan as antimicrobial therapy for pulp capping

a. Disruption of cellular membranes of microorganisms

Electrostatic interaction between the positively charged amine groups (NH3+) of glucosamine of chitosan with the negatively charged microbial cell surface microrganisms, disturbed the cellular components leading to cell death (Feng et al., 2021). Teichoic acid which is negatively charged present in Gram-negative bacteria will separate the intracellular and extracellular membrane. As a result, periplasmic space was formed and causing damage to the cell membrane. Besides that, elevation of electrical conductivity of cellular lysate and β -galactosidase activity led to hydrolysis of peptidoglycans and release of cellular components. In contrast, chitosan neutralizes the negative charges on Gram-negative bacteria due to the presence of lipopolysaccharides (LPS). This will disintegrate the cellular membrane allowing pathway for chitosan entry and cause cell death.

b. Interference of cellular transcription and translation

Synthesis of mRNA and proteins functions for cellular replication via transcription and translation process. Chitosan can disturb the process by penetrating the nucleic component of microorganisms. Interaction of positively charged NH₂ groups in oleoyl-chitosan nanoparticles (OCNPs) with the negatively charged phosphate groups of DNA/RNA inhibited the transcription and translation in *E. coli* (Xing et al., 2009). The results were similar with other studies which utilize chitosan oligomers for inhibition of DNA transcription and protein biosynthesis in *E. coli* (Galván Márquez et al., 2013).

c. Chitosan as a chelating agent

The NH³⁺ group of glucosamine in chitosan selectively interact with metal ions present in the bacterial cell surface. The interaction creates a chelating activity which inhibits the microbial growth. The process occurs by the donation of electrons from NH3+ to the metal ions of phosphate groups in LPS or teichoic acid on the cellular membrane. These formed a stabilized polymer and metal complex resulting in the instability of the cell surface potential and mutual repulsion among negatively charged phosphate group, ultimately leading to the rupture of the cell membrane (Feng et al., 2021).

d. Blockage of oxygen pathway

A dense layer of chitosan obstructs the porins on the microbial cell surface's, inhibiting the exchange of gases and nutrients between the microbial cell and its surroundings and impeding its ability to proliferate (Feng et al., 2021). The effect of this activity is more pronounce in aerobic microorganisms, in which depletion of oxygen may hinder their growth and leads to cell death.

3.4 Adhere to restorative material and dentin

Adhesive dentistry has made significant strides in the last few decades. Even with advancements in adhesive dentistry, the durability of adhesive-bonded restorations remains uncertain. Present of unorganised matrix and differs morphologically from healthy dentin cause a challenge to achieve bioadhesive interface in partially deminaralized substrate (Perdigão, 2009). Hydrolysis of exposed collagen due to bacterial byproducts and enzymatic activity lead to dentin-resin interface degradation and decreased bond strength. Even though calcium hydroxide has been used in dentistry since several decades due to its various advantages, there are also some limitations. Calcium hydroxide-based sealer has been found to have poor cohesive strength, greater solubility and finally lead to high incidence of marginal leakage (Desai & Chandler, 2009).

A paper from Restorative Dentistry & Endodontics has evaluated the bond strength of adhesive interface on demineralized dentin after application of chitosan solution. They suggested that chitosan-induced biomodification may improve the adhesive interface on demineralized dentin (Ziotti et al., 2022). The *in vitro* study involves 80 extracted third molars which then divided into two groups, control using distilled water and 2.5% chitosan solution. The specimen was then further divided into immediate test and aged. The microtensile bond strength demonstrated that chitosan treatment on demineralized dentin improved the bond strength immediately and after aging of the specimens. However, chemical and morphological compositions found no significant differences. The hypotheses behind it can be due to the chitosan treatment are able to retain the organic and inorganic components of the samples and thus maintaining its compositions before and after the procedure.

