

Darianna Masih

dariannaprimelles@gmail.com

Education

- University of Maryland School of Dentistry, AEGD Program, 2022-Current.
- University of Maryland School of Dentistry, Master of Biomedical Science, Spring 2024.
- Higher Institute of Medical Sciences of Camaguey Carlos J Finlay, 2005-2009.
- 3.91 GPA

Work Experience/Dental Assistant

- Garbis Dental Associates: July 2019 - Present.
 - Delivering restorative material with dentist at chairside
 - Applying fluoride varnish
 - Instructing in oral hygiene
 - Placement and removal of materials for the isolation of the dentition, provided that the material is not retained by the dentition.
 - Take and record vital signs
 - Rinsing and aspiration of the oral cavity
 - Retraction of the lips, cheek, tongue and flaps
 - Place and remove cotton rolls
 - Applying topical anesthesia
 - Performing intraoral photography
 - Cleaning and disinfecting environmental surfaces and equipment
 - Sterilizing instruments
 - Chart existing restorations or conditions
 - Perform mouth mirror inspection of the oral cavity
 - Receive and prepare patients for treatment, including seating, positioning chair and placing napkin
 - Complete laboratory authorization forms
 - Dry canals
 - Remove sutures
 - Select and manipulate gypsums and waxes
 - Expose radiographs
 - Provide pre and postoperative instructions
 - Place and remove dental dam and clasps
 - Take preliminary and final impressions
 - Fabricate, place, and remove temporary crowns

Monitor nitrous oxide/oxygen analgesia
Maintain emergency kit
Remove permanent cement from supragingival surfaces
Place post extraction dressings
Fabricate custom trays, to include impression and bleaching trays, and athletic mouthguards.
Recognize and respond to basic dental emergencies.

- Montpellier Family Dentistry: April 2017 – July 2019.
Delivering restorative material with dentist at chairside
Applying fluoride varnish
Instructing in oral hygiene
Placement and removal of materials for the isolation of the dentition, provided that the material is not retained by the dentition.
Take and record vital signs
Rinsing and aspiration of the oral cavity
Retraction of the lips, cheek, tongue and flaps
Place and remove cotton rolls
Applying topical anesthesia
Performing intraoral photography
Cleaning and disinfecting environmental surfaces and equipment
Sterilizing instruments
Chart existing restorations or conditions
Perform mouth mirror inspection of the oral cavity
Receive and prepare patients for treatment, including seating, positioning chair and placing napkin
Complete laboratory authorization forms
Dry canals
Remove sutures
Select and manipulate gypsums and waxes
Expose radiographs
Provide pre and postoperative instructions
Place and remove dental dam and clasps
Take preliminary and final impressions
Fabricate, place, and remove temporary crowns
Monitor nitrous oxide/oxygen analgesia
Maintain emergency kit
Remove permanent cement from supragingival surfaces
Place post extraction dressings
Fabricate custom trays, to include impression and bleaching trays, and athletic mouthguards.
Recognize and respond to basic dental emergencies.
Perform clerical work

- Family Dental Group: April 2016 – April 2017.

Delivering restorative material with dentist at chairside
Applying fluoride varnish
Instructing in oral hygiene
Placement and removal of materials for the isolation of the dentition, provided that the material is not retained by the dentition.
Take and record vital signs
Rinsing and aspiration of the oral cavity
Retraction of the lips, cheek, tongue and flaps
Place and remove cotton rolls
Applying topical anesthesia
Performing intraoral photography
Cleaning and disinfecting environmental surfaces and equipment
Sterilizing instruments
Chart existing restorations or conditions
Perform mouth mirror inspection of the oral cavity
Receive and prepare patients for treatment, including seating, positioning chair and placing napkin
Complete laboratory authorization forms
Dry canals
Remove sutures
Select and manipulate gypsums and waxes
Expose radiographs
Provide pre and postoperative instructions
Place and remove dental dam and clasps
Take preliminary and final impressions
Fabricate, place, and remove temporary crowns
Monitor nitrous oxide/oxygen analgesia
Maintain emergency kit
Remove permanent cement from supragingival surfaces
Place post extraction dressings
Fabricate custom trays, to include impression and bleaching trays, and athletic mouthguards.
Recognize and respond to basic dental emergencies.
Perform clerical work

- Precision Endodontics: March – April 2016
Take and record vital signs
Rinsing and aspiration of the oral cavity
Retraction of the lips, cheek, tongue and flaps

Place and remove cotton rolls
Applying topical anesthesia
Performing intraoral photography
Cleaning and disinfecting environmental surfaces and equipment
Sterilizing instruments
Perform mouth mirror inspection of the oral cavity
Receive and prepare patients for treatment, including seating, positioning chair and placing napkin
Dry canals
Remove sutures
Expose radiographs
Provide pre and postoperative instructions
Place and remove dental dam and clasps
Recognize and respond to basic dental emergencies
Perform clerical work

Work Experience/Non – Dental Related

- Thakkar, Patel and Avalos Mds Lc: (October 2013-February 2016)
Posting Pathology Billing and Reports
Answering the phone when needed
Posting senior-home billing
Processing pathology lab slides
Requesting second opinions from Moffitt Cancer Center

Work Experience/Dentist

- Barrio Adentro Mission (October 2010-December 2012): General Dentist.
Prescribe medication as needed
Educate patients on proper brushing techniques, flossing and fluoride use
Promote good oral health and preventive dental care
Exam patients' medical records
Prepare patients for treatment
Check teeth, gums and other parts of the mouth, along with x-rays and tests, to diagnose dental problems
Dental prophylaxis
Identify tooth decay and replace cavities with fillings
Conduct tooth extractions
Recognize and respond to basic dental emergencies
Perform clerical work

Remove sutures
Expose radiographs
Provide pre and postoperative instructions
Performing intraoral photography
Cleaning and disinfecting environmental surfaces and equipment
Sterilizing instruments

- Minas City Dental Clinic (September 2009-October 2010): Director of General Dentistry Department and Clinical Assistant Professor of General Dentistry Department.
Provide hands on patient care as schedule every week
Develop, maintain and implement formal clinical policies and procedures; guide dentists and professional staff to deliver consistent standards of care according policies and assuring clinical practices are above reproach and of the highest quality.
Orient and train new professional staff
Evaluate the professional staff to insure performance goals related to production, clinical quality and regulatory compliance are met
Participate in board and management meetings and annual planning processes and implementation of the strategic plan
Ensure smooth operation of clinic and programs including staffing and patient ratios, scheduling.
Represent dentists at community meetings and events, as requested.
Spend about 75% of my time seeing patients
Participate in course administration and instruction for the dental school curriculum.
Exam patients' medical records
Prepare patients for treatment
Check teeth, gums and other parts of the mouth, along with x-rays and tests, to diagnose dental problems
Dental prophylaxis
Identify tooth decay and replace cavities with fillings
Design and fit dental prosthetics, such as bridges, crowns and dentures
Apply teeth-whitening treatments
Conduct tooth extractions and root canal procedures
Perform surgeries on diseased tissues and bones
Prescribe medication as needed
Educate patients on proper brushing techniques, flossing and fluoride use
Promote good oral health and preventive dental care.

- Love Heal Free Clinic: October 14th 2023.
- Adventist Medical Evangelism Network (AMEN): November 5th 2017, October 6th 2019.
- Dr. Steven Guelff DMD-Orthodontics: 2015-2016.
- Heartland Oral and Maxillofacial Surgery: 2016.

Research/Presentation/General Dentistry Experience

- Higher Institute of Medical Sciences of Camaguey Carlos J Finlay General Dentistry Department (2007-2008); Mentor: Dr. Jackeline Leganoa. Purpose of the study was how to address acute periapical infections to reduce root canal treatment failure.
- Higher Institute of Medical Sciences of Camaguey Carlos J Finlay Student Research-Presentation Day – Acute Periapical Infection (2008).

Teaching/General Dentistry Experience

- Minas Hospital General Dentistry Department (2009-2010); Clinical Assistant Professor.

Leadership/Achievement/Award

- Minas City (2009-2010)
 - Director of General Dentistry Department
- Higher Institute of Medical Sciences of Camaguey Carlos J Finlay (2005-2009)
 - Dean's list
 - Placed within the top ten percent of my class
 - 3.91 GPA
 - Summa Cum Laude
 - Captain of kickball team

Certification

- Maryland RDA
- CPR
- OSHA
- Invisalign
- MGE Management Experts

Dental Software

- Itero Scanner
- Softdent
- Eaglesoft

- PBS Endo
- DTX Studio Implant
- DSD-Smile Design
- Cerec CAD-CAM
- Meshmixer

Language

- English
- Spanish

ABSTRACT

Title: Enhancing Cervical Composite Restorations Management.

Darianna M. , Master of Biomedical Science, 2024.

Thesis Directed by: Mary Anne S. Melo, DDS, M.Sc, PhD, FADM, FACD, Clinical Professor and Chair Department of Comprehensive Dentistry.

The management of cervical composite restorations, also known as Class V restorations, represents a challenge in contemporary dentistry. This difficulty arises from the complex nature of cervical lesions and unfavorable location, close to soft tissues prone biofilm accumulation. Additionally, cervical composite restorations have significant failure rates, further complicating their management and longevity. The etiological factors contributing to cervical cavitation are diverse, ranging from mechanical trauma, abrasion, and erosion leading to non-carious cervical lesions (NCCLs), to cariogenic biofilms responsible for root caries. The combination of these factors, high failure rates, varied etiology, and the technical difficulty of restoring dentin-margin cavities underscores the need for advanced understanding of proper management and techniques in treating cervical composite restorations. This master thesis is presented in chapters with the following aims: 1) perform a scoping review as a preliminary assessment to identify how restorative protocols (surface pre-treatment, such as etching with different agents and techniques, like bevel) influence the longevity outcome of non-carious cervical lesions (NCCLs) restorations, and, 2) to comprehensively analyze and compare the biofilm composition grown over healthy and cavitated root caries lesions in two distinct age groups: patients younger than 65 years old (healthy and cavitated) and those older than 65 years old (healthy and cavitated). As the methodology approached, the scoping review and clinical studies were performed according to the cited

objectives. For the results, a collective of the literature showed that the most frequently dentin pre-treatment method studied was 'Application mode - Etching', with over 35% of studies focusing on this area, followed by 'Application mode - Adhesive' in 34% of the studies. 'Pre-treatment of surface with substance' was the focus of around 14% 'Pre-treatment of surface with lasers' accounted for approximately 11% of studies (Chapter 1.) In our clinical study the analysis of predominant bacteria in control and cavitated root caries lesions across two age groups (<65 years old and >65 years old) reveals notable variations in microbial composition. Cavitated lesions exhibit higher bacterial diversity compared to control ones, as indicated by the Shannon diversity index, suggesting a more complex microbial ecosystem within these lesions. Furthermore, the Simpson's dominance index suggests that control lesions are characterized by a few dominating species, contrasting with the more evenly distributed bacterial community in cavitated lesions. Age-related differences further modify these patterns, with older individuals displaying distinct bacterial profiles. These findings underscore the impact of lesion type and patient age on the oral microbiota's diversity and dominance, highlighting the complexity of microbial ecosystems in root caries lesions. (chapter 2).

Enhancing Cervical Composite Restorations Management

by
Darianna Masih

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Master of Science
2024.

©Copyright 2024 by Darianna Masih
All rights reserved.

Acknowledgment

Foremost, I would like to thank the good Lord, almighty creator of the universe. I am humbled by your grace and mercy in my life. Your love and guidance have sustained me through every challenge and have brought me to where I am today. Also, I would like to express my sincere gratitude to my advisor, Dr. Mary Anne Melo and Dr. Qoot Alkhubaizai, for the continuous support of my master's research. As well as their patience, motivation and mentorship.

Furthermore, I extend my gratitude to PhD. Lamia Mokeen for her help during this project.

Besides my advisors, I would like to thank the rest of my thesis committee: Dr. Dr. Guilherme Arossi and Dr. Isadora Garcia for their encouragement and insightful comments.

My sincere thanks also go to the Institutional Review Board (IRB) and the Department of General Dentistry for funding this study

I thank my lab team, Mr. Mike Humphrys, Lisa Bilski, and my fellow classmates, specially Dr. Lindsay Diaz for their help processing and collecting my research samples.

Most of all, I am grateful to my life and husband, Rohit Masih, for his love, prayers, caring and sacrifices.

TABLE OF CONTENTS

Acknowledgments	iii
List of tables	vi
List of figures	vii
List of abbreviations	viii
Introduction	1
Specific aims	4
Chapters:	
Chapter 1	
1 Introduction.....	8
2 Materials and methods.....	11
2.1 Study Design.....	11
2.2 Stage I: The Research Question.....	11
2.3 Stage II: Searching for Relevant Studies.....	11
2.4 Stage III: Study Selection.....	14
2.5 Stage IV: Charting the Data.....	17
2.6 Stage V: Data Extraction	17
3 Results and Discussion.....	18
3.1 Study Selection.....	18
3.2 Types of Study Design.....	20
3.3 Outcome Measures.....	25
4. Limitations.....	28
5. Conclusion.....	29
Acknowledgments.....	39

Chapter 2

Introduction.....	34
Materials and Methods.....	36
2.1 Clinical assessment.....	37
2.2 Sample collection.....	38
2.3 Data extraction and purification.....	40
2.4 Results.....	41
2.5 Discussion.....	44
2.6 Conclusion.....	48
Overall Discussion.....	50
Conclusion and Remarks.....	52
References.....	53

LIST OF TABLES

Chapter 1:

PAGE

Table 1. Summary the keywords and subject heading method utilized in the search strategy 11

Table 2. Summary of the bonding protocols utilized in the studies 24

Chapter 2:

Table 3. Characteristics of subjects per each experimental group 37

LIST OF FIGURES

<u>Introduction</u>	PAGE
Figure 1. Clinical image of several class V restorations after six months of service inside the mouth.....	1
Figure 2. Illustrates root caries lesions restored after 2 years in the mouth.....	2
<u>Chapter 1:</u>	
Figure 3. PRISMA flow diagram of studies in this scoping review.....	16
Figure 4. Risk of bias graph	18
Figure 5. Frequency distribution graph	19
Figure 6. Global distribution of publications per country.....	20
Figure 7. Type of study design distribution graph	20
Figure 8. Characteristics of selected studies	21
Figure 9. Frequency of dentin pre-treatment studies	22
<u>Chapter 2:</u>	
Figure 10. Flow chart of patient visit procedures to sequencing	39
Figure 11. Illustrate a comparison of bacterial alpha diversity across the four groups.....	42
Figure 12. Illustrate the Shannon's index alpha diversity by subjects group.....	43
Figure 13. Simpson's dominance index measuring the dominance of particular species within the community.....	44

List of Abbreviations:

NCCLs	Non-cariou cervical lesions
HL	Hybrid layer
SE	Self-etch
E&R	Etch-and-rinse
MMPs	Metalloproteinases
CPs	Cysteine cathepsins
CHX	Chlorhexidine
EDTA	Ethylenediaminetetraacetic acid
RC	Root Caries

1. Introduction

The management of cervical composite restorations, also known as Class V restorations, is a formidable challenge in contemporary dentistry (Lee, Burrow, and Botelho 2023). This complexity stems from the intricate nature of cervical lesions and their unfavorable placement adjacent to soft tissues that are susceptible to plaque accumulation, elevating the risk of further dental complications, such as periodontal disease, esthetic, de-bonding and recurrent decay. As a result, cervical composite restorations exhibit considerable failure rates (20% to 30%), adding another layer of difficulty to their management and raising concerns over their clinical longevity (X. Chen et al. 2020; L. Chen et al. 2015).



Figure 1. Clinical image of several class V restorations after six months of service inside the mouth.

The etiology of cervical cavitation encompasses a wide array of etiological factors, including mechanical trauma, abrasion, and erosion, which collectively contribute to the formation of

non-carious cervical lesions (NCCLs) (Tomasik 2006; Faye et al. 2005; Warreth et al. 2020; Osborne-Smith, Burke, and Wilson 1999). Root caries and non-carious cervical lesions (NCCLs) represent significant concerns in dental health, particularly in the United States, where the prevalence (25% to 50%) of these conditions has been a focus of ongoing research and public health initiatives.



Figure 2. clinical images of root caries lesions restored after 2 years of service inside the mouth. Note the presence of marginal infiltration around the class V composite restorations (Yang et al. 2016; Smith, Marchan, and Rafeek 2008; Teixeira et al. 2020).

