Journal of Pioneering Medical Sciences
Received: February 21, 2025 | Accepted: April 01, 2025 | Published: April 27, 2025
Volume 14, Issue S01, Pages 77-84

DOI https://doi.org/10.47310/jpms202514S0110

Comparative Analysis of Acetylated Hydroxyethyl Methacrylate (Ac-HEMA) as a Novel Direct Restorative Material in Dentistry: An In vitro Study on Mechanical Properties and Microleakage Performance

Sivashankari Boobalan[1], Jayashri Prabakar[2*], Jishnu Krishna Kumar[3] and Meignana Arumugham. I[4]
[1,2,3,4]Department of Public Health Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University, 600077 Chennai, India

Author Designation: [1]Postgraduate Student, [2]Associate Professor, [3]Associate Professor, [4]Professor

*Corresponding author: Jayashri Prabakar (e-mail: jayashriprabakar@yahoo.com).

Abstract **Background:** Hydroxyethyl Methacrylate (HEMA) has shown potential as a restorative material due to its biocompatibility, chemical stability and adhesive properties. However, challenges such as polymerization shrinkage and limited mechanical strength have prompted efforts to improve its performance. Acetylated Hydroxyethyl Methacrylate (Ac-HEMA) has been developed with the aim of enhancing mechanical strength, marginal integrity and resistance to microleakage. This study investigates the performance of Ac-HEMA as a restorative material in comparison with conventional Glass Ionomer Cement (GIC) and composite resins. **Materials and Methods:** Ac-HEMA was synthesized through an acetylation process confirmed via Fourier Transform Infrared Spectroscopy (FTIR). The material's microleakage and fracture resistance were evaluated using thermocycling and Scanning Electron Microscopy (SEM) analysis. Dye penetration methods were used to assess microleakage and compressive strength testing was conducted to determine mechanical performance. Comparative analysis with GIC and composite resins was performed under identical conditions and statistical analysis was applied to validate the findings. **Results:** Ac-HEMA demonstrated a maximum force of 474.24 N and a compressive stress of 8.98 MPa. The compressive displacement and strain were recorded at 3.84 mm and 3.84%, with a compressive strain value of 1.37%. The GIC exhibited a maximum force of 1130.59 N and a compressive stress of 11.64 MPa, while composite resins recorded superior results with a maximum force of 2198.49 N and compressive stress of 21.85 MPa. Microleakage assessment revealed increased dye penetration in Ac-HEMA compared to GIC and composite resins, indicating higher microleakage levels. **Conclusion:** Although Ac-HEMA demonstrated moderate mechanical strength and promising adhesive properties, its increased microleakage presents a significant limitation in its current formulation. Further refinement of Ac-HEMA's chemical composition and bonding characteristics is recommended to improve its sealing ability and clinical performance. Future research should focus on enhancing Ac-HEMA's hydrophilic balance, improving structural durability and conducting long-term clinical trials to assess its viability for minimally invasive dental treatments. Ac-HEMA holds potential as a restorative material with continued development.

Key Words Acetylated Hydroxyethyl Methacrylate, restorative material, microleakage, fracture resistance, dental restoration, *in vitro* study

INTRODUCTION

In modern dentistry, restorative materials play a crucial role in maintaining oral health by preserving tooth structure, enhancing functionality and improving aesthetics. An ideal restorative material should be biocompatible, durable and capable of mimicking the natural appearance of teeth while ensuring minimal risk of secondary caries [1,2].

Dental amalgam has been widely used for many years due to its exceptional durability, reliability and affordability for tooth restoration. Its ability to withstand strong occlusal forces makes it suitable for use in posterior teeth. However, amalgam has several drawbacks, including poor aesthetics, mercury toxicity, thermal conductivity, delayed expansion and susceptibility to microleakage, which limit its appeal in contemporary restorative dentistry [3,4].

In recent years, composite resins have emerged as a preferred choice for restorative procedures among both patients and dentists. Their primary advantage lies in their ability to closely match the natural appearance of teeth, providing superior aesthetics, particularly in anterior restorations [5]. Furthermore, composite resins often require less removal of healthy tooth structure, aligning well with the principles of minimally invasive dentistry. However, composite materials are prone to polymerization shrinkage during curing, which can compromise marginal integrity and increase the risk of microleakage unless incremental layering techniques are employed [6].

Glass Ionomer Cement (GIC) is another commonly used restorative material due to its unique chemical bonding properties, which make it suitable as a liner, bonding agent, or cement. While GIC offers strong adhesion to dental tissues, it exhibits lower wear resistance compared to composite resins, especially in areas with high occlusal forces, limiting its suitability for high-stress applications [7,8].