Another paper also reported similar results on the bond strength upon incorporation of chitosan and nanochitosan in universal adhesive system (El-Din et al., 2023). The objective of their study is to assess the wettability, pH, and microtensile bond strength of universal adhesive after incorporation of 0.5% and 1% of bulk-chitosan and nanochitosan. Similar method as previous study were conducted in which premolars (n=50) were involved. The results on microtensile bond strength showed that addition of nanochitosan produced greater bond strength. On the contrary, no effect was found on the immediate bond strength upon application of bulk-chitosan. The inclusion of filler nanoparticles is thought to enhance the mechanical characteristics of the adhesive monomers and strengthen the hybrid layer and bond strength (Alhenaki et al., 2021).

3.5 Resist forces during restoration placement

In different *in vitro* study, the paper also reported similar findings on the durability of resin-dentin interface with additional of chitosan gel on different etching system. Initially, they reported that there is no significant difference was observed between etch-and-rinse and self-etch adhesive system. Following that, chitosan treated dentin had been found to has better immediate bond strength with the dentin and adhesive materials. The science behind it was well explained by Baena et al. Chemical structure of chitosan contain free amino and hydroxyl groups besides positive charges that form an ionic interaction tp produce a cross-

linkage with dentin collagen. This results in a mechanically robust fibril chain and improves the mechanical performance of restorations (Baena et al., 2020).

There is minimal research that observe the bonding strength between chitosan and other restorative materials. Two papers have reported on the application of chitosan-incorporated composite resin and its evaluation on bond strength and microleakage. First paper found that application of 0.25% chitosan nanoparticles (CSN) incorporated in universal composite and eight-generation dentin bondin agent (DBA) provide greater bond strength compared to other percentage of chitosan. Nevertheless, the result is not significant towards the control group which use only DBA and composite, without chitosan incorporation (Halkai et al., 2022).

The outcome aligns with the findings of the second paper, in which they discovered that there was no statistically significant difference in microleakage score between chitosan-composite group and unmodified composite group upon immediate placement of restoration. Yet, chitosan-incorporated composite appears to have better mechanical qualities and create a more stable bond than unmodified composite. It can be seen from the reported evidence of microleakage after 3 months of storage in artificial saliva. The paper stated that the occurrence of microleakage appears to be considerably lower in the chitosan-composite group after 3 months of storage in artificial saliva (Nadig et al., 2021).

Based on the multiple reported studies, it is belief that chitosan may act as a component to improve dentin adhesive system, bonding, and dental restoration. However, there are limited number of research and most of the studies done are limited to *in vitro* technique using extracted teeth. The actual intraoral environment could be different compared to the laboratory settings. Therefore, future research is required to investigate the outcome of chitosan adhesion and bond strength in actual intraoral conditions using *in vivo* or clinical studies.

4. RECENT STUDIES ON CHITOSAN FOR DENTIN-PULP REGENERATION

Title / Author	Materials	Conclusion
Design And Characterization of a Chitosan-Enriched Fibrin Hydrogel for Human Dental Pulp Regeneration. (Ducret et al., 2019)	Chitosan-enriched fibrin hydrogel	'Comparative analysis of fibrin-alone and fibrin- chitosan hydrogels revealed a potent antibacterial effect of the chitosan in the fibrin network, and similar DP-MSC viability, fibroblast-like morphology, proliferation rate and type I/III collagen production capacity.'
Evaluation of Chitosan Hydrogel for Sustained Delivery of VEGF for Odontogenic Differentiation of Dental Pulp Stem Cells. (Wu et al., 2019)	Chitosan hydrogel with vascular endothelial growth factor (VEGF)	'CS/β-GP hydrogel could continually release VEGF and contribute to odontogenic differentiation of DPSCs, thus may become a potential carrier of bioactive molecules in pulp capping therapy.'
Silver-Doped Bioactive Glass/Chitosan Hydrogel with Potential Application in Dental Pulp Repair. (Zhu et al., 2019)	Silver-doped bioactive glass/chitosan hydrogel	'The results showed that Ag-BG/CS induced stronger reparative dentin formation and enhanced preservation of vital pulp tissue when compared to the MTA. It also enhanced pulpal repair through the mitogen-activated protein kinase (MAPK) pathway. This material may represent a superior solution for dental pulp-capping clinical scenarios with specific advantages for cases of early diffuse pulpitis in immature permanent teeth.'
Dental Pulp Inflammatory/ Immune	Chitosan-enriched	'These data collectively demonstrated that fibrin-
Response to a Chitosan-Enriched Fibrin	fibrin hydrogel	chitosan hydrogels induced an inflammatory/

