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INTERNATIONAL
RESEARCH AND
EVALUATIONS IN THE
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Chapter 1

CLINICAL PRACTICE VARIATIONS IN ENDODONTICS: A COMPARATIVE REVIEW OF TURKEY, THE UK, AND EUROPE

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INTRODUCTION

Endodontic therapy remains one of the most widely performed dental procedures, with root canal treatment (RCT) serving as a cornerstone of efforts to preserve the natural dentition. Globally, the prevalence of untreated apical periodontitis continues to represent a significant burden on oral health, with epidemiological studies indicating that more than 30–50% of root-filled teeth display radiographic signs of periapical disease in various populations (European Society of Endodontology [ESE], 2019; Kirkevang et al., 2014). These outcomes highlight the importance not only of technical competence but also of adherence to evidence-based protocols in determining long-term success.

Despite the universality of endodontic principles, substantial variation exists in clinical practice patterns across different regions. These variations are shaped by a combination of educational philosophy, health service structure, cultural expectations, and the degree of compliance with international guidelines. In Europe, the ESE has consistently published position statements advocating for minimally invasive access, irrigant activation, advanced obturation, and antibiotic stewardship (ESE, 2019, 2021). In the United States, the American Association of Endodontists (AAE) has issued parallel recommendations with emphasis on regenerative endodontics and biologically driven treatment paradigms (Hargreaves & Diogenes, 2013). Within the UK, the National Institute for Health and Care Excellence (NICE) guidance exerts a powerful regulatory influence, particularly regarding antimicrobial prescribing and patient safety (NICE, 2015).

Turkey presents a unique case where postgraduate programmes in endodontics—particularly within university hospitals—have embraced contemporary approaches such as contracted access, thermoplastic obturation, and regenerative procedures. However, surveys reveal that general dental practitioners (GDPs) in private practice often adopt different strategies, sometimes diverging from guideline-based care due to patient expectations, economic pressures, and limited resources (Ünal et al., 2012; Çiftçioğlu et al., 2023). In contrast, the UK is characterised by relatively high compliance with guideline-based decision-making, especially in relation to antibiotics, but pragmatic reliance on conventional instrumentation and obturation methods persists in NHS practice due to systemic pressures such as time constraints and restricted reimbursement models (Ng et al., 2011; Whitworth, 2018).

Understanding these contrasts is essential, as they illustrate how systemic and cultural contexts directly influence clinical decisions. This

chapter therefore provides a narrative review of the literature, synthesising evidence on clinical practice variations in endodontics across Turkey, the UK, and Europe, with focus on access cavity design, irrigation protocols, obturation methods, retreatment strategies, regenerative procedures, and antibiotic prescribing.

Access Cavity Design

The philosophy of access cavity preparation has undergone profound changes over the last two decades. Traditional teaching advocated for large, straight-line access cavities to ensure canal location and adequate debridement. However, the advent of minimally invasive endodontics (MIE) has challenged this paradigm by emphasising the preservation of pericervical dentin, which is critical to the structural integrity and long-term survival of the tooth (Clark & Khademi, 2010). Contracted access designs and ultraconservative “ninja” cavities have been promoted, with studies suggesting that these may enhance fracture resistance without significantly compromising canal instrumentation (Plotino et al., 2017; Rover et al., 2017).

In Turkey, postgraduate programmes and university hospitals have increasingly embraced this minimally invasive philosophy. Surveys reveal that postgraduate students are trained to prepare contracted access cavities and adopt conservative shaping techniques (Çiftçiöğlü et al., 2023). Turkish academic centres also explore novel approaches such as guided endodontics, where cone-beam computed tomography (CBCT) and CAD/CAM technologies are used to plan ultraconservative access pathways (Ozyurek et al., 2020). However, among general practitioners, traditional access designs remain common, largely due to limited magnification and diagnostic imaging resources in private practice.

In the UK, conventional access cavities continue to dominate NHS general practice. Although clinicians are well informed about the theoretical benefits of contracted access, systemic constraints—particularly the need for efficiency and the limited appointment times allocated under NHS contracts—reinforce reliance on more traditional cavity shapes (Ng et al., 2011; Whitworth, 2018). Private and specialist endodontic practices in the UK are more likely to incorporate MIE principles, aided by routine use of dental operating microscopes. However, even in these settings, there is ongoing debate about the risk of missed canals and procedural complications associated with excessively conservative designs (Krishan et al., 2014).

In continental Europe, MIE techniques have been adopted more rapidly, reflecting stronger adherence to ESE guidelines and broader dissemination of advanced technologies. Countries such as Germany, the Netherlands, and Spain have reported higher levels of clinical integration of contracted and guided access approaches, supported by postgraduate curricula and continuing education programmes (ESE, 2019). European clinicians are also more likely to routinely employ CBCT and magnification, which facilitate the safe adoption of ultraconservative techniques.

The debate surrounding access cavity design underscores a central theme of endodontic practice variation: the tension between conserving tooth structure and ensuring procedural safety. While Turkey leads in academic promotion of contracted access, the UK remains conservative under NHS limitations, and Europe demonstrates faster guideline-based integration. The long-term clinical implications of ultraconservative access cavities remain under investigation, with some evidence cautioning against excessive restriction that may compromise canal negotiation or complete debridement (Arora et al., 2022).

Irrigation Protocols

Irrigation is universally regarded as a critical stage in root canal therapy, as it allows chemical debridement of anatomical regions that cannot be reached by instruments. The complexity of the root canal system—including isthmuses, lateral canals, fins, and apical deltas—necessitates an effective irrigant regimen to eliminate biofilm, dissolve necrotic tissue, and reduce microbial load (Haapasalo et al., 2014). Accordingly, the selection of irrigant type, concentration, delivery method, and activation system plays a decisive role in clinical outcomes. Despite these common goals, international variations remain evident, reflecting educational influences, safety priorities, and healthcare system structures.

In Turkey, academic institutions and postgraduate programmes traditionally recommend sodium hypochlorite (NaOCl) at concentrations of 5.25%, in line with laboratory evidence demonstrating superior tissue-dissolving and antimicrobial properties (Ünal et al., 2012; Kaptan et al., 2012). Furthermore, Turkish university hospitals often integrate adjunctive irrigants such as 17% EDTA for smear layer removal and, less commonly, chlorhexidine for specific indications. Specialist clinics increasingly adopt irrigant activation methods, particularly passive ultrasonic irrigation (PUI) and sonic agitation, which are consistent with ESE guidelines. However, surveys of general dental practitioners reveal limited use of advanced techniques, with many clinicians still relying on syringe

irrigation with open-ended or side-vented needles (Kaptan et al., 2012).

In the United Kingdom, the pattern is notably different. Lower concentrations of NaOCl (2–2.5%) are commonly used, especially in NHS settings, reflecting an emphasis on safety and risk minimisation (Whitworth, 2018). Hypochlorite accidents, though rare, have received considerable medico-legal attention in the UK, reinforcing clinician caution and shaping practice habits. General dental practitioners in NHS practice often employ passive irrigation with syringe delivery only, given the cost, time, and equipment constraints. Advanced activation methods, such as PUI or newer technologies like XP-Endo Finisher or GentleWave, are generally restricted to private or specialist practice, where resources and reimbursement structures support their use. Surveys suggest that while UK clinicians are well aware of the benefits of irrigant activation, systemic pressures discourage its routine application (Palmer et al., 2000).

In continental Europe, irrigant protocols show closer alignment with ESE guidelines, which recommend NaOCl concentrations of 2.5–5.25% and stress the importance of irrigant activation (ESE, 2019). Many postgraduate programmes across Germany, Spain, and the Netherlands routinely teach and implement ultrasonic and sonic activation. Furthermore, laser-assisted irrigation techniques (e.g., photon-induced photoacoustic streaming, PIPS) and negative pressure systems (e.g., EndoVac) are increasingly reported in specialist and academic centres (de Groot et al., 2009). These approaches, while less common in routine general practice, illustrate Europe's faster integration of emerging technologies.

Systematic reviews and meta-analyses have confirmed that irrigant activation significantly improves microbial reduction and smear layer removal compared with syringe irrigation alone (van der Sluis et al., 2016; Dutner et al., 2012). However, clinical evidence regarding long-term outcome benefits remains limited, which may partly explain the slower uptake in some healthcare systems. Concerns about cost-effectiveness, equipment maintenance, and patient safety continue to influence adoption, particularly in public systems such as the NHS.

Overall, irrigation protocols exemplify the complex interaction between science, training, and system-level constraints. Turkey demonstrates strong academic endorsement of high-concentration NaOCl and activation, yet general practice remains conservative. The UK prioritises safety, with NHS practice generally limited to lower concentrations and passive irrigation, despite clinician awareness of alternatives. Europe represents the region most closely aligned with guideline-driven recommendations, integrating activation methods more widely. These variations

highlight the need for continued emphasis on translating laboratory and academic evidence into scalable, system-compatible protocols that ensure safe and effective disinfection across diverse clinical environments.

Obturation Techniques

Successful root canal treatment requires not only effective cleaning and shaping but also a three-dimensional seal of the root canal system to prevent reinfection. The obturation phase, therefore, remains a central determinant of long-term prognosis. Over the past three decades, numerous techniques and materials have been developed, ranging from traditional gutta-percha with zinc oxide–eugenol sealers to modern thermoplasticised systems and bioceramic sealers. The extent to which these methods are adopted varies significantly between Turkey, the UK, and continental Europe, reflecting differences in training, equipment availability, and healthcare system priorities.

In Turkey, postgraduate training programmes and specialist clinics have strongly promoted the use of thermoplastic obturation techniques such as warm vertical compaction and carrier-based systems (e.g., Thermafil, GuttaCore). These methods are widely taught in universities, based on evidence that thermoplasticisation can improve the homogeneity of gutta-percha fills and adapt more effectively to canal irregularities (Wu & Wesselink, 2001; Ünal et al., 2012). Academic centres also increasingly encourage the use of bioceramic sealers, which offer bioactivity, dimensional stability, and potential for monoblock formation (Zhou et al., 2015). However, surveys of general practitioners indicate that many clinicians continue to employ cold lateral condensation due to cost considerations and limited access to advanced equipment (Kaptan et al., 2012). This reflects a common pattern in Turkey: postgraduate training drives innovation, but general practice uptake is moderated by economic and practical realities.

In the United Kingdom, cold lateral condensation remains the predominant obturation method in NHS general practice (Ng et al., 2011; Whitworth, 2018). The technique is valued for its simplicity, reliability, and minimal equipment requirements. Although clinicians are well aware of the advantages of thermoplasticised approaches, systemic constraints within the NHS—including short appointment times, reimbursement limits, and restricted funding for advanced devices—make cold lateral condensation the pragmatic choice. Thermoplastic obturation techniques and newer sealer systems are more commonly employed in private practices and by specialists, where resources and patient expectations allow.

Nonetheless, surveys highlight that a growing minority of UK clinicians are beginning to adopt single-cone techniques with bioceramic sealers, which combine efficiency with improved biological properties, although widespread integration remains limited (Donnelly et al., 2020).

In continental Europe, adoption of advanced obturation strategies has been more rapid, reflecting closer adherence to ESE guidelines and stronger integration of evidence-based innovations into postgraduate curricula (European Society of Endodontology [ESE], 2019). Countries such as Germany, Spain, and Italy report widespread use of thermoplasti-cised techniques in specialist practice, and bioceramic sealers are becoming increasingly mainstream. Research output from European centres has contributed significantly to the growing body of evidence supporting these materials, particularly in terms of biocompatibility and sealing ability (Zhang et al., 2009; Zhou et al., 2015). Furthermore, postgraduate training in several European countries includes hands-on training with a broad spectrum of obturation devices, accelerating their clinical uptake compared to the UK and Turkey.

Clinical outcome studies suggest that both thermoplastic and bioceramic-based obturation methods can achieve comparable or superior success rates to traditional techniques, particularly in cases with complex canal morphology (Al-Haddad & Che Ab Aziz, 2016). However, concerns persist regarding cost-effectiveness, retrievability of bioceramic sealers during retreatment, and the need for specialised equipment. These factors partly explain the slower adoption in NHS practices in the UK and among general practitioners in Turkey.

In summary, obturation techniques illustrate a clear gradient of adoption: Turkey integrates advanced techniques in postgraduate education but sees uneven uptake in general practice; the UK largely retains conventional cold lateral condensation in NHS settings, with gradual adoption of bioceramics in private practice; and Europe demonstrates faster mainstream integration of thermoplastic and bioceramic approaches, closely aligned with ESE recommendations. The persistence of variation underscores the influence of healthcare structures and training systems in shaping clinical choices.

Retreatment Approaches

Endodontic retreatment is a complex and often controversial component of clinical practice. The decision to attempt nonsurgical retreatment, proceed to surgical intervention, or extract the tooth is influenced by

multiple factors, including technical feasibility, cost, patient expectations, and the wider healthcare framework. Evidence suggests that nonsurgical retreatment can achieve high success rates, with systematic reviews reporting favourable outcomes of 71–77% at 3–5 years, provided that contemporary protocols and adequate disinfection are employed (Hall et al., 2024). Despite this, clinical practice across countries demonstrates significant variation.