2-Hydroxyethyl methacrylate (HEMA) is a monomer derived from methacrylic acid (MAA) and is known for its excellent biocompatibility and adhesive properties. HEMA, a colorless and transparent liquid with the chemical formula $C6H10O3$, is widely utilized in the synthesis of various polymers and resins. Its ability to form strong chemical bonds with the tooth structure is a key factor contributing to the success and durability of dental restorations [9,10]. HEMA's biocompatibility, low toxicity and chemical stability ensure its safety and longevity in dental applications [11,12]. Additionally, HEMA's compatibility with various dental materials, such as composites, bonding agents and sealants, makes it a versatile component in restorative dentistry. By mitigating polymerization shrinkage in dental composites, HEMA helps minimize marginal gaps and reduces the risk of secondary caries [13].

When HEMA-based materials are applied to tooth surfaces, they adhere effectively to dentin and enamel, forming stable and long-lasting restorations. HEMA undergoes polymerization through light-curing (photo polymerization) or self-curing methods, forming strong polymers or copolymers that reinforce marginal integrity [14]. Despite its advantages, polymerization shrinkage remains a concern in HEMA-based restorations, potentially compromising marginal adaptation [15].

To address these limitations, Acetylated Hydroxyethyl Methacrylate (Ac-HEMA) was developed as a novel restorative material designed to improve mechanical strength, enhance marginal integrity and minimize microleakage. Ac-HEMA combines the chemical bonding properties of HEMA with improved hydrophilic characteristics, enhancing its adhesion to tooth structures. By forming a well-characterized matrix, Ac-HEMA is designed to improve structural durability and reduce microleakage [16].

This in-vitro study was undertaken to evaluate Ac-HEMA as a direct restorative material and compare its mechanical and chemical properties with established materials such as composite resins and GIC. The study aims to determine whether Ac-HEMA can provide superior mechanical strength, reduced microleakage and improved marginal integrity, thereby advancing restorative techniques in modern dentistry.

MATERIALS AND METHODS

Synthesis of Acetylated Hydroxyethyl Methacrylate (Ac-HEMA)

Ac-HEMA was synthesized through a controlled acetylation process. Hydroxyethyl methacrylate (HEMA), dichloromethane and ethylene triamine were reacted to form chloroacetic acid. The mixture was maintained at -20°C for 15 minutes, followed by incubation at 20°C for 2 hours. Subsequently, 30 ml of distilled water was added and the solution was stirred overnight to ensure complete acetylation, resulting in the formation of Acetylated Hydroxyethyl Methacrylate (Table 1). This controlled acetylation process was chosen to enhance the chemical stability and structural integrity of the material.

Fourier Infrared Spectroscopy (FTIR)

To confirm the acetylation of HEMA, Fourier Transform Infrared Spectroscopy (FTIR) analysis was conducted. FTIR spectroscopy utilizes infrared light to analyze the chemical properties of the sample. The spectra of both HEMA and Ac-HEMA were recorded, revealing a distinct shift in peaks and an additional peak at 1632 cm^{-1} and 1453 cm^{-1}, confirming the successful acetylation of HEMA. The FTIR spectra of Ac-HEMA were acquired three times using a Perkin Elmer Spectrum Two spectrometer equipped with a Universal Diamond attenuated total reflectance attachment (Perkin Elmer, London, UK). Spectra were collected with 16 scans accumulated over a range of 4000 cm^{-1} to 450 cm^{-1} with a resolution of 4 cm^{-1} (Figure 1). This rigorous confirmation ensured the precise identification of Ac-HEMA and its acetylated structure.

Characterization of Acetylated Hydroxyethyl Methacrylate

Ac-HEMA was characterized for its adhesive properties, marginal integrity and hydrophilic nature. Ac-HEMA bonds

Table 1: Composition of HEMA

Composition	Percentage
BIS GMA	0.75
HEMA	0.75
Ac-HEMA	0.3
Methacrylic acid	0.03
Titanium dioxide	0.003
Sodium monofluorophosphate	0.002
Tricalcium silicate	200 mg

