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Chitosan as a Bioactive Agent for Enhancing Coronal and Radicular Dentin Interfaces

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Abstract: ***Chitosan, a naturally derived biopolymer, has gained attention in adhesive and endodontic dentistry for its bioactive, antimicrobial, and chelating properties. This review evaluates its role in dentin conditioning and root canal therapies, emphasizing its ability to enhance smear layer removal, promote collagen stability, and support hybrid layer formation. Chitosan's antimicrobial action and compatibility with dental materials make it a promising adjunct in adhesive systems and intracanal applications. Despite encouraging laboratory evidence, further research is needed to standardize formulations and validate long-term clinical efficacy. The integration of chitosan offers a biologically driven approach to improve restorative and endodontic outcomes.***

Keywords: chitosan, dentin bonding, hybrid layer, endodontic therapy, antimicrobial agent

1. Introduction

Chitosan and its organic derivatives, particularly chitosan citrate, have recently gained attention as innovative biomaterials in dentistry. Owing to its chelating and biomimetic properties, chitosan citrate functions as an effective dentin conditioner that promotes remineralization of dentin and optimizes the collagen fibril network for enhanced adhesive bonding. In the context of root canal retreatment, its application on radicular dentin improves the adhesion of the sealer to the dentinal surface and exerts a favorable biomimetic influence on periapical tissues [0].

The longevity of adhesive direct and indirect restorations is intrinsically linked to the stability of the hybrid layer. Establishing a bioadhesive interface on a partially demineralized substrate requires careful consideration, as caries-affected dentin exhibits a disorganized organic matrix and morphological characteristics distinct from those of sound dentin [**Error! Reference source not found.**]. Bacterial by-products generated during caries formation activate matrix metalloproteinases (MMPs) within the dentin. Combined with hydrolytic activity, these enzymes accelerate degradation of the adhesive interface. [0]. Methacrylate polymers present in adhesive systems are susceptible to chemical hydrolysis and enzymatic breakdown mediated by MMPs [[3]]. To inhibit MMP activity and prevent hybrid layer deterioration, it is essential to premedicate the cavity surface and employ adhesive systems resistant to collagenolytic enzymatic activity [[4]]. Moreover, the incorporation of bioactive agents capable of modulating enzymatic activity and promoting remineralization has emerged as a strategic approach to reinforce the hybrid layer and extend restoration durability [[5]]. The development of novel adhesive formulations with enhanced resistance to biodegradation and improved interaction with dentin substrates remains a key focus in restorative dentistry.

In parallel, the predictable elimination of microorganisms from the microcanal system of radicular dentin has become achievable through the combined use of appropriate irrigation solutions and antimicrobial agents, alongside conventional mechanical root canal instrumentation. The dissolution of the smear layer over time may enhance permeability, allowing various irrigants and materials to penetrate the dentinal tubules and potentially influence the periodontal status of the tooth. The extent of irrigant diffusion is dependent on the physicochemical properties of the solutions used [[6]]. Current literature supports the use of irrigation protocols that incorporate broad-spectrum antimicrobial agents capable of dissolving endotoxins and removing the smear layer [[7],[8]]. Additionally, the synergistic use of chelating agents and surfactants has shown promise in optimizing canal cleanliness and improving the efficacy of subsequent obturation procedures. The integration of nanotechnology-based irrigants and medicaments is also being explored to enhance antimicrobial action and reduce cytotoxicity.

This review critically explores the application of chitosan in dental adhesives, irrigation solutions, intracanal medicaments, and sealers, highlighting its advantages, limitations, and future perspectives, with particular emphasis on addressing hybrid layer degradation over time. Given chitosan's multifunctional properties and its ability to interact favorably with dentin substrates, its integration into endodontic materials represents a promising avenue for enhancing clinical outcomes. The incorporation of chitosan-citrate formulations offers a multifaceted approach to endodontic therapy by enhancing antimicrobial efficacy, promoting biocompatibility, and improving the physicochemical properties of dental materials [[9]]. Furthermore, chitosan's chelating capacity, film-forming ability, and potential to inhibit MMPs position it as a versatile component in the development of next-generation bioactive dental products. Continued research into its molecular interactions and long-term performance in clinical settings will be essential to fully harness its therapeutic potential. Understanding the potential of chitosan in clinical protocols may lead to more durable, biologically integrated restorative and endodontic treatments.