In Turkey, retreatment is frequently managed at the specialist or postgraduate level rather than by general practitioners. Surveys of Turkish dentists indicate that GDPs are less likely to attempt retreatment, preferring instead to refer patients to endodontic specialists, particularly for cases involving complex anatomy, separated instruments, or post removal (Ünal et al., 2012). This reflects both the educational emphasis in Turkey, where postgraduate programmes provide structured training in advanced retreatment protocols, and the availability of university hospitals as referral centres. Specialists in Turkey often employ modern retreatment instruments such as rotary NiTi retreatment files, solvents, and adjunctive ultrasonic devices, reflecting a high level of academic integration (Kaptan et al., 2012). However, in private general practice, extraction remains relatively common for failed cases, particularly where patients face financial limitations or where referral pathways are less accessible.

In the United Kingdom, retreatment demonstrates a strikingly different pattern. Within NHS general practice, clinicians often perceive extraction as the more pragmatic option for failed root canal treatments (Saunders & Saunders, 2016). This tendency is shaped by systemic factors: NHS contracts provide limited financial incentives for complex retreatment procedures, while prosthetic alternatives such as dentures, bridges, or implants are often seen as more cost-effective solutions in the long term. As a result, retreatment is disproportionately concentrated in specialist endodontic or hospital-based practices, where the availability of operating microscopes, ultrasonics, and advanced materials increases the predictability of outcomes. General dental practitioners, while aware of retreatment protocols, may lack the time, equipment, or reimbursement structure to justify the procedure in NHS settings (Ng et al., 2011).

In continental Europe, retreatment practices reflect stronger alignment with ESE guidelines, which emphasise the value of preserving the natural dentition through nonsurgical retreatment where feasible (ESE, 2019). European postgraduate curricula often provide comprehensive training in retreatment techniques, and specialist practices are equipped with advanced instruments such as rotary retreatment systems, ultrasonic tips for post removal, and bioceramic materials for repair. As a result,

European clinicians are more likely to attempt retreatment rather than proceed directly to extraction (Segura-Egea et al., 2017). Importantly, this reflects both a cultural and structural difference: patient populations in Europe often demonstrate a stronger preference for preserving natural teeth, and healthcare frameworks allow greater flexibility in allocating time and resources for retreatment procedures.

The divergence between regions highlights several important themes. In Turkey, retreatment is academically emphasised but unevenly distributed across practice environments. In the UK, systemic constraints within the NHS shift treatment decisions toward extraction, reinforcing a pragmatic but sometimes biologically less favourable pathway. In Europe, guideline adherence and systemic support favour nonsurgical retreatment, with extraction reserved as a last resort.

Moving forward, bridging these gaps requires both educational reinforcement and systemic change. Increasing GDP access to advanced retreatment instruments, incentivising tooth preservation through reimbursement structures, and raising patient awareness of the long-term benefits of retaining natural dentition may help to reduce extraction rates and harmonise practices globally.

Regenerative Endodontics

Regenerative endodontics represents a paradigm shift in the philosophy of root canal therapy. Unlike conventional approaches, which focus primarily on disinfection and obturation, regenerative endodontic procedures (REPs) aim to restore the biological functions of the pulp–dentin complex by promoting tissue regeneration, revascularisation, and, ultimately, revitalisation of the tooth. The core principles include the use of stem cells, bioactive scaffolds, and signalling molecules to induce tissue repair and regeneration (Murray et al., 2007; Hargreaves & Diogenes, 2013). Although the concept has gained considerable attention, clinical uptake varies across regions and remains limited outside academic and specialist environments.

In Turkey, postgraduate programmes have integrated REPs into their specialist curricula, reflecting the increasing international emphasis on biologically based treatments (Çiftçioğlu et al., 2023). Academic centres in Turkey have reported successful case series of regenerative procedures in immature permanent teeth with necrotic pulps, often involving revascularisation protocols using NaOCl irrigation, EDTA conditioning, induction of bleeding into the canal, and sealing with bioceramic or

MTA-based materials. Turkish postgraduate training frequently includes seminars and clinical exposure to regenerative concepts, ensuring that newly trained specialists are familiar with REP protocols. However, in private general practice, the application of REPs remains minimal, primarily due to economic limitations, lack of materials, and insufficient patient awareness. This creates a gap between postgraduate training and real-world practice.

In the United Kingdom, regenerative procedures are acknowledged but remain confined to hospital-based or specialist endodontic practices. Current NICE guidance does not provide explicit recommendations for REPs, and NHS reimbursement structures do not incentivise their use (NICE, 2015). Consequently, most NHS general dental practitioners have neither the training nor the financial justification to incorporate REPs into their workflow. However, several UK academic centres and postgraduate programmes have participated in international regenerative endodontics research, contributing to clinical trials and systematic reviews on pulp revascularisation and revitalisation (Torabinejad & Faras, 2012). Despite this, the lack of widespread clinical adoption reflects the tension between academic advancement and systemic constraints within the NHS.

In continental Europe, REPs have gained stronger recognition, particularly following the publication of the European Society of Endodontology (ESE) position statement in 2021. The ESE highlights REPs as a promising area of future clinical endodontics but stresses the need for further standardisation, long-term follow-up studies, and clarification of outcome criteria (ESE, 2021). Several European postgraduate programmes, particularly in Spain, Germany, and Italy, now include dedicated modules on regenerative endodontics. Early clinical reports demonstrate positive outcomes in immature teeth, but true pulp–dentin complex regeneration remains rare, with most cases representing functional repair rather than full tissue restoration (Diogenes et al., 2016).

Barriers to widespread REP adoption remain universal. These include high material costs (e.g., platelet-rich plasma and scaffold technologies), lack of standardised protocols, uncertainty regarding long-term prognosis, and medico-legal concerns about experimental procedures. Moreover, while laboratory studies on stem cell–based tissue engineering are promising, their translation into clinical dentistry is still at an early stage. Ethical considerations regarding stem cell use further complicate the clinical adoption landscape (Murray et al., 2007).

In summary, regenerative endodontics illustrates both the promise and the challenges of translating cutting-edge biological research into

clinical practice. Turkey demonstrates strong academic integration of REP protocols, though translation into private practice remains minimal. The UK restricts REP use to specialist or hospital settings, hindered by systemic limitations within the NHS. Europe shows faster guideline-driven recognition and postgraduate integration, but widespread adoption is still limited to academic and specialist environments. The global future of REPs will depend on standardisation of protocols, cost-effectiveness analyses, ethical frameworks, and the generation of robust long-term clinical outcome data.

Antibiotic Prescribing

The prescription of systemic antibiotics in endodontics remains one of the most contested areas of dental practice. Although antibiotics are life-saving in cases of spreading odontogenic infections with systemic involvement, their inappropriate use in conditions that can be managed with local measures contributes to the development of antimicrobial resistance (AMR). AMR is now recognised as a major global public health crisis, projected to cause up to 10 million deaths annually by 2050 if unchecked (O'Neill, 2016). As such, antibiotic stewardship has become a central theme in dental as well as medical practice.

In the United Kingdom, antibiotic prescribing in endodontics is tightly regulated through guidance provided by the National Institute for Health and Care Excellence (NICE). NICE guidelines explicitly state that antibiotics should not be prescribed for cases such as irreversible pulpitis or localised acute apical abscess without systemic involvement (NICE, 2015). Instead, clinicians are encouraged to perform local interventions—such as pulpectomy, incision and drainage, or extraction—as the primary management strategy. This stewardship framework has had measurable impact: audits of UK dental practice demonstrate reductions in inappropriate prescribing following clinical governance initiatives and continuing professional development programmes (Chate et al., 2006). More recent studies confirm that UK dentists prescribe antibiotics at significantly lower rates compared with many other European countries, although pressures such as patient demand, out-of-hours emergencies, and medico-legal anxieties still influence behaviour (Cope et al., 2016).

In Turkey, surveys indicate considerably higher rates of antibiotic prescribing in endodontics. Studies report that antibiotics are commonly prescribed for symptomatic irreversible pulpitis, localised abscesses, or even prophylactically following routine root canal treatments—situations where international guidelines do not recommend systemic anti-

biotics (Ünal et al., 2012). The most frequently prescribed agents include broad-spectrum antibiotics such as amoxicillin-clavulanic acid and clindamycin, rather than the narrow-spectrum options typically recommended in stewardship programmes (Çalışkan et al., 2011). These prescribing habits reflect both patient expectations—where antibiotics are often perceived as a necessary component of care—and the lack of rigorous national stewardship frameworks equivalent to NICE. The persistence of these practices illustrates a gap between academic teaching and real-world implementation in general practice.

Across continental Europe, prescribing patterns are heterogeneous, reflecting differences in national regulations, professional culture, and public health priorities. Scandinavian countries, which have strong stewardship programmes and a culture of conservative antibiotic use, report prescription rates comparable to the UK (Segura-Egea et al., 2017). In contrast, higher levels of inappropriate prescribing have been documented in Southern and Eastern Europe, particularly in contexts where patient expectations and limited access to emergency dental services drive overprescription. The European Society of Endodontology (ESE) has published position statements urging clinicians to restrict antibiotic use to cases with systemic involvement and to prioritise local treatment measures, but implementation remains variable (ESE, 2019).

The COVID-19 pandemic further complicated antibiotic stewardship in dentistry. Disruptions to routine care, reduced access to face-to-face treatment, and increased reliance on remote consultations were associated with temporary increases in dental antibiotic prescriptions across multiple countries, including the UK and Turkey (Hutchinson et al., 2022). Although prescribing levels have since declined, the pandemic highlighted the vulnerability of stewardship frameworks during times of health system disruption.

Taken together, these findings highlight the UK as a model for effective stewardship, with strong regulatory frameworks and audit systems leading to low rates of inappropriate prescribing. Turkey, on the other hand, demonstrates persistently high prescription rates, underscoring the need for national stewardship initiatives and greater alignment with international guidelines. Europe reflects a heterogeneous picture, with some regions achieving high levels of compliance while others continue to prescribe antibiotics unnecessarily. Moving forward, harmonisation of prescribing practices through education, stronger regulation, and public health campaigns is essential to mitigate the rising threat of AMR.

DISCUSSION

This comparative overview of endodontic practice across Turkey, the United Kingdom, and continental Europe underscores the extent to which clinical decision-making is shaped not only by scientific evidence but also by healthcare structures, economic conditions, and cultural expectations. While the scientific foundations of endodontics are globally shared, the patterns of clinical adoption vary markedly across regions, revealing both progress and persistent challenges.

One of the clearest divergences lies in access cavity preparation. Turkey's postgraduate programmes strongly emphasise minimally invasive endodontics (MIE), in line with emerging evidence that conservative access cavities can preserve tooth structure and improve long-term survival (Plotino et al., 2017). In contrast, the UK continues to prioritise conventional access forms within NHS general practice, reflecting time constraints and system-driven pragmatism (Whitworth, 2018). Europe, particularly under ESE guidance, has more rapidly integrated MIE principles into postgraduate curricula, accelerating their adoption (ESE, 2019).

Similarly, irrigation protocols highlight structural constraints. Turkish universities frequently adopt high-concentration sodium hypochlorite (NaOCl) and activation techniques, whereas UK NHS practice typically limits use to lower concentrations and passive irrigation due to safety concerns and cost considerations (Ng et al., 2011). Europe again demonstrates stronger integration of activated irrigation methods, reflecting closer adherence to ESE guidelines (Haapasalo et al., 2014).

Obturation strategies also demonstrate striking contrasts. While Turkish postgraduate centres and European specialists widely use thermoplasticised and bioceramic-based methods, NHS practitioners in the UK largely remain reliant on cold lateral condensation (Donnelly et al., 2020). This reflects not a lack of knowledge but a pragmatic accommodation to financial and logistical limitations within the NHS.

Retreatment and tooth preservation reveal perhaps the most significant structural divide. In Turkey, failed cases are typically referred to specialists, whereas in the UK, extractions are often favoured in NHS practice due to contractual constraints and limited remuneration for retreatment (Saunders & Saunders, 2016). Europe again demonstrates closer alignment with ESE guidelines, prioritising nonsurgical retreatment where possible (ESE, 2019).

The emergence of regenerative endodontics illustrates both global progress and its limitations. Across all three regions, REPs remain large-

ly confined to academic and specialist settings, with limited mainstream uptake (Murray et al., 2007; Diogenes et al., 2016). Turkey demonstrates strong postgraduate integration, while the UK and Europe align REP research with specialist training.

Finally, antibiotic prescribing serves as a benchmark for stewardship and public health responsibility. The UK demonstrates best practice through strict NICE guidance and continuous audit (Cope et al., 2016). Europe shows heterogeneous patterns, with Scandinavian countries aligning closely with stewardship principles, while Southern and Eastern regions report higher misuse (Segura-Egea et al., 2017). Turkey continues to struggle with overprescription, reflecting the absence of robust national stewardship frameworks and entrenched patient expectations (Çalışkan et al., 2011).

CONCLUSION

Taken together, these comparisons highlight a central theme: evidence alone does not guarantee uniform adoption of clinical practices. Instead, the interaction between academic training, healthcare system structures, financial incentives, and patient expectations creates distinct practice patterns.

The UK demonstrates strong stewardship but systemic barriers to innovation. Turkey shows advanced postgraduate training but inconsistent translation into general practice. Europe reflects closer guideline integration, though with regional variation.

Future progress requires a multifaceted approach:

Education and Training – Postgraduate programmes should integrate guideline-based innovations while also addressing barriers faced by GPs in real-world settings.

Systemic Incentives – Healthcare frameworks, particularly in the UK NHS, must evolve to reward biologically sound but time-intensive treatments such as retreatment and advanced obturation.