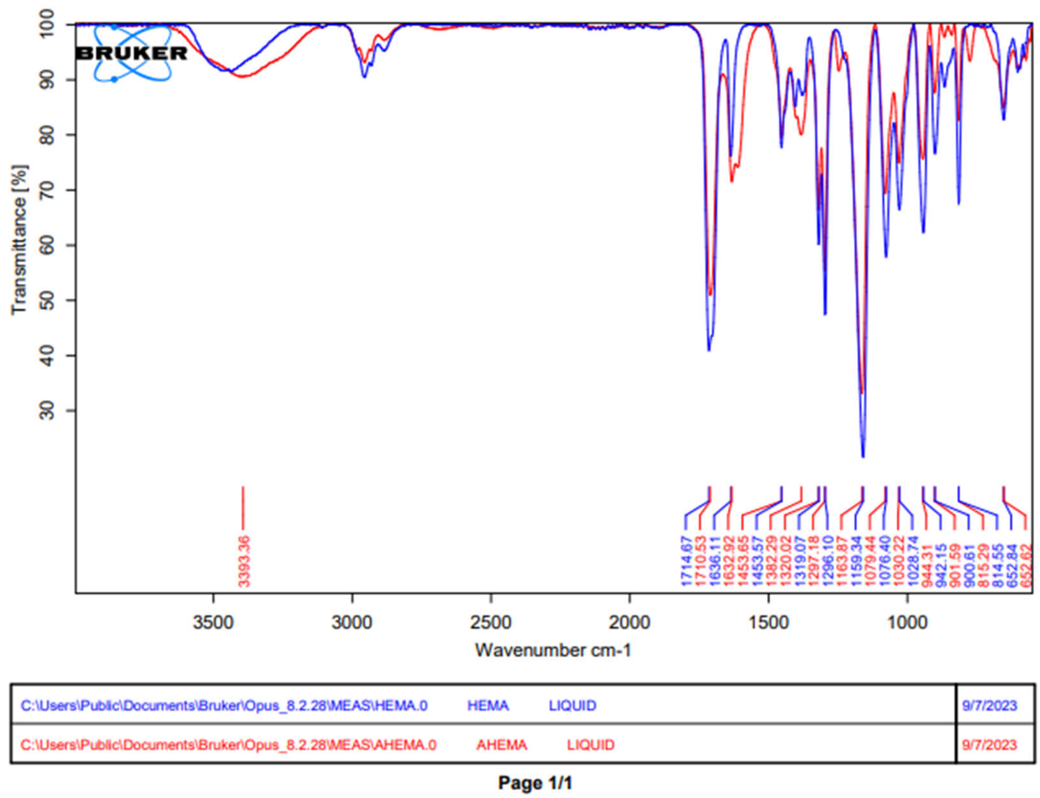

Figure 1: Ac-HEMA confirmation by Fourier Infrared Spectroscopy (FTIR)

chemically to the tooth surface, enhancing adhesion and improving marginal integration. To assess its practical application, Ac-HEMA was evaluated only in small controlled amounts, as its adhesive efficiency diminishes when applied in excessive quantities [17].

For this study, ten extracted teeth (six premolars and four molars) were collected from the Oral Biology Department of Saveetha Dental College. The teeth were manually debrided using scaling instruments, cleaned with pumice paste and stored in distilled water for a maximum of 14 days to maintain hydration and minimize bacterial growth.

The teeth were divided into three groups for comparative evaluation:

- **Group I:** The occlusal surface of one premolar was etched with 35% phosphoric acid gel, air-dried and treated with a bonding agent followed by composite resin restoration using light curing
- **Group II:** The occlusal surface of one premolar was restored with Glass Ionomer Cement (GIC)
- **Group III:** The occlusal surface of one molar was etched with 35% phosphoric acid gel, air-dried and treated with Ac-HEMA, which was cured using a 380 nm light source

The use of identical preparation protocols ensured consistency in restorative application across all groups, minimizing procedural variability.

Scanning Electron Microscopy (SEM) Analysis

To evaluate the structural morphology and fracture characteristics of Ac-HEMA, Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) analyses were performed. Ac-HEMA samples, cured in disc form, were crushed between steel plates using controlled low-impact force to generate fracture surfaces. For SEM observation, the fractured samples were affixed to carbon tabs and analyzed using a JEOL JSM5410 LV electron microscope. An Oxford Instruments X-MaxN EDX detector was employed for elemental analysis.

SEM imaging was conducted in low vacuum mode at an accelerating voltage of 1.0 kV with an 8.4 mm working distance to reduce charging artifacts and improve image clarity. This method allowed for high-resolution analysis of Ac-HEMA's surface morphology and elemental composition, providing insights into its bonding characteristics and potential defects [18].

Microleakage Analysis

To evaluate microleakage, teeth restored with Ac-HEMA, GIC and composite resins were immersed in distilled water at 37°C for 24 hours. Following this, the samples were subjected to 2500 thermal cycles between 5°C and 55°C, with a 10-second transfer time and a 30-second dwell time in each temperature phase.