2. Chitosan as dentin conditioner in adhesive dentistry

2.1 Biocompatibility and Biodegradability

Chitosan has emerged as a promising biopolymer in both adhesive and endodontic applications. Discovered by Rouge in 1859 through the alkaline treatment of chitin, and later named by Hoppe-Seyler in 1894, chitosan is a linear polysaccharide derived from the deacetylation of chitin [[10]]. Chitosan naturally occurs in the cell walls of fungi, yeasts, insects, and crustacean shells [[11]]. Structurally, chitosan consists of β(1→4)-linked 2-amino-2-deoxy-D-glucose (60%–100%) and 2-acetamido-2-deoxy-D-glucoside (0%–50%) [[12]]. Its biocompatibility, biodegradability, non-toxicity, and bioadhesiveness make it suitable for a wide range of biomedical and dental applications [[13],[14],[15]].

Theory 1: Adhesion is attributed to electrostatic interactions between the amino group of chitosan (NH_3^+) and the carboxyl group of collagen (COO^-) [[16]].

Theory 2: Covalent immobilization of chitosan onto dentinal collagen is hypothesized to induce remineralization of exposed and demineralized dentin structures, as its functional phosphate groups can bind calcium ions to create a favorable surface for crystal nucleation, leading to the formation of a calcium phosphate layer [[17]].

Although chitosan is highly soluble in acidic solutions with pH below 6.0, its solubility is suboptimal for biological applications. Enhancing solubility expands its utility as a nanomaterial. Practical approaches include chitin deacetylation and chemical modification via attachment of hydrophilic biomolecules to amino or hydroxyl groups [[18]].

2.2 Collagen Cross-linking

Chitosan's molecular structure contains free amino- and hydroxyl- groups, along with positively charged sites that facilitate ionic interactions with dentin collagen, leading to cross-link formation [[19]] This process generates mechanically robust fibril chains, thereby enhancing the structural integrity of dental restorations[[20],[21]].Previous studies have demonstrated that collagen fibril crosslinking can occur within hours following application of crosslinking agents, with measurable improvements in bond strength observed as early as 24 hours. This suggests that the enhanced immediate bond strength in our evaluation may be attributed to early crosslinking mechanisms [[22], [23], [24], [25]].

2.3 Protection Against Degradation

Chitosan treatment has been shown to improve the chemical and mechanical resistance of dentin surfaces to enzymatic degradation by reducing MMP activity [[26],[27]]. Additionally, chitosan exhibits chelating capacity and antimicrobial properties [[28],[29],[30]]. In vitro studies have demonstrated the superior efficacy of chitosan nanoparticles (ChNPs), which, exhibit improved absorption, strong adhesion, and deep penetration into oral biofilms compared to chitosan solutions [[31],[32]].

2.4 Antibacterial Properties

Chitosan's cationic nature allows it to disrupt bacterial membranes, granting it potent antimicrobial capabilities, particularly against Streptococcus mutans and other cariogenic pathogens [[33],[34],[35]]. Yao et al. found that adhesives incorporating carboxymethyl chitosan significantly inhibited bacterial growth while maintaining bonding efficacy [[33]]. Dobrzyński et al.'s systematic review confirmed that chitosan integration at low concentrations into resin composites improved their antibacterial activity without negatively affecting mechanical performance [[35]]. Shen et al. also reported that arginine–chitosan-modified adhesives showed both bioactivity and MMP inhibition, preventing bond degradation [[36]]. Chitosan, incorporated into an experimental adhesive system at concentrations of 0.2% and 0.5%, exhibited antimicrobial activity against Streptococcus mutans and Lactobacillus casei, comparable to the effects observed with the conventional adhesive system Adper Single Bond 2[[37]].