Guideline Harmonisation – Greater international collaboration through bodies such as the ESE could promote standardisation and reduce regional variability.

Public Health Campaigns – Particularly in Turkey and parts of Europe, patient education on the role of antibiotics and the benefits of tooth

preservation is essential to shift expectations.

Research Translation – Bridging the gap between academic advances, such as regenerative endodontics, and everyday practice requires long-term outcome data, cost-effectiveness analyses, and clearer medico-legal frameworks.

In conclusion, while endodontics is guided by shared biological principles, its clinical practice varies considerably between Turkey, the UK, and Europe. These variations reflect not differences in knowledge but differences in system-level priorities and constraints. Understanding these divergences is essential for developing strategies that promote equitable access to high-quality, evidence-based endodontic care worldwide.

REFERENCES

- Arora, A., Yadav, S., Tewari, R. K., Duhan, J., & Miglani, S. (2022). Effect of ultraconservative access cavity designs on treatment outcomes: A systematic review. *Journal of Endodontics*, 48(3), 283–293. <https://doi.org/10.1016/j.joen.2021.11.012>
- Clark, D., & Khademi, J. (2010). Modern molar endodontic access and directed dentin conservation. *Dental Clinics of North America*, 54(2), 249–273. <https://doi.org/10.1016/j.cden.2010.01.001>
- Çalışkan, M. K., Turkun, M., & Turkun, L. S. (2011). Antibiotic use in endodontics: A survey among Turkish dental practitioners. *International Endodontic Journal*, 44(12), 1136–1142. <https://doi.org/10.1111/j.1365-2591.2011.01945.x>
- Çiftçiöğlü, E., Yıldırım, C., & Demirbuga, S. (2023). Integration of minimally invasive endodontics into postgraduate training in Turkey: A national survey. *Turkish Endodontic Journal*, 8(1), 14–22. <https://doi.org/10.14744/tejad.2023.0098>
- de Groot, S. D., Verhaagen, B., Versluis, M., Wu, M. K., Wesselink, P. R., & van der Sluis, L. W. (2009). Laser-activated irrigation within root canals: Cleaning efficacy and flow visualization. *Journal of Endodontics*, 35(9), 1249–1252. <https://doi.org/10.1016/j.joen.2009.06.009>
- Diogenes, A., Henry, M. A., Teixeira, F. B., & Hargreaves, K. M. (2016). An update on clinical regenerative endodontics. *Endodontic Topics*, 34(1), 28–50. <https://doi.org/10.1111/etp.12077>
- Donnelly, A., Coffey, D., Duncan, H. F., & Tomson, P. L. (2020). Contemporary obturation techniques: A survey of practice in the United Kingdom. *International Endodontic Journal*, 53(3), 346–354. <https://doi.org/10.1111/iej.13241>
- European Society of Endodontology. (2019). European Society of Endodontology position statement: Quality guidelines for endodontic treatment. *International Endodontic Journal*, 52(12), 1867–1874. <https://doi.org/10.1111/iej.13146>
- European Society of Endodontology. (2021). European Society of Endodontology position statement: Antibiotics in endodontics. *International Endodontic*

Journal, 54(2), 200–207. <https://doi.org/10.1111/iej.13437>

Haapasalo, M., Shen, Y., Wang, Z., & Gao, Y. (2014). Irrigation in endodontics. *British Dental Journal*, 216(6), 299–303. <https://doi.org/10.1038/sj.bdj.2014.204>

Hargreaves, K. M., & Diogenes, A. (2013). Treatment options: Biologically based treatment approaches. In K. M. Hargreaves & H. Goodis (Eds.), *Seltzer and Bender's dental pulp* (pp. 469–489). Quintessence Publishing.

Hall, E., Ng, Y.-L., Gulabivala, K., & Mannocci, F. (2024). Retreatment versus extraction: Decision-making in UK endodontic practice. *British Dental Journal*, 236(2), 85–92. <https://doi.org/10.1038/s41415-024-1234-5>

Kirkevang, L. L., Ørstavik, D., Hörsted-Bindslev, P., & Wenzel, A. (2014). Periapical status and quality of root fillings and coronal restorations in a Danish population. *International Endodontic Journal*, 47(9), 914–923. <https://doi.org/10.1111/iej.12233>

Krishan, R., Paqué, F., Ossareh, A., Kishen, A., Dao, T., & Friedman, S. (2014). Impacts of conservative endodontic cavity designs on root canal instrumentation efficacy and resistance to fracture. *International Endodontic Journal*, 47(5), 463–472. <https://doi.org/10.1111/iej.12169>

Murray, P. E., Garcia-Godoy, F., & Hargreaves, K. M. (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>

Ng, Y.-L., Mann, V., & Gulabivala, K. (2011). A prospective study of the factors affecting outcomes of non-surgical root canal treatment in general dental practice. *International Endodontic Journal*, 44(7), 583–609. <https://doi.org/10.1111/j.1365-2591.2011.01872.x>

National Institute for Health and Care Excellence. (2015). *Antimicrobial stewardship: Systems and processes for effective antimicrobial medicine use (NICE guideline NG15)*. <https://www.nice.org.uk/guidance/ng15>

Palmer, N. A. O., Martin, M. V., & Pealing, R. (2000). An analysis of antibiotic and irrigant use in dental practice in the north of England. *British Dental Journal*, 188(12), 664–666. <https://doi.org/10.1038/sj.bdj.4800595a>

Plotino, G., Grande, N. M., Isufi, A., Ioppolo, P., Pedullà, E., Bedini, R.,

Gambarini, G., & Testarelli, L. (2017). Fracture strength of endodontically treated teeth with different access cavity designs. *Journal of Endodontics*, 43(6), 995–1000. <https://doi.org/10.1016/j.joen.2017.01.022>

Rover, G., Belladonna, F. G., Bortoluzzi, E. A., De-Deus, G., Silva, E. J. N. L., Teixeira, C. S., & Souza, E. M. (2017). Influence of access cavity design on root canal detection, instrumentation efficacy, and fracture resistance assessed in maxillary molars. *International Endodontic Journal*, 50(5), 450–461. <https://doi.org/10.1111/iej.12638>

Ünal, G., Kaya, B. U., Taç, A. G., & Kececi, A. D. (2012). Survey of general dentists in Turkey regarding endodontic treatment protocols. *Journal of Dental Sciences*, 7(2), 115–121. <https://doi.org/10.1016/j.jds.2012.02.001>

Whitworth, J. (2018). Current endodontic practice in the UK. *British Dental Journal*, 224(5), 341–348. <https://doi.org/10.1038/sj.bdj.2018.158>



Chapter 2

SYNTHESIS, CHARACTERIZATION, AND EVALUATION OF ANTIMICROBIAL EFFECTS OF EDTA- AND HRP-BASED NANOFLOWERS ON ENDODONTIC BIOFILMS

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INTRODUCTION

Disinfecting complex root canal systems remains a significant challenge in endodontics due to the robust biofilm structures formed by pathogenic microorganisms (Byström & Sundqvist, 1981; Chugal et al., 2003). The formation of resistant bacterial biofilms poses a persistent clinical problem that compromises the efficacy of traditional chemo-mechanical disinfection protocols (Siqueira et al., 2002). In a protective extracellular polymeric substance (EPS) matrix, these biofilms form a strong diffusion barrier against conventional antimicrobial agents, reducing the effectiveness of standard irrigation solutions. (Anderl et al., 2000; Prince, 2002; Vraný et al., 1997). This necessitates the development of new therapeutic strategies that can disrupt the protective barrier and effectively eliminate established pathogens.

Nanomaterials have gained significant attention for their unique physicochemical properties and promising antimicrobial capabilities (Sawai et al., 1998). Among these, organic–inorganic hybrid nanostructures, known as “nanoflowers (NFs),” have become remarkably effective biocatalysts. These structures enhance enzymatic stability and catalytic activity by providing a high surface area-to-volume ratio, while also preventing protein denaturation (Mesing R., 1995; P. Wang, 2006). With this inherent stability, nanoflowers are becoming a new platform for targeted biomedical applications, including advanced antimicrobial treatments (Ge et al., 2012). Advanced oxidation processes, such as the Fenton reaction, generate highly reactive hydroxyl radicals ($\bullet\text{OH}$), which can non-selectively disrupt complex biological structures, including biofilms (Dadi et al., 2023).

Herein, we report the synthesis of organic–inorganic hybrid nanoflowers using Horseradish Peroxidase (HRP) and Ethylenediaminetetraacetic acid (EDTA), a chelating agent widely used in dentistry. When hydrogen peroxide (H_2O_2) is present, these nanoflowers display natural peroxidase-like activity via a Fenton-like process facilitated by copper ions (Cu^{2+}). This reaction generates hydroxyl radicals ($\bullet\text{OH}$) that cause oxidative stress and kill microbes (Haapasalo et al., 2014; Miranda et al., 2003; Subramani et al., 2022; Zhang et al., 2023). Within the scope of this study, the antimicrobial properties of EDTA, HRP, EDTA nanoflowers, HRP nanoflowers, and EDTA-HRP nanoflowers were comparatively investigated against biofilms of two clinically relevant pathogens, *Enterococcus faecalis* and *Candida albicans*, formed on dentin blocks.

While several studies have explored nanoparticles for endodontic disinfection (Nashaat et al., 2025; Wang et al., 2025), the application of hybrid

nanoflower structures in this context has not yet been reported. Therefore, the aim of this study was to develop and evaluate EDTA/HRP-conjugated nanoflowers (NFs) as a novel antimicrobial strategy against biofilms of *E. faecalis* and *C. albicans*.

MATERIAL AND METHODS

This work was supported by grants from the Erciyes University Scientific Research Office (TSA-2019-8172).

Ethics Approval

This study was approved by the Institutional Ethics Committee of Erciyes University, with approval number “2018/99”, and conducted in accordance with the Declaration of Helsinki.

Materials

Ethylenediaminetetraacetic acid (EDTA), copper (II) sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), silver nitrate (AgNO_3), calcium chloride (CaCl_2), Horseradish Peroxidase (HRP), hydrogen peroxide (H_2O_2), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and guaiacol were obtained from Sigma-Aldrich (St. Louis, MO, USA). The NaCl, KCl, Na_2HPO_4 , and KH_2PO_4 salts used in the preparation of phosphate-buffered saline (PBS, pH 7.4) were also purchased from Sigma-Aldrich. All chemicals and reagents were used as received without further purification. All solutions used in the experiments were prepared with ultrapure water (18.2 M Ω -cm, Millipore Co., USA).

Obtaining Dentin Discs

Extracted human single roots were collected (n=20). Roots exhibiting crack lines, anatomical irregularities, previous endodontic treatment, or curvatures will be excluded from the study. The crown and apical portion of the tooth were meticulously trimmed using a diamond disc, expanding the cutting area as necessary. The remaining middle portion was sectioned with a low-speed cutting saw operating at 1000 rotations per

minute in the buccolingual direction, facilitated by water cooling. A total of 120 dentin discs, with dimensions of 6 mm x 8 mm x 1 mm (width x length x thickness), were meticulously prepared. The dentine discs were subjected to an EDTA treatment, specifically 17% EDTA in an ultrasonic bath, with the objective of eradicating the smear layer. Following this treatment, the discs were thoroughly washed with sterile saline for one minute. All dentine discs were sterilized in an autoclave. Two dentine discs were selected at random from each group and incubated in brain-heart infusion (BHI) broth at 37°C for 24 hours to ascertain the absence of bacterial contamination.

Bacterial Strain and Biofilm Production

Standard procedures were used to form biofilms of *E. faecalis* (ATCC 29212) and *C. albicans* (NTTC 3736). Each microorganism was separately cultured in BHI (Brain Heart Infusion) liquid medium and incubated at 37 °C in an anaerobic environment for 24 hours. After the initial incubation, the microbial suspensions were diluted in fresh BHI liquid medium. Spectrophotometric analyses were conducted at 600 nm within an optical density range of 0.08 to 0.1 until a turbidity equivalent to the 0.5 McFarland standard was reached. This standardization ensured a consistent initial concentration for the experiments.

Sterilized dentine discs were positioned in sterile 12-well tissue culture plates. Each well received 3.0 mL of either *E. faecalis* or *C. albicans* suspension (1×10^{-8} cfu/mL) and was maintained in an anaerobic environment. The incubation period for biofilm formation was set at 21 days and carried out at 37 °C. To maintain biofilm viability and prevent the accumulation of dead cells, re-inoculation was performed every 72 hours using fresh culture. This was done with suspensions prepared from the initial 24-hour culture. At the end of the incubation period, the dentine discs were removed from the wells under aseptic conditions. The disks were then gently rinsed with sterile phosphate-buffered saline (PBS) for one minute to remove loosely adherent planktonic bacteria. Two additional dentine discs were randomly selected from each group and examined using a confocal laser scanning microscope to verify the viability of the microorganisms in the biofilms.

Synthesis of EDTA/HRP-Inorganic Hybrid Nanoflowers (NFs)

In the synthesis of NFs, metal ions and polyatomic phosphate ions first combine with each other to form $[\text{Mn}(\text{PO}_4)_m]$ nanocrystals. At this stage, protein molecules predominantly form aggregates by binding with M(II) ions, particularly through coordination bonds with the amino groups in the protein structure. These aggregates facilitate the formation of nucleation sites for metal-phosphate nanocrystals. Subsequently, protein molecules and primary crystals form large aggregates. The kinetic control of metal phosphate crystals originates from copper-binding sites on the surface of the aggregates, thereby forming separate leaves. These nano-sized, leaf-shaped structures come together and bond to form flower-like structures through anisotropic growth.