To isolate the restorative margins, a protective layer of nail varnish was applied to the samples, leaving a 2 mm uncovered area around the restoration. The roots were embedded in acrylic resin cylinders (Meliodent, Bayer Co., Leverkusen, Germany) for stability. Each sample underwent 5-minute immersion in 0.5% basic fuchsin dye to evaluate dye penetration patterns. Following dye immersion, the samples were sectioned in the bucco-lingual direction using a water-cooled diamond saw to obtain three precise slices for analysis [19].

RESULTS

Fracture resistance of Ac-HEMA

The fracture resistance of Acetylated Hydroxyethyl Methacrylate (Ac-HEMA) was evaluated through compressive strength testing. Ac-HEMA demonstrated a maximum force of 474.24 N, with a corresponding compressive stress of 8.98 MPa. The recorded compressive displacement was 3.84 mm and the compressive strain was 3.84%, with an additional recorded compressive strain value of 1.37%. These values indicate that while Ac-HEMA exhibits moderate mechanical strength, it falls short when compared to conventional restorative materials such as Glass Ionomer Cement (GIC) and composite resins (Figure 2, 3, Table 2).

In comparison, GIC demonstrated a maximum force of 1130.59 N with a compressive stress of 11.64 MPa, while composite resins exhibited the highest performance with a maximum force of 2198.49 N and a compressive stress of 21.85 MPa. These findings suggest that while Ac-HEMA offers moderate fracture resistance, its performance is inferior to that of GIC and composite resins, particularly in high-stress areas.

Microleakage

Microleakage assessment was performed using dye penetration methods to evaluate the sealing ability of Ac-HEMA in comparison to GIC and composite resins. The results revealed that Ac-HEMA exhibited dye penetration extending beyond half of the fissure involvement, indicating increased microleakage compared to the other materials tested (Figure 3, Table 3).

Table 2: Fracture resistance of Ac-HEMA

Maximum force (N)	Compressive stress at maximum force (Mpa)	Compressive displacement at break (standard) (mm)	Compressive strain (displacement) at break (standard) (%)	Compressive stress at break (standard) (Mpa)
474.24	8.98	3.84	3.84	1.37