Hydrogel in the Pulpotomised Rat Incisor. (Renard et al., 2020)		immune response similar to that of the fibrin hydrogel. The results confirmed the potential clinical use of fibrin-chitosan hydrogel as a new scaffold for vital-pulp therapies.'
In Vitro Evaluation of Injectable Tideglusib-Loaded Hyaluronic Acid Hydrogels Incorporated with Rg1-Loaded Chitosan Microspheres for Vital Pulp Regeneration. (Atila et al., 2021)	Tideglusib-loaded hyaluronic acid hydrogels and Rg1-loaded chitosan microspheres	'Injectable Tideglusib-loaded hyaluronic acid hydrogels incorporated with Rg1-loaded chitosan microspheres hydrogel formulation has potential to improve strategies for vital pulp regeneration.'
Physical and Biological Properties of a Chitosan Hydrogel Scaffold Associated to Photobiomodulation Therapy for Dental Pulp Regeneration: An In Vitro and In Vivo Study. (Moreira et al., 2021)	Chitosan hydrogel scaffold with photobiomodulation therapy	'Chitosan hydrogel when applied with a blood clot and PBMT could in the future improve previous results of dental pulp regeneration through cell homing approaches.'
Tunable Chitosan-Calcium Phosphate Composites As Cell Instructive Dental Pulp Capping Agents. (Osmond & Krebs, 2021)	Chitosan-calcium phosphate hydrogel	'These composites provide sufficient compressive modulus, biocompatibility, and odontogenic potential to potentially be used as a regenerative dental composite in future studies.'
Chitosan In Association with Osteogenic Factors as A Cell Homing Platform for Dentin Regeneration: Analysis in A Pulp- In-A-Chip Model. (Bordini et al., 2022)	Calcium hydroxide, β- glycerophosphae (βGP) and porous chitosan scaffold	'Direct interaction of pulp cells in a 3D matrix simulating clinical pulp exposure also revealed that the microenvironment created by this innovative scaffold is capable of mobilizing pulp cells to its surface, inducing odontoblastic differentiation even in the absence of osteogenic medium supplementation, along with stimulation of mineralized matrix deposition in an environment surrounded by dentin.'
The Effect of Chitosan on the Formation of Odontoblast-Like Cells in Reversible Pulpitis (in Vivo Study on Sprague Dawley Rats). (Tifani et al., 2023)	Chitosan- coated red snapper fish scales paste	'Chitosan red snapper fish scales used as a direct pulp capping material influence the formation of odontoblast-like cells in reversible pulpitis.'
Potency of the Combination of Chitosan and Hydroxyapatite on Angiogenesis and Fibroblast Cell Proliferation in Direct Pulp Capping of <i>Rattus norvegicus</i> . (Sularsih et al., 2024)	Chitosan and hydroxyapatite paste	'The combination of chitosan and hydroxyapatite could promote healing of direct pulp capping treatment by increasing the expression of VEGF, blood vessel, and fibroblast cell proliferation.'
The Effect of Nanochitosane of Red Snapper Fish Scales (Lutjanus Sp.) on Pain and Pulp Inflammation. (Widyastuti et al., 2024)	Nanochitosan	'The results of this study showed that redfish scale nanochitosane can decrease inflammation by lowering TNF- and pain levels.'
Bioinspired Smart Dentin ECM-Chitosan Hydrogels for Dentin-Pulp Complex Regeneration. (Osman et al., 2025)	ECM-chitosan hydrogels	'The proposed dentin-ECM-chitosan hydrogels have demonstrable microarchitecture, biophysical characteristics, biocompatibility, and antibacterial capacity to produce cell-inducing scaffolds for use in regenerative dentistry.'