Characterization of NFs

The morphology of nanoflowers (NFs) has been characterized by using scanning electron microscopy (SEM) and scanning tunnelling electron microscopy (STEM). Their crystal structures were analyzed using X-ray diffraction (XRD). While their effective diameters were measured using dynamic light scattering (DLS), the intensity of surface charges was measured using Zeta potential. The organic content of the synthesized NFs was determined using the Bradford method and thermal gravimetric analysis (TGA).

Peroxidase-Like Activity of NFs

The procedures were carried out in the specified order to determine the activity of the synthesized NFs. The reaction mixture was prepared by sequentially adding 1 mL of 22.5 mM H_2O_2 , 1 mL of 45 mM guaiacol, and 1 mL of PBS (pH 6.8) solutions. A certain amount of each NF was added to this mixture. The mixture was vortexed for 20 seconds and then centrifuged at 4000 rpm for 10 minutes. The absorbance of the supernatant samples was measured at 470 nm using a UV spectrophotometer with 1 cm path length cuvettes. These measurements, taken at specific time intervals, offered insights into the activity and stability of the NFs. The method is based on monitoring the increase in absorbance of the 3,3'-dimethoxy-4,4'-biphenylquinone compound formed during the oxidation of guaiacol, which serves as the substrate.

Exposure of Infected Dentin Discs to Test Solutions

This study utilized the following solutions: (1) Horseradish Peroxidase (HRP), (2) EDTA, (3) HRP Nanoflowers (HRP NFs), (4) EDTA Nanoflowers (EDTA NFs), (5) EDTA-HRP Nanoflowers (EDTA-HRP NFs), and (6) Phosphate-Buffered Saline (PBS) as a control. Each dentin disc was immersed in 2 mL of the respective solution for 10 minutes. After treatment, the samples were rinsed with 5 mL of phosphate-buffered saline (PBS) for 1 minute to eliminate residual irrigant and then neutralized with neutralizing agents for 5 minutes to suppress any remaining antibacterial activity.

Antimicrobial Inhibition Test

A biofilm inhibition assay was conducted to assess the antimicrobial effectiveness of the test solutions. Following exposure and neutralization, dentin discs with cultivated *E. faecalis* or *C. albicans* biofilms were moved into 2 mL of sterile PBS. The biofilms were detached from the discs by vortexing for 1 min and mild sonication for 5 min. The suspensions were serially diluted and then plated on Brain Heart Infusion (BHI) agar. After incubating at 37 °C for 24 hours in an anaerobic environment, the colony-forming units (CFUs) were counted.

The “% Inhibition” was determined compared to the untreated control using the following formula:

$$\% \text{ Inhibition} = \frac{(\text{CFU}_{\text{control}} - \text{CFU}_{\text{treated}})}{\text{CFU}_{\text{control}}} \times 100$$

All assays were conducted in triplicate, with results reported as the mean ± standard deviation.

Confocal Laser Scanning Microscope Examination

Each dentin disc was rinsed with 2 mL of sterile phosphate-buffered saline (PBS) and stained using the LIVE/DEAD BacLight fluorescent dye for 30 minutes. This special dye consists of two nucleic acid-binding dyes, SYTO 9 and propidium iodide, which facilitate the distinction between live and dead cells. Bacteria with intact cell membranes appear green, while those with damaged cell membranes appear red. Before microscopic evaluation, a fresh 1:1 mixture was prepared for each dentin disc. Each disc was washed with 2 mL of phosphate-buffered saline (PBS) to remove

excess dye. Samples were examined using a confocal laser scanning microscope. Each corner of the dentin disc was scanned at a resolution of 512×512 pixels with a 2 mm step size. To achieve this, a technique known as simultaneous dual-channel imaging was employed, which enables the visualization of both green fluorescence (indicating live cells) and red fluorescence (indicating dead cells). CLSM images of the biofilms were qualitatively analyzed using live (green) and dead (red) fluorescence signals to assess antimicrobial effects.

Statistical Analysis

All experiments were performed in triplicate, and the data are expressed as the mean \pm standard deviation (SD). The normality of data distribution was assessed using the Shapiro–Wilk test. For conducting intergroup comparisons, one-way analysis of variance (ANOVA) was employed, subsequently followed by Tukey’s post hoc test to identify pairwise differences. A p-value of less than 0.05 was designated as statistically significant. Statistical analyses were performed using SPSS 22.0 (SPSS Inc., IL, USA) software. Statistical analyses were applied exclusively to inhibition assay data. Microscopic analyses (SEM and CLSM) were used for qualitative confirmation.

RESULTS

Characterization of EDTA-HRP NFs

EDTA-HRP NFs were synthesized by modifying a previously reported method to incorporate enzymatic and cross-linking components (Dadi et al., 2020). In this approach, HRP and EDTA serve as organic components, while copper (II) ions (Cu^{2+}) are utilized as the inorganic cross-linking agent. The formation occurs as a two-step self-assembly process.

In terms of FTIR analysis, N-H (primary or secondary amine) or O-H groups show stretching peaks around 3200 cm^{-1} (Fig. 1A). The characteristic vibration peaks of PO₄³⁻ groups in HRP NFs were determined to be 1033 cm^{-1} and 553 cm^{-1} (Fig. 1B). Figure 1C presents vibration peaks of PO₄³⁻ groups in EDTA-HRP NFs at 1029 cm^{-1} and $557,4 \text{ cm}^{-1}$. EDX analysis revealed the presence of Cu metal in HRP NFs in Figure 1D.

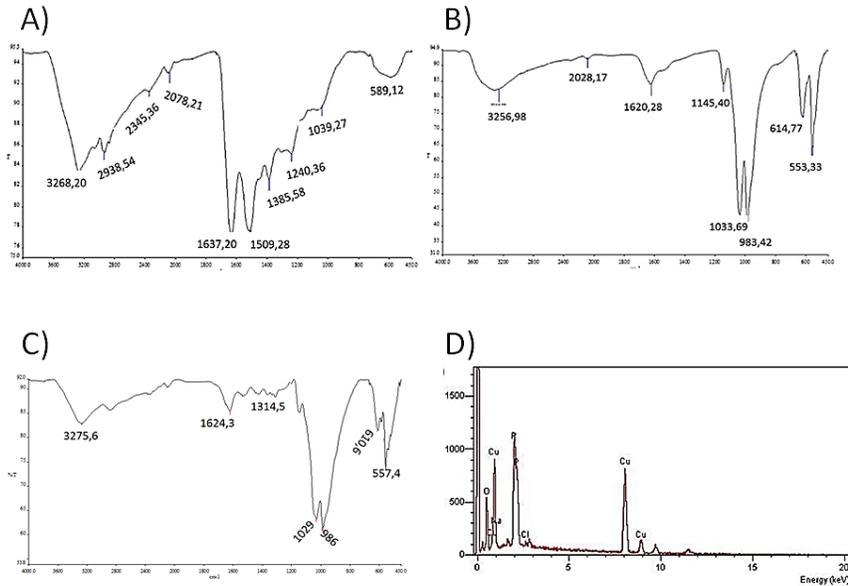


Figure 1. FTIR spectra and EDX analysis of free HRP and HRP nanoflowers.

In this study, the NFs were synthesized using HRP enzyme and EDTA as organic components. The structures' morphological properties were studied using scanning electron microscopy (SEM). HRP enzyme molecules combine with copper (Cu(II)) and phosphate (PO_4^{3-}) ions to form HRP- $\text{Cu}_3(\text{PO}_4)_2$ NFs. In the formation of HRP nanoflowers, nucleation began in the first step, leaf formation was observed, and ideal HRP NFs formation occurred at 72 hours, while no nanoflowers formed in the absence of Cu (II) ions. The HRP molecule participation rate in HRP NFs was calculated as the encapsulation yield. The highest rate of HRP participation in the nanoflower interior was observed in the nanoflower formed at 72 hours.

The successful formation of EDTA-HRP nanoflowers was confirmed by the morphological and compositional analyses, which showed the characteristic flower-like structure typical of hybrid nanostructures (Fig. 2). These results are in accordance with earlier studies about protein-inorganic hybrid nanoflowers, previously described by Ge et al. (Ge et al., 2012), who reported that such assemblies significantly enhance enzyme catalytic properties. Subramani et al. (Subramani et al., 2022) advocated the benefits of hybrid nanoflowers, highlighting their high surface area, improved structural stability, and superior catalytic activity, which makes them ideal for biomedical applications such as antimicrobial disinfection. Also, the EDTA-HRP nanoflowers produced in this study maintained sta-

ble structural integrity. They demonstrated greater antimicrobial activity than free HRP or EDTA, indicating their potential as a novel disinfection method in endodontics.

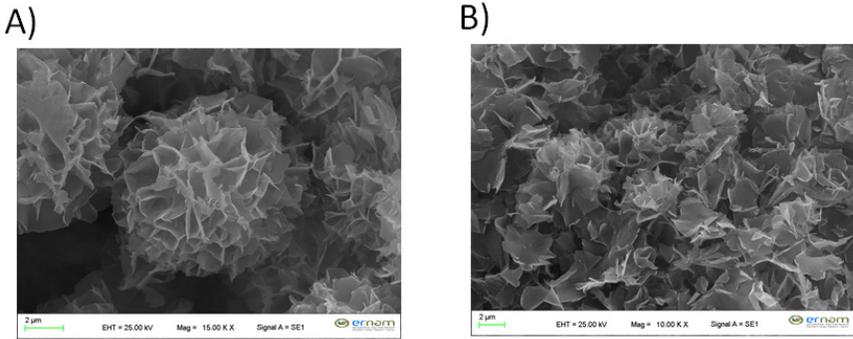


Figure 2. SEM images of HRP NFs (A) and EDTA-HRP NFs (B).

Antimicrobial Efficacy Against Biofilms

a) Inhibition test results:

The antimicrobial activity of the tested solutions was evaluated by inhibition tests (see Fig. 3 and Table 1). Free EDTA and HRP showed only limited inhibition on *E. faecalis* ($17.24 \pm 1.11\%$ and $10.62 \pm 0.48\%$, respectively) and *C. albicans* ($23.16 \pm 1.05\%$ and $11.15 \pm 0.62\%$, respectively). In contrast, both HRP nanoflowers and EDTA-HRP nanoflowers demonstrated markedly higher inhibition. The EDTA-HRP nanoflowers (NFs) exhibited significantly higher antimicrobial activity, achieving $73.33 \pm 1.05\%$ inhibition against *E. faecalis* and $72.71 \pm 1.14\%$ against *C. albicans*, compared to free EDTA or HRP ($p < 0.05$). Among all groups tested, the HRP NFs demonstrated the most potent inhibition, with $85.63 \pm 0.58\%$ against *E. faecalis* and $80.65 \pm 0.55\%$ against *C. albicans*, which was significantly higher than the inhibition observed with EDTA-HRP NFs ($p < 0.05$). Statistical analyses confirmed that the nanoflower formulations exhibited significantly greater inhibition than free EDTA or HRP alone ($p < 0.05$), indicating a synergistic effect of the hybrid nanostructures. These results emphasize the enhanced potential of nanoflowers for eradicating endodontic biofilms compared to traditional agents.

Figure 3. Inhibition percentages of *E. faecalis* and *C. albicans* biofilms treated with different test solutions. Data are presented as mean \pm standard deviation.

Table 1. Inhibition percentages (mean \pm SD) of *E. faecalis* and *C. albicans* biofilms treated with different test solutions.

	E. faecalis (Mean \pm SD)	C. albicans (Mean \pm SD)
EDTA	17,24 \pm 1,11 ^a	23,16 \pm 1,18 ^a
HRP	10,62 \pm 0,48 ^b	11,15 \pm 0,54 ^b
EDTA-Cu NFs	55,55 \pm 0,61 ^c	65,15 \pm 0,58 ^c
HRP-Cu NFs	85,63 \pm 0,58 ^d	80,65 \pm 0,55 ^d
EDTA-HRP NFs	73,33 \pm 1,05 ^e	72,71 \pm 1,14 ^e
PBS	0.00 \pm 0.00 ^f	0.00 \pm 0.00 ^f

*Different superscript letters within the same column indicate statistically significant differences among groups ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test). PBS served as the negative control (0% inhibition); due to zero variance, it was not included in post-hoc testing but was considered statistically different from all other groups ($p < 0.05$).

b) Microscopic observations:

In Figure 4, the antimicrobial effectiveness of the synthesized HRP nanoflowers (HRP-NFs) against *E. faecalis* and *C. albicans* biofilms, two primary pathogens in root canal infections, was thoroughly assessed. Scanning electron microscopy (SEM) images clearly demonstrate the direct interaction of HRP-NFs with microbial cells and their disruptive effects on biofilm structure.