On the other hand, NCCLs involve the loss of dental hard tissue at the tooth's cervical area and are not caused by decay. The etiology of NCCLs is multifactorial, with abrasion, erosion, and abfraction being leading factors (Warreth et al. 2020). These lesions are prevalent across various age groups but are particularly observed in middle-aged adults and seniors. The increase in NCCLs has been associated with dietary habits, such as the consumption

of acidic foods and beverages, as well as mechanical factors like toothbrushing technique or localized occlusal trauma (Yoshizaki et al. 2017; Teixeira et al. 2020).

In parallel, cariogenic biofilms play a pivotal role in the onset of root caries, further complicating the clinical scenario. Root caries, a form of tooth decay that occurs on the root surfaces of teeth, has been increasingly prevalent, especially among older adults. This uptick in cases is largely attributed to the aging population, with a more significant number of individuals retaining their natural teeth into later life (Reddy et al. 2021; Qutieshat et al. 2021). Factors such as gingival recession, which exposes root surfaces to the oral environment, and changes in saliva production and composition contribute to the heightened risk (up to 50%) of root caries in the elderly population. Studies have shown that root caries affects 90% of adults over the age of 65, making it a notable concern for geriatric dental care (Fee et al. 2020; Zhang et al. 2019; Reddy et al. 2021).

Given the high failure rates, the varied etiology of cervical lesions, and the technical challenges associated with restoring dentin-margin cavities, there is a pressing need for an advanced comprehension of the appropriate management strategies and techniques applicable to cervical composite restorations. This understanding is crucial for enhancing the longevity of such restorations and ensuring their optimal performance over time. Addressing these challenges requires a deep dive into the existing body of knowledge and the exploration of innovative approaches and methodologies tailored to the unique requirements of cervical composite restorations.

This master thesis undertakings to shed light on these critical aspects by setting forth a structured inquiry into the prevailing practices and outcomes associated with managing cervical composite restorations. The research is organized into two chapters, each designed to address

specific objectives that collectively aim to advance the understanding of how to address these lesions better.

SPECIFIC AIMS

Our hypothesis in this master's thesis project is addressed with the following specific aims:

1) **Perform a scoping review as a preliminary assessment to identify how various restorative surface pre-treatment protocols** such as etching with different agents, and techniques such as application of bevel, per example, influence the longevity outcomes of non-carious cervical lesions (NCCLs) restorations.

2) **Perform a preliminary clinical study to comprehensively analyze and compare the biofilm composition grown over control and cavitated root caries lesions across two distinct age groups:** patients younger than 65 and those older than 65. This analysis is instrumental in understanding the interplay between age, the physiological environment of the oral cavity, and the development of carious and non-carious lesions.

Assessing the Impact of Restorative Protocols on the Longevity of Non-Carious Cervical Lesions (NCCLs) Restorations: A Scoping Review

Darianna Masih¹, Lamia Sami Mokeem², Qoot Alkhubaizi¹, Mary Anne S. Melo^{2,3}

¹ Advanced Education in General Dentistry Program, Department of Comprehensive Dentistry
University of Maryland School of Dentistry, Baltimore, MD 21201, USA;

² Ph.D. Program in Biomedical Sciences, University of Maryland School of Dentistry, Baltimore,
MD, 21201, USA;

³ Operative Dentistry & Cariology Division, Department of Comprehensive Dentistry, University
of Maryland School of Dentistry, Baltimore, MD 21201, USA

Correspondence: Mary Anne Sampaio de Melo, Operative Dentistry & Cariology Division,
Department of Comprehensive Dentistry, University of Maryland School of Dentistry, Baltimore,
MD 21201, USA

Email: Mmelo@umaryland.edu

Key words: Cervical lesions, Non-cervical carious lesions, restorative protocols, dental composites, surface pre-treatment.

Abstract

Despite the high prevalence of NCCLs and their impact on dental health, there remains a significant gap in understanding the optimal restorative protocols to enhance the durability of such interventions.

Objectives: This scoping review aims to systematically assess the existing literature on the influence of surface pre-treatment techniques, including etching with various agents and applying bevel, on the longevity of restorations for non-carious cervical lesions (NCCLs).

Methods: Studies investigating surface pre-treatment techniques and /or protocols were screened. Risk bias was assessed, and the following data were extracted: study characteristics, including Authors, year of publication, country of study, pre-treatment methods, adhesive types, retention rates, and study quality. Arksey and O'Malley's five stages framework using Medline (OVID), EMBASE, and Scopus (Elsevier) databases guided this review.

Results: Findings suggest a large variation in protocols for bonding, with about 60% of studies concentrating on 'Etching and Adhesive Application Mode'. Approximately 15% of studies focus on 'Surface Pre-treatment with Substance', while 'Surface Pre-treatment with Lasers' accounts for roughly 10%. Lastly, 'Combined Bevel with Enamel Margins' is the least studied method, with only about 5% of investigations exploring this technique.

Conclusion: This scoping review maps the current knowledge landscape regarding NCCL restorative protocols and identifies key areas for further investigation. The results indicate that various dentin pre-treatment agents, application techniques, and enamel beveling exhibit comparable outcomes in adhesion processes when considered individually, yet their cumulative impact may be significant. Moreover, the review highlights a diversity in methodological

approaches and outcome measures across studies, indicating a need for standardization in future research.

Key Words: Non-carious cervical lesions, hybrid layer, metalloproteinases, cysteine cathepsins, chlorhexidine, ethylenediaminetetraacetic acid.

Introduction

Non-carious cervical lesions (NCCLs) present a unique challenge in restorative dentistry, given their location at the tooth's cervical margin where the enamel is thin or absent, and dentin is often exposed (Teixeira et al. 2020; Smith, Marchan, and Rafeek 2008). Caries does not cause these lesions but they result from a combination of factors, including abrasion, erosion, and occlusal stress, leading to the loss of hard dental tissue (Tomasik 2006; Osborne-Smith, Burke, and Wilson 1999). The restoration of NCCLs is crucial for preventing further tissue loss, reducing sensitivity, and restoring aesthetics and function. However, the longevity of these restorations is influenced by various factors, including the restorative material used, the application technique, and notably, the protocols for surface pre-treatment before bonding. (de Albuquerque et al. 2022; Digole et al. 2020)

The complexity of restoring NCCLs is compounded by their proximity to the gingival tissue and the variable nature of the exposed surface, which may include both sclerotic dentin and cementum (Balbinot et al. 2020; Tomasik 2006). These factors make adhesion more challenging and can affect the durability of the restoration. Consequently, the choice of the adhesive system, its application method, and the tooth surface's pre-treatment are all critical considerations that can significantly impact the restoration outcome.(de Almeida et al. 2023; de Paris Matos et al. 2020)

Despite the advancements in adhesive systems, hybrid layer degradation and microleakage over time jeopardize the longevity of the restorations. Fluid infiltration along the cavity walls has harmful effects, such as disruption of the marginal integrity, discoloration, and postoperative sensitivity (de Almeida et al. 2023; Digole et al. 2020). Hybrid layer degradation is a combination of hydrolysis and percolating of adhesive from the demineralized dentin matrix, which makes it

vulnerable to the attack of host-proteolytic enzymes, metalloproteinases (MMPs) and cysteine cathepsins (CPs). (Boushell et al. 2016; de Souza et al. 2020)

To prolong the resin-bond durability, dentin pre-treatment with protease inhibitors has been advised, such as, Chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), and Q-mix. (Alencar et al. 2023; Dutra-Correa et al. 2013; Luque-Martinez et al. 2015)

Chlorhexidine inhibits the action of MMP-2 (gelatinase A), MMP-8 (collagenase 2), and MMP-9 (gelatinase B) by different mechanisms. A cation-chelating mechanism impedes MMP-2 and MMP-9 by binding to the active site's zinc or calcium metal ions. On the other hand, MMP-8 is inhibited by interacting with the sulfhydryl and/or cysteine group. In the literature, it has been reported that CHX has inactivated MMPs at concentrations as low as 0.02%. (Gendron et al. 1999; Dutra-Correa et al. 2013). Similarly, EDTA inhibits MMPs by chelating the calcium ions and removing the smear layer. Furthermore, it is a weak acid that produces a gentle and shallowed demineralization of the dentin, which is considered accountable for the strong bonding to sound and sclerotic dentin in self-etch adhesives.(Alencar et al. 2023; Hazra et al. 2012)

Over the years, surface pre-treatment techniques have been developed to improve the bonding of restorative materials to the tooth structure. These techniques include mechanical (surface roughening and sandblast) as well as using various etching agents to alter the tooth surface's chemistry and topography, and applying different adhesive systems designed to optimize bond strength and longevity (Verma et al. 2023; Rocha et al. 2019). Additionally, modifications such as incorporating a bevel at the lesion margin have been proposed to enhance restorations' esthetic integration.(Da Costa, Loguercio, and Reis 2013; Baratieri et al. 2003; Verma et al. 2023)

Despite the multitude of techniques available, there is a notable variability in the clinical outcomes of NCCL restorations, with studies reporting a wide range of survival rates (20% to

80%). This variability underscores the need for a comprehensive understanding of how different restorative protocols affect the longevity of NCCL restorations (de Paris Matos et al. 2020; de Almeida et al. 2023). Furthermore, patient-related outcomes such as postoperative sensitivity, satisfaction with the aesthetic results, and the incidence of secondary caries or periodontal responses also merit consideration, as they contribute to the overall success of the treatment. (de Albuquerque et al. 2022; Patricia Manarte-Monteiro et al. 2021; Jorge Perdigão et al. 2020)

Given the evolving landscape of dental materials and adhesive technologies, alongside the ongoing debate regarding the optimal strategies for NCCL restoration, a systematic exploration of the current evidence is both timely and necessary (Barceleiro et al. 2022; Hass et al. 2022). The field of restorative dentistry continues to seek improvements in clinical protocols to enhance the durability, functionality, and aesthetics of NCCL restorations, making it imperative to assess the impact of various factors on restoration longevity. (Oz, Ergin, and Canatan 2019; Heyder et al. 2022)

Hence, this scoping review seeks to methodically evaluate and amalgamate the available literature concerning the influence of restorative protocols, particularly emphasizing surface pre-treatment methods, on the durability of restorations for non-carious cervical lesions. By examining the evidence on clinical performance, patient satisfaction, and the incidence of complications, this review seeks to identify knowledge gaps and inform future research directions, ultimately contributing to the refinement of best practices in the management of NCCLs.

2. Material and methods

This section provides background information and related works, including the scoping review method and design strategy.

2.1 Study Design

This paper applied the Arksey and O'Malley framework (Arksey and O'Malley 2005) and enhanced by Levac, Colquhoun, and O'Brien's recommendations (Levac, Colquhoun, and O'Brien 2010). The methodological framework comprises determining the research question and relevant studies, selecting the type of study, mapping the data, outlying and reporting the results.

2.2 Stage I: Research Question

The research questions proposed to answer in this scoping review are mainly in the following aspects:

- What is known from the literature about the influence of dentin pre-treatments in the longevity outcome of NCCLs restorations?

2.3 Stage II: Relevant Studies Search

Two independent reviewers (D. M. and L.M) searched literature to identify papers in three databases that included references in one language. English publications in Ovid Medline[®], EMBASE (Elsevier) and SCOPUS (Elsevier) were searched using the keywords and subject heading methods and was logically connected with Boolean operator "AND" and "OR". Publication-year limit was set, from 1946 to January 12, 2024. The search strategy (**Table 1**) was adapted to the other databases.

Table 1 Literature Search Strategy

Final Search Strategy	Date
-----------------------	------

Table 1 continued

<p>1 (Non-carious cervical lesion\$1 or Noncarious cervical lesion\$1 or NCCL\$1 or Class-V).ti,ab,kw.</p> <p>2 exp Tooth Wear/ or ((Tooth or dental) adj (wear\$1 or attrition or erosion or abrasion)).ti,ab. or Abfraction.ti,ab.</p> <p>3 Gingival Recession/ or ((Gingiva or Gingival) adj (recession or atroph\$3)).ti,ab.</p> <p>4 exp Dental Restoration, Permanent/ or Dental Restoration, Temporary/ or (restoration or marginal-adaption).ti,ab.</p> <p>5 Adhesiveness/ or Adhesives/ or Dental Cements/ or Compomers/ or exp Glass Ionomer Cements/ or (Adhesive\$1 or Adhesiveness\$ or Adhesion\$1 or dental-cement\$1 or luting-agent\$1 or compomer\$1 or glass-ionomer\$1).ti,ab.</p> <p>6 Dental bonding/ or Self-Curing of Dental Resins/ or Light-Curing of Dental Adhesives/ or exp Dentin-bonding agents/ or bond\$1.ti,ab. or bonding.ti,ab. or self-curing.ti,ab. or light-curing.ti,ab.</p> <p>7 Dental Materials/ or exp Composite Resins/ or exp Resins, Synthetic/ or Photoinitiators, Dental/ or (resin-modified-glass-ionomer\$1 or restorative-material\$1 or dental-material\$1 or composite\$1 or synthetic-resin\$1 or photoinitiator\$1).ti,ab.</p>	<p>1/15/2024</p>
--	------------------

<p>Table 1 continued</p> <p>8 exp surface properties/ or exp Dental Etching/ or exp Tooth preparation/ or Lasers, Solid-State/ or Lasers/ or Air Abrasion, Dental/ or exp Aluminum Oxide/ or Chlorhexidine/ or (surface-treatment\$1 or surface- propert\$3 or Dentin-conditioning or tooth-preparation\$ or lasers or air- abrasion or aluminum-oxide or Chlorhexidine).ti,ab.</p> <p>9 Dental Restoration Failure/ or exp Treatment Outcome/ or exp Treatment Failure/ or Outcome Assessment, Health Care/ or Patient Outcome Assessment/ or Patient Reported Outcome Measures/ or Patient Satisfaction/ or (Outcome\$1 or longevity or failure\$1 or assessment\$1 or measure\$1 or survival or retention or satisfaction).ti,ab.</p> <p>10 1 and 2</p> <p>11 1 and 3</p> <p>12 1 and 4</p> <p>13 1 and 5</p> <p>14 1 and 6</p> <p>15 1 and 7</p> <p>16 1 and 8</p> <p>17 1 and 9</p> <p>18 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17</p>	
---	--

Table 1 continued	
19 limit 18 to english language	

2.4 Stage III: Study Selection

The overall inclusion criteria for study selection were based on stages I and II methodologies. Studies eligible for inclusion encompass randomized controlled trials (RCTs), cohort studies, case-control studies, and cross-sectional studies. The population of interest includes patients undergoing restorative treatment for non-carious cervical lesions younger and older than 65 years old.

Regarding interventions, the review will include studies that evaluate various restorative protocols for NCCLs. This encompasses investigations into different 1- adhesive modes, 2- application of compounds, and 3-surface pre-treatments. Comparative studies examining the differences in techniques or materials used in NCCL restorations are also sought. The primary outcome of interest is the longevity of restorations, measured through survival rate, failure rate, or the need for re-intervention. Secondary outcomes may include postoperative sensitivity, esthetic outcomes, and patient satisfaction.

Finally, the review will only include studies with a follow-up period of at least one year to assess restoration' longevity objectively. The language criterion stipulates that studies must be published in English or have available English translations to ensure data accessibility for analysis.

We omitted studies categorized as case reports, editorials, opinion articles, and animal studies based on our exclusion criteria. Additionally, in vitro studies were also not included. Regarding the study population, the review excludes studies focusing on carious lesions or other dental conditions unrelated to NCCLs. Regarding intervention focus, studies that do not

specifically assess restorative protocols relevant to NCCLs, such as studies that focus on the type of composite or glass ionomers used as restorative materials.

Studies with incomplete data or lacking sufficient detail to assess the outcomes are excluded to maintain the quality and reliability of the review findings. Lastly, language limitations play a role in study selection. Studies published in languages other than English without available translations are excluded.

A flowchart of the review process was drawn per the PRISMA flow diagram for scoping reviews along with reasons for excluded sources (**Figure 3**).