2.5 Improved Bonding

Efforts to inhibit MMP activity and collagen degradation have also aimed to improve the durability of the resin–dentin interface, with MMP inhibitors applied as a separate dentin pretreatment step [[38]]. Discussion Pascioini et al. reported that application of chitosan in dental treatment, combined with an etch-and-rinse or self-etch adhesive system, improved the immediate and preserved the 6-month bond strength of the adhesive interface [[39]]. Fig.1 represent structural comparison of dentin conditioned with or without 0,1 % and 0,6 % chitosan solution. The selection of a 2.5% chitosan solution concentration was informed by some studies, which demonstrated its ability to induce calcium phosphate layer formation on dentin without altering the adhesive's wettability on the substrate [[40],[41],[42]].

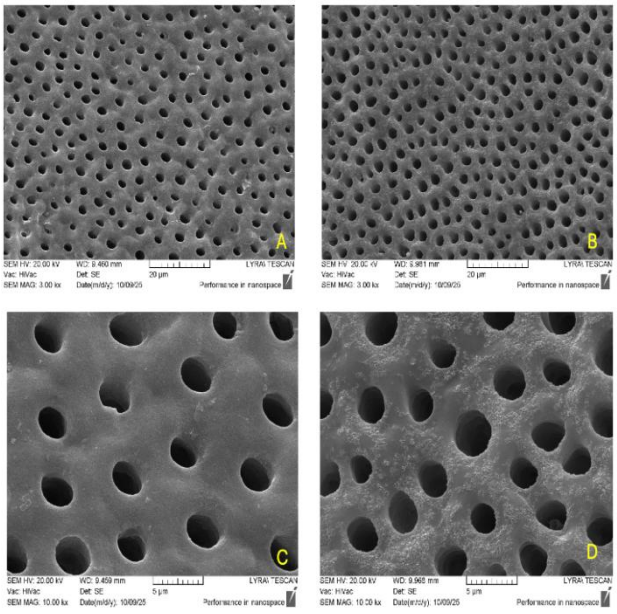

Figure 1: SEM of prepared coronal dentin
A.conditioned with 0,1 % chitosan solution(magnification x 3000)
B.conditioned with physiological saline (magnification x 3000)
C.conditioned with 0,1 % chitosan solution (magnification x10000)
D.conditioned with physiological saline (magnification x 10000)

2.6 Nanocomplexes:

When combined with hydroxyapatite, chitosan forms chitosan- hydroxyapatite (CS-HA) nanocomplexes, which further enhance the fracture resistance of root dentin and offer increased antibiofilm efficacy. Recent studies have explored the use of nanocomplexes composed of carboxymethyl chitosan and amorphous calcium phosphate (CMC/ACP) for dentin remineralization via a biomimetic strategy. Remineralization in dentin is more challenging than in enamel, which mechanism Simeonov at al. presented [[43]], due to its organic matrix and occurs primarily through the growth of residual crystals. Using a monolayer model, CMC/ACP releases ACP nanoparticles to achieve intrafibrillar mineralization of collagen—where minerals are deposited within the gap zones of collagen fibrils and microfibrillar spaces—following a bottom-up strategy. This approach enables remineralization of demineralized dentin while preserving the dentin tissue to the greatest extent possible [[44]].

One of the most advantageous properties of chitosan is its potential in the development of novel biomaterials due to its high bioactivity, enabling broader applications in dentistry. Advances in biomaterial science have led to significant progress across various clinical disciplines [[45]]. Chitosan has been used to promote biomimetic enamel reconstruction and inhibit biofilm formation on titanium implant surfaces [[29],[32]]. Its incorporation into experimental adhesive systems improves the biological and mechanical properties of collagen matrices and enhances antibacterial activity through ionic interactions with bacterial cells [[30]]. Chitosan nanoparticles at neutral pH have been shown to damage cariogenic microorganisms such as *Streptococcus mutans* [[46]]. To enhance adhesive strength and develop robust dentin bonding systems, researchers have investigated antioxidant chitosan hydrogels infused with propolis, nystatin, and β-carotene [[45]]. The hypothesis of the referenced study focused on evaluating the effect of various antibacterial cleansing agents on dentin microshear bond strength, indicating the need for further analyses and investigations in this direction.