5. LIMITATION OF CHITOSAN

Despite of the wide application of chitosan in biomedical field, there are some limitations of chitosan. The challenges include poor solubility of the polymer especially in neutral pH and alkaline pH condition. This

might limit its applications in many fields (Younes & Rinaudo, 2015). Commonly, chitosan will be dissolved in other dilute acid solutions such as 1% of acetic acid, formic acid or lactic acid (Pardo-Castaño & Bolaños, 2019). To further overcome the issue, various techniques of chemical modifications were applied. These includes oligomerization, alkylation, acylation, quaternization, hydroxyalkylation, carboxyalkylation, thiolation, sulfation, phosphorylation, enzymatic modifications and graft copolymerization. The process involved mainly the alteration of functional groups present in chitosan (eg: -OH and -NH₂ groups). These chemical changes have the potential to modify the structure of chitosan while also enhancing its physicochemical and biological properties, including adsorption, solubility, and biocompatibility.

Besides that, chitosan polymer also exhibits inferior mechanical properties. These may result in brittleness of the material and may impaired its longevity and function. To solve the drawbacks, chitosan is commonly blended with other synthetic or natural polymer such as polyethylene oxide (PEO), polyethylene glycol (PEG), carboxy methyl cellulose (CMC), polyvinyl alcohol (PVA) and others (Kaur et al., 2023). Combination of two or more biopolymers along with inorganic materials may form a superior properties of hybrid biocomposites. Laboratory research on the improvement of mechanical properties of chitosan films by the addition of PEO was conducted. The result suggested that blended films of chitosan/PEO produced a better mechanical property hence, it is suitable to be utilised in biomedical application (Alexeev et al., 2000).

6. CONCLUSION

Chitosan is a biocompatible biopolymer with a unique chemical structure that offers exceptional physiochemical and biological properties such as less toxicity, biodegradability, biocompatibility, low cost, hemostatic, and mucoadhesive. The rising market perspective and the volume of papers and patents that arise annually indicate the potential interest in these polymers. In the field of dentistry, chitosan is a good candidate for the future dental materials. Based on the various studies, chitosan is found to have a great potential as a pulp capping material. With the migration towards regenerative dentistry, chitosan has the ability to stimulate reparative dentin formation, maintain pulpal vitality with a good antimicrobial property.

The main disadvantages of chitosan are poor solubility in aqueous solution and inferior mechanical properties. To overcome the limitations, chemical modification of chitosan can be applied by the alteration of its functional group. Chitosan can also be blended with other polymers to improve its qualities and mechanical properties. Current trend of nanotechnology and tissue engineering have gained significant attention and provide a wide application of chitosan in biomedical field. Therefore, frequent review and studies should be conducted to discover a lot more novel biomedical uses of chitosan.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

AUTHORS' CONTRIBUTIONS

Nurul Izzani Zulkifle carried out the research, wrote and revised the article. Nurul Izzani Zulkifle, Afzan Adilah Ayoub, Siti Aisyah Roslan and Amalina Amir conceptualised the central research idea and provided the theoretical framework. Afzan Adilah Ayoub, Siti Aisyah Roslan and Amalina Amir designed the research, supervised research progress; Afzan Adilah Ayoub act as corresponding author, anchored the review, revisions and approved the article submission.