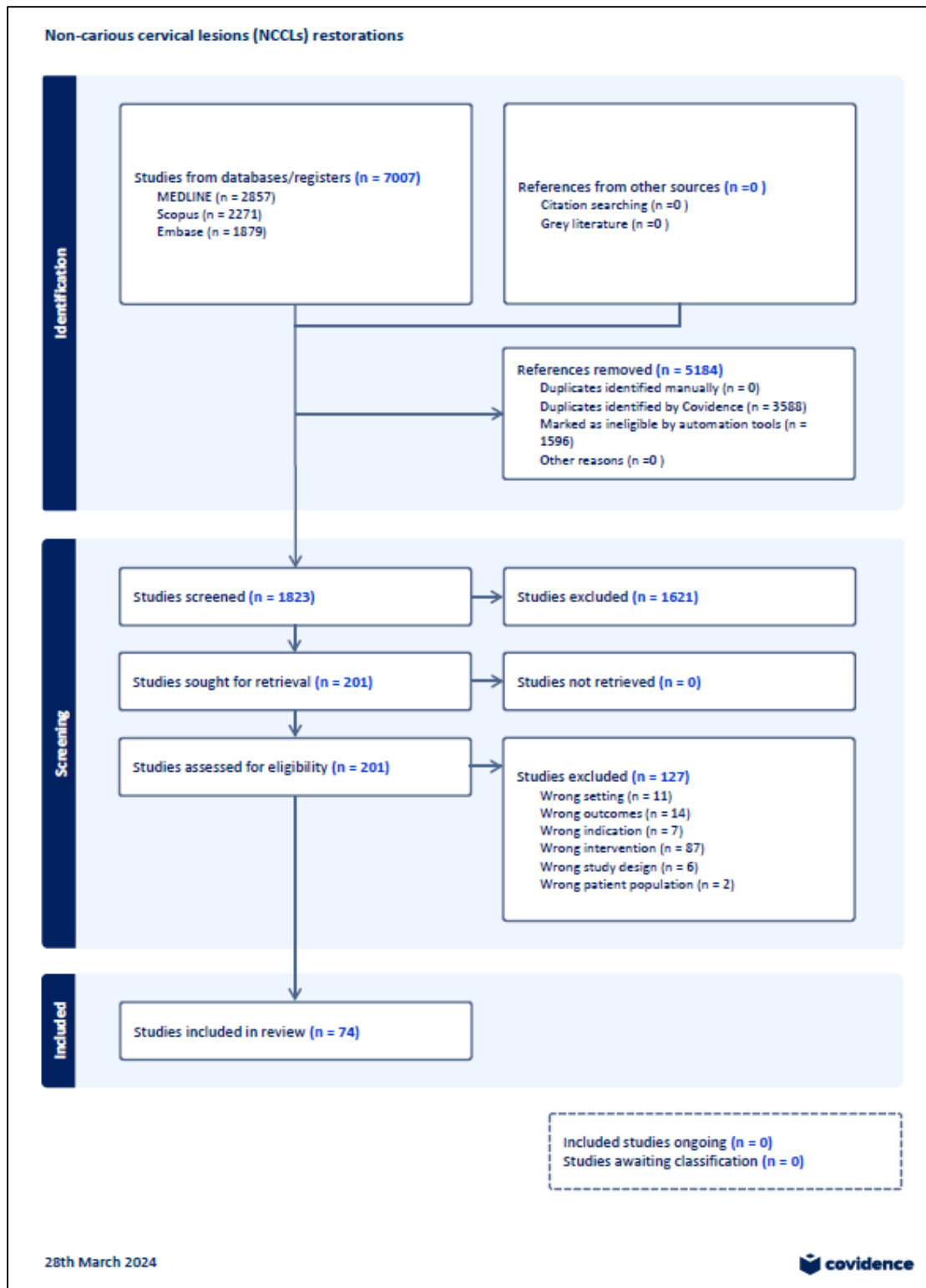


Figure 3. PRISMA flow diagram of studies in the systematic scoping review based on " Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement.

If consensus between the two sets of articles was achieved, the selected articles progressed to assessing the abstract via the same method. The same two reviewers then assessed full-text articles. If there were disagreements between the reviewers, the paper in question was discussed until a consensus was achieved.

2.5 Stage IV: Data Charting

A model for extracting data was created in Microsoft Excel and evaluated by all authors involved to identify and document variables required for accurate data extraction.

2.6 V: Data Extraction

In the selected studies, authors extracted the data (D.M and M.M). The data was organized based on authors, 1) year of publication, 2) country of study, 3) study designs, 3) surface pre-treatment (substance/compound or technique), follow-up years, longevity outcome, and level of evidence. Additionally, the studies included were evaluated for bias risk using the Cochrane collaborative network bias tool into high, low or not clear risk across various criteria:

- **Sampling Bias – Sample Size:** if the study's authors described how they determined the sample size, it indicated low risk. If they did not report justification for the sample size, it posed high risk, or if they did not present clear justification for assessment, it was considered not clear.
- **Sampling Bias – Randomization:** the study authors detailed a random process assigning enamel, dentin, or tooth samples, indicated low risk. If the process of selecting samples was not randomized, represented a high risk, or no clear information was provided to determine a level of risk, it was considered not clear.
- **Reporting Bias:** the outcomes described in the methods sections align with those presented in the results section, indicated low risk. If there were discrepancies or inconsistencies

between the methods and results sections regarding outcomes, indicated high risk. When insufficient information was provided to determine, it was considered unclear.

- Other Bias: if no conflict of interest was disclosed, suggested a low risk. If a conflict of interest was reported, it indicated a high risk. In cases where there was insufficient information provided to determine the presence or absence of a conflict of interest, the risk level was considered unclear.

3. Results and Discussion:

3.1 Study Selection

The initial literature was searched in Ovid Medline[®], EMBASE and SCOPUS from 1946 up to 2024. The total number of citations retrieved was 7,007. After the duplication (3588 studies) and ineligible auto-marked (1596 studies) by Covidence the total number of citations was 1823. After screening the titles and abstracts, 127 that were excluded due to classified as wrong setting, intervention, outcome or indication. Full text was obtained for the remaining 74 publications.

Figure 4 provides an outline of the risk of bias.

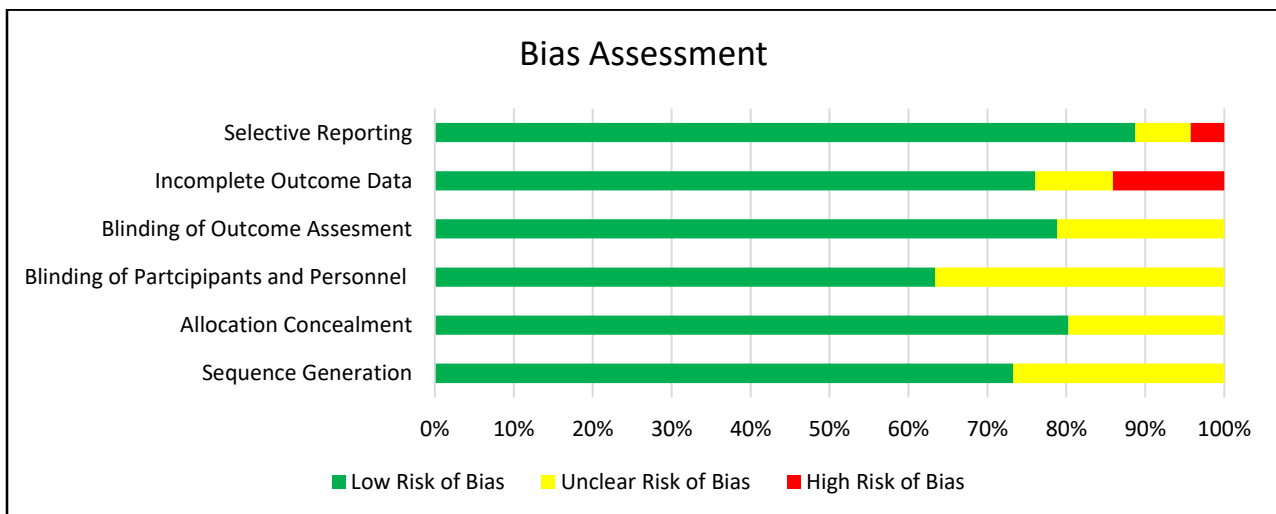


Figure 4 Risk of bias graph: review author’s judgments about each risk of bias item presented as percentage across all included studies (a plot of the distribution of judgments low risk of bias, unclear risk of bias, and high risk of bias) across studies for each risk of bias item.

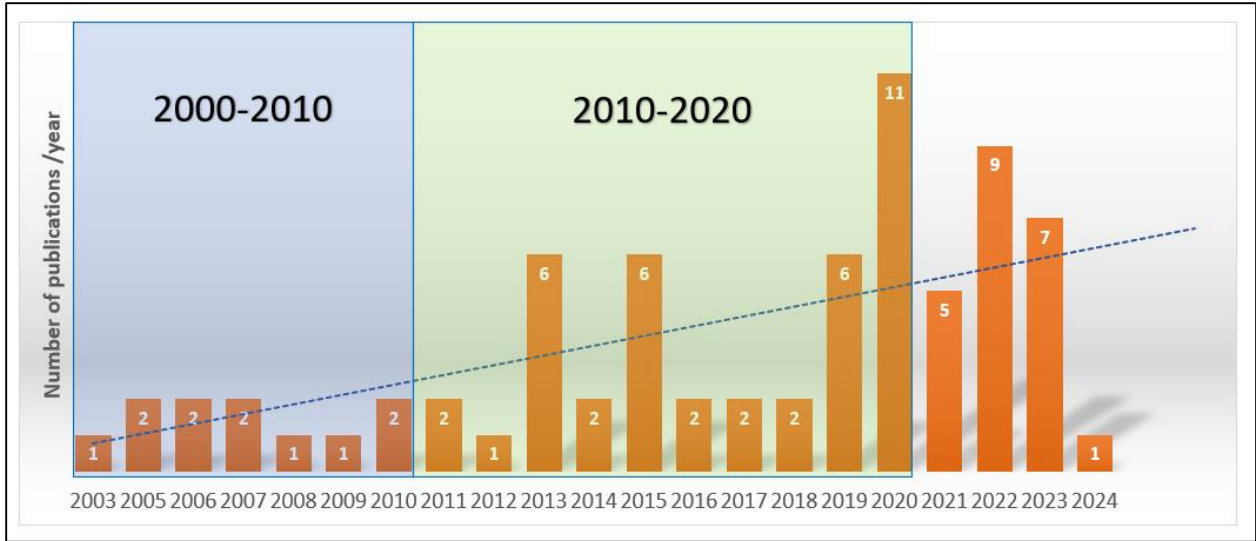


Figure 5. Frequency distribution graph. Publication frequency of papers that met the inclusion criteria plotted against the year of publication. Trendline forecast represents a straight line to a set of data points in a way that best represents the data's overall direction or pattern.

The frequency of publications has been rising, reflecting the increasing attention on this subject due to their broad applicability. Most studies were conducted in Brazil followed by Portugal, Germany and United States, as illustrated in **Figure 6**.

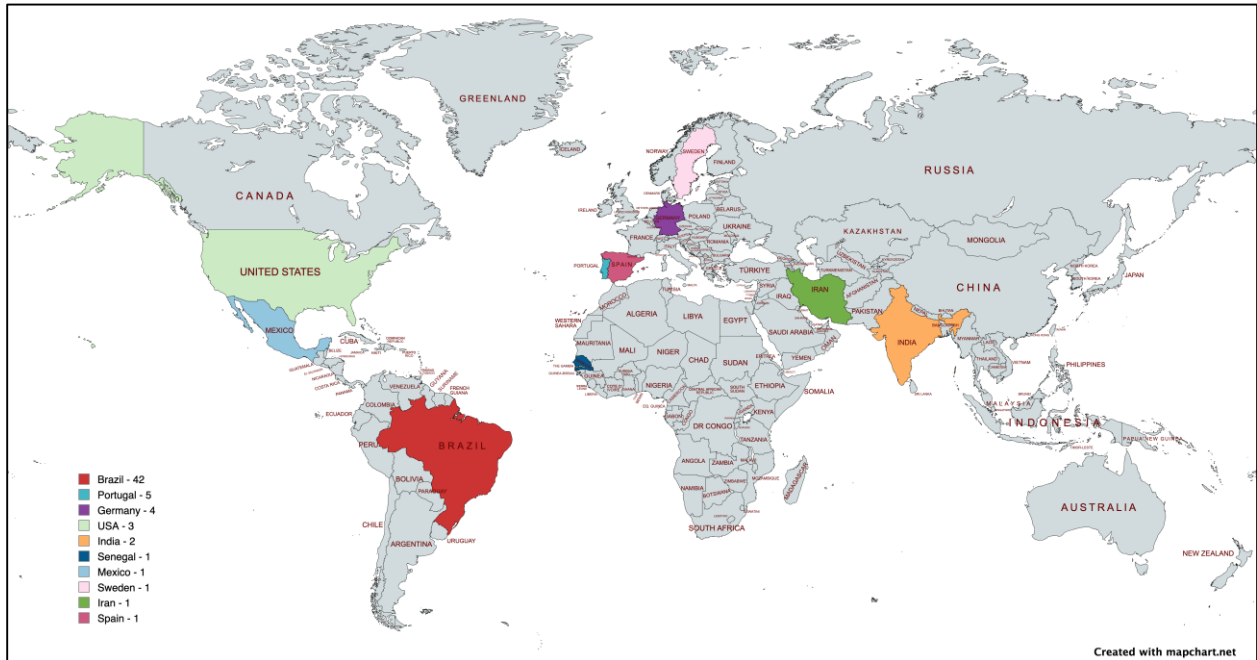


Figure 6. The global distribution of publications per country.

The outcomes were categorized according to retention rate, marginal integrity, color stability, recurrent caries, tooth integrity, contact surface, surface texture, loss of anatomical form, post-operative sensitivity, patient complaints and patient's satisfaction.

3.2 Types of Study Design

Our analysis identified 79% (n = 74) of the studies as randomized clinical trials, highlighting their adherence to rigorous experimental protocols. The remaining 21% did not specify their randomization procedures and were therefore categorized as non-randomized clinical trials. Notably, we did not observe any cross-sectional studies or studies employing a mixed-design that combines randomized and cohort elements. Given the hierarchical structure of study designs, randomized clinical trials are one of the most reliable forms of scientific evidence, underscoring the robustness of our dataset's evidence base.

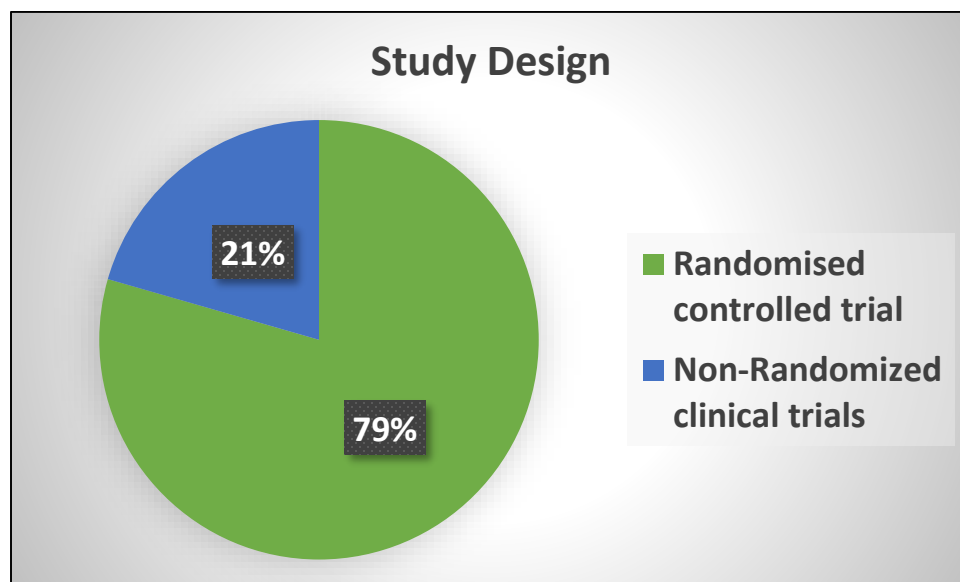


Figure 7. Distribution of studies according to the type of study design.