3. Chitosan as dentin conditioner in endodontics

Based on expansive clinical experience, recommendations from guidelines, treatment protocols, and current evidence, debridement of root canals is recommended in most endodontic treatment [[47]]. Mechanical preparation alone cannot suffice to create a sterile space within the infected root canals due to complex nature of the human root canal systems [[48]]. As per recent consensus statements on endodontics, both traditional stainless steel hand instruments and the use of nickel-titanium equipment leave nearly half of the root canal walls untreated [[48]]. Thus, it is critical to have an irrigation system or intervention as part of the conventional root canal treatment. The sole objective of the irrigation is for cleansing that does not take place with mechanical preparation [[49]].

3.1 Antibacterial effect

Chitosan solutions (especially chitosan citrate) have garnered attention in endodontics due to their potent antibacterial properties. Chitosan, a natural polysaccharide derived from chitin, exhibits broad-spectrum antimicrobial activity, which is further enhanced when combined with citric acid [[50]]. This combination disrupts bacterial cell walls and membranes, leading to cell death. Studies have demonstrated the efficacy of chitosan-citrate solutions against common endodontic pathogens. For instance, a study by Silva et al. showed that a 0.2% chitosan solution effectively eliminated Enterococcus faecalis biofilms, a bacterium often associated with persistent root canal infections. The study emphasized the solution's capacity to infiltrate biofilms and compromise bacterial cell integrity [[51]]. Another investigation by Zhang et al. evaluated the antibacterial activity of chitosan-citrate solutions against Streptococcus mutans and Lactobacillus acidophilus [[52]]. The results indicated significant bacterial reduction, attributing the effect to the chelating action of citric acid and the antimicrobial properties of chitosan. Furthermore, chitosan-citrate solutions have been compared to traditional irrigants like sodium hypochlorite. While sodium hypochlorite remains a gold standard, chitosan-citrate offers a biocompatible alternative with fewer cytotoxic effects, making it suitable for patients with sensitivities [[51]]. L. S. Gu et al. reported in their study that live/dead staining assays demonstrated the antibacterial efficacy of acetic acid-solubilized chitosan against three single-species biofilms—Streptococcus mutans, Actinomyces naeslundii, and Enterococcus faecalis—highlighting its antibacterial potential as a bioactive component in endodontic disinfection protocols [[21]].

3.2 Haemostasis and pulpotomy

Chitosan solutions have emerged as promising agents in achieving haemostasis and facilitating pulpotomy procedures in endodontic therapy. Chitosan exhibits haemostatic properties due to its positive charge, which promotes platelet adhesion and aggregation, leading to rapid blood clot formation. When combined with citric acid, the resultant chitosan-citrate solution enhances these effects, providing an effective means to control bleeding during dental procedures. In the context of pulpotomy, chitosan-citrate solutions have been investigated as alternatives to traditional agents like formocresol [[53]]. A clinical pilot study by Kothari et al. compared the efficacy of chitosan and formocresol as pulpotomy medicaments in primary molars. The study found that chitosan exhibited comparable clinical success rates and superior radiographic outcomes, suggesting its potential as a safer and effective alternative [[54]].

Additionally, chitosan's biocompatibility and antimicrobial properties make it suitable for use in pulpotomy procedures. Its ability to inhibit bacterial growth reduces the risk of postoperative infections, while its biodegradability ensures that it does not interfere with the natural healing process. These attributes position chitosan-citrate solutions as advantageous in managing pulpal exposures and promoting tissue repair.

3.3 Smear layer removal

In a systematic review of *ex vivo* studies Ferreira-Reguera A, et al. concluded that 0.2% chitosan may be considered as a promising irrigation solution when employed as a final irrigant to remove the smear layer in endodontic protocol. Gusiyiska et al. reported that chitosan-citrate solution effectively removed the smear layer and opened dentinal tubules without causing excessive erosion. The SEM images showed clean dentin surfaces with well-defined tubule openings (Fig.2, Fig.3, Fig.4, Fig.5- courtesy by A.Gusiyska) [[55]]. Standardized protocols for the application of various chelators in the protocol of modern endodontic treatment should be investigated and refined in future studies, regardless of the results of this literature review [[56]]