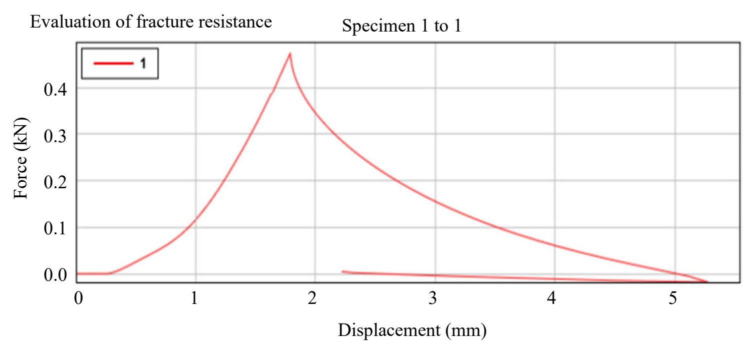

Figure 2: Compressive stress and strain of Ac-HEMA

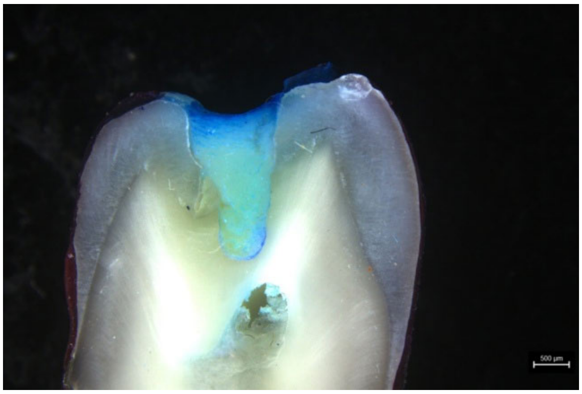

Figure 3: Tooth restored with AC-HEMA

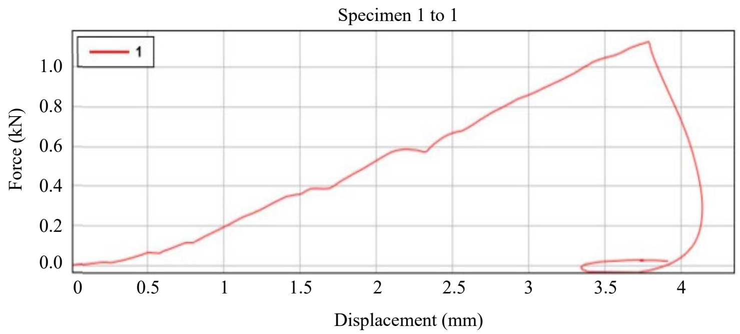

Figure 4: Compressive stress and strain of GIC

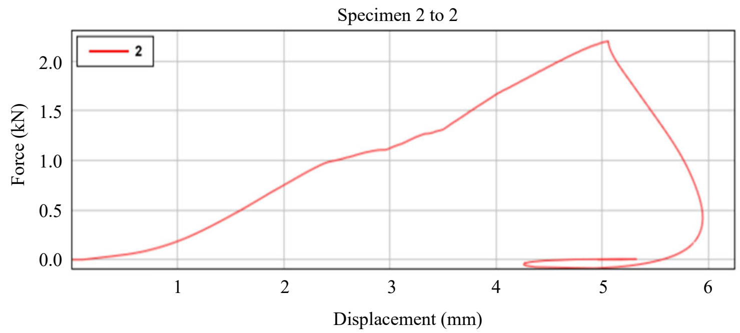

Figure 5: Compressive stress and strain of composite

Table 3: Dye penetration

Score	Dye penetration
0	There is no dye penetration
1	Dye penetration upto ½ of the fissure
2	Dye penetration beyond ½ of the fissure without total involvement
3	Dy penetration seen till the base

In contrast, both GIC and composite restorations displayed minimal dye penetration, with dye infiltration limited to less than half of the fissure involvement. These results highlight Ac-HEMA's vulnerability to marginal leakage, suggesting that modifications to its formulation are required to improve its sealing properties.

DISCUSSION

This study assessed the mechanical and chemical properties of Acetylated Hydroxyethyl Methacrylate (Ac-HEMA) as a restorative material, comparing it to Glass Ionomer Cement (GIC) and composite resins. The findings highlight that while Ac-HEMA shows potential as a restorative material, it requires further refinement to improve its clinical performance.

The results demonstrated that Ac-HEMA exhibited a maximum force of 474.24 N with a compressive stress of 8.98 MPa, compressive displacement of 3.84 mm and compressive strain of 3.84%. In comparison, GIC recorded a maximum force of 1130.59 N with a compressive stress of 11.64 MPa and a compressive displacement of 3.91 mm. Composite resins outperformed both materials with a maximum force of 2198.49 N, compressive stress of 21.85 MPa and a compressive displacement of 5.19 mm (Figure 4, 5, Table 4). These results confirm that Ac-HEMA offers moderate mechanical strength, limiting its suitability for areas subject to high occlusal forces, particularly in posterior teeth. Microleakage analysis revealed that Ac-HEMA showed increased dye penetration beyond half of the fissure involvement, indicating greater microleakage compared to GIC and composite resins. Both GIC and composite restorations demonstrated minimal dye penetration, suggesting superior marginal sealing properties. The increased microleakage observed in Ac-HEMA raises concerns about its long-term durability and ability to protect against secondary caries (Figure 3, Table 3) [20].

The superior performance of composite resins in both compressive strength and microleakage resistance reinforces their status as the preferred material for high-stress areas. While GIC showed lower compressive strength compared to composite resins, its minimal microleakage makes it a viable option for moderate-stress restorations [21] (Figure 6, 7).

Despite its limitations, Ac-HEMA exhibits some promising attributes. The acetylation process appears to enhance its chemical bonding potential when compared to non-acetylated HEMA. Ac-HEMA's ability to bond chemically with dental substrates may help reduce polymerization shrinkage, improving marginal integrity.