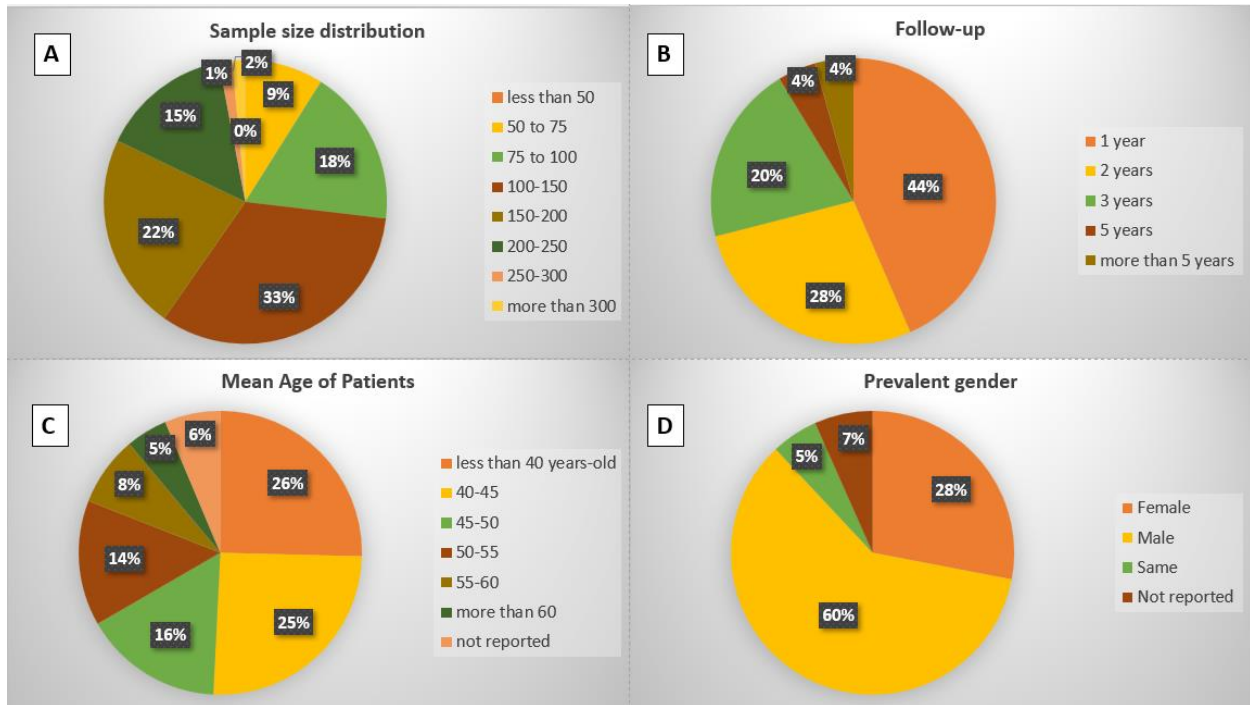


Figure 8. Characteristics of selected studies represented by **A.** Sample size distribution, **B.** Follow-up, **C.** Age of patients, and **D.** prevalent gender.

Regarding sample size distribution (A), most of the studies had a sample size of less than 100, constituting 33% of the total. This was followed by sample sizes ranging from 150 to 200 and 75 to 100, making up 22% and 18% of the studies, respectively. A smaller proportion of studies had sample sizes between 200 to 250 (15%), and 50 to 75 (9%). Very few studies had sample sizes between 250 to 300 (2%), and an even smaller fraction fell within more than 300 (1%).

In terms of follow-up duration (B), the most common was a 1-year follow-up, reported in 44% of the studies. The next most frequent follow-up period was 2 years, observed in 28% of the cases. A 3-year follow-up was noted in 20% of the studies, while follow-ups of 5 years and more than 5 years were the least common, each accounting for 4%.

The mean age of patients (C) in the studies was most commonly reported to be less than 40 years old, representing 26% of the studies, followed by the age group of 40 to 45 years, accounting for 25%. Age groups of 45 to 50 and 50 to 55 years were represented in 16% and 14% of the

studies, respectively. Fewer studies included patients aged 55 to 60 (8%) and more than 60 years old (5%). In 6% of the studies, the mean age of patients was not reported.

Lastly, the prevalent gender (D) among the study participants was predominantly male, making up 60% of the total, while females were prevalent in 28% of the studies. A minority of studies (5%) reported the same prevalence for both genders, and in 7% of the studies, the gender was not reported.

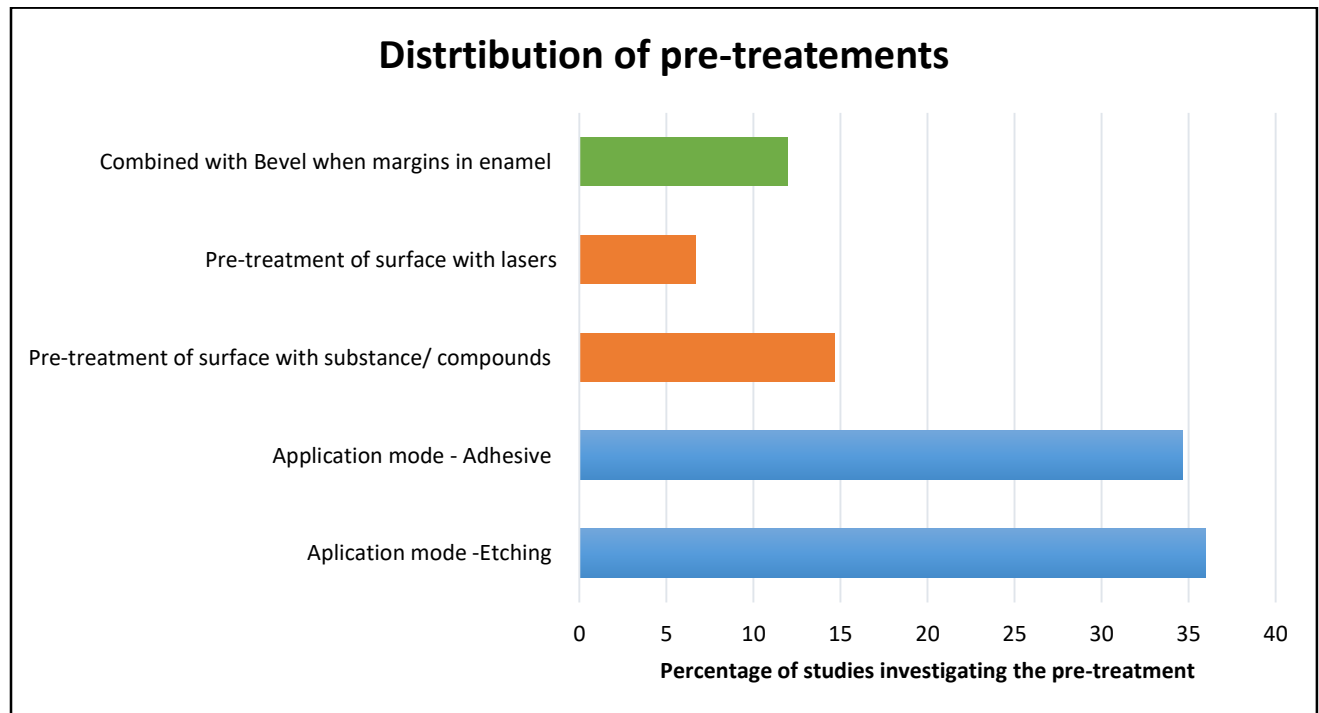


Figure 9 Distribution of pre-treatments by percentage of studies.

The pre-treatment distribution graph shows that the most frequently studied pre-treatment method is 'Application mode - Etching', with just over 35% of studies focusing on this area. This suggests that etching is a primary area of interest and possibly considered a critical factor in the success of dental treatments.

The next most commonly researched method is 'Application mode - Adhesive', which constitutes about 34% of the studies. This indicates a significant research interest in applying adhesives, which are crucial for the bonding process in dental restorations.

'Pre-treatment of surface with substance' is the focus of 14% of the studies. The exact substance or technique is not fully visible in the image, but it implies a moderate interest in this particular type of pre-treatment.

The method 'Combined with Bevel when margins in enamel' 'Pre-treatment of surface with lasers' accounts for approximately 11% of studies, showing that while enamel beveling is of interest, it is less frequently explored compared to the application methods of etching and adhesives.

Finally, the method 'Pre-treatment of surface with lasers' is represented in the least number of studies, with about 6% investigating this technique. This suggests that laser, while still of interest, is considered a more specialized or less essential technique compared to the others represented in the graph.

Regarding the protocols, a range of procedures utilized in dental studies for applying etchants, adhesives, and conditioners is described in **Table 2**.

A common sequence found across most protocols begins with the etching of enamel and sometimes dentin using phosphoric acid, with concentrations ranging from 7% to 40% and application times varying, usually between 15 and 30 seconds. Following etching, the protocols uniformly call for rinsing and drying, typically lasting from 15 seconds to a minute, with some specifying the need to keep the dentin surface slightly wet.

The application of adhesive systems is a crucial next step, with protocols diverging in the types of adhesives used, techniques (such as scrubbing or applying under finger pressure), and the

time frame for application, which can range from 10 to 20 seconds. In several protocols, a preparation step before the adhesive application involves mixing two liquids in equal ratios to form a homogeneous mixture.

Air drying and light curing of the adhesive layer is another shared step, although the duration of air drying (ranging from 2 to 10 seconds) and the settings for light curing (often 600 to 1000 mW/cm²) may vary. These steps are critical for ensuring proper adhesion and setting of the dental material.

Notable differences among the protocols include additional conditioning steps that some employ, such as NAOCL, Chlorhexidine, or epigallocatechin-3-gallate. These conditioners may influence the adhesive process and the longevity of the dental treatment. Specificities like using a laser for surface preparation or a sonic applicator for adhesive application illustrate the vast array of techniques being researched and implemented.

Table 2. Summary table of protocols.

Step	Action	Specifics
1	Beveled enamel	Enamel of occlusal margin
2	Moisture Control	Rubber dam, cotton roll and cheek retractor
3	Dentin pre-treatment (mechanical and /or chemical)	<p>Mechanical</p> <ul style="list-style-type: none"> - Sandblast - Surface roughening = fine diamond bur <p>Chemical</p> <ul style="list-style-type: none"> - 2% Chlorhexidine - EDTA - 10% NAOCL - Epigallocatechin-3-gallate
4	Enamel etching	Phosphoric acid 40% for 15 seconds.

Step	Action	Specifics
5	Self-etch approach	According to manufacturer instructions
6	Adhesive Application	Vigorous rubbing for 10-20 seconds
7	Air Drying	For 2-10 seconds with glossy finish
8	Light Curing	For 10-40 seconds
9	Composite Application	Thin layer

Table 2 continued

Outcomes

Follow-up of 1 Year:

Etching enamel with phosphoric acid (selective etching or total etching including dentin) delivered promising outcomes, with a 100% retention rate for most studies (Balbinot et al. 2020; Digole et al. 2020; Ranjitha et al. 2020; Patrícia Manarte-Monteiro et al. 2019; Loguercio and Reis 2008; Reis et al. 2009; Moosavi et al. 2013; Shinohara et al. 2020).

Applying adhesive systems with vigorous rubbing, specifically Clearfil SE Bond, showcased a retention rate of 100% at the 12-month, suggesting its effectiveness in clinical applications. Similarly, the use of Adper Easy One, also known as Adper Easy Bond, achieved an equally impressive retention rate of 100%, in the studies with 1 year-follow-up (Jorge Perdigão et al. 2005; Reis et al. 2009; J. Perdigão et al. 2012; Jf et al. 2013; N. Sartori et al. 2013; Luque-Martinez et al. 2015; De Almeida et al. 2024).

A universal adhesive system like Scotchbond Universal was tested using self-etch and etch-and-rinse approaches. The self-etch approach yielded a retention rate of 97% after 18 months, whereas the etch-and-rinse strategy demonstrated a retention rate of 100% at 1-year follow-up, indicating that both methods are highly effective, albeit with potentially different long-term outcomes (Jf et al. 2013; N. Sartori et al. 2013; Follak et al. 2021; de Almeida et al. 2023).

In the groups where enamel bevel and dentin roughening was applied the outcomes were similar in comparison to control groups ($p > 0.05$) (Digole et al. 2020; Verma et al. 2023; Da Costa, Loguercio, and Reis 2013; Loguercio et al. 2018).

The application chlorhexidine prior to the dentin adhesive did not affect the clinical longevity of the composite restorations (Dutra-Correa et al. 2013). On the other hand, significantly higher retention rates were observed for the EDTA group (95.5%) than the control group (79.6%) (Luque-Martinez et al. 2015).

When sonic was applied the retention rate were 50% for manual application and 84.2% for sonic application, which appears to be an alternative method to improved the clinical outcome of NCCLs (Hass et al. 2022).

Follow-up of 2 Years:

The studies with a two-year follow-up included a comparison of the performance of different adhesives (Clearfil SE, GLUMA, Scotchbond and Bisco) in non-carious cervical lesions using FDI criteria with retention rates of 70% to 97%. In any case, no improvement in the retention rate was detected ($p > 0.05$), and the clinical performance of all adhesives deteriorated over time for marginal adaptation and discoloration (Patricia Manarte-Monteiro et al. 2021; Christiana Zander-Grande et al. 2011; C. Zander-Grande et al. 2014; Pena et al. 2016; Van Meerbeek et al. 2005; Cruz et al. 2021; Rouse et al. 2020; Oz, Ergin, and Canatan 2019; Zanatta et al. 2019; Lawson et al. 2015; Christiana Zander-Grande et al. 2011).

Saboia et al.,2006 evaluated the dentin pre-treatment with 10% NAOCL. It showed no statistical significance between etching and not etching groups (Saboia et al. 2006). Also, dentin

pre-treatment with epigallocatechin-3-gallate provided no benefit in the clinical performance of the adhesive applied, regardless of the adhesive technique utilized. The retention rate for ER was 97% and SE 94.2% (Costa et al. 2020).

Passive and various rubbing applications were applied for Xeno and Adapter adhesives. The study yielded that Adapter adhesive and the passive application mode showed significantly higher marginal staining than XE and active application ($p < 0.05$) (C. Zander-Grande et al. 2014). Also, the two-step etch and rinse approach in Loguercio et al.'s study showed difference in the retention rate with 92.5% for the vigorous rubbing group (Loguercio et al. 2011).

Follow-up of 3 Years:

Even though the application of the self-etch and etch-and-rinse approach did not yield a powerful difference in the retention rate (84% to 90%) (Jorge Perdigão et al. 2020; Dutra-Correa et al. 2019; Rocha et al. 2019; Gonçalves et al. 2021; Haak et al. 2023; Barceleiro et al. 2022; Atalay, Ozgunaltay, and Yazici 2020; de Albuquerque et al. 2022).

The dentine pre-treatment with 2% Chlorhexidine and 10% NaOCL solution showed no difference in the survival rate among the groups (Morgana Favetti et al. 2017; M. Favetti et al. 2022; Neimar Sartori et al. 2013).

Beveled enamel margins did not improve the clinical longevity of composite restorations after three years of follow up (Baratieri et al. 2003).

Follow-up of 5 Years:

In the 13-year follow-up study by Van Dijken et al. 2007, continuous degradation of the resin-dentin bond was observed for all bonding systems (Allbond, Clearfil, Denthesive, and GLUMA regardless of the approach applied.(van Dijken, Sunnegårdh-Grönberg, and Lindberg 2007). In contrast, Peumans found a clinical success rate of 86% to 93% of Clearfil SE Bond with selective acid-etching of the enamel cavity margins (Peumans et al. 2015).

The total-etch adhesive system with or without collagen removal with 10% sodium hypochlorite (NaOCl) after five years did not change the clinical performance of resin composite restorations (Rocha Gomes Torres et al. 2014).

After 5 years, the clinical behavior of the universal adhesive in the etch-and-rinse strategy was better when compared to the self-etch strategy when presence of enamel at the occlusal margin. (de Paris Matos et al. 2020; Ñaupari-Villasante et al. 2023; Boushell et al. 2016).

Limitations

Even though this scoping review was conducted according to the scoping review methodology, some limitations are worth noting. First, scoping reviews aim to provide a broad overview of the available evidence on a specific topic, which may lead to a superficial analysis of individual studies. Second, instead of conducting a meta-analysis or quantitative data synthesis, a scoping review typically emphasizes descriptive analysis and mapping of the available evidence. This may limit the ability to draw solid conclusions or make conclusive recommendations based on the review's findings. A final limitation is that this field is advancing rapidly, so it is important to acknowledge that this scoping review is a snapshot at a particular time.

Conclusions

This scoping review outlines the current understanding of NCCL restorative protocols while pinpointing areas that warrant deeper investigation. By identifying evidence gaps, this study lays the groundwork for refining clinical strategies and improving patient outcomes. Firstly, it highlights the variability in study designs, treatment protocols, and outcome assessments. Notably, the etching application method emerged as the most studied intervention, while aspects such as loss of anatomical form, surface texture, and tooth integrity received limited attention. Surprisingly, patient complaints and satisfaction remained unexplored in the reviewed studies. Secondly, the majority of investigations had relatively short follow-up periods, typically up to one year. Thirdly, some studies lacked clear diagnoses of NCCLs, particularly concerning their extent and severity. Lastly, the majority of participants were under 60 years old, raising questions about the generalizability of findings to older populations. Given these concerns regarding evidence heterogeneity, future research should aim to elucidate the factors influencing the effectiveness of NCCL restorations and refine treatment approaches accordingly.

Acknowledgments

We thank Mary Ann Williams from the Health Sciences and Human Services Library, University of Maryland, Baltimore, for her assistance with the systematic review database search

Authors' Contributions

DM and MM were involved in screening studies. DA was involved in full-text screening and responsible for data extraction. All authors were involved in the study's design, conduct, and preparation and review of the manuscript.

Conflicts of Interest

There are no other conflicts of interest to declare.