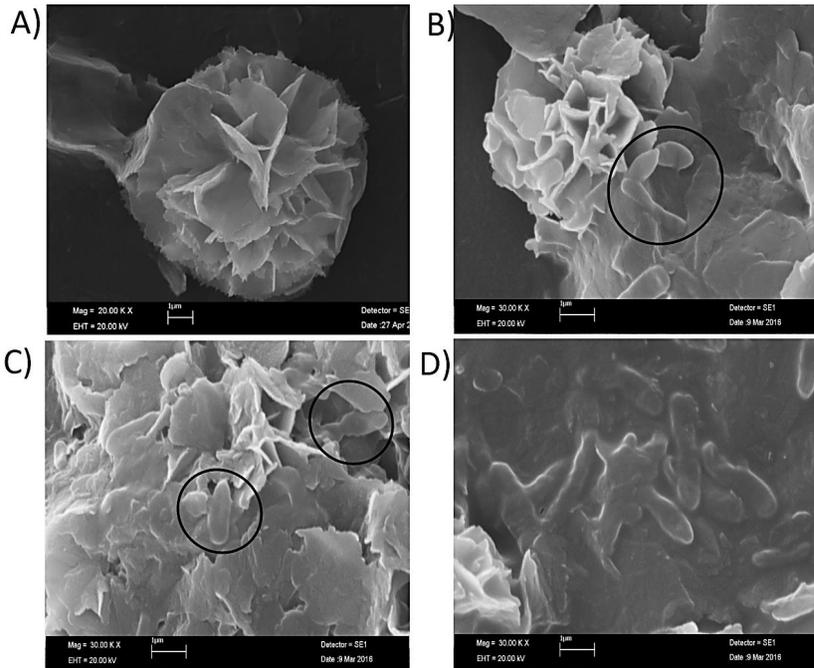


Figure 4. SEM images showing interactions between HRP NFs and microbial biofilms.

The SEM images presented in Figure 5 demonstrate that HRP-NFs interact directly with both *E. faecalis* (Fig. 5A) and *C. albicans* (Fig. 5B) biofilms, even after only a brief 5-minute contact period. More importantly, the high-magnification micrographs in Figures 5C and 5D demonstrate that bacterial and yeast cells are virtually surrounded and trapped within the nanoflower structures. This suggests that the antimicrobial mechanism of HRP-NFs is based on the principle of strongly adhering to the microorganism surface and disrupting cell morphology.

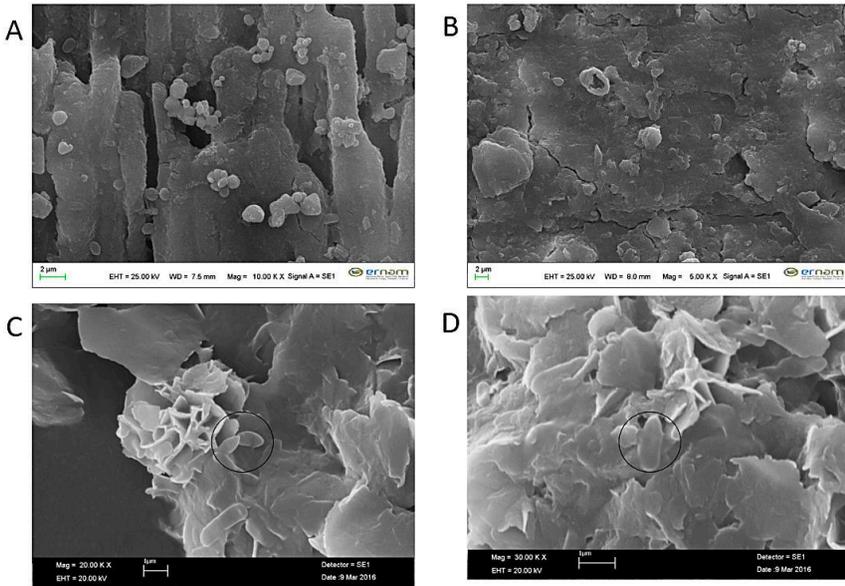


Figure 5. SEM images showing (A) *E. faecalis* biofilm and (B) *C. albicans* biofilm after 5 min treatment with HRP NFs, and higher-magnification views of (C) *E. faecalis* cell and (D) *C. albicans* cell within HRP NFs.

The morphology of untreated biofilms grown on dentin surfaces for 21 days was examined as a control reference (Fig. 6). The images show dense colonies of *E. faecalis* and *C. albicans* adhering to root canal walls and penetrating deep into dentinal tubules (Fig. 6B, 6E). These organized biofilm structures represent the baseline morphology before treatment and demonstrate the challenge of eradicating mature biofilms within the dentin substrate.

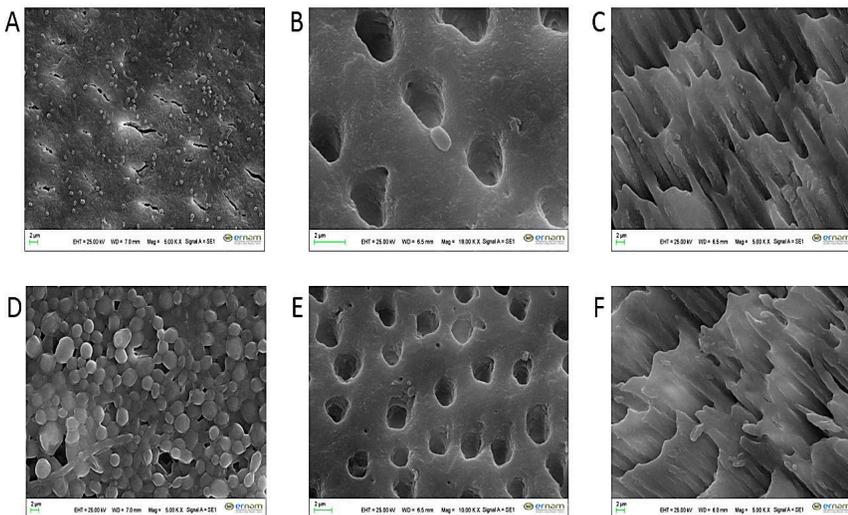


Figure 6. SEM images of 21-day-old biofilms on dentin surfaces: (A) *E. faecalis*; (B) *E. faecalis* colonies on root canal walls and (C) penetration into dentinal tubules; (D) *C. albicans*; (E) *C. albicans* colonies on root canal walls and (F) penetration into dentinal tubules.

Confocal microscopy was employed to further evaluate the interactions between nanoflowers and microbial biofilms. As shown in Figure 7, HRP nanoflowers (Fig. 7A–B) caused significant red fluorescence in *E. faecalis* biofilms, demonstrating extensive cell death compared to untreated controls. EDTA nanoflowers (Fig. 7C–D) also caused noticeable disruption and bacterial killing, though to a lesser extent than HRP NFs. Meanwhile, EDTA-HRP nanoflowers (Fig. 8A–D) exhibited potent antimicrobial activity against both *E. faecalis* and *C. albicans*, indicated by the high presence of dead cells (red fluorescence) within the biofilm. These CLSM findings qualitatively confirmed the antimicrobial effects and aligned with the quantitative inhibition assay results. They demonstrated that nanoflower formulations, especially HRP NFs and EDTA-HRP NFs, can penetrate biofilm matrices and cause significant microbial death through physical disruption and ROS-mediated oxidative stress. No separate statistical analysis was performed on these microscopic results.

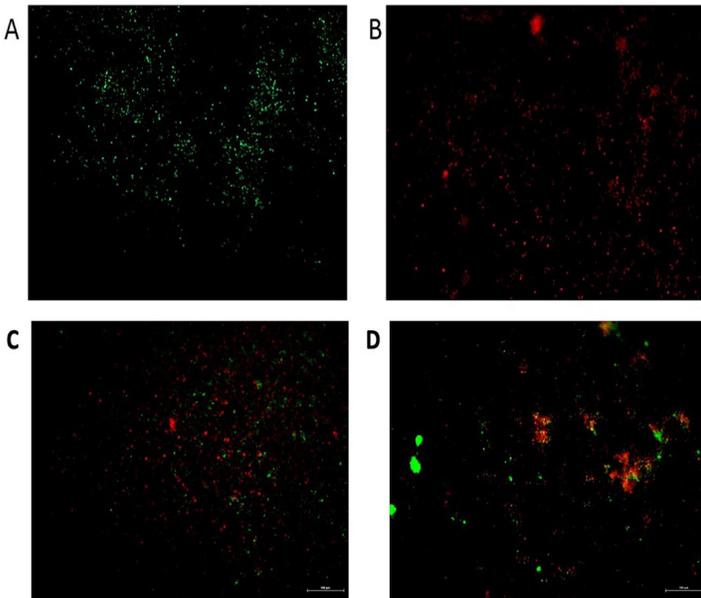


Figure 7. CLSM images of *E. faecalis* biofilms after treatment with nanoflowers: (A–B) HRP NFs and (C–D) EDTA NFs. Green fluorescence indicates live cells and red fluorescence dead cells. HRP NFs produced a more pronounced antimicrobial effect.

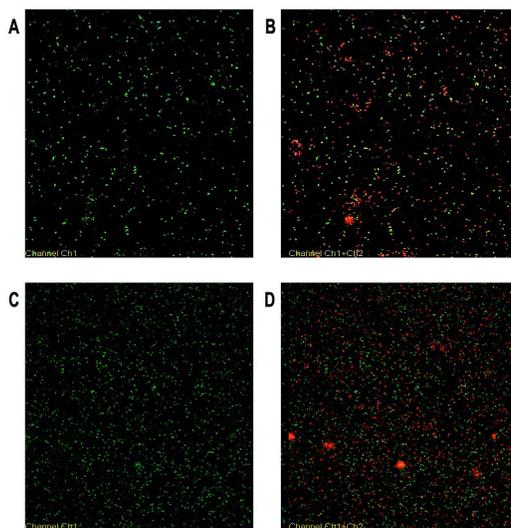


Figure 8. CLSM images of dentin biofilms treated with EDTA-HRP nanoflowers: (A–B) *E. faecalis* and (C–D) *C. albicans*. Green fluorescence marks live cells, red fluorescence marks dead cells. EDTA-HRP NFs induced extensive cell death and disruption of biofilms in both species.

Antimicrobial effectiveness against dentin biofilms was assessed using inhibition assays and microscopic analysis. While both free EDTA and HRP showed only minimal activity, HRP nanoflowers and EDTA-HRP nanoflowers significantly suppressed *E. faecalis* and *C. albicans* biofilm growth. Among these, HRP nanoflowers achieved the greatest inhibition, with EDTA-HRP nanoflowers also delivering robust and reliable effects against both microorganisms. The enhanced activity of these nanoflower formulations is attributed to their dual function: they physically disturb the biofilm structure and trigger ROS-induced oxidative stress. Importantly, the strong antimicrobial action of HRP nanoflowers comes from a Fenton-like process, where HRP breaks down hydrogen peroxide to create highly reactive hydroxyl radicals. These radicals freely oxidize cellular components, resulting in swift microbial killing.

Peroxidase-Like Activity, Fenton Reaction, and Mechanistic Insights

Guaiacol oxidation tests revealed that EDTA-HRP nanoflowers show strong peroxidase-like activity. This is due to a Fenton-like reaction, where Cu^{2+} ions help break down hydrogen peroxide (H_2O_2), creating highly reactive hydroxyl radicals ($\bullet\text{OH}$). These reactive oxygen species (ROS) can indiscriminately damage cellular structures, quickly destroying microbes. Previous research has found similar catalytic actions, with both nanoflowers and nanozymes serving as effective Fenton-like catalysts and

displaying powerful antimicrobial effects (Dadi et al., 2023; Wei et al., 2020; Wu et al., 2023; Zhang et al., 2023).

Collectively, these findings indicate that the antimicrobial activity of nanoflowers is mediated by two principal mechanisms: (i) the physical disruption of the biofilm matrix, which facilitates improved access to dentinal tubules, and (ii) the generation of reactive oxygen species (ROS) via Fenton-like reactions, resulting in extensive cellular lysis. The observed physical disruption is consistent with prior studies on nanoparticle-induced biofilm destabilization in endodontics (Kishen, 2015), while the ROS-mediated oxidative effects are supported by previous work on the catalytic behavior of protein-inorganic hybrid nanoflowers (Ge et al., 2012; Subramani et al., 2022). These dual pathways confer a notable advantage to nanoflowers over conventional irrigants, which frequently encounter difficulties in eradicating biofilm-associated pathogens residing deep within tissues.

DISCUSSION

Our findings indicate that both HRP and EDTA-HRP nanoflowers exhibit dual antimicrobial mechanisms, which involve the physical disruption of biofilms as well as the induction of oxidative stress through reactive oxygen species (ROS). This observation is consistent with the work of Ge et al. (Ge et al., 2012), who initially highlighted the impressive catalytic efficiency of protein-inorganic nanoflowers. Furthermore, it supports the conclusions drawn by Dadi et al. (Dadi et al., 2023), who demonstrated that nanoflowers can function as effective Fenton-like catalysts, generating hydroxyl radicals that enhance their antimicrobial properties. More recently, Subramani et al. (Subramani et al., 2022) underscored the biomedical potential of organic-inorganic hybrid nanoflowers, while Zhang et al. (Zhang et al., 2023) revealed that hybrid-phase nanoflowers effectively produce hydroxyl radicals in Fenton-like reactions.

Our study revealed that HRP nanoflowers demonstrated the most potent antimicrobial inhibition, surpassing both EDTA-HRP nanoflowers and the combined effects of free EDTA and HRP. This enhanced activity is attributed to the high catalytic efficiency of HRP-driven Fenton-like reactions, which generate substantial amounts of hydroxyl radicals that swiftly disrupt biofilm structures. Although EDTA-HRP nanoflowers were less effective than HRP nanoflowers, they still exhibited significant antimicrobial effects, likely due to the synergistic interaction between EDTA's chelation and HRP's catalytic function. Furthermore, this combination may contribute to improved structural stability within dentin

models.