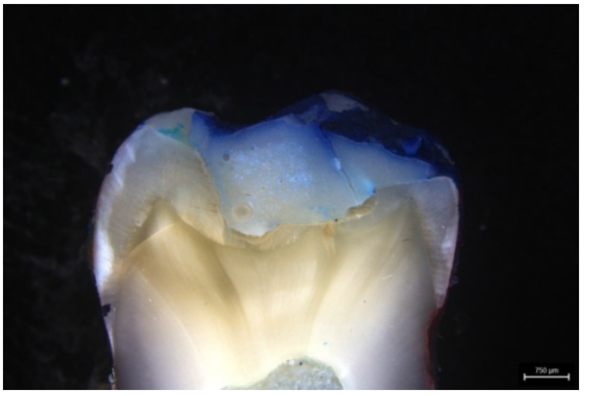

Figure 6: Microleakage of GIC

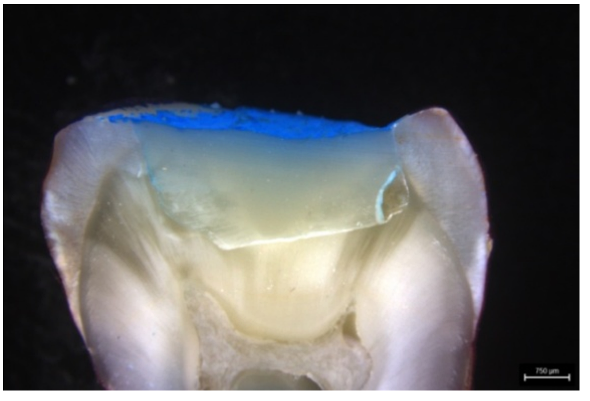

Figure 7: Microleakage of composite

Table 4: Fracture resistance of GIC and composite

	Maximum force (N)	Compressive stress at maximum force (Mpa)	Compressive displacement at break (standard) (mm)	Compressive strain (displacement) at break (standard) (%)	Compressive stress at break (standard) (Mpa)
GIC	1130.59	11.64	3.91	3.91	0.23
Composite	2198.49	21.85	5.19	5.19	0.01

However, its hydrophilic nature may contribute to increased microleakage by promoting moisture absorption at the restorative margins. While this hydrophilic property can enhance adhesion in controlled conditions, it may compromise the material's sealing ability in moist clinical environments [22].

To address this limitation, future formulations of Ac-HEMA should focus on optimizing its hydrophilic balance. Modifications aimed at improving its resistance to moisture absorption may reduce microleakage while preserving its adhesive strength. Furthermore, enhancing Ac-HEMA's mechanical properties, particularly its compressive strength and wear resistance, could improve its suitability for high-stress regions such as molars and premolars [23].

CONCLUSION

This study evaluated Acetylated Hydroxyethyl Methacrylate (Ac-HEMA) as a novel restorative material in dentistry, comparing its performance to established materials such as composite resins and Glass Ionomer Cement (GIC). While Ac-HEMA exhibited moderate compressive strength and promising adhesive properties, it demonstrated increased microleakage, which raises concerns about its long-term durability and sealing ability. The material's hydrophilic nature, while beneficial for adhesion, appears to contribute to this microleakage, indicating a need for improved formulation to optimize its moisture resistance and bonding stability. Despite these limitations, the acetylation process in Ac-HEMA offers potential advantages in enhancing chemical bonding with dental substrates. To improve its clinical efficacy, future research should focus on refining Ac-HEMA's composition to enhance its mechanical strength, reduce microleakage and achieve better marginal integrity. Developing specialized bonding agents or surface treatments tailored to Ac-HEMA may further improve its performance in restorative applications. Additionally, comprehensive long-term clinical trials are essential to assess the material's performance in real-world conditions, particularly in areas subjected to high occlusal forces. With continued refinement and improved formulation, Ac-HEMA holds promise as an innovative restorative material that may contribute to advancing minimally invasive approaches in dental treatments.

Acknowledgment

The authors express their sincere gratitude to the Department of Public Health Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, for providing the necessary facilities and support to conduct this study. Special thanks are extended to the faculty and technical staff for their valuable guidance and assistance throughout the research process. The authors also acknowledge the Oral Biology Department for providing the extracted teeth samples used in this study.

Limitations

The study identified several limitations. Ac-HEMA's increased microleakage compared to GIC and composite resins indicates a significant challenge in achieving optimal marginal sealing. This limitation may compromise the material's longevity and increase the risk of secondary caries. Additionally, the study's sample size was limited to ten extracted teeth, which may not fully represent the variability seen in clinical conditions. Expanding the sample size and ensuring broader patient representation would improve the reliability of future findings.

Another limitation involves the study's focus on short-term performance parameters. Long-term factors such as wear resistance, thermal cycling and aging effects were not evaluated. These factors are crucial for determining the material's ability to withstand dynamic oral conditions over time.

The study also relied on an *in vitro* design, which, although controlled, may not fully replicate the complexities of the oral environment. Real-world factors such as salivary flow, pH fluctuations and variable occlusal forces may influence Ac-HEMA's performance in clinical settings.

Future Recommendations

To improve Ac-HEMA's clinical viability, future research should focus on enhancing its bonding strength to reduce microleakage. This may involve developing advanced bonding agents or surface treatments specifically designed to complement Ac-HEMA's unique properties.