Data availability statement

Data are available on reasonable request. All data presented have been previously published. Our data will be made available on reasonable request to the corresponding author.

Age-Related Differences in Biofilm Composition on Control vs. Cavitated Root Caries Lesions: A Preliminary Clinical Study

Darianna Mashi¹ Lamia Sami Mokeem², Qoot Alkhubaizi ¹, Mary Anne S. Melo^{2,3}

¹ Advanced Education in General Dentistry Program, Department of Comprehensive Dentistry University of Maryland School of Dentistry, Baltimore, MD 21201, USA;

² Ph.D. Program in Biomedical Sciences, University of Maryland School of Dentistry, Baltimore, MD, 21201, USA;

³ Operative Dentistry & Cariology Division, Department of Comprehensive Dentistry, University of Maryland School of Dentistry, Baltimore, MD 21201, USA

Correspondence: Mary Anne Sampaio de Melo, Operative Dentistry & Cariology Division, Department of Comprehensive Dentistry, University of Maryland School of Dentistry, Baltimore, MD 21201, USA

Email: Mmelo@umaryland.edu

Abstract

Objective: This study aims to comprehensively analyze the biofilm composition over healthy root surfaces (control group) and cavitated root caries lesions (study group) among patients in two distinct age groups: those younger than 65 (control and cavitated) and older than 65 years (control and cavitated).

Materials and Methods: Forty-four adults (27 females and 17 males) were recruited from the University of Maryland School of Dentistry Advanced Education in General Dentistry clinic. The study employed 16S rRNA sequencing to analyze the microbial composition, focusing on diversity and dominance across healthy and cavitated root surfaces. Samples were collected directly from these surfaces to ensure an accurate representation of the biofilm present.

Results: The microbiological analysis revealed variations in biofilm composition. Cavitated lesions exhibited a higher bacterial diversity compared to non-cavitated surfaces, as indicated by the Shannon diversity index, suggesting a more complex microbial ecosystem within these lesions. Furthermore, the Simpson's dominance index showed that non-cavitated surfaces were characterized by a few dominating species, contrasting with the more evenly distributed bacterial community in cavitated lesions. Additionally, variances with age altered these trends, with older individuals exhibiting distinct bacterial compositions.

Conclusion: This study highlights the significance of age and lesion presence on the composition of the biofilm in root caries lesions. The observed differences in microbial diversity and dominance suggest that both factors play a crucial role in shaping the biofilm ecosystem. These insights could inform future research and clinical strategies aimed at managing root caries through targeted

biofilm management, particularly in older populations. Further research is needed to explore the clinical implications of these microbial patterns and their potential impact on treatment outcomes.

Keywords: Root Caries, Biofilm Composition, Age-Related Differences, Control Lesions, Cavitated Lesions, Oral Microbiota, Microbial Diversity.

Introduction

By 2060, 98 million Americans will be over the age of 65 years old. Currently, 70% of older adults have periodontal disease, and 20% have untreated tooth decay ("Oral Health for Older Americans | Adult Oral Health | Basics | Division of Oral Health | CDC" 2023). In aged individuals, decreased manual dexterity, physical or mental impairment, and immune system alterations are associated with increased susceptibility to root caries (Tonetti et al. 2017).

Root caries lesions represent a significant concern in dental health, affecting a broad spectrum of the population across various age groups (Reddy et al. 2021; W. Chen, Zhu, and Zhang 2023). These lesions, which occur on the root surfaces of teeth, can be broadly categorized into non-cavitated and cavitated lesions, each presenting unique challenges in terms of prevention, management, and treatment. The distinction between non-cavitated and cavitated lesions is crucial, as it reflects the progression of the disease and has implications for treatment strategy (Qutieshat et al. 2021; Fee et al. 2020). Non-cavitated lesions, dentin is sound, whereas cavitated lesions exhibit actual structural damage.

The biofilm composition on the tooth surface plays a pivotal role in the development and progression of root caries (Zhang et al. 2019; Chen et al. 2015). Dental biofilms are complex communities of microorganisms that adhere to dental surfaces and can contribute to both the initiation and advancement of carious lesions (Chen et al. 2020; Chen et al. 2015). The composition of these biofilms is influenced by dietary habits, oral hygiene practices, saliva composition, and, importantly, the individual's age. Aging characteristics related to the patients can alter the oral microbiome, potentially affecting the susceptibility to and nature of dental caries (W. Chen, Zhu, and Zhang 2023).

Age-related changes in oral health status, including increased prevalence of root exposure due to gum recession and alterations in salivary flow and composition, make the elderly population particularly vulnerable to root caries (Tonetti et al. 2017; Tan et al. 2017). Furthermore, the presence of cavitated lesions in older adults can complicate oral hygiene practices, providing protected niches for biofilm accumulation and making these lesions more challenging to treat effectively (Tokumoto et al. 2022; Duke and MacInnes 2021). On the other hand, younger individuals, although not immune, typically exhibit a different pattern of susceptibility to root caries, influenced by factors such as diet, oral hygiene habits, and the overall health of gingival tissues (Ogwo et al. 2023).

Understanding the biofilm composition of root caries lesions is essential for developing targeted preventive and therapeutic strategies. The microbial composition of biofilms can indicate the potential pathogenicity of the lesion and inform the choice of antimicrobial agents or other interventions aimed at controlling or eliminating the biofilm. Differences in biofilm composition between control and cavitated lesions and across different age groups can provide insights into the dynamics of root caries progression and the factors influencing the effectiveness of various treatment approaches.

Research into the comparative biofilm composition of control versus cavitated root caries lesions remains sparse, especially across distinct age demographics. Such information is critical for tailoring prevention and treatment strategies to the specific needs and challenges presented by different stages of lesion development and patient ages. The evolving understanding of the oral microbiome and its role in dental diseases highlights the need for more focused investigations in this area.

Therefore, this study aims to comprehensively analyze the biofilm composition over control and cavitated root caries lesions in two distinct age groups: patients younger than 65 and those older than 65. By elucidating the differences in biofilm composition associated with lesion type and age, this study seeks to contribute to developing more effective, age-specific strategies for the prevention and management of root caries.

Materials and Methods

Participants

Patients under comprehensive care enrolled in the Advanced Education in General Dentistry (AEGD) dental clinic at the University of Maryland School of Dentistry were screened for the presence of cavitated root caries lesions. The Institutional Review Board (IRB) and University of Maryland School of Dentistry (Baltimore, MD) approved the study (HP-00090352). Forty-four adults (27 females and 17 males) met the inclusion criteria after completing the clinical exam. The inclusion criteria were high caries risk, presence of cavity located in the root surface, 18 years or older, have not taken any antibiotics in the last six months or received any active periodontal treatment in the last 12 months causing bleeding on the collecting site. Patients that use orthodontic appliances or denture attached to the tooth presenting RC lesion, alteration in the motor condition that modifies tooth brushing habits, use of commercial antiseptic mouthwash on the day of sample collection, history of current or past smoking, tobacco use, presence of gingival bleeding in the site of collection, and being pregnant or lactating were excluded. All patients were randomly selected, informed of the research objectives and signed the informed consent. The mean age was 61.4 years, ranging from 34 to 84 (**Table 3**).

Characteristics of research subjects	Number of patients
Gender distribution	
Male	17
Female	27
Age distribution (years)	
30-49	5
50-69	26
>70	13

Table 3: Characteristics of the research subjects

Clinical Assessment

The patients were subjected to visual examination, seeking signs indicative of root caries, such as discoloration or changes in the texture on the root surface. Lesions may appear as dark spots or shadows that contrast with the lighter color of healthy root surfaces, ranging in color from light brown to black, depending on the lesion's age and activity level.

The tactile examination was performed following the visual examination to evaluate suspected root caries lesions. Using a dental explorer, the operator (D.M) gently touches the identified spots to test their texture. Healthy root surfaces were typically hard and smooth, while cavitated lesions, with broken surface integrity, formed a detectable pit or groove carious lesions that might feel soft, sticky, or leathery due to demineralization.

Subsequently, patients were divided into four groups: two controls group (B65C= 13, UP65C=12) and two root caries group (B65RC=13, UP65RC=6). The control groups were non-

cavitated, and the RC patients had one or more root caries lesions when clinical examination was completed. Root surface lesions were categorized as carious lesions when they present a dentin cavitation with soft. The American Dental Association Caries Classification System (Young et al. 2015) was used to diagnose and define RC lesions.

Sample Collection

Supragingival dental plaque samples of control groups were collected by gently swabbing using sterile micro brush applicator over the tooth (**Figure 10**). All samples were taken by one examiner (D.M). All specimens were placed into sterile 1.5 ml microcentrifuge tubes containing C 2.1 buffer solution (133 mM ammonium acetate, 0.04% sodium azide, and .04% bromophenol blue) and stored at -80⁰C. All samples were frozen in UMSOD laboratory until they were transported on ice to the Genomics Resource Center for further processing.

DNA Extraction and Purification.

The QIAamp DNA mini kit (Qiagen, Hilden, Renania, Germany) was used to extract bacterial DNA. The V3-V4 regions of the 16S rRNA gene was amplified and then sequenced using an Illumina HiSeq 2500 (San Diego, CA, USA), which was adapted to produce 300 pb paired-end. This sequencing was conducted at the Genomic Resource Center of the Institute of Genome Sciences, University of Maryland School of Medicine, located in Baltimore, MD, USA. Afterward, using the SILVA rRNA database, the obtained cleaned reads were grouped into operational taxonomic units (OTUs) based on their similarity (97%). The richness of the biofilm samples was assessed by analyzing the percentage of OTUs representatives at the genus level.

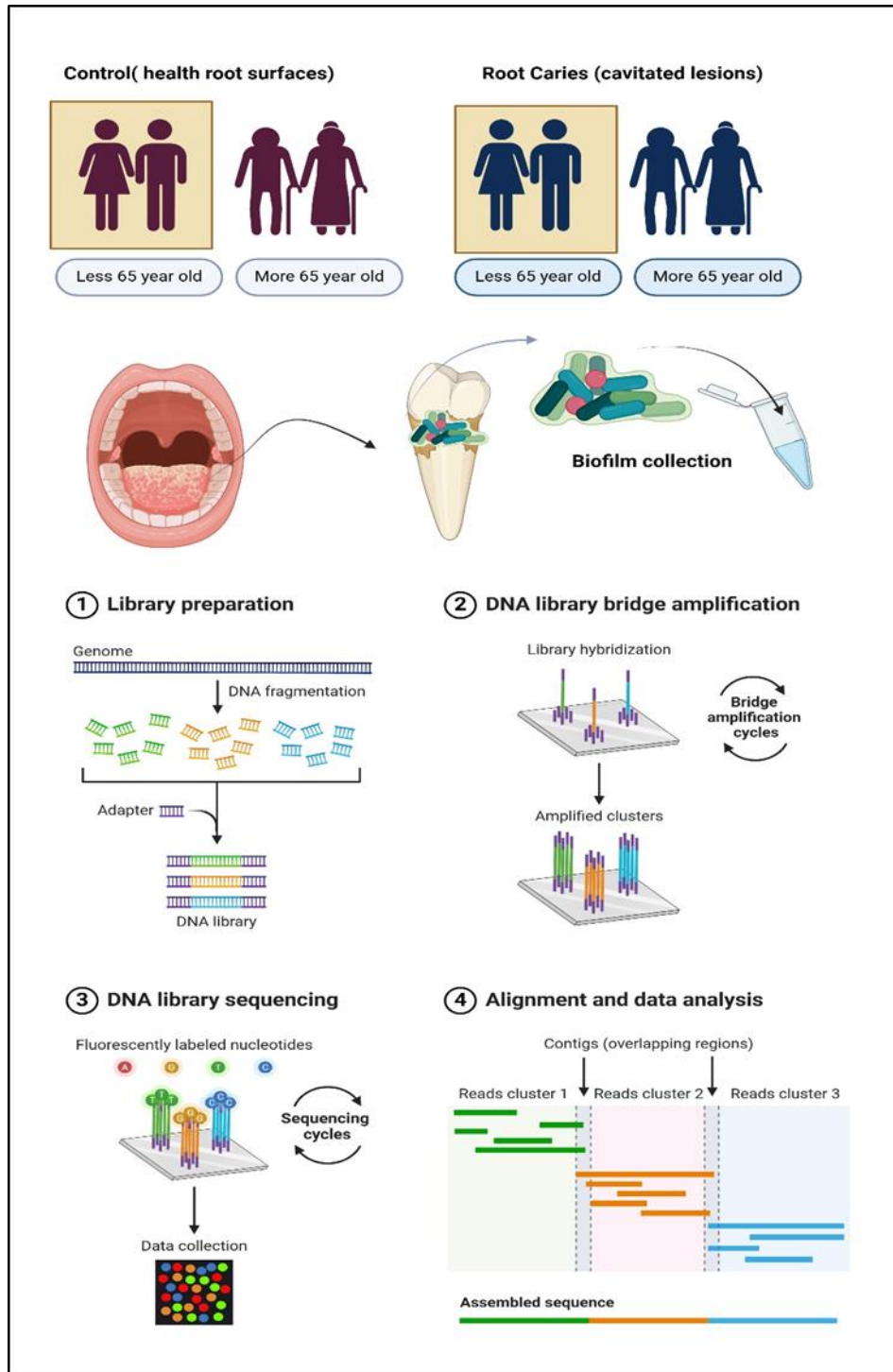


Figure 10. Flowchart describing the groups, sample collection and 16S RNA Sequencing.

Statistical Analysis

For the biofilm composition on non-cavitated vs. cavitated root surfaces across the two age groups, only descriptive statistics were used to summarize the 16S rRNA sequencing findings.

1)Bacterial Diversity: Reporting each group's average Shannon diversity index to describe the biofilm's biodiversity. Higher values indicate a more diverse microbial community.

2)Bacterial Dominance: Utilizing the Simpson's dominance index to describe how evenly the bacteria are distributed within the biofilm. Lower values suggest a more equitable distribution among bacterial species.

3)Most Prevalent Bacteria: Listing the most commonly identified bacteria in the biofilm of cavitated lesions and healthy root surfaces, along with their relative abundance percentages.

Results

The analysis of the microbial composition at the genus level revealed distinct variations in the prevalence of bacteria among the studied groups. Genus *Streptococcus* was consistently identified as the dominant bacteria among the most prevalent genera across all participant groups. However, notable differences were observed in its relative abundance between individuals with cavitated root caries lesions and those with non-cavitated root surfaces.

In participants above 65 with non-cavitated lesions, Genus *Streptococcus* accounted for an average of 80% of the bacterial composition, compared to 54% in those under 65, highlighting an age-related difference in microbial prevalence. Conversely, in cavitated root surfaces, Genus *Actinomyces* constituted only 5% of the bacterial population in older participants and 21% in younger participants, suggesting that the presence of cavitation significantly influences the microbial ecosystem.

Other prevalent genera identified include Genus *Veillonella*, Genus *Corynebacterium*, Genus *Granulicatella* and Genus *Capnocytophaga*, each showing distinct patterns of distribution between the different age groups and root surface conditions. For instance, Genus *Corynebacterium* was more abundant in cavitated lesions of older individuals, while Genus *Veillonella* was predominantly found in the younger cohort. Genus *Lactobacilli* was not found, this suggests that they exist below the detection level of a 16S rRNA gene survey with bacterial universal primers.