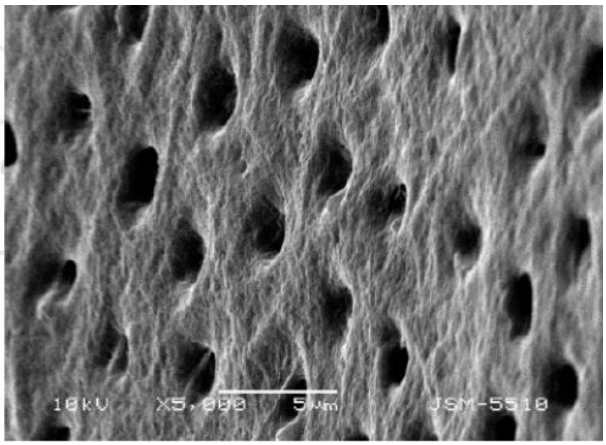

Figure 2: SEM of prepared radicular dentin irrigated with 0,1 % chitosan solution (magnification x 5000)

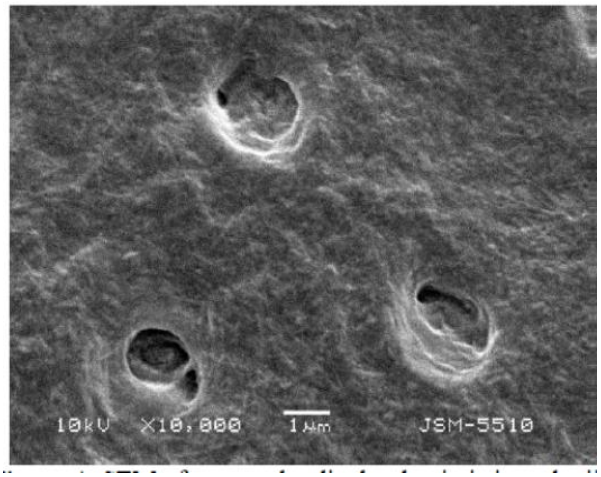

Figure 3: SEM of prepared radicular dentin irrigated with 0, 1 % chitosan solution (magnification x10 000)

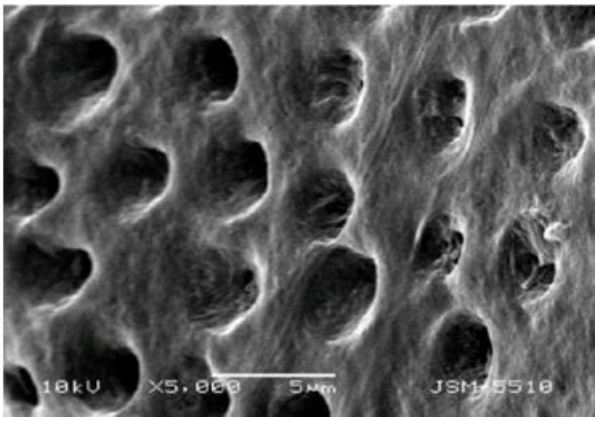

Figure 4: SEM of prepared radicular dentin irrigated with 0, 6 % chitosan solution(magnification x 5000)

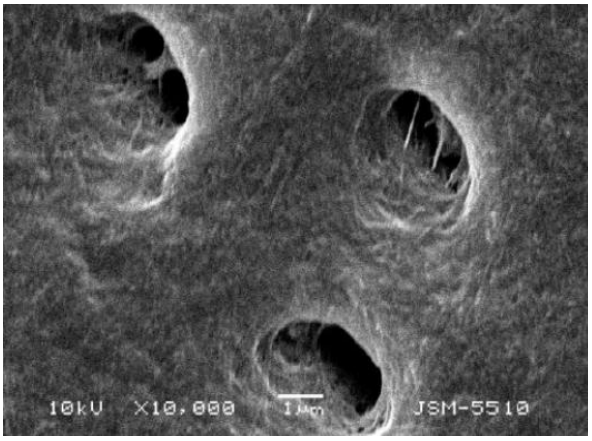

Figure 5: SEM of prepared radicular dentin irrigate with 0,6 % chitosan solution (magnification x10 000)

3.4 Intracanal medication and sealers

The persistent challenge in endodontic therapy is the eradication of resilient microorganisms such as Enterococcus faecalis and Candida albicans, which are often implicated in root canal treatment failures. Chitosan-citrate solutions have emerged as promising intracanal medicaments and sealer additives due to their potent antimicrobial properties, biocompatibility, and ability to enhance the physical characteristics of endodontic materials [[57]].