REFERENCES

- Alex, G. (2018). Direct and Indirect Pulp Capping: A Brief History, Material Innovations, and Clinical Case Report. *Compendium of continuing education in dentistry*, 39(3), 182-189.
- Alexeev, V. L., Kelberg, E. A., Evmenenko, G. A., & Bronnikov, S. V. (2000). Improvement of the mechanical properties of chitosan films by the addition of poly (ethylene oxide). *Polymer Engineering and Science*, 40(5), 1211–1215. https://doi.org/10.1002/pen.11248.
- Alhenaki, A. M., Attar, E. A., Alshahrani, A., Farooq, I., Vohra, F., & Abduljabbar, T. (2021). Dentin Bond Integrity of Filled and Unfilled Resin Adhesive Enhanced with Silica Nanoparticles—An SEM, EDX, Micro-Raman, FTIR and Micro-Tensile Bond Strength Study. *Polymers*, 13(7), 1093. https://doi.org/10.3390/polym13071093.
- American Academy of Pediatric Dentistry. (2023). Pulp therapy for primary and immature permanent teeth. In The Reference Manual of Pediatric Dentistry (Vols. 457–65). https://www.aapd.org/media/Policies Guidelines/BP PulpTherapy.pdf.
- Atila, D., Chen, C., Lin, C., Lee, Y., Hasirci, V., Tezcaner, A., & Lin, F. (2021). In vitro evaluation of injectable Tideglusib-loaded hyaluronic acid hydrogels incorporated with Rg1-loaded chitosan microspheres for vital pulp regeneration. *Carbohydrate Polymers*, 278, 118976. https://doi.org/10.1016/j.carbpol.2021.118976.
- Baena, E., Cunha, S. R., Maravić, T., Comba, A., Paganelli, F., Alessandri-Bonetti, G., Ceballos, L., Tay, F. R., Breschi, L., & Mazzoni, A. (2020). Effect of Chitosan as a Cross-Linker on Matrix Metalloproteinase Activity and Bond Stability with Different Adhesive Systems. *Marine Drugs*, 18(5), 263. https://doi.org/10.3390/md18050263.
- Bordini, E., Cassiano, F., Bronze-Uhle, E., Alamo, L., Hebling, J., De Souza Costa, C., & Soares, D. (2022). Chitosan in association with osteogenic factors as a cell-homing platform for dentin regeneration: Analysis in a pulp-in-a-chip model. *Dental Materials*, 38(4), 655–669. https://doi.org/10.1016/j.dental.2022.02.004.
- Bu, W., Wu, Y., Ghaemmaghami, A. M., Sun, H., & Mata, A. (2022). Rational design of hydrogels for immunomodulation. *Regen Biomater*, *9*, rbac009. https://doi: 10.1093/rb/rbac009.
- Chen, C., & Chung, Y. (2012). Antibacterial effect of water-soluble chitosan on representative dental pathogens Streptococcus mutans and Lactobacilli brevis. *Journal of Applied Oral Science*, 20(6), 620–627. https://doi.org/10.1590/s1678-77572012000600006.
- Chen, Z., Cao, S., Wang, H., Li, Y., Kishen, A., Deng, X., Yang, X., Wang, Y., Cong, C., Wang, H., & Zhang, X. (2015). Biomimetic Remineralization of Demineralized Dentine Using Scaffold of CMC/ACP Nanocomplexes in an In Vitro Tooth Model of Deep Caries. *PLoS ONE*, 10(1), e0116553. https://doi.org/10.1371/journal.pone.0116553.