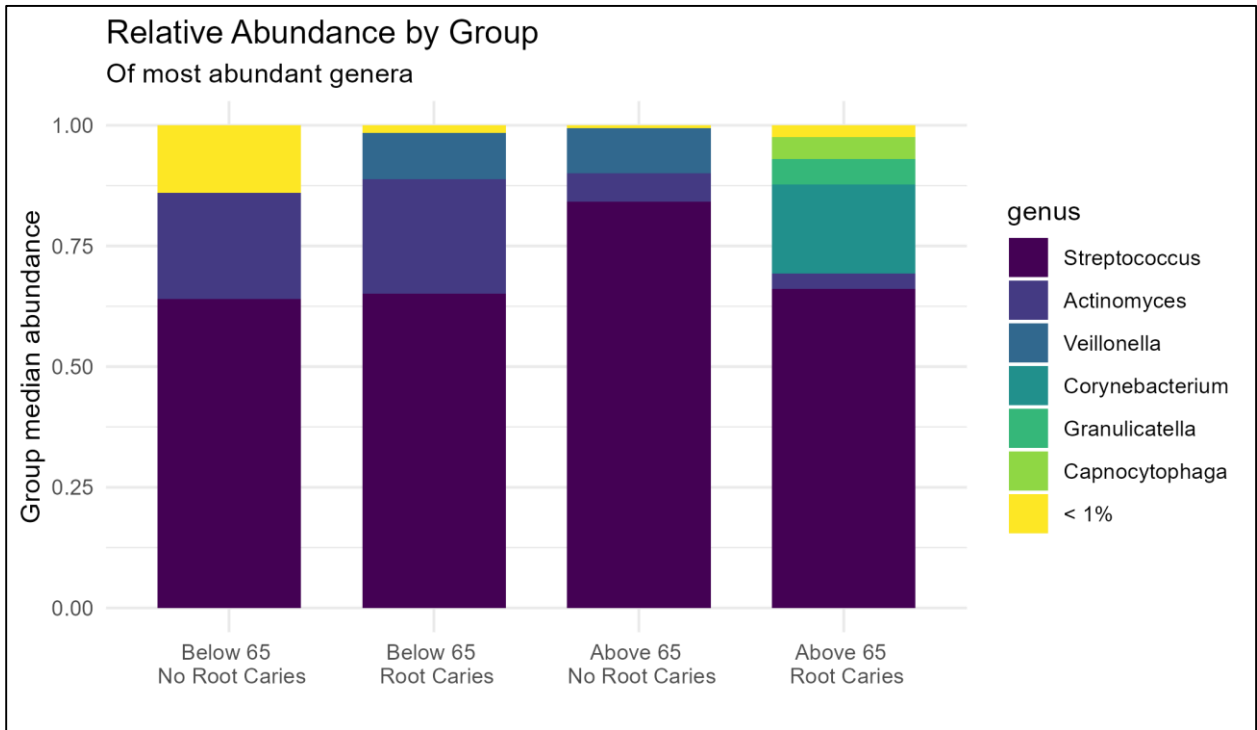


Figure 11. Comparison of Shannon’s index (alpha diversity) across the four groups.

The comparison between patients younger than 65 years old and those older than 65 years old reveals interesting trends. In general, older patients tend to show a different bacterial profile in their root caries lesions, possibly due to changes in saliva composition, pH, and other age-related oral health changes. These shifts could influence the microbial ecosystem within the oral cavity, leading to variations in the predominant bacteria between the two age groups.

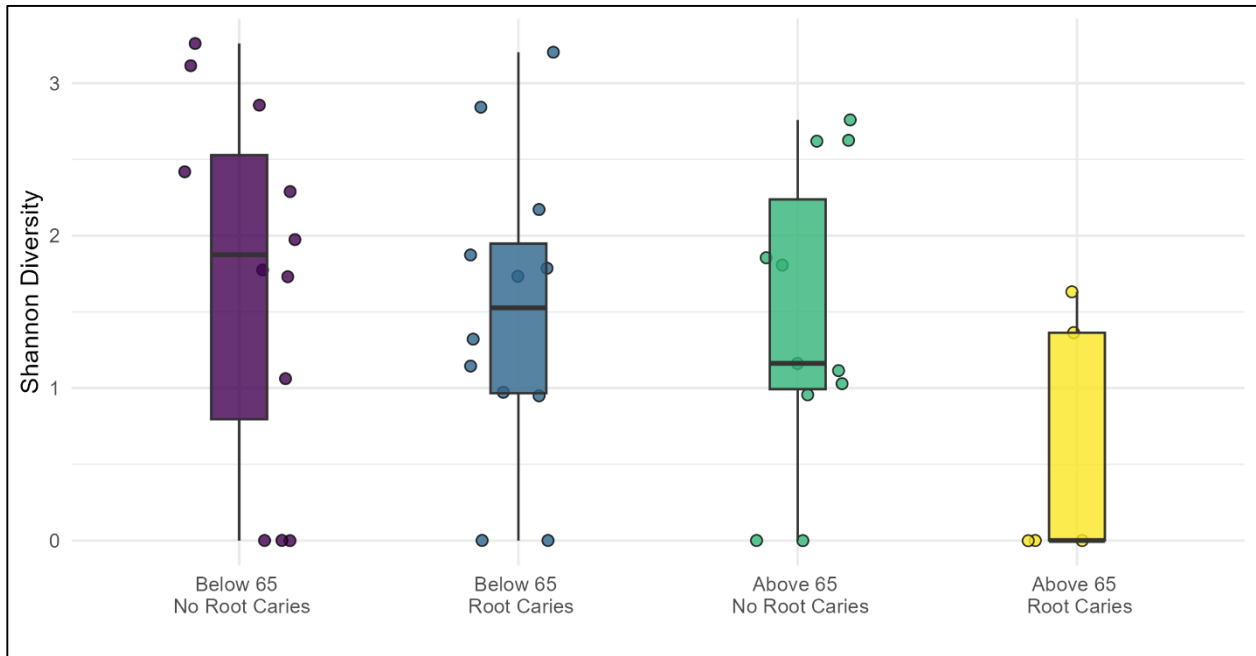


Figure 12. Shannon’s index by subjects group.

The Shannon diversity index (**figure 12**), which measures the diversity of the bacterial community, indicates a greater diversity in cavitated lesions across both age groups. This increased diversity within cavitated lesions suggests that the environment within these lesions supports a wider range of bacterial species. Moreover, the Shannon index might slightly differ between the two age groups, potentially reflecting age-related changes in oral microbiota composition.

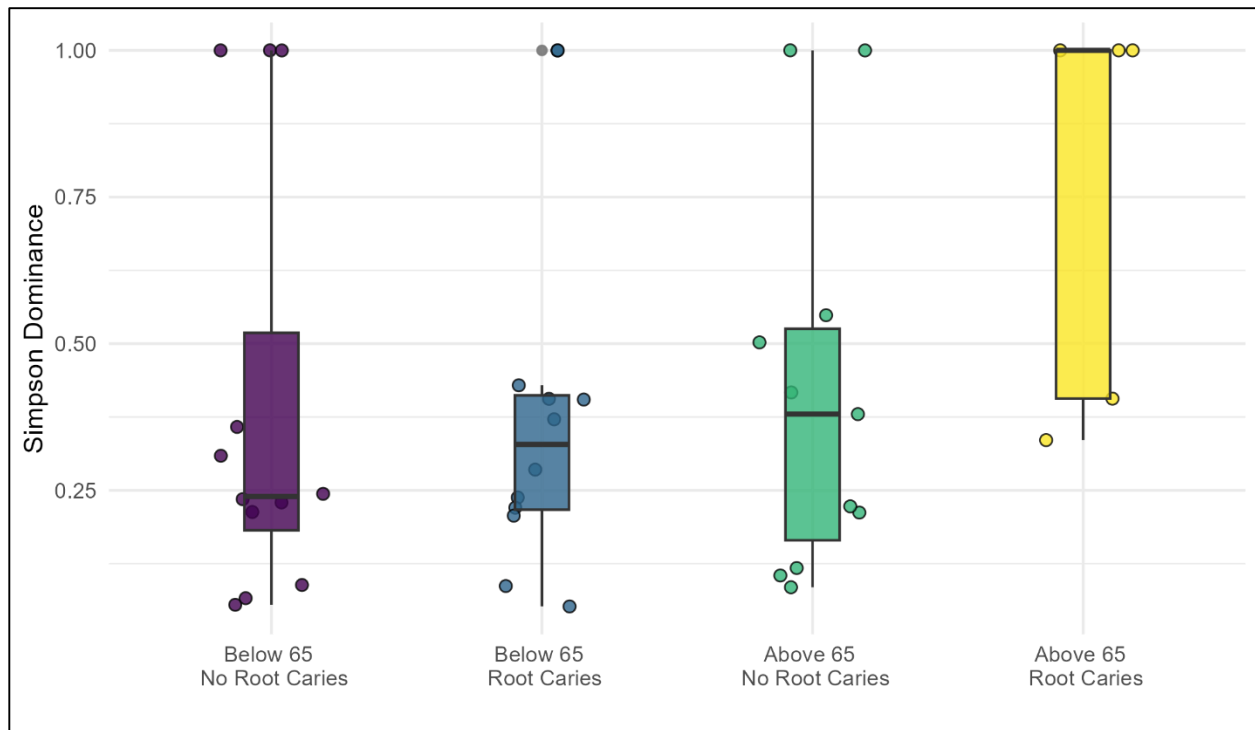


Figure 13. Simpson's dominance index of specific species inside the biofilm community across the four groups.

Simpson's dominance index (**Figure 13**) is a measure of the dominance of particular species within the community. The figures imply that non-cavitated lesions tend to have a higher dominance index, suggesting a few species predominate in these environments. In contrast, with their higher bacterial diversity, cavitated lesions would have a lower dominance index, indicating no single bacterial species overwhelmingly dominates the community.

Discussion

The findings from this study contribute to the growing body of literature on the role of microbial communities in the development and progression of root caries, with a specific focus on age-related differences in biofilm composition. The observed dominance of *Streptococcus* in

cavitated root caries lesions across all age groups identified this genus as a key player in the cariogenic process. However, the marked difference in its prevalence between olderer (80%) and younger individuals (54%) with non-cavitated lesions underscores the influence of age on oral microbial dynamics. This variation suggests that the microbial ecosystem associated with root caries is not static but evolves with age, possibly due to physiological changes, alterations in immune response, or differences in oral hygiene practices over time.

The increased abundance of Actinomyces in cavitated root surfaces among younger adults (21%) compared to older individuals (5%) is particularly noteworthy. Actinomyces is often associated with the initial stages of dental plaque formation and has a recognized role in the development of root caries as indicated by the results.

The presence of other bacteria such as Veilonella, Corynebacterium, Granulicatella, and Capnocytophaga, and their distinct distribution patterns between age groups and lesion types, further illustrates the complexity of the oral microbiome. Corynebacterium's predominance in older individuals' cavitated lesions, for instance, may reflect its adaptation to the altered microenvironment within these carious sites. Conversely, the prevalence of Veilonella in the younger cohort's lesions might be influenced by dietary factors or the biochemical milieu specific to their oral cavity.

Each genus contributes differently to the microbial ecosystem of cavitated lesions and non-cavitated root surfaces, affecting the disease's development and progression.

Streptococcus, particularly Streptococcus mutans, is a well-documented protagonist in the initiation and progression of dental caries, including root caries. Its ability to adhere to dental surfaces, form biofilms, and produce acid from fermentable carbohydrates makes it a key player in

enamel demineralization and the subsequent development of carious lesions. The high prevalence of *Streptococcus* in cavitated root caries lesions across all age groups, underscores its significant role in the cariogenic process. This dominance also highlights the potential for interventions aimed at reducing *Streptococcus* levels, such as the use of antimicrobial agents or probiotics, to prevent or mitigate root caries development.

Actinomyces is another crucial genus, known for its role in the early stages of dental plaque formation and its association with root caries. The higher prevalence of *Actinomyces* on cavitated root surfaces in younger adults suggests its role in predisposing these surfaces to carious lesions. This finding points to the importance of maintaining diligent oral hygiene practices, especially for young adults, to prevent *Actinomyces* from contributing to the initiation of root caries. Moreover, understanding the conditions that favor *Actinomyces* overgrowth could lead to targeted preventive strategies, such as altering the oral pH or disrupting biofilm formation.

Veillonella, found predominantly in younger individuals with cavitated lesions, is notable for its lactate-utilizing ability, which may influence the caries process by modulating the oral microbial ecosystem's pH. Its presence reflects the complex interactions within the biofilm that can either protect against or promote caries development, depending on the broader microbial context.

Corynebacterium, more abundant in cavitated lesions of older individuals, and other genera like *Granulicatella* and *Capnocytophaga*, demonstrate the diversity of bacteria involved in root caries beyond the traditional culprits. These bacteria may contribute to the progression of caries through various mechanisms, such as influencing the biofilm's structural integrity, acid production, or interactions with other microbes. The specific roles of these genera in root caries suggest that a

multifaceted approach to managing the oral microbiome could be beneficial for preventing or controlling the disease.

The variations in the Shannon diversity index and Simpson's dominance index observed in this study offer profound insights into the microbial ecology of cavitated root caries lesions versus non-cavitated root surfaces, underlining the complexity of the biofilm's microbial community. The Shannon index, which quantifies the diversity within a community by considering both the abundance and evenness of species present, revealed a greater diversity in cavitated lesions across all age groups. This increased diversity suggests a more complex microbial ecosystem within cavitated lesions, possibly due to a more varied array of ecological niches or a higher level of nutrient availability that supports a wider range of bacterial species. This complexity could contribute to the resilience of the biofilm, potentially complicating efforts to disrupt cariogenic bacteria through traditional oral hygiene practices.

Conversely, the Simpson's dominance index, which emphasizes the concentration of dominance in a community, pointing to how evenly spread the bacterial population is, illustrates another aspect of microbial dynamics. A lower dominance index in cavitated lesions implies a more equitable distribution of species, contrary to what one might expect in a disease-associated biofilm, where a few pathogenic species often predominate. This finding suggests that root caries lesions provide a habitat that supports a broad spectrum of microbial life, rather than being dominated by a single or a few cariogenic pathogens. This diversity and distribution pattern might contribute to the pathogenicity of the lesion, affecting disease progression and the efficacy of antimicrobial treatments.

The differences observed in the Shannon and Simpson indices between age groups also warrant attention. These differences could reflect age-related changes in the oral environment, such as alterations in saliva composition, pH, and nutrient availability, which might influence the microbial community structure. Additionally, these findings suggest that the oral microbiome's response to the cariogenic challenge is modulated by the host's age, pointing to the need for age-specific preventive and therapeutic strategies that consider the microbial diversity and dominance patterns.

Conclusion

Within the limitation of this being a preliminary study, it can be concluded that the significant age-related differences in the prevalence and diversity of these genera underscore the need for age-tailored oral healthcare strategies. For younger adults, who may experience a shift towards a microbiome that includes higher levels of *Actinomyces* and *Corynebacterium*, preventive measures might focus on interventions that modulate the biofilm composition to favor non-cariogenic bacteria. For older individuals, strategies that target *Streptococcus* and *Veillonella* might be more effective in preventing root caries.

Acknowledgments

The success of this study is indebted to the invaluable contributions of our participants. We extend our deepest gratitude to each volunteer who generously dedicated their time and effort to participate in this research.

Authors' Contributions: Conceptualization, M/M.; Methodology, M.M.; Validation, Q.A.; Formal analysis, D.M.; Investigation, D.M, Q.A.; Resources, M.M.; Data curation, M.M; Writing—original draft preparation, D/M.; Writing—review and editing, M/M.: Visualization, Q.A.; Supervision, M/M.; Project administration, Q.A, and M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the ICTR Award (M.M)

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of University of Maryland Baltimore (HP-00090352; 11 April 2021).

Informed Consent Statement: Informed consent was obtained from all participants included in the study.

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest

Overall Discussion

The thesis presented offers a profound exploration of two pivotal realms within dental medicine: the challenges associated with managing cervical composite restorations, and the intricate biofilm composition dynamics on root caries lesions across different age demographics. The investigation into non-carious cervical lesions (NCCLs) underscores the intricate balance required in selecting and applying restorative protocols to enhance the longevity of dental restorations. (de Albuquerque et al. 2022; Digole et al. 2020). The scoping review within the first chapter casts light on the diverse outcomes stemming from various surface pre-treatment techniques, revealing the critical nature of adhesion and the subsequent impact on restoration durability. (Costa et al. 2020; Morgana Favetti et al. 2017; Saboia et al. 2006). This foundational understanding serves as a prelude to the deeper examination of microbial influences on root caries, merging mechanical restoration considerations with biological dynamics.

Transitioning to the microbial ecosystem's role in root caries progression, Chapter 2 delves into the age-related variations in biofilm composition, particularly focusing on cavitated and non-cavitated lesions. The detailed analysis of microbial diversity, dominated by genera such as *Streptococcus* and *Actinomyces*, reveals a complex interplay within the oral microbiome (Tonetti et al. 2017; Tan et al. 2017). These findings highlight how the microbial landscape shifts with the presence of cavitation and the patient's age. This nuanced understanding of biofilm composition underlines the necessity for a dynamic approach to dental care, responsive to the changing microbial profiles associated with different stages of life and dental health statuses (Reddy et al. 2021; W. Chen, Zhu, and Zhang 2023).

The juxtaposition of these detailed investigations into restorative challenges and microbial dynamics offers a comprehensive view that bridges the gap between clinical dentistry and

microbiological research. This dual perspective is crucial for developing holistic dental care strategies that are not only effective in restoring dental aesthetics and function but also in preventing the onset and progression of root caries through biofilm management (Qutieshat et al. 2021; Fee et al. 2020). The research underscores the importance of considering the oral microbiome's role in dental diseases, advocating for a preventive approach that takes into account the microbial factors contributing to lesion development.