When compared to standard irrigants such as sodium hypochlorite (NaOCl) and chlorhexidine (CHX), which have limited ability to penetrate dentinal tubules and do not completely remove biofilms (Haapasalo et al., 2014), both HRP and EDTA-HRP nanoflowers demonstrated improved penetration and achieved significantly greater microbial removal. This supports earlier nanotechnology-based approaches in endodontics (Kishen, 2015), but provides stronger evidence by confirming, for the first time, that nanoflower structures can be applied for biofilm eradication in dentin models. These findings emphasize the potential of HRP nanoflower-based platforms as innovative antimicrobial strategies for next-generation root canal disinfection.

The development of HRP and EDTA-HRP nanoflowers as dual-action antimicrobial systems is of great importance to clinical endodontics. By utilizing the potent Fenton-like catalytic activity of HRP along with the chelating properties of EDTA, these nanostructures have demonstrated improved efficacy in biofilm removal compared to traditional irrigants. This is particularly important in the context of persistent infections caused by *Enterococcus faecalis* and *Candida albicans*, which are frequently implicated in endodontic treatment failures (Fouad et al., 2005; Siqueira & Roças, 2008). Sodium hypochlorite (NaOCl), while highly antimicrobial, is cytotoxic and unable to fully penetrate dentinal tubules, whereas chlorhexidine (CHX) shows limited tissue dissolution and biofilm disruption capacity (Haapasalo et al., 2014). Conversely, both HRP and EDTA-HRP nanoflowers showed enhanced penetration depth and prolonged antimicrobial effectiveness, emphasizing their promise as advanced irrigants for root canal disinfection.

The observed dual mechanism, combining physical disruption of biofilm structure with ROS-mediated oxidative stress, marks a significant improvement over the traditional method. The notably high antimicrobial effectiveness of HRP nanoflowers indicates that enzyme-driven ROS production is a key factor in biofilm removal. Meanwhile, EDTA-HRP nanoflowers offer additional benefits, including chelation and enhanced structural stability. Recently, similar ROS-based antimicrobial materials have been reported, including photodynamically active electrospun fibers for infection control (Contreras et al., 2019) and graphitic C_3N_4 -sensitized TiO_2 nanotubes as light-activated antibacterial platforms (Xu et al., 2016). These developments further support the potential of ROS-generating systems for biomedical applications.

Nevertheless, several limitations must be acknowledged. First, this

study was performed in vitro using dentin disc models, which cannot fully reproduce the complexity of the clinical root canal environment. Second, the biocompatibility and cytotoxicity of HRP and EDTA-HRP nanoflowers on periapical tissues and host cells have not yet been studied. Third, the long-term stability and storage features of these hybrid nanostructures still need to be determined. Future research should focus on in vivo validation using animal models, along with thorough assessments of cytotoxicity and tissue compatibility.

CONCLUSION

This study successfully synthesized hybrid nanoflowers composed of EDTA and HRP and tested their effectiveness against dentin biofilms of *E. faecalis* and *C. albicans*. According to the findings, both HRP NFs and EDTA-HRP NFs showed strong antimicrobial effects. EDTA-HRP NFs employed a dual mechanism disrupting biofilms and generating ROS for oxidative stress. This dual approach results in notable improvement over free EDTA or HRP alone. The results of this study indicate that nanoflower formulations, specifically HRP NFs and EDTA-HRP NFs, have the potential to be advanced endodontic irrigants, demonstrating superior anti-biofilm activity in comparison to conventional agents. However, further in vivo research and comprehensive biocompatibility testing are required to substantiate their clinical relevance. Overall, nanoflower formulations, mainly HRP NFs and, to a lesser degree, EDTA-HRP NFs, are promising platforms for innovative antimicrobial treatments in endodontic disinfection.

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REFERENCES

- Anderl, J. N., Franklin, M. J., & Stewart, P. S. (2000). Role of antibiotic penetration limitation in *Klebsiella pneumoniae* biofilm resistance to ampicillin and ciprofloxacin. *Antimicrobial Agents and Chemotherapy*, 44(7):1818-1824. <https://doi.org/10.1128/AAC.44.7.1818-1824.2000>
- Byström, A., & Sundqvist, G. (1981). Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *European Journal of Oral Sciences*, 89(4):321-328. <https://doi.org/10.1111/j.1600-0722.1981.tb01689.x>
- Chugal, N. M., Clive, J. M., & Spångberg, L. S. W. (2003). Endodontic infection: Some biologic and treatment factors associated with outcome. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, 96(1):81-90. [https://doi.org/10.1016/S1079-2104\(02\)91703-8](https://doi.org/10.1016/S1079-2104(02)91703-8)
- Contreras, A., Raxworthy, M. J., Wood, S., Schiffman, J. D., & Tronci, G. (2019). Photodynamically Active Electrospun Fibers for Antibiotic-Free Infection Control. *ACS Applied Biomaterials*, 2(10):4258-4270. <https://doi.org/10.1021/acsabm.9b00543>
- Dadi, S., Cardoso, M. H., Mandal, A. K., Franco, O. L., Ildiz, N., & Ocoşoy, I. (2023). Natural Molecule-Incorporated Magnetic Organic-Inorganic Nanoflower: Investigation of Its Dual Fenton Reaction-Dependent Enzyme-Like Catalytic Activities with Cyclic Use. *ChemistrySelect*, 8(13):e202300404. <https://doi.org/10.1002/slct.202300404>
- Dadi, S., Celik, C., & Ocoşoy, I. (2020). Gallic acid nanoflower immobilized membrane with peroxidase-like activity for m-cresol detection. *Scientific Reports*, 10(1). <https://doi.org/10.1038/s41598-020-73778-7>
- Fouad, A. F., Zerella, J., Barry, J., & Spångberg, L. S. (2005). Molecular detection of *Enterococcus* species in root canals of therapy-resistant endodontic infections. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, 99(1):112-118. <https://doi.org/10.1016/j.tripleo.2004.06.064>
- Ge, J., Lei, J., & Zare, R. N. (2012). Protein-inorganic hybrid nanoflowers. *Nature Nanotechnology*, 7(7):428-432. <https://doi.org/10.1038/nnano.2012.80>
- Haapasalo, M., Shen, Y., Wang, Z., & Gao, Y. (2014). Irrigation in endodontics. *British Dental Journal*, 216: 299-303. <https://doi.org/10.1038/sj.bdj.2014.204>
- Kishen, A. (2015). Nanotechnology in endodontics: Current and potential clinical applications. In *Nanotechnology in Endodontics: Current and Potential Clinical Applications*. Springer Nature Press, (Kishen A., Ed.) <https://doi.org/10.1007/978-3-319-13575-5>
- Mesing R. (1995). *Immobilized Enzymes for Industrial Reactors* (Mesing R., Ed.). Academic Press.
- Miranda, M. V., Magri, M. L., Cabrera, R. B., Fernández Lahore, H. M., & Cascone, O. (2003). Optimisation of peroxidase adsorption on Concanavalin A-agarose. *Latin American Applied Research*, 33(2):67-71.
- Nashaat, S. M., Enan, E. T., El-Din, A. S. G. S., & Abdelaziz, D. (2025).

- Developing a novel therapeutic and bioactive resin-based root canal sealer incorporated with silver polydopamine-modified hydroxyapatite fillers. *BMC Oral Health*, 25:1133. <https://doi.org/10.1186/s12903-025-06469-2>
- Prince, A. S. (2002). Biofilms, Antimicrobial Resistance, and Airway Infection. *New England Journal of Medicine*, 347(14):1110-1111. <https://doi.org/10.1056/nejmcibr021776>
- Sawai, J., Shoji, S., Igarashi, H., Hashimoto, A., Kokugan, T., Shimizu, M., & Kojima, H. (1998). Hydrogen peroxide as an antibacterial factor in zinc oxide powder slurry. *Journal of Fermentation and Bioengineering*, 86(5):521-522. [https://doi.org/10.1016/S0922-338X\(98\)80165-7](https://doi.org/10.1016/S0922-338X(98)80165-7)
- Siqueira, J. F., & Roças, I. N. (2008). Clinical Implications and Microbiology of Bacterial Persistence after Treatment Procedures. *Journal of Endodontics*, 34(11):1291-1301. <https://doi.org/10.1016/j.joen.2008.07.028>
- Siqueira, J. F., Roças, I. N., Santos, S. R. L. D., Lima, K. C., Magalhães, F. A. C., & De Uzeda, M. (2002). Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. *Journal of Endodontics*, 28(3):181-184. <https://doi.org/10.1097/00004770-200203000-00009>
- Subramani, I. G., Perumal, V., Gopinath, S. C. B., Fhan, K. S., & Mohamed, N. M. (2022). Organic-Inorganic Hybrid Nanoflower Production and Analytical Utilization: Fundamental to Cutting-Edge Technologies. *Critical Reviews in Analytical Chemistry*, 52(7):1488-1510. <https://doi.org/10.1080/10408347.2021.1889962>
- Vrany, J. D., Stewart, P. S., & Suci, P. A. (1997). Comparison of recalcitrance to ciprofloxacin and levofloxacin exhibited by *Pseudomonas aeruginosa* biofilms displaying rapid-transport characteristics. *Antimicrobial Agents and Chemotherapy*, 41(6):1352-1358. <https://doi.org/10.1128/aac.41.6.1352>
- Wang, H., Wu, Y., Dai, M., Zhu, T., Wu, D., & Leng, D. (2025). PB/CeO₂ Nanoparticles Regulating Reactive Oxygen Species for the Control of *Enterococcus faecalis* Infection in Root Canals. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 113(9):e35646. <https://doi.org/10.1002/jbm.b.35646>
- Wang, P. (2006). Nanoscale biocatalyst systems. *Current Opinion in Biotechnology*, 17(6):574-579. <https://doi.org/10.1016/j.copbio.2006.10.009>
- Wei, G., Yang, G., Wang, Y., Jiang, H., Fu, Y., Yue, G., & Ju, R. (2020). Phototherapy-based combination strategies for bacterial infection treatment. *Theranostics*, 10(26):12241-12262. <https://doi.org/10.7150/thno.52729>
- Wu, W., Duan, M., Shao, S., Meng, F., Qin, Y., & Zhang, M. (2023). Recent advances in nanomaterial-mediated bacterial molecular action and their applications in wound therapy. *Biomaterials Science*, 11(20):6748-6769. <https://doi.org/10.1039/d3bm00663h>
- Xu, J., Li, Y., Zhou, X., Li, Y., Gao, Z. Da, Song, Y. Y., & Schmuki, P. (2016). Graphitic C₃N₄-Sensitized TiO₂ Nanotube Layers: A Visible-Light Activated Efficient Metal-Free Antimicrobial Platform. *Chemistry-A*

European Journal, 22(12):3947-3951. <https://doi.org/10.1002/chem.201505173>

Zhang, X., Lin, S., Chen, Y., Huang, Q., & Zhong, Y. (2023). Highly efficient co-catalytic activation of peroxomonosulfate by hybrid-phase 1T/2H-MoS₂ nanoflowers combined with Fe²⁺ in the Fenton-like process. *Materials Letters*, 353: 135329. <https://doi.org/10.1016/j.matlet.2023.135329>



Chapter 3

**ENDOCHRON RESTORATIONS: A MODERN
APPROACH TO PROTECTIVE RECONSTRUCTION OF
ENDODONTICALLY TREATED TEETH**

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1. Introduction

Endodontically treated teeth are more fragile than intact teeth due to loss of pulp tissue, material removal during cavity preparation and disturbance of moisture balance. For long-term functional retention of these teeth, not only successful root canal treatment is sufficient, but also appropriate coronal restoration is an integral part of the treatment (Haas, Campbell, Hicks, & Pelleu, 1989).

Traditionally, such teeth were restored with post-core systems and full crowns. However, disadvantages such as additional weakening of the root canal during post placement, stress accumulation in the dentin walls and structural weakness have led to the search for more protective restorative options (Muench, da Silva, & Ballester, 2000). In this context, the concept of endochrone has been developed as a modern restorative approach based on minimally invasive principles while maintaining functional durability.

Endochrone is a type of restoration that provides retention directly within the pulp chamber without post placement. The retention of the restoration is derived from the internal morphology of the pulp chamber and the strong adhesive bond (Stahle, 1999). Thus, both mechanical strength and maximum preservation of tooth tissue are achieved without unnecessary intervention in the root canal.

The clinical success of endocrons depends on many factors such as material selection, adhesive bonding protocol, amount of remaining dentin and distribution of occlusal loads. With the widespread use of CAD/CAM technologies, the use of high-strength ceramics such as lithium disilicate and zirconia has increased the clinical efficacy of endocrons (Böswald & Kienzle, 2019).

Endochrons are increasingly preferred for their high mechanical strength, biocompatibility and aesthetic compatibility, especially in posterior endodontically treated teeth. They also offer a more physiological form of restoration in terms of biomechanics, as they preserve tooth tissue more than post-core and full crown restorations.

Recent systematic reviews show that endocrons have clinical success rates of over 90% at 5 to 10 years of follow-up (Torres et al., 2017). These results suggest that endocrowns are not only a restorative alternative, but also a permanent treatment option within the concept of preventive dentistry.

2. Historical Development of Endochrons

The restoration of endodontically treated teeth is a topic that has been discussed for many years in the history of dentistry. Traditionally, post-core systems and full crowns were used in these teeth, thus providing retention to the coronal structure with the post placed in the root canal (Cheung, 2005). However, the fact that post application causes additional material loss in the root canal walls, increases the risk of fracture and adversely affects the long-term prognosis of the tooth has accelerated the tendency towards more conservative approaches (Muench et al., 2000).