Further studies should aim to refine Ac-HEMA's chemical composition to enhance its compressive strength and structural durability. Modifying the material's formulation to achieve a better balance between hydrophilic and hydrophobic properties may improve its sealing capacity and overall clinical performance.

Conducting long-term clinical trials is essential to evaluate Ac-HEMA's durability, wear resistance and capacity to withstand temperature fluctuations in real-world dental practice. These studies should include varied clinical conditions, such as differing occlusal forces, saliva exposure and diverse patient demographics.

Incorporating comprehensive statistical analyses, including confidence intervals, effect sizes and significance testing, will strengthen the reliability of future findings. Expanding visual data presentation with clear bar charts and comparative tables would improve the clarity and impact of result interpretation.

By addressing these aspects, future developments in Ac-HEMA's formulation and clinical research can improve its potential as an effective restorative material. With enhanced bonding properties, reduced microleakage and improved strength, Ac-HEMA may contribute significantly to minimally invasive dental treatments.

Conflict of Interest

The authors declare no conflict of interest related to this study. All results presented are unbiased and have been interpreted with scientific integrity. No financial support, grants, or external funding was received that could have influenced the study's outcomes.

Ethical Consideration

This study was conducted in accordance with ethical guidelines and principles outlined in the Declaration of Helsinki. Ethical approval for the use of extracted teeth was obtained from the Institutional Ethical Review Board of Saveetha Dental College and Hospitals. All extracted teeth samples used in the study were collected with proper consent from patients and the samples were de-identified to ensure confidentiality and privacy. The study followed strict protocols to maintain ethical standards throughout the research process.

REFERENCES

1. Baygin, Ozgul *et al.*, "The effect of different enamel surface treatments on the microleakage of fissure sealants." *Lasers in Medical Science,* vol. 27, no. 2, November 2013, pp. 153-160. https://pubmed.ncbi.nlm.nih.gov/24308402/.
2. Beun, Sébastien *et al.*, "Physical, mechanical and rheological characterization of resin-based pit and fissure sealants compared to flowable resin composites." *Dental Materials,* vol. 28, no. 4, April 2012, pp. 349-359. https://pubmed.ncbi.nlm.nih.gov/22119547/.
3. Borsatto,, Maria Cristina *et al.*, "Microleakage of a Resin Sealant after Acid-Etching, Er:YAG Laser Irradiation and Air-Abrasion of Pits and Fissures." *Journal of Clinical Laser Medicine & Surgery,* vol. 19, no. 2, July 2004, pp. 83-87. https://www.liebertpub.com/doi/abs/10.1089/104454701750285403?journalCode=pho.1.
4. Brinker, Shannon Pace, "Preventing carious lesions. Clinical steps for applying a newly introduced hydrophilic sealant." *Dentistry Today,* vol. 32, no. 10, October 2013, pp. 82-83. https://pubmed.ncbi.nlm.nih.gov/24245004/.