Furthermore, the thesis brings to light the critical role of patient-specific factors, particularly age, in determining the most effective treatment and prevention strategies. The distinction between younger and older populations in terms of biofilm composition and the response to restorative procedures emphasizes the need for age-tailored dental care. This approach is paramount for addressing the unique challenges presented by the dental health landscape of an aging population, which is increasingly retaining natural dentition into later life and, as a result, facing a higher risk of root caries ("Oral Health for Older Americans | Adult Oral Health | Basics | Division of Oral Health | CDC" 2023) .

The exploration of NCCLs and the microbial composition associated with root caries within this thesis contributes significantly to the dental field. It provides a scaffold for future research aimed at refining dental restoration techniques and developing targeted interventions to manage and prevent root caries. By highlighting the intricate relationship between mechanical restorative efforts and the biological environment of the oral cavity, the research underscores the need for an integrated approach that leverages advancements in both dental materials science and microbiology.

Conclusion & Remarks

The thesis summarizes the exploration of dental restorative challenges and microbial dynamics, advocating for a nuanced, patient-centered approach to dental care. This comprehensive perspective is instrumental in advancing the field of restorative dentistry, guiding clinical practice towards more personalized, effective, and preventative strategies. Through a deeper understanding of both the material and microbial aspects of dental health, dental professionals can better navigate the complexities of restoring and maintaining the oral cavity, ultimately enhancing patient outcomes and oral health quality of life.

REFERENCES

- Albuquerque, Elisa Gomes de, Flavio Warol, Chane Tardem, Fernanda Signorelli Calazans, Luiz Augusto Poubel, Thalita P. Matos, Jullian J. Souza, Alessandra Reis, Marcos O. Barceleiro, and Alessandro D. Loguercio. 2022. "Universal Simplified Adhesive Applied under Different Bonding Technique's: 36-Month Randomized Multicentre Clinical Trial." *Journal of Dentistry* 122 (July): 104120. <https://doi.org/10.1016/j.jdent.2022.104120>.
- Alencar, Christiane de M., Gabriela C. S. Fernandes, Antonio Patrícia O. Barros, Joissi F. Zaniboni, Aryvelto M. Silva, Fernanda F. de A. Jassé, Cecy M. Silva, and Edson A. de Campos. 2023. "Clinical Evaluation of Surface Treatment on Clinical Performance of Non-Carious Sclerotic Cervical Lesions: 18-Month Follow-Up." *American Journal of Dentistry* 36 (3): 143–50.
- Almeida, Rossana Aboud Matos de, Suellen Nogueira Linares Lima, Maria Vitória Nassif, Natanael Henrique Ribeiro Mattos, Thalita Paris de Matos, Rudys Rodolfo de Jesus Tavarez, Andres Felipe Millan Cardenas, Matheus Coelho Bandeca, and Alessandro D. Loguercio. 2023. "Eighteen-Month Clinical Evaluation of a New Universal Adhesive Applied in the 'No-Waiting' Technique: A Randomized Clinical Trial." *Clinical Oral Investigations* 27 (1): 151–63. <https://doi.org/10.1007/s00784-022-04703-7>.
- Atalay, Cansu, Gul Ozguntay, and Ayse Ruya Yazici. 2020. "Thirty-Six-Month Clinical Evaluation of Different Adhesive Strategies of a Universal Adhesive." *Clinical Oral Investigations* 24 (4): 1569–78. <https://doi.org/10.1007/s00784-019-03052-2>.
- Balbinot, Carlos Eduardo Agostini, Raquel Pippi Antoniazzi, Juliana Tabim Parode, Luciana d'Avila Farias, Camila Zamboni, and Jovito Adiel Skupien. 2020. "Clinical Evaluation of a Self-Etch and an Etch-and-Rinse Adhesive System in Class V Noncarious Composite Restorations." *European Journal of General Dentistry* 9 (1): 23–27. https://doi.org/10.4103/ejgd.ejgd_102_19.
- Baratieri, Luiz Narciso, Simone Canabarro, Guilherme C. Lopes, and André V. Ritter. 2003. "Effect of Resin Viscosity and Enamel Beveling on the Clinical Performance of Class V Composite Restorations: Three-Year Results." *Operative Dentistry* 28 (5): 482–87.
- Barceleiro, Marcos O., Leticia S. Lopes, Chane Tardem, Fernanda S. Calazans, Thalita P. Matos, Alessandra Reis, Abraham Lincoln Calixto, and Alessandro D. Loguercio. 2022. "Thirty-Six-Month Follow-up of Cervical Composite Restorations Placed with an MDP-Free Universal Adhesive System Using Different Adhesive Protocols: A Randomized Clinical Trial." *Clinical Oral Investigations* 26 (6): 4337–50. <https://doi.org/10.1007/s00784-022-04397-x>.

- Boushell, Lee W., Harald O. Heymann, Andre V. Ritter, John R. Sturdevant, Edward J. Swift, Aldridge D. Wilder, Yunro Chung, Cynthia A. Lambert, and Ricardo Walter. 2016. "Six-Year Clinical Performance of Etch-and-Rinse and Self-Etch Adhesives." *Dental Materials: Official Publication of the Academy of Dental Materials* 32 (9): 1065–72. <https://doi.org/10.1016/j.dental.2016.06.003>.
- Chen, Lin, Bingcai Qin, Minquan Du, Huanzi Zhong, Qingan Xu, Yuhong Li, Ping Zhang, and Mingwen Fan. 2015. "Extensive Description and Comparison of Human Supra-Gingival Microbiome in Root Caries and Health." *PLoS ONE* 10 (2): e0117064. <https://doi.org/10.1371/journal.pone.0117064>.
- Chen, Weixing, Tianer Zhu, and Denghui Zhang. 2023. "The Prevalence and Common Risk Indicators of Root Caries and Oral Health Service Utilization Pattern among Adults, a Cross-Sectional Study." *PeerJ* 11 (November): e16458. <https://doi.org/10.7717/peerj.16458>.
- Chen, Xiuqin, Eric Banan-Mwine Daliri, Namhyeon Kim, Jong-Rae Kim, Daesang Yoo, and Deog-Hwan Oh. 2020. "Microbial Etiology and Prevention of Dental Caries: Exploiting Natural Products to Inhibit Cariogenic Biofilms." *Pathogens* 9 (7): 569. <https://doi.org/10.3390/pathogens9070569>.
- Costa, Caga, Nlg Albuquerque, J. S. Mendonça, A. D. Loguercio, Vpa Saboia, and S. L. Santiago. 2020. "Catechin-Based Dentin Pretreatment and the Clinical Performance of a Universal Adhesive: A Two-Year Randomized Clinical Trial." *Operative Dentistry* 45 (5): 473–83. <https://doi.org/10.2341/19-088-C>.
- Cruz, Joana, Ana Luisa Silva, Raquel Eira, Catarina Coito, Bernardo Romão Sousa, Maria Manuela Lopes, and Alexandre Cavalheiro. 2021. "24-Month Clinical Performance of a Universal Adhesive on Non-Carious Cervical Lesions: Self-Etch and Etch-and-Rinse Techniques." *The Journal of Adhesive Dentistry* 23 (5): 379–87. <https://doi.org/10.3290/j.jad.b2000173>.
- Da Costa, Thays Regina Ferreira, Alessandro Dourado Loguercio, and Alessandra Reis. 2013. "Effect of Enamel Bevel on the Clinical Performance of Resin Composite Restorations Placed in Non-Carious Cervical Lesions." *Journal of Esthetic and Restorative Dentistry: Official Publication of the American Academy of Esthetic Dentistry ... [et Al.]* 25 (5): 346–56. <https://doi.org/10.1111/jerd.12042>.
- De Almeida, Rab, Fsf Siqueira, Thiago Verde, R. Ñaupari-Villasante, A. Reis, A. D. Loguercio, and Afm Cardenas. 2024. "Prolonged Application Time Effects on Universal Adhesives in Non-Carious Cervical Lesions: An 18-Month Split Mouth Randomized Clinical Trial." *Journal of Dentistry* 140 (January): 104800. <https://doi.org/10.1016/j.jdent.2023.104800>.

- Digole, Vasanta Ramesh, Manjusha M. Warhadpande, Parag Dua, and Darshan Dakshindas. 2020. "Comparative Evaluation of Clinical Performance of Two Self-Etch Adhesive Systems with Total-Etch Adhesive System in Noncarious Cervical Lesions: An in Vivo Study." *Journal of Conservative Dentistry: JCD* 23 (2): 190–95. https://doi.org/10.4103/JCD.JCD_166_20.
- Dijken, Jan W. V. van, Karin Sunnegårdh-Grönberg, and A. Lindberg. 2007. "Clinical Long-Term Retention of Etch-and-Rinse and Self-Etch Adhesive Systems in Non-Carious Cervical Lesions. A 13 Years Evaluation." *Dental Materials: Official Publication of the Academy of Dental Materials* 23 (9): 1101–7. <https://doi.org/10.1016/j.dental.2006.10.005>.
- Duke, Alice, and Andrew MacInnes. 2021. "What Are the Main Factors Associated with Root Caries?" *Evidence-Based Dentistry* 22 (1): 16–17. <https://doi.org/10.1038/s41432-021-0148-4>.
- Dutra-Correa, Maristela, Vanessa Harumi Kiyam, Marcia Tonetti Ciaramicoli, Vanessa Pecorari, Flávia Pires Rodrigues, and Cintia Helena Coury Saraceni. 2019. "Randomized Clinical Trial of Four Adhesion Strategies: A 42 Month Study." *Indian Journal of Dental Research: Official Publication of Indian Society for Dental Research* 30 (4): 487–95. https://doi.org/10.4103/ijdr.IJDR_466_16.
- Dutra-Correa, Maristela, Cintia Helena Saraceni, Márcia Tonetti Ciaramicoli, Vanessa Harumi Kiyam, and Celso Silva Queiroz. 2013. "Effect of Chlorhexidine on the 18-Month Clinical Performance of Two Adhesives." *The Journal of Adhesive Dentistry* 15 (3): 287–92. <https://doi.org/10.3290/j.jad.a29533>.
- Favetti, M., T. Schroeder, A. F. Montagner, R. R. Moraes, T. Pereira-Cenci, and M. S. Cenci. 2022. "NaOCl Application after Acid Etching and Retention of Cervical Restorations: A 3-Year Randomized Clinical Trial." *Operative Dentistry* 47 (3): 268–78. <https://doi.org/10.2341/20-166-C>.
- Favetti, Morgana, Thaianne Schroeder, Anelise Fernandes Montagner, Marcos Britto Correa, Tatiana Pereira-Cenci, and Maximiliano Sergio Cenci. 2017. "Effectiveness of Pre-Treatment with Chlorhexidine in Restoration Retention: A 36-Month Follow-up Randomized Clinical Trial." *Journal of Dentistry* 60 (May): 44–49. <https://doi.org/10.1016/j.jdent.2017.02.014>.
- Faye, B., M. Sarr, A. W. Kane, B. Toure, F. Leye, F. Gaye, and M. B. Dieng. 2005. "[Prevalence and etiologic factors of non-carious cervical lesions. A study in a Senegalese population]." *Odonto-Stomatologie Tropicale = Tropical Dental Journal* 28 (112): 15–18.

- Fee, Patrick A, Richard Macey, Tanya Walsh, Janet E Clarkson, and David Ricketts. 2020. "Tests to Detect and Inform the Diagnosis of Root Caries." *The Cochrane Database of Systematic Reviews* 2020 (12): CD013806. <https://doi.org/10.1002/14651858.CD013806>.
- Follak, Andressa Cargnelutti, Bruna Dias Ilha, Julia Oling, Thais Savian, Rachel de Oliveira Rocha, and Fabio Zovico Maxnuck Soares. 2021. "Clinical Behavior of Universal Adhesives in Non-Carious Cervical Lesions: A Randomized Clinical Trial." *Journal of Dentistry* 113 (October): 103747. <https://doi.org/10.1016/j.jdent.2021.103747>.
- Gendron, R., D. Grenier, T. Sorsa, and D. Mayrand. 1999. "Inhibition of the Activities of Matrix Metalloproteinases 2, 8, and 9 by Chlorhexidine." *Clinical and Diagnostic Laboratory Immunology* 6 (3): 437–39. <https://doi.org/10.1128/CDLI.6.3.437-439.1999>.
- Gonçalves, Diego Felipe Mardegan, Mirela Sanae Shinohara, Paulo Roberto Marão de Andrade Carvalho, Fernanda de Souza E. Silva Ramos, Laryssa de Castro Oliveira, Érika Mayumi Omoto, and Ticiane Cestari Fagundes. 2021. "Three-Year Evaluation of Different Adhesion Strategies in Non-Carious Cervical Lesion Restorations: A Randomized Clinical Trial." *Journal of Applied Oral Science: Revista FOB* 29: e20210192. <https://doi.org/10.1590/1678-7757-2021-0192>.
- Haak, Rainer, Gesa Stache, Hartmut Schneider, Matthias Häfer, Gerhard Schmalz, and Ellen Schulz-Kornas. 2023. "Effect of the Adhesive Strategy on Clinical Performance and Marginal Integrity of a Universal Adhesive in Non-Carious Cervical Lesions in a Randomized 36-Month Study." *Journal of Clinical Medicine* 12 (18): 5776. <https://doi.org/10.3390/jcm12185776>.
- Hass, Viviane, Andres F. Cartagena, Thalita P. Matos, Jullian J. de Souza, Patricia E. Toyotani, Alessandra Reis, Abraham L. Calixto, and Alessandro D. Loguercio. 2022. "Sonic Application of One-Step Self-Etch Adhesive in Composite Restorations of Non-Carious Cervical Lesions: A Double-Blind Randomized Clinical Trial." *Journal of Esthetic and Restorative Dentistry* 34 (4): 689–98. <https://doi.org/10.1111/jerd.12885>.
- Hazra, Sarbani, Rajdeep Guha, Geram Jongkey, Himangshu Palui, Akhilesh Mishra, Geeta K. Vemuganti, Samar K. Basak, Tapan Kumar Mandal, and Aditya Konar. 2012. "Modulation of Matrix Metalloproteinase Activity by EDTA Prevents Posterior Capsular Opacification." *Molecular Vision* 18 (June): 1701–11.
- Heyder, Markus, Bernd Sigusch, Christoph Hoder-Przyrembel, Juliane Schuetze, Stefan Kranz, and Markus Reise. 2022. "Clinical Effects of Laser-Based Cavity Preparation on Class V Resin-Composite Fillings." *PloS One* 17 (6): e0270312. <https://doi.org/10.1371/journal.pone.0270312>.

- Jf, Araújo, Barros Ta, Braga Em, Loretto Sc, Silva e Souza Pde A, and Silva e Souza Mh. 2013. "One-Year Evaluation of a Simplified Ethanol-Wet Bonding Technique: A Randomized Clinical Trial." *Brazilian Dental Journal* 24 (3). <https://doi.org/10.1590/0103-6440201302128>.
- Lawson, Nathaniel C., Augusto Robles, Chin-Chuan Fu, Chee Paul Lin, Kanchan Sawlani, and John O. Burgess. 2015. "Two-Year Clinical Trial of a Universal Adhesive in Total-Etch and Self-Etch Mode in Non-Carious Cervical Lesions." *Journal of Dentistry* 43 (10): 1229–34. <https://doi.org/10.1016/j.jdent.2015.07.009>.
- Lee, Johnson Chun Ming, Michael Francis Burrow, and Michael George Botelho. 2023. "A Qualitative Analysis of Dentists' Understanding and Management of Non-Carious Cervical Lesions (NCCL)." *Journal of Dentistry* 136 (September): 104640. <https://doi.org/10.1016/j.jdent.2023.104640>.
- Loguercio, Alessandro D., Issis Virginia Luque-Martinez, Sebastian Fuentes, Alessandra Reis, and Miguel Angel Muñoz. 2018. "Effect of Dentin Roughness on the Adhesive Performance in Non-Carious Cervical Lesions: A Double-Blind Randomized Clinical Trial." *Journal of Dentistry* 69 (February): 60–69. <https://doi.org/10.1016/j.jdent.2017.09.011>.
- Loguercio, Alessandro D., Jovani Raffo, Fabrício Bassani, Heloiza Balestrini, Dalvan Santo, Roberto César do Amaral, and Alessandra Reis. 2011. "24-Month Clinical Evaluation in Non-Carious Cervical Lesions of a Two-Step Etch-and-Rinse Adhesive Applied Using a Rubbing Motion." *Clinical Oral Investigations* 15 (4): 589–96. <https://doi.org/10.1007/s00784-010-0408-8>.
- Loguercio, Alessandro D., and Alessandra Reis. 2008. "Application of a Dental Adhesive Using the Self-Etch and Etch-and-Rinse Approaches: An 18-Month Clinical Evaluation." *Journal of the American Dental Association* (1939) 139 (1): 53–61. <https://doi.org/10.14219/jada.archive.2008.0021>.
- Luque-Martinez, Issis, Miguel Angel Muñoz, Alexandra Mena-Serrano, Viviane Hass, Alessandra Reis, and Alessandro D. Loguercio. 2015. "Effect of EDTA Conditioning on Cervical Restorations Bonded with a Self-Etch Adhesive: A Randomized Double-Blind Clinical Trial." *Journal of Dentistry* 43 (9): 1175–83. <https://doi.org/10.1016/j.jdent.2015.04.013>.
- Manarte-Monteiro, Patrícia, Joana Domingues, Liliana Teixeira, Sandra Gavinha, and Maria Conceição Manso. 2019. "Multi-Mode Adhesives Performance and Success/Retention Rates in NCCLs Restorations: Randomised Clinical Trial One-Year Report." *Biomaterial Investigations in Dentistry* 6 (1): 43–53. <https://doi.org/10.1080/26415275.2019.1684199>.