- Cicciù, N., Fiorillo, N., & Cervino, N. (2019). Chitosan use in dentistry: A Systematic review of recent clinical studies. *Marine Drugs*, 17(7), 417. https://doi.org/10.3390/md17070417.
- Cohen, B. D., & Combe, E. C. (1994). Development of new adhesive pulp capping materials. *Dental Update*, 21(2), 57–62.
- Colombo, J. S., Moore, A. N., Hartgerink, J. D., & D'Souza, R. N. (2014). Scaffolds to Control Inflammation and Facilitate Dental Pulp Regeneration. *Journal of Endodontics*, 40(4), S6–S12. https://doi.org/10.1016/j.joen.2014.01.019.
- Desai, S., & Chandler, N. (2009). Calcium Hydroxide–Based Root Canal Sealers: A Review. *Journal of Endodontics*, 35(4), 475–480. https://doi.org/10.1016/j.joen.2008.11.026.
- Ducret, M., Montembault, A., Josse, J., Pasdeloup, M., Celle, A., Benchrih, R., Mallein-Gerin, F., Alliot-Licht, B., David, L., & Farges, J. (2019). Design and characterization of a chitosan-enriched fibrin hydrogel for human dental pulp regeneration. *Dental Materials*, 35(4), 523–533. https://doi.org/10.1016/j.dental.2019.01.018.
- El-Din, Y. E., El-Banna, A., & Hussein, T. S. (2023). Bonding of chitosan and nanochitosan modified universal adhesive to dentin. *International Journal of Adhesion and Adhesives*, 125, 103432. https://doi.org/10.1016/j.ijadhadh.2023.103432.
- Erpaçal, B., Adigüzel, Ö., Cangül, S., & Acartürk, M. (2019). A General Overview of Chitosan and its Use in Dentistry. *In IBBJ* (Journal-Article No. 1; pp. 1–3) [Journal]. https://ibbj.org.
- Feng, P., Luo, Y., Ke, C., Qiu, H., Wang, W., Zhu, Y., Hou, R., Xu, L., & Wu, S. (2021). Chitosan-based functional materials for skin wound repair: Mechanisms and applications. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.650598.
- Franzin, N. R. S., Sostena, M. M. D. S., Santos, A. D. D., Moura, M. R., De Camargo, E. R., Hosida, T. Y., Delbem, A. C. B., & Moraes, J. C. S. (2022). Novel pulp capping material based on sodium trimetaphosphate: synthesis, characterization, and antimicrobial properties. *Journal of Applied Oral Science*, *30*. https://doi.org/10.1590/1678-7757-2021-0483.
- Galván Márquez, I., Akuaku, J., Cruz, I., Cheetham, J., Golshani, A., & Smith, M. L. (2013). Disruption of protein synthesis as antifungal mode of action by Chitosan. *International Journal of Food Microbiology*, 164(1), 108–112. https://doi.org/10.1016/j.ijfoodmicro.2013.03.025.
- Halkai, K., Halkai, R., Gopinagaruri, S., Hussain, A., Rangappa, J., & Reshma, S. (2022). Evaluation of push-out bond strength of different concentrations of chitosan nanoparticles incorporated composite resin and eighth-generation bonding agent for class II restoration: An in vitro study. *Journal of Conservative Dentistry*, 25(6), 666. https://doi.org/10.4103/jcd.jcd 336 22.
- Hermann, B. W. (1952). On the reaction of the dental pulp to vital amputation and calxyl capping. *Dtsch Zahnarztl Z*, 7(24), 1446–7.
- Kaur, M., Sharma, A., Puri, V., Aggarwal, G., Maman, P., Huanbutta, K., Nagpal, M., & Sangnim, T. (2023). Chitosan-Based Polymer Blends for Drug Delivery Systems. *Polymers*, 15(9), 2028. https://doi.org/10.3390/polym15092028.
- Kmiec, M., Jr, Pighinelli, L., Tedesco, M., F., Silva, M., M., Reis, V., & Universidade Luterana do Brasil. (2017). Chitosan-properties and applications in dentistry. *Advance in Tissue Engineering and Regenerative Medicine Open Access*, 2(4), 205–211. https://doi.org/10.15406/atroa.2017.02.00035.