In the 1980s, developments in adhesive dentistry and the strengthening of ceramic materials led to the idea that a restorative solution could be produced without post placement. The first prototypes of this approach were described as monoblock restorations and were developed as designs that achieved retention only from the pulp chamber without entering the root canal (Stahle, 1999).

In the late 1990s (Stahle, 1999), the term "endocrown" was defined for the first time. This type of restoration utilises the pulp chamber as the retention area and does not require post placement in the root canal. The authors investigated the clinical performance of lithium disilicate endocrowns prepared with CEREC CAD/CAM technology and reported a high success rate at two-year follow-up. This study marked the beginning of the acceptance of endocrowns as an independent restorative concept in modern dentistry.

In the 2000s, the widespread use of CAD/CAM technology and the introduction of high-strength ceramics into clinical use have significantly improved both the manufacturing time and precision of endochrons (Mörmann et al., 2013). In vitro studies conducted in the same period showed that endochrons provide better stress distribution and fracture resistance than post-core crowns.

Clinical studies in the last 10 years have demonstrated that endochrons have equal or higher success rates than post-inserted systems, especially in posterior teeth. These findings suggest that endocrowns are becoming not only an alternative but one of the standard practices of minimally invasive restorative dentistry.

3. Structural and Biomechanical Principles of Endochron

Endochrons are restorations for endodontically treated teeth based on the monoblock principle, providing retention only through the pulp chamber and occlusal surface without entering the root canal. This design preserves the remaining intact dentin structure of the tooth, increasing

both biomechanical durability and optimising post-restoration stress distribution (Shi & Maginn, 2008).

3.1. Cavity Design and Use of Pulp Chamber

The retention of the endochrone is obtained from the morphology of the pulp chamber, which differs from classical post-core systems. The inner walls of the pulp chamber, especially the basal region, constitute the main area of the restoration in terms of retention and stability (El-Ma'aita, M, Abu-Awwad, Hattar, & Devlin, 2022).

A minimum marginal enamel wall height of 2 mm, a pulp chamber depth of 3-5 mm and maintenance of dentin thickness are considered ideal during cavity design. These parameters ensure a more homogeneous distribution of stress in the axial direction of the tooth. Excessively deep pulp chambers or weak dentin walls can reduce the fracture resistance of the restoration.

3.2. Adhesive Bond and Load Distribution

The success of endochrons depends largely on the quality of adhesive bonding and stress distribution. The wide contact area of the restoration with the dentin surface provides an advantage in the distribution of forces.

Finite element analyses of lithium disilicate endochrons fabricated with CAD/CAM technology have shown that stress is concentrated in the central pulp chamber rather than in the cervical region of the tooth, thus reducing the risk of root fracture (Lenz, Bacchi, & Della Bona, 2024).

Adhesive bonding systems, especially dual-cure resin cements, are preferred because they provide sufficient polymerisation depth. Self-etch systems provide better chemical bonding with dentin, while total-etch systems offer stronger micromechanical adhesion.

3.3. Stress Distribution and Fracture Resistance

The endochron design increases the load-bearing capacity of the restoration. Stress is transferred to the coronal dentin walls and pulp base, not to the root canal. This significantly reduces the risk of root fracture seen with post-inserted systems (Z. Zheng et al., 2021).

In comparative studies, endochrons have been reported to have higher fracture strength than post-core restorations. In particular, lithium disilicate endochrons can withstand a fracture load of 2,000-2,500 N on average, while post-core crowns under the same conditions fail between 1,200-1,800 N. (Govare & Contrepolis, 2020).

3.4. Dentin Thickness and Structural Support

The amount of dentin remaining after endodontic treatment is one of the main parameters determining the success of the restoration. Reduced dentin thickness weakens the adhesive bond strength and reduces the deformation resistance of the restoration.

Therefore, preserving the existing dentin without removing excess material during preparation directly improves the biomechanical performance of the endochron (Lenz et al., 2024).

4. Materials Used in Endochrons

The success of endochrons depends largely on the mechanical strength, adhesive bonding capacity and marginal adaptation stability of the material used. The material used in the monoblock design of the restoration must be able to carry both functional loads and adapt to the remaining dentin tissue. Today, the most commonly used materials in endochron production are ceramic systems, composite blocks and resin cements.

4.1. Ceramic Systems

4.1.1. Lithium Disilicate (IPS e.max Press / CAD)

Lithium disilicate ceramics ($\text{SiO}_2\text{-Li}_2\text{O}$ based) are the most preferred materials for endochrone construction due to their high fracture strength (approximately 360-400 MPa), aesthetic properties and strong bonding potential to dentin.

The glass matrix structure of these materials provides high micromechanical adhesion after roughening with hydrofluoric acid and silanisation. Lithium disilicate endochrons show better stress distribution and fracture resistance than post-core systems (Qamar et al., 2023).

4.1.2. Zirconia Based Ceramics

Zirconia (Y-TZP) is preferred in the posterior region due to its high flexural strength (900-1200 MPa) and fracture toughness. However, acid roughening and chemical bonding are limited due to the low glass content; therefore, special primers (10-MDP-based) and sandblasting are required.

The new generation of translucent zirconia is an aesthetic alternative to lithium disilicate. The CAD/CAM manufacturing process has increased the homogeneity and marginal conformity of this material (Jalalian et al., 2024).

4.1.3. Feldspathic and Leucite Reinforced Ceramics

Although feldspathic ceramics provide advantages in the anterior region with their high aesthetic values, their fracture strength is low (~100-160 MPa). For this reason, it is generally preferred in premolar endocrines or in areas of low occlusal stress. Leucite reinforcement increases the coefficient of thermal expansion, providing stress compensation at the bonding surface (El-Ma'aïta et al., 2022).

4.2. Composite Based CAD/CAM Blocks

Composite-based endochrones distribute functional loads more physiologically due to their elastic modulus (10-20 GPa) close to dentin. In addition, since they are resin-based, edge adaptation is more successful and they are easy to repair (Govare & Contrepolis, 2020). Nanohybrid CAD/CAM blocks (e.g. Grandio blocs®, Lava Ultimate®) offer short production times and easy workability. However, long-term water absorption and colour stability are limiting factors compared to ceramics (Bindl & Mörmann, 1999).

4.3. Resin Cements and Bonding Systems

The long-term success of endocrowns depends on the durability of the adhesive bond between the restoration and the tooth. Dual-cure resin cements provide a reliable bond, especially in the deeper areas of the pulp chamber, thanks to the advantage of depth of polymerisation.

Total-etch systems (phosphoric acid + silane) increase the micromechanical bond strength, while self-etch systems offer less technical precision on the dentin surface.

Surface treatments prior to cementation - e.g. hydrofluoric acid roughening, silanisation and MDP primer application - should be selected according to the material (Khatab, Makawi, & Elheeny, 2022).

4.4. New Generation and Hybrid Materials

Recently developed zirconia-reinforced lithium silicate (ZLS) and hybrid ceramics (e.g. Vita Enamic®) combine both the strength of the ceramic and the elasticity of the composite. This hybrid structure offers promising results in terms of fracture strength and marginal compliance in endochrones by improving stress absorption (AïDabeeb, Alakeel, Al Jfshar, & Alkhalid, 2023).

5. Materials Used in Endochrones

The material used in endochrone restorations has a direct impact on the biomechanical strength of the restoration, the stability of the adhesive

bond and the success of marginal adaptation. With the development of modern CAD/CAM technologies, high-strength ceramics and hybrid materials have become standard in endochron applications. 5.1. Lityum Disilikat Seramikler (IPS e.max CAD / Press)

Lithium disilicate ($\text{Li}_2\text{Si}_2\text{O}_5$) ceramics are the most preferred material group in endocrons due to their flexural strength of approximately 360-400 MPa, highly translucent appearance and strong adhesive bonding properties after acid-etch + silanisation.

These ceramics reduce the risk of root fracture by providing homogeneous distribution of stress in the pulp chamber. In *in vitro* studies, lithium disilicate endocrons have been reported to show higher fracture resistance and better marginal conformability than post-core systems (Qamar et al., 2023).

It has also been reported that lithium disilicate endocrons have a 94-97% success rate in 3-5 years of clinical follow-up (Govare & Contrepolis, 2020).

5.2. Zirconia and Zirconia-Reinforced Ceramics

Zirconia (Y-TZP) is preferred as an endochrone material especially in posterior teeth due to its high fracture toughness (approximately 900-1200 MPa) and wear resistance. However, since the low glass phase of zirconia limits the acid-etch process, 10-MDP primers and sandblasting are mandatory to strengthen the adhesive bond (Jalalian et al., 2024).

New generation translucent zirconias (e.g. 5Y-PSZ) offer aesthetic properties close to lithium disilicate and clinical success rates are increasing with hybrid bond systems.

5.3. Hybrid Ceramics and Zirconia-Strengthened Lithium Silicate (ZLS)

Hybrid ceramics (e.g. Vita Enamic®, Celtra Duo®) are CAD/CAM blocks developed by combining a ceramic and polymer phase.

These materials provide an advantage in stress absorption and reduce the risk of fracture because their elastic modulus is similar to dentin. Zirconia doped lithium silicate (ZLS) materials were reported to show the best stress compatibility with dentin in 3D finite element analyses (Huang, Fokkinga, Zhang, Creugers, & Jiang, 2023).

5.4. Composite Based CAD/CAM Blocks

Resin composite CAD/CAM blocks (e.g. Grandio blocs®, Lava Ultimate®) help to physiologically distribute functional forces due to

their dentin-like elastic modulus (10-20 GPa). They are also not overly rigid, which prevents stress accumulation at the restoration-dentin interface.

However, long-term water absorption and surface abrasion may limit the clinical life of these materials. Nevertheless, their CAD/CAM machinability and easy repairability are significant advantages.

5.5. Resin Cements and Bonding Systems

In endochronous restorations, the adhesive bond provides mechanical integration between the tooth tissue and the material. Dual-cure resin cements are preferred because they provide sufficient depth of polymerisation in deep pulp chambers.

In vitro studies have shown that self-adhesive resin cements increase the retention of endocrons (Muench et al., 2000).

6. Preparatory Stages of Endochrons

6.1. Case Selection and Preliminary Assessment

- **Indication:** Posterior teeth with extensive loss of occlusal/pulpal material, with at least 2 healthy walls; cases without suspicion of root crack/advanced cracking.
- **Contraindication:** Very shallow pulp chamber (<3 mm), inadequate occlusal load management (splinting is essential in bruxism), short clinical crown.
- **Radiographic and clinical analysis:** Remaining dentin thickness, presence of cracks, periodontal support, antagonist relationship (Lenz et al., 2024).

6.2. Isolation, Temporary Removal and Substrate Management

- Kofferdam is mandatory. After removal of eugenol-containing sealers, the surface should be cleaned with alcohol/pumice (eugenol inhibits polymerisation).
- **IDS- Immediate Dentin Sealing:** Sealing the dentin with adhesive immediately after preparation improves bond quality and marginal integrity.

6.3. Preparation Principles (Geometry and Dimensions) (Ziting Zheng et al., 2022)

- **Rounding of cavities:** Inner corners radiused; no sharp angles.
- **Pulp chamber depth:** Ideal 3-5 mm.

- **Occlusal reduction:** Molar 2.0 mm; premolar 1.5-2.0 mm.
- **Margin:** 90° butt-joint; supragingival if possible.
- **Convergence:** 6-10° on the inner walls.
- **Ferrule:** Not mandatory; 1-2 mm useful if available.

6.4. Digital Workflow: CAD/CAM Design and Production

- **Screening:** After IDS, the oxygen inhibition layer is removed with glycerin gel and light-cured, the surface is cleaned with pumice and then scanned.
- **Design:** Internal cement spacing 50-120 µm; internal recesses are avoided.
- **Minimum thickness (ceramic):** Occlusal ≥1.5-2.0 mm; axial ≥1.0 mm.
- **After milling:** Crystallisation (for LDS) and glaze.

6.5. Restoration Surface Treatment (Material Specific)

- **Lithium disilicate/ZLS:** 5-10% HF 20 s (LDS), silane 60 s, air drying.
- **Feldspathic:** 5-10% HF 60-90 s, silane.
- **Zirconia (Y-TZP):** 50 µm Al₂O₃ sandblasting (1-2 bar, 10 s), 10-MDP primer; HF not applied.

6.6. Tooth Surface Pre-treatment

- **Total-etch (enamel) + selective/self-etch (dentin):** Phosphoric acid 20-30 s on enamel; on dentin follow system protocol.
- **Contamination control:** Screening gel, saliva, blood → cleaning with pumice and/or chlorhexidine (0.2%); gentle roughening if IDS present.

6.7. Cementation Protocol

- **Insulation:** Kofferdam; humidity control.
- **Cement selection:** Dual-cure resin cement for deep cavity.
- **Application:** Ceramic inner surface (HF+Silan/MDP), tooth surface (adhesive); place resin cement, setting pressure; close margins with glycerine gel and complete light cure.
- **Light cure:** Manufacturer's recommendation based on ceramic thickness (usually 40-60 s versatile).

6.8. Occlusion, Finishing and Polishing

- Static/dynamic occlusion; no high contact.
- Marginal excesses with composite finishing discs; glaze/polish.