- Manarte-Monteiro, Patricia, Joana Domingues, Liliana Teixeira, Sandra Gavinha, and Maria Conceição Manso. 2021. "Universal Adhesives and Adhesion Modes in Non-Carious Cervical Restorations: 2-Year Randomised Clinical Trial." *Polymers* 14 (1): 33. <https://doi.org/10.3390/polym14010033>.
- Moosavi, H., S. Kimyai, M. Forghani, and R. Khodadadi. 2013. "The Clinical Effectiveness of Various Adhesive Systems: An 18-Month Evaluation." *Operative Dentistry* 38 (2): 134–41. <https://doi.org/10.2341/12-110-CR>.
- Ñaupari-Villasante, Romina, Thalita P. Matos, Elisa Gomes de Albuquerque, Flavio Warol, Chane Tardem, Fernanda Signorelli Calazans, Luiz Augusto Poubel, Alessandra Reis, Marcos Oliveira Barceiro, and Alessandro D. Loguercio. 2023. "Five-Year Clinical Evaluation of Universal Adhesive Applied Following Different Bonding Techniques: A Randomized Multicenter Clinical Trial." *Dental Materials: Official Publication of the Academy of Dental Materials* 39 (6): 586–94. <https://doi.org/10.1016/j.dental.2023.04.007>.
- Ogwo, Chukwuebuka, Grant Brown, John Warren, Daniel Caplan, and Steven Levy. 2023. "Dental Caries Incidence and Associated Factors in Young Adults." *Journal of Public Health Dentistry* 83 (4): 347–54. <https://doi.org/10.1111/jphd.12586>.
- "Oral Health for Older Americans | Adult Oral Health | Basics | Division of Oral Health | CDC." 2023. January 4, 2023. https://www.cdc.gov/oralhealth/basics/adult-oral-health/adult_older.htm.
- Osborne-Smith, K. L., F. J. Burke, and N. H. Wilson. 1999. "The Aetiology of the Non-Carious Cervical Lesion." *International Dental Journal* 49 (3): 139–43. <https://doi.org/10.1002/j.1875-595x.1999.tb00898.x>.
- Oz, Fatma Dilsad, Esra Ergin, and Simge Canatan. 2019. "Twenty-Four-Month Clinical Performance of Different Universal Adhesives in Etch-and-Rinse, Selective Etching and Self-Etch Application Modes in NCCL - a Randomized Controlled Clinical Trial." *Journal of Applied Oral Science: Revista FOB* 27: e20180358. <https://doi.org/10.1590/1678-7757-2018-0358>.
- Paris Matos, Thalita de, Jorge Perdigão, Eloisa de Paula, Fabiana Coppla, Viviane Hass, Rafael F. Scheffer, Alessandra Reis, and Alessandro D. Loguercio. 2020. "Five-Year Clinical Evaluation of a Universal Adhesive: A Randomized Double-Blind Trial." *Dental Materials: Official Publication of the Academy of Dental Materials* 36 (11): 1474–85. <https://doi.org/10.1016/j.dental.2020.08.007>.

- Pena, C. E., J. A. Rodrigues, C. Ely, M. Giannini, and A. F. Reis. 2016. "Two-Year Randomized Clinical Trial of Self-Etching Adhesives and Selective Enamel Etching." *Operative Dentistry* 41 (3): 249–57. <https://doi.org/10.2341/15-130-C>.
- Perdigão, J., M. Dutra-Corrêa, C. H. C. Saraceni, M. T. Ciaramicoli, V. H. Kiyan, and C. S. Queiroz. 2012. "Randomized Clinical Trial of Four Adhesion Strategies: 18-Month Results." *Operative Dentistry* 37 (1): 3–11. <https://doi.org/10.2341/11-222-C>.
- Perdigão, Jorge, André R. P. Carmo, Camillo Anauate-Netto, Ricardo Amore, Hugo R. Lewgoy, Hiram João D. Cordeiro, Maristela Dutra-Corrêa, and Natália Castilhos. 2005. "Clinical Performance of a Self-Etching Adhesive at 18 Months." *American Journal of Dentistry* 18 (2): 135–40.
- Perdigão, Jorge, Laura Ceballos, Isabel Giráldez, Bruno Baracco, and Ma Victoria Fuentes. 2020. "Effect of a Hydrophobic Bonding Resin on the 36-Month Performance of a Universal Adhesive—a Randomized Clinical Trial." *Clinical Oral Investigations* 24 (2): 765–76. <https://doi.org/10.1007/s00784-019-02940-x>.
- Peumans, M., J. De Munck, K. Van Landuyt, and B. Van Meerbeek. 2015. "Thirteen-Year Randomized Controlled Clinical Trial of a Two-Step Self-Etch Adhesive in Non-Carious Cervical Lesions." *Dental Materials: Official Publication of the Academy of Dental Materials* 31 (3): 308–14. <https://doi.org/10.1016/j.dental.2015.01.005>.
- Qutieshat, Abubaker, Abdurahman Salem, Rayhana Aouididi, Juliana Delatorre Bronzato, Haider Al-Waeli, Mousa Abufadallah, Saleem Shaikh, et al. 2021. "Perspective and Practice of Root Caries Management: A Multicountry Study – Part I." *Journal of Conservative Dentistry : JCD* 24 (2): 141–47. https://doi.org/10.4103/jcd.jcd_19_21.
- Ranjitha, G. R., R. Vikram, N. Meena, L. Vijayalakshmi, and Chethana S. Murthy. 2020. "Clinical Efficacy of Universal Adhesives for the Restoration of Noncarious Cervical Lesions: A Randomized Clinical Trial." *Journal of Conservative Dentistry: JCD* 23 (3): 227–32. https://doi.org/10.4103/JCD.JCD_51_20.
- Reddy, Lingaladinne Swetha, Satti Venkata Lakshmi, Yenugandula Vijaya Lakshmi, K Priya Deepa Lakshmi, Yadlapalli Sravanthi, and Manupreet Kaur. 2021. "Root Caries Experience and Its Association with Risk Indicators among Middle-Aged Adults." *Journal of Pharmacy & Bioallied Sciences* 13 (Suppl 2): S1523–29. https://doi.org/10.4103/jpbs.jpbs_271_21.
- Reis, Alessandra, Tatiana Moroz Leite, Karina Matte, Roberto Michels, Roberto C. Amaral, Saulo Geraldeli, and Alessandro Dourado Loguercio. 2009. "Improving Clinical

- Retention of One-Step Self-Etching Adhesive Systems with an Additional Hydrophobic Adhesive Layer.” *Journal of the American Dental Association* (1939) 140 (7): 877–85. <https://doi.org/10.14219/jada.archive.2009.0281>.
- Rocha, A. C., Mm Suca Salas, A. S. Masotti, Wlo da Rosa, C. H. Zanchi, and R. G. Lund. 2019. “A Randomized Double-Blind Clinical Trial of Dentin Surface Treatments for Composite Restorations in Noncarious Cervical Lesions: A 36-Month Evaluation.” *Operative Dentistry* 44 (2): 114–26. <https://doi.org/10.2341/17-308-C>.
- Rocha Gomes Torres, Carlos, Daphne Camara Barcellos, Graziela Ribeiro Batista, César Rogério Pucci, Maria Julia Sala Antunes, Daniela Barone de La Cruz, and Alessandra Bühler Borges. 2014. “Five-Year Clinical Performance of the Dentine Deproteinization Technique in Non-Carious Cervical Lesions.” *Journal of Dentistry* 42 (7): 816–23. <https://doi.org/10.1016/j.jdent.2014.04.004>.
- Rouse, Matthew A., Jaren T. May, Jeffrey A. Platt, N. Blaine Cook, Oriana R. Capin, Brooke N. Adams, Michele L. Kirkup, and Kim E. Diefenderfer. 2020. “Clinical Evaluation of a Universal Adhesive in Non-Carious Cervical Lesions.” *Journal of Esthetic and Restorative Dentistry: Official Publication of the American Academy of Esthetic Dentistry ... [et Al.]* 32 (7): 691–98. <https://doi.org/10.1111/jerd.12622>.
- Saboia, Vicente de Paulo Aragão, Paulo César Almeida, André V. Rittet, Edward J. Swift, and Luiz André Freire Pimenta. 2006. “2-Year Clinical Evaluation of Sodium Hypochlorite Treatment in the Restoration of Non-Carious Cervical Lesions: A Pilot Study.” *Operative Dentistry* 31 (5): 530–35. <https://doi.org/10.2341/05-119>.
- Sartori, N., L. D. Peruchi, J. C. Guimarães, S. B. Silva, S. Monteiro, L. N. Baratieri, and R. Belli. 2013. “Clinical Effectiveness of a Hydrophobic Coating Used in Conjunction with a One-Step Self-Etch Adhesive: An 18-Month Evaluation.” *Operative Dentistry* 38 (3): 249–57. <https://doi.org/10.2341/12-014-C>.
- Sartori, Neimar, Sheila C. Stolf, Silvana B. Silva, Guilherme C. Lopes, and Marcela Carrilho. 2013. “Influence of Chlorhexidine Digluconate on the Clinical Performance of Adhesive Restorations: A 3-Year Follow-Up.” *Journal of Dentistry* 41 (12): 1188–95. <https://doi.org/10.1016/j.jdent.2013.09.004>.
- Shinohara, Mirela Sanae, Paulo Roberto Marão de Carvalho, Laercio Neves Marcon, Diego Felipe Mardegan Gonçalves, Fernanda de Souza E. Ramos, and Ticiane Cestari Fagundes. 2020. “Randomized Clinical Trial of Different Adhesion Strategies in Noncarious Cervical Lesion Restorations: 1-Year Follow-Up.” *Quintessence International (Berlin, Germany)* 51 (5): 352–63. <https://doi.org/10.3290/j.qi.a44367>.

- Smith, W. a. J., S. Marchan, and R. N. Rafeek. 2008. "The Prevalence and Severity of Non-Carious Cervical Lesions in a Group of Patients Attending a University Hospital in Trinidad." *Journal of Oral Rehabilitation* 35 (2): 128–34. <https://doi.org/10.1111/j.1365-2842.2007.01763.x>.
- Souza, Lidiane Costa de, Nara Sousa Rodrigues, Diana Araújo Cunha, Victor Pinheiro Feitosa, Sérgio Lima Santiago, Alessandra Reis, Alessandro D. Loguercio, Jorge Perdigão, and Vicente de Paulo Aragão Saboia. 2020. "Two-Year Clinical Evaluation of a Proanthocyanidins-Based Primer in Non-Carious Cervical Lesions: A Double-Blind Randomized Clinical Trial." *Journal of Dentistry* 96 (May): 103325. <https://doi.org/10.1016/j.jdent.2020.103325>.
- Tan, Haiping, Lindsay Richards, Tanya Walsh, Helen V Worthington, Jan E Clarkson, Linda Wang, and Marília Mattar de Amoedo Campos Velo. 2017. "Interventions for Managing Root Caries." *The Cochrane Database of Systematic Reviews* 2017 (8): CD012750. <https://doi.org/10.1002/14651858.CD012750>.
- Teixeira, Daniela Navarro Ribeiro, Renske Z. Thomas, Paulo Vinicius Soares, Marco S. Cune, Marco M. M. Gresnigt, and Dagmar Else Slot. 2020. "Prevalence of Noncarious Cervical Lesions among Adults: A Systematic Review." *Journal of Dentistry* 95 (April): 103285. <https://doi.org/10.1016/j.jdent.2020.103285>.
- Tokumoto, Kana, Aya Kimura-Ono, Takuya Mino, Suguru Osaka, Ken Numoto, Eri Koyama, Yoko Kurosaki, et al. 2022. "Risk Factors for Root Caries Annual Incidence and Progression among Older People Requiring Nursing Care: A One-Year Prospective Cohort Study." *Journal of Prosthodontic Research* 66 (2): 250–57. https://doi.org/10.2186/jpr.JPR_D_20_00272.
- Tomasik, Małgorzata. 2006. "[Analysis of etiological factors involved in noncarious cervical lesions]." *Annales Academiae Medicae Stetinensis* 52 (3): 125–36.
- Tonetti, Maurizio S., Peter Bottenberg, Georg Conrads, Peter Eickholz, Peter Heasman, Marie-Charlotte Huysmans, Rodrigo López, et al. 2017. "Dental Caries and Periodontal Diseases in the Ageing Population: Call to Action to Protect and Enhance Oral Health and Well-Being as an Essential Component of Healthy Ageing - Consensus Report of Group 4 of the Joint EFP/ORCA Workshop on the Boundaries between Caries and Periodontal Diseases." *Journal of Clinical Periodontology* 44 Suppl 18 (March): S135–44. <https://doi.org/10.1111/jcpe.12681>.
- Van Meerbeek, Bart, Padmini Kanumilli, Jan De Munck, Kirsten Van Landuyt, Paul Lambrechts, and Marleen Peumans. 2005. "A Randomized Controlled Study Evaluating the Effectiveness of a Two-Step Self-Etch Adhesive with and without Selective

- Phosphoric-Acid Etching of Enamel.” *Dental Materials: Official Publication of the Academy of Dental Materials* 21 (4): 375–83.
<https://doi.org/10.1016/j.dental.2004.05.008>.
- Verma, Sanjana, Rakesh Singla, Gurdeep Singh Gill, and Namita Jain. 2023. “Effect of Dentin Roughening and Type of Composite Material on the Restoration of Non-Carious Cervical Lesions: An in Vivo Study with 18 Months of Follow-Up.” *Restorative Dentistry & Endodontics* 48 (4): e35. <https://doi.org/10.5395/rde.2023.48.e35>.
- Warreth, Abdulhadi, Eyas Abuhijleh, Mohammad Adel Almaghribi, Ghanim Mahwal, and Ali Ashawish. 2020. “Tooth Surface Loss: A Review of Literature.” *The Saudi Dental Journal* 32 (2): 53–60. <https://doi.org/10.1016/j.sdentj.2019.09.004>.
- Yang, J., D. Cai, F. Wang, D. He, L. Ma, Y. Jin, and K. Que. 2016. “Non-Carious Cervical Lesions (NCCLs) in a Random Sampling Community Population and the Association of NCCLs with Occlusive Wear.” *Journal of Oral Rehabilitation* 43 (12): 960–66.
<https://doi.org/10.1111/joor.12445>.
- Zanatta, R. F., T. M. Silva, Malr Esper, E. Bresciani, Sep Gonçalves, and Tmf Caneppele. 2019. “Bonding Performance of Simplified Adhesive Systems in Noncarious Cervical Lesions at 2-Year Follow-up: A Double-Blind Randomized Clinical Trial.” *Operative Dentistry* 44 (5): 476–87. <https://doi.org/10.2341/18-049-C>.
- Zander-Grande, C., R. C. Amaral, A. D. Loguercio, L. P. Barroso, and A. Reis. 2014. “Clinical Performance of One-Step Self-Etch Adhesives Applied Actively in Cervical Lesions: 24-Month Clinical Trial.” *Operative Dentistry* 39 (3): 228–38. <https://doi.org/10.2341/12-286-C>.
- Zander-Grande, Christiana, Sabrina Queji Ferreira, Thays Regina Ferreira da Costa, Alessandro Dourado Loguercio, and Alessandra Reis. 2011. “Application of Etch-and-Rinse Adhesives on Dry and Rewet Dentin under Rubbing Action: A 24-Month Clinical Evaluation.” *Journal of the American Dental Association (1939)* 142 (7): 828–35.
<https://doi.org/10.14219/jada.archive.2011.0272>.
- Zhang, Jingyang, Katherine C. M. Leung, Divesh Sardana, May C. M. Wong, and Edward C. M. Lo. 2019. “Risk Predictors of Dental Root Caries: A Systematic Review.” *Journal of Dentistry* 89 (October): 103166. <https://doi.org/