- Komabayashi, T., Zhu, Q., Eberhart, R., & Imai, Y. (2016). Current status of direct pulp-capping materials for permanent teeth. *Dental Materials Journal*, 35(1), 1-12. https://doi.org/10.4012/dmj.2015-013.
- Li, Z., Cao, L., Fan, M., & Xu, Q. (2015). Direct pulp capping with calcium hydroxide or mineral trioxide aggregate: A meta-analysis. *Journal of Endodontics*, 41(9), 1412–1417. https://doi.org/10.1016/j.joen.2015.04.012
- Moreira, M. S., Sarra, G., Carvalho, G. L., Gonçalves, F., Caballero-Flores, H. V., Pedroni, A. C. F., Lascala, C. A., Catalani, L. H., & Marques, M. M. (2021). Physical and biological properties of a Chitosan Hydrogel Scaffold Associated to Photobiomodulation Therapy for dental pulp regeneration: An In Vitro and In Vivo study. *BioMed Research International*, 2021, 1–10. https://doi.org/10.1155/2021/6684667.
- Murray, P. E., Garcia-Godoy, F., Hargreaves, K. M., & American Association of Endodontists. (2007). Regenerative endodontics: A review of current status and a call for action. *Journal of endodontics*, 33(4), 377–390. https://doi.org/10.1016/j.joen.2006.09.013.
- Nadig, R. R., Pai, V., & Deb, A. (2021). Evaluation of immediate and delayed microleakage of Class V cavities restored with Chitosan-incorporated composite resins: An In Vitro study. *International Journal of Clinical Pediatric Dentistry*, 14(5), 621–627. https://doi.org/10.5005/jp-journals-10005-2043.
- Osman, M., Sharmin, Z., Suchy, S., Gao, F., Kaminski, A., Mitchell, J. C., Sigar, I. M., & Carrilho, M. R. (2025). Bioinspired smart dentin ECM-chitosan hydrogels for dentin-pulp complex regeneration. *Journal of Dentistry*, 159, 105811. https://doi.org/10.1016/j.jdent.2025.105811.
- Osmond, M. J., & Krebs, M. D. (2021). Tunable chitosan-calcium phosphate composites as cell-instructive dental pulp capping agents. *Journal of Biomaterials Science Polymer Edition*, 32(11), 1450–1465. https://doi.org/10.1080/09205063.2021.1925390.
- Pardo-Castaño, C., & Bolaños, G. (2019). Solubility of chitosan in aqueous acetic acid and pressurized carbon dioxide-water: Experimental equilibrium and solubilization kinetics. *The Journal of Supercritical Fluids*, 151, 63–74. https://doi.org/10.1016/j.supflu.2019.05.007.
- Perdigão, J. (2009). Dentin bonding—Variables related to the clinical situation and the substrate treatment. *Dental Materials*, 26(2), e24–e37. https://doi.org/10.1016/j.dental.2009.11.149.
- Renard, E., Amiaud, J., Delbos, L., Charrier, C., Montembault, A., Ducret, M., Farges, J., David, L., Alliot-Licht, B., & Gaudin, A. (2020). Dental pulp inflammatory/immune response to a chitosan-enriched fibrin hydrogel in the pulpotomised rat incisor. *European Cells and Materials*, 40, 74–87. https://doi.org/10.22203/ecm.v040a05.
- Sangwan, P., Sangwan, A., Duhan, J., & Rohilla, A. (2012). Tertiary dentinogenesis with calcium hydroxide: A review of proposed mechanisms. *International Endodontic Journal*, 46(1), 3–19. https://doi.org/10.1111/j.1365-2591.2012.02101.x.
- Siqueira, J. F., Jr, & Rôças, I. N. (2008). Clinical implications and microbiology of bacterial persistence after treatment procedures. *Journal of Endodontics*, 34(11), 1292-1301. https://doi.org/10.1016/j.joen.2008.07.028.
- Sularsih, S., Fransiska, W., Salsabila, S., Rahmitasari, F., Soesilo, D., & Prananingrum, W. (2024). Potency of the combination of Chitosan and Hydroxyapatite on Angiogenesis and Fibroblast cell proliferation in direct pulp capping of rattus norvegicus. *European Journal of Dentistry*, 18(4), 1135-1141.