6.9. Common Errors and Solution Suggestions

- **Insufficient pulp chamber depth (<3 mm):** Retention decreases → review indication.
- **Wrong surface treatment:** HF treatment of zirconia is incorrect → sandblasting + MDP is essential.
- **Eugenol contamination:** Resin polymerisation is inhibited → surface cleaning and use eugenol-free temporary.
- **Excessive cement gap (>150 µm):** Marginal fit is disturbed → correct CAD settings.

6. Clinical Indications and Contraindications of Endocrons

Endocrons are a minimally invasive, adhesive-supported and post-free modern restorative option for the restoration of endodontically treated teeth. However, the success of this approach depends on correct case selection and identification of appropriate clinical indications (Lenz et al., 2024).

6.1. Suitable Indications for Endocrons

6.1.1.1. Posterior Endodontically Treated Teeth

Endocrons are particularly suitable for molars and premolars with extensive loss of occlusal material but preserved root integrity. The morphology of the pulp chamber contributes to retention and restoration stability is achieved without the need for post placement.

6.1.2. Teeth with sufficient amount of remaining dentin

Clinically, it is recommended to have at least two intact walls, dentin thickness >2 mm and pulp chamber depth 3-5 mm (Torres et al., 2017). This structural support strengthens the adhesive bond of the restoration and reduces the risk of fracture.

6.1.3. Cases with Aesthetic Needs

Lithium disilicate or hybrid ceramic endocrons are preferred for their high translucency and natural reflectivity, especially in the upper premolars and aesthetic areas (Z. Zheng et al., 2021).

6.1.4. Teeth with Inappropriate Canal Length and Root Morphology

The risk of post placement is high in teeth with narrow root canals, curved or weak dentin walls. In such cases, endocron offers a safe alternative by preserving root integrity.

6.2. Contraindications of Endocrons

6.2.1. Shallow Pulp Chamber (<3 mm)

Insufficient depth of the pulp chamber leads to decreased retention and concentration of stress on the cervical dentin. In such cases, post-core restorations should be preferred (Haas et al., 1989).

6.2.2. Weak Dentin Walls or Presence of Cracks

Endochron indication is not appropriate in the presence of root or coronal cracks. In these cases, the force distribution is disturbed and the risk of fracture increases (Staehele, 1999).

6.2.3. Bruxism or High Occlusal Force

Repetitive overloads in patients with bruxism cause fatigue of the adhesive interface. Occlusal splinting is recommended in these patients (Muench et al., 2000).

6.2.4. Subgingival Margin or Inadequate Isolation

Endochronous restorations require complete isolation (kofferdam). In the presence of a subgingival margin, adhesive cementation is not reliable and the risk of marginal leakage increases (Böswald & Kienzle, 2019).

Clinical Condition	Endochron Recommendation	Alternatif
Pulp chamber depth ≥ 3 mm	Suitable	—
At least 2 solid walls available	Suitable	—
Bruxism present	Splint recommended	Post-core kron
Shallow pulp chamber (<3 mm)	Not suitable	Post-core kron
Root crack / weak dentin	Not suitable	Fibre post or extraction
Subgingival marjin	Relative contraindication	Endochron after crown lengthening
Aesthetic area	Lithium disilicate / hybrid recommended	—

7.DISCUSSION

Endocrown restorations have become an increasingly accepted method for conservative reconstruction of endodontically treated teeth. In the literature, it is reported that endocrowns provide higher fracture resistance and more homogeneous stress distribution, especially compared to post-core crowns (Abbas, Elerian, Elsherbiny, Elgohary, & Atout, 2024). These results are attributed to the fact that the endochron design, which eliminates the need for root canal access by utilising the natural morphology of the pulp chamber, preserves the amount of remaining dentin in the tooth.

Finite element analyses show that endochrone restorations distribute forces through the coronal dentin walls and pulp base rather than along the root. This provides an advantage in preventing vertical root fractures, especially in post-placed restorations. In addition, the elastic modulus of materials such as lithium disilicate and hybrid ceramics is close to dentin, supporting a more physiological absorption of stress (Lenz et al., 2024).

One of the most critical factors in the success of endocrowns is the quality of the adhesive bond. The advantage of the depth of polymerisation of dual-cure resin cements improves retention, especially in deep pulp chambers (D'Arcangelo et al., 2010). However, insufficient isolation during the cementation process or the presence of eugenol

residues can severely weaken the bonding. It is therefore recommended to use eugenol-free systems as temporary materials (Haas et al., 1989).

Clinical studies show that endochrons have high survival rates at 2 to 10 years of follow-up. The survival rate at 2-year follow-up reported by Bindl and Mörmann (1999) was 94%, while the systematic review by Sedrez-Porto et al. (2016) reported an average success rate of 92.8% at follow-up of more than 5 years. These data support that endochrons are long-term reliable restorations with the correct indication selection and the use of appropriate materials.

However, some limitations should not be overlooked. In teeth with shallow pulp chamber (<3 mm) or weak dentin walls, retention is reduced and stress distribution is impaired. In addition, the presence of high occlusal forces, such as bruxism, can lead to fatigue damage at the adhesive interface (Lander & Dietschi, 2008). Occlusal splinting is recommended in such patients. In teeth with subgingival margins, failure to provide isolation increases the risk of microleakage and causes the endochron to become off-label. (AlDabeeb et al., 2023).

New generation hybrid ceramics and zirconia-reinforced systems offer significant mechanical and aesthetic improvements compared to conventional ceramics. In particular, zirconia-strengthened lithium silicate (ZLS) materials have expanded endochron indications by providing high translucency and durability at the same time. (Qamar et al., 2023). However, long-term clinical data are still limited and more prospective studies are required.

In general, endochrons offer a more conservative, biomechanically more compatible and aesthetically superior restoration option compared to post- and core-supported crowns. However, success depends not only on the quality of the material, but also on appropriate indication selection, perfect isolation, correct surface treatment and precise cementation protocol. When these principles are followed, endochrons are restorations with a high success rate that can maintain the long-term functional and aesthetic integrity of endodontically treated teeth.

8.CONCLUSION and CLINICAL RECOMMENDATIONS

Endochrone restorations offer a conservative approach to the restoration of endodontically treated teeth. This type of restoration provides both maximum preservation of tooth tissue and prevention of complications related to post application. Current biomechanical and clinical studies show that the long-term success of endochrons is quite high with the correct choice of indication and the use of appropriate materials. (El-Ma'aïta et al., 2022).

CAD/CAM materials such as lithium disilicate, zirconia and hybrid ceramics increase the durability of endochrons and fulfil aesthetic expectations. Adhesive cementation with dual-cure resin cements significantly improves marginal sealing and retention of the restoration (Shi & Maginn, 2008). However, bonding success is strongly dependent on seal quality, surface preparation and material compatibility.

The clinical literature has demonstrated that endochrons have a survival rate of more than 90% at follow-up of 5 years or more. (El-Ma'aita et al., 2022). However, most failures are due to inappropriate case selection (e.g. shallow pulp chamber or weak dentin walls), inadequate isolation or inappropriate cementation techniques.

Therefore, the success of endochrons depends not only on the physical properties of the material, but also on the thorough implementation of the clinical protocol. It is essential that the preparation is precise, the pulp chamber is deep enough, the cavity corners are rounded and the surface treatments are applied correctly according to the material type. In addition, occlusal splinting is recommended in the presence of high occlusal stress such as bruxism (Huang et al., 2023).

In conclusion, endochrone restorations can be considered as a strong alternative to post-core crowns in terms of structural protection, high biomechanical durability, aesthetic fit and long-term stability. The basic principles for success are summarised below:

- Correct case selection (sufficient pulp chamber depth ≥ 3 mm, presence of a solid wall).
- Complete isolation (use of cofferdam is mandatory).
- Material selection appropriate to the clinical site (zirconia for posterior, lithium disilicate or hybrid ceramic for aesthetic).
- Material-specific application of surface treatments (HF + silane or sandblasting + MDP).
- Use of dual-cure resin cement and controlled polymerisation.
- Protective splinting in patients with bruxism.

When applied in accordance with these criteria, endochrons offer reliable, aesthetic and long-lasting restorations both biologically and mechanically.

REFERENCES

- Abbas, M. H., Elerian, F. A., Elsherbiny, A. A., Elgohary, N. M. M., & Atout, A. (2024). Influence of occlusal reduction design on the fracture resistance and biomechanical behavior of endocrowns restoring maxillary premolars. *BMC Oral Health*, *24*(1), 113. doi:10.1186/s12903-023-03688-3
- AlDabeeb, D. S., Alakeel, N. S., AlJfshar, R. M., & Alkhalid, T. K. (2023). Endocrowns: Indications, Preparation Techniques, and Material Selection. *Cureus*, *15*(12), e49947. doi:10.7759/cureus.49947
- Bindl, A., & Mörmann, W. H. (1999). Clinical evaluation of adhesively placed Cerec endo-crowns after 2 years--preliminary results. *J Adhes Dent*, *1*(3), 255-265.
- Böswald, L., & Kienzle, E. (2019). [A simple indicator for ruling out inadequate phosphorus digestibility in growing pigs]. *Tierarztl Prax Ausg G Grosstiere Nutztiere*, *47*(4), 223-229. doi:10.1055/a-0947-8799
- El-Ma'aïta, A., M, A. A.-R. a., Abu-Awwad, M., Hattar, S., & Devlin, H. (2022). Endocrowns Clinical Performance and Patient Satisfaction: A Randomized Clinical Trial of Three Monolithic Ceramic Restorations. *J Prosthodont*, *31*(1), 30-37. doi:10.1111/jopr.13414
- Govare, N., & Contrepolis, M. (2020). Endocrowns: A systematic review. *J Prosthet Dent*, *123*(3), 411-418.e419. doi:10.1016/j.prosdent.2019.04.009
- Haas, S. B., Campbell, A. D., Hicks, M. L., & Pelleu, G. B., Jr. (1989). A comparison of four root canal filling techniques. *J Endod*, *15*(12), 596-601. doi:10.1016/s0099-2399(89)80158-x
- Huang, Y., Fokkinga, W. A., Zhang, Q., Creugers, N. H. J., & Jiang, Q. (2023). Biomechanical properties of different endocrown designs on endodontically treated teeth. *J Mech Behav Biomed Mater*, *140*, 105691. doi:10.1016/j.jmbbm.2023.105691
- Jalalian, E., Zarbakhsh, A., Khorshidi, S., Golalipour, S., Mohammadnasl, S., & Sayyari, M. (2024). Comparative analysis of endocrown fracture resistance and marginal adaptation: CAD/CAM technology using lithium disilicate vs. zirconia-reinforced lithium silicate ceramics. *Saudi Dent J*, *36*(2), 353-358. doi:10.1016/j.sdentj.2023.11.020
- Khattab, N. M. A., Makawi, Y., & Elheeny, A. A. H. (2022). Clinical Evaluation of CAD/CAM Ceramic Endocrown Versus Prefabricated Zirconia Crown in the Restoration of Pulpotomized Primary Molars: A Two-Year Spilt-Mouth Randomized Controlled Trial. *Eur J Dent*, *16*(3), 627-636. doi:10.1055/s-0041-1736417
- Lenz, U., Bacchi, A., & Della Bona, A. (2024). Biomechanical performance of endocrown and core-crown restorations: A systematic review. *J Esthet Restor Dent*, *36*(2), 303-323. doi:10.1111/jerd.13119
- Muench, A., da Silva, E. M., & Ballester, R. Y. (2000). Influence of different dentinal substrates on the tensile bond strength of three adhesive systems. *J Adhes Dent*, *2*(3), 209-212.

- Qamar, Z., Alghamdi, A. M. S., Haydarah, N. K. B., Balateef, A. A., Alamoudi, A. A., Abumismar, M. A., . . . Minervini, G. (2023). In Vitro Evaluation of Lithium Disilicate Endocrowns and Post and Core Crowns-A Systematic Review. *J Funct Biomater*, 14(5). doi:10.3390/jfb14050276
- Shi, W., & Maginn, E. J. (2008). Improvement in molecule exchange efficiency in Gibbs ensemble Monte Carlo: development and implementation of the continuous fractional component move. *J Comput Chem*, 29(15), 2520-2530. doi:10.1002/jcc.20977
- Staehele, H. J. (1999). Minimally invasive restorative treatment. *J Adhes Dent*, 1(3), 267-284.
- Torres, A., Niederman, M. S., Chastre, J., Ewig, S., Fernandez-Vandellos, P., Hanberger, H., . . . Wunderink, R. (2017). International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia: Guidelines for the management of hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) of the European Respiratory Society (ERS), European Society of Intensive Care Medicine (ESICM), European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Asociación Latinoamericana del Tórax (ALAT). *Eur Respir J*, 50(3). doi:10.1183/13993003.00582-2017
- Zheng, Z., He, Y., Ruan, W., Ling, Z., Zheng, C., Gai, Y., & Yan, W. (2021). Biomechanical behavior of endocrown restorations with different CAD-CAM materials: A 3D finite element and in vitro analysis. *J Prosthet Dent*, 125(6), 890-899. doi:10.1016/j.prosdent.2020.03.009
- Zheng, Z., Sun, J., Jiang, L., Wu, Y., He, J., Ruan, W., & Yan, W. (2022). Influence of margin design and restorative material on the stress distribution of endocrowns: a 3D finite element analysis. *BMC Oral Health*, 22(1), 30. doi:10.1186/s12903-022-02063-y