3.5 Antimicrobial Efficacy

Chitosan's polycationic nature allows it to interact with the negatively charged microbial cell membranes, leading to increased permeability and eventual cell death. When formulated as a paste, chitosan has demonstrated significant antimicrobial activity against E. faecalis and C. albicans. A study by Siqueira et al. showed that chitosan paste exhibited superior efficacy in reducing viable E. faecalis biofilms compared to calcium hydroxide after a 7-day intracanal application. The study highlighted chitosan's potential as an effective alternative intracanal medicament, particularly in cases involving resistant microbial strains [[58]]. An in vitro study by Gyulbenkiyan et al. confirms that a 0.6% chitosan-citrate solution exhibits significant antibacterial activity against Enterococcus faecalis, suggesting its potential as an alternative root canal irrigant[[59]].

3.6 Biocompatibility and Cytotoxicity

Biocompatibility is a crucial consideration for any intracanal medicament or sealer. Chitosan has been shown to be biocompatible, promoting cell proliferation and exhibiting low cytotoxicity. Studies have indicated that chitosan-containing sealers support the viability of periapical tissues and may facilitate tissue regeneration. This contrasts with some traditional sealers, associated with adverse tissue reactions [[51]].

3.7 Smear Layer Removal and Dentin Interaction

Chitosan-citrate solutions have also been evaluated for their ability to remove the smear layer and interact favorably with dentin. Gusiyska et al. reported that chitosan-citrate effectively removed the smear layer, especially in the coronal and middle thirds of the canal [[60]]. The chelating action of citric acid combined with chitosan's antimicrobial properties results in effective smear layer removal without causing significant dentin erosion [[61]]. This property enhances the penetration of sealers into dentinal tubules, potentially improving the seal and longevity of root canal fillings. The integration of chitosan-citrate solutions into intracanal medications and sealers offers a multifaceted approach to endodontic therapy, combining antimicrobial efficacy, biocompatibility, and improved material properties. These attributes position chitosan-citrate as a valuable component in the development of advanced endodontic materials aimed at enhancing treatment outcomes and reducing the incidence of root canal failures [[50],[51]].

4. Future research/ Prospective investigations

Future research should prioritize the refinement of chitosan formulations, the development of standardized application protocols, and rigorous long-term clinical evaluations to validate its efficacy across diverse dental substrates and clinical scenarios. Particular emphasis should be placed on optimizing molecular weight, degree of deacetylation, and carrier systems to enhance chitosan's bioactivity, substantivity, and compatibility with existing adhesive and endodontic materials. Investigations into its synergistic interactions with other bioactive compounds—such as calcium phosphates, nanoparticles, and enzymatic inhibitors—may unlock new therapeutic potentials [[62],[62]]. Interdisciplinary collaboration—bridging materials science, microbiology, and clinical dentistry—will be essential to fully realize the potential of chitosan as a reliable and biologically active component in adhesive and endodontic therapies. Moreover, in vivo studies and randomized controlled trials are needed to assess its long-term performance, safety profile, and influence on treatment outcomes such as bond durability, pulp vitality, and periapical healing. The integration of chitosan into minimally invasive and regenerative protocols may also pave the way for its use in biomimetic approaches to tissue preservation and repair.

5. Conclusion

Chitosan offers a promising solution in both adhesive and endodontic dentistry due to its antimicrobial, bioactive, and biocompatible nature. Its potential to strengthen the hybrid layer and improve root canal disinfection aligns with modern minimally invasive practices. Future integration into clinical protocols depends on overcoming formulation, stability, and compatibility challenges through standardized research and trials.

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