- https://doi.org/10.1055/s-0044-1782212.
- Supotngarmkul, A., Panichuttra, A., Ratisoontorn, C., Nawachinda, M., & Matangkasombut, O. (2020). Antibacterial property of chitosan against E. faecalis standard strain and clinical isolates. *Dental Materials Journal*, 39(3), 456–463. https://doi.org/10.4012/dmj.2018-343.
- Tifani, A. S., Widyastuti, N. H., Nugrahani, N. A., & Cahyani, C. (2023). The effect of Chitosan on the formation of Odontoblast-Like cells in reversible Pulpitis (in Vivo Study on Sprague Dawley Rats). *Advances in Health Sciences Research/Advances in Health Sciences Research*, 33-39. https://doi.org/10.2991/978-94-6463-184-5.
- Tomson, P. L., & Duncan, H. F. (2021). Pulp capping materials for the maintenance of pulp vitality. Endodontic Materials in Clinical Practice, 15–45. https://doi.org/10.1002/9781119513568.ch2.
- Urgiles, C. D., Urgiles, U. C. D., Urgiles Esquivel, C. E., Bravo, M. I., Gonzalez, F., & San Martin, D. (2024). Biomaterials for regeneration of the dentin-pulp complex [Chapter]. In Biomaterials for Regeneration of the Dentin-Pulp Complex. https://doi.org/10.5772/intechopen.114895.
- Widyastuti, N. H., Prayitno, A., Cilmiaty, R., & Wasita, B. (2024). Effect of chitosan nanoparticles from red snapper scales (lutjanus sp.) on pulp repair: In vivo study. *Journal of Pharmacy & Pharmacognosy Research*, 12(s1). https://doi.org/10.56499/jppres23.1769 12.s1.69.
- Wu, S., Zhou, Y., Yu, Y., Zhou, X., Du, W., Wan, M., Fan, Y., Zhou, X., Xu, X., & Zheng, L. (2019). Evaluation of Chitosan hydrogel for sustained delivery of VEGF for odontogenic differentiation of dental pulp stem cells. *Stem Cells International*, 2019, 1–14. https://doi.org/10.1155/2019/1515040.
- Xing, K., Chen, X. G., Liu, C. S., Cha, D. S., & Park, H. J. (2009). Oleoyl-chitosan nanoparticles inhibits escherichia coli and Staphylococcus aureus by damaging the cell membrane and putative binding to extracellular or intracellular targets. *International Journal of Food Microbiology*, 132(2–3), 127–133. https://doi.org/10.1016/j.ijfoodmicro.2009.04.013.
- Yang, X., Han, G., Pang, X., & Fan, M. (2012). Chitosan/collagen scaffold containing bone morphogenetic protein-7 DNA supports dental pulp stem cell differentiation in vitro and in vivo. *Journal of Biomedical Materials Research Part A*, 108(12), 2519–2526. https://doi.org/10.1002/jbm.a.34064.
- Yao, S., Chen, S., Wang, R., Zhang, K., Lin, X., & Mai, S. (2022). Antibacterial activity and bonding performance of carboxymethyl chitosan—containing dental adhesive system. *International Journal of Adhesion and Adhesives*, 119, 103269. https://doi.org/10.1016/j.ijadhadh.2022.103269.
- Yildirim, Z., Öncül, N., & Yildirim, M. (2016). Kitosan ve antimikrobiyal özellikleri. *Ömer Halisdemir Üniversitesi Mühendislik Bilimleri Dergisi*, 5(1), 19–36. https://doi.org/10.28948/ngumuh.239351.
- Younes, I., & Rinaudo, M. (2015). Chitin and Chitosan preparation from marine sources. Structure, Properties and Applications. *Marine Drugs*, 13(3), 1133–1174. https://doi.org/10.3390/md13031133.
- Zhu, C., Ju, B., & Ni, R. (2015) Clinical outcome of direct pulp capping with MTA or calcium hydroxide: a systematic review and meta-analysis. *International Journal of Clinical and Experimental Medicine*, 8(10):17055-60. PMID: 26770296; PMCID: PMC4694196.
- Zhu, N., Chatzistavrou, X., Papagerakis, P., Ge, L., Qin, M., & Wang, Y. (2019). Silver-doped bioactive glass/Chitosan hydrogel with potential application in dental pulp repair. *ACS Biomaterials Science & Engineering*, 5(9), 4624–4633. https://doi.org/10.1021/acsbiomaterials.9b00811.

Ziotti, I. R., Paschoini, V. L., Corona, S. A. M., & Souza-Gabriel, A. E. (2022). Chitosan-induced biomodification on demineralized dentin to improve the adhesive interface. *Restorative Dentistry & Endodontics*, 47(3). https://doi.org/10.5395/rde.2022.47.e28.



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