5. Ciucchi, Philip *et al.*, "Evaluation of different types of enamel conditioning before application of a fissure sealant." *Lasers in Medical Science,* vol. 30, no. 1, January 2015, pp. 1-9. https://pubmed.ncbi.nlm.nih.gov/23636296/.
6. Eliades, Anna *et al.*, "Self-adhesive restoratives as pit and fissure sealants: a comparative laboratory study." *Dental Materials,* vol. 29, no. 7, July 2013, pp. 752-762. https://pubmed.ncbi.nlm.nih.gov/23669197/.
7. Ferracane, Jack L., "Correlation between hardness and degree of conversion during the setting reaction of unfilled dental restorative resins." *Dental Materials,* vol. 1, no. 1, February 1985, pp. 11-14. https://pubmed.ncbi.nlm.nih.gov/3160625/.
8. Gooch, Barbara F. *et al.*, "Preventing Dental Caries Through School-Based Sealant Programs: Updated Recommendations and Reviews of Evidence." *The Journal of the American Dental Association,* vol. 140, no. 11, November 2009, pp. 1356-1365. https://pubmed.ncbi.nlm.nih.gov/19884392/.
9. Hossain, Mozammal *et al.*, "Removal of organic debris with Er:YAG laser irradiation and microleakage of fissures sealants in vitro." *Lasers in Medical Science,* vol. 27, no. 5, September 2012, pp. 895-902. https://pubmed.ncbi.nlm.nih.gov/ 21968762/.
10. Cueto, Eriberto I. and Michael G. Buonocore, "Sealing of pits and fissures with an adhesive resin: its use in caries prevention." *The Journal of the American Dental Association,* vol. 75, no. 1, July 1967, pp. 121-128. https://pubmed.ncbi.nlm.nih.gov/5338243/.
11. Ahovuo-Saloranta, Anneli *et al.*, "Pit and fissure sealants for preventing dental decay in the permanent teeth of children and adolescents." *Cochrane Database of Systematic reviews,* vol. 3, July 2004. https://pubmed.ncbi.nlm.nih.gov/15266455/.
12. Beauchamp, Jean *et al.*, "Evidence-based clinical recommendations for the use of pit-and-fissure sealants: a report of the American Dental Association Council on Scientific Affairs." *The Journal of the American Dental Association,* vol. 139, no. 3, March 2008, pp. 257-268. https://pubmed.ncbi.nlm. nih.gov/18310730/.
13. Burrow, J.F. *et al.*, "Pits and fissures: Relative space contribution in fissures from sealants, prophylaxis pastes and organic remnants." *Australian Dental Journal,* vol. 48, no. 3, March 2008, pp. 175-179. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1834-78 19.2003.tb00028.x.
14. Raadal, M. *et al.*, The Caries Lesion and its Management in Children and Adolescents. In: Pediatric Dentistry – A Clinical Approach, 1st edn., Koch, G. and S. Poulsen, (eds), Copenhagen: Munksgaard, 2001, pp. 173-212.
15. Burrow, Michael F. *et al.*, "Pits and fissures: etch resistance in prismless enamel walls." *Australian Dental Journal,* vol. 46, no. 4, December 2001, pp. 258-262. https://pubmed.ncbi.nlm.nih.gov/11838872/.
16. Keller, Ulrich and Raimund Hibst, "Effects of Er:YAG laser in caries treatment: A clinical pilot study." *Lasers in Surgery and Medicine,* vol. 20, no. 1, 1997, pp. 32-38. https://pubmed.ncbi.nlm.nih.gov/9041505/.
17. Matson, Juliana R. *et al.*, "Er:YAG laser effects on enamel occlusal fissures: an in vitro study." *Journal of Clinical Laser Medicine & Surgery,* vol. 20, no. 1, February 2002, pp. 27-35. https://pubmed.ncbi.nlm.nih.gov/11902351/.
18. Lupi-Pégurier, Laurence *et al.*, "Microleakage of resin-based sealants after Er:YAG laser conditioning." *Lasers in Medical Science,* vol. 22, no. 3, September 2007, pp. 183-188. https://pubmed.ncbi.nlm.nih.gov/17256104/.
19. Feigal, Robert J., "The use of pit and fissure sealants." *Pediatric Dental,* vol. 24, no. 5, September 2007, pp. 415-422. https://www.aapd.org/globalassets/media/publications/archives/feigal 5-02.pdf.
20. Govindaraju, Lavanya *et al.*, "Clinical and radiographic success rate of the root canal filling materials used in primary teeth: A systematic review." *Dental and Medical Problems,* vol. 61, no. 3, June 2024, pp. 447-455. https://pubmed.ncbi.nlm.nih.gov/38963396/.
21. Pratheebha, C. *et al.*, "Knowledge, awareness, and perception on root canal treatment among South Indian population - A survey." *Journal of Advanced Pharmaceutical Technology amp Research,* vol. 13, no. Suppl 1, November 2022, pp. S302-S307. https://pubmed.ncbi.nlm.nih.gov/36643105/.
22. Govindaraju, Lavanya and Ganesh Jeevanandan, "Evaluation of the antimicrobial efficacy of different concentrations of a novel root canal filling material for primary teeth - An *in vitro* study." *Dental Research Journal,* vol. 20, no. 1, February 2023. https://pubmed.ncbi.nlm.nih.gov/36960024/.
23. Mukundan, Divya *et al.*, "Antimicrobial Efficacy of Different Concentrations of Sodium Hypochlorite in the Elimination of *Enterococcus Faecalis*: An In-vitro Study." *Journal of Clinical & Diagnostic Research,* vol. 18, no. 1, January 2024. https://www.jcdr.net/article_abstract.asp?issn=0973-709x&year=2024&volume=18&issue=1&page=ZC11&issn=0973-709x&id=18884.
24. Chandran, Neena and Sindhu Ramesh, "Antibacterial activity and smear layer removal efficiency of silver nanoparticles as a final irrigant against Enterococcus faecalis using confocal laser scanning microscopy and scanning electron microscopy." *Saudi Endodontic Journal,* vol. 15, no. 1, April 2025, pp. 9-16. https://journals.lww.com/senj/fulltext/2025/15010/antibacterial_activity_and_smear_layer_removal.2.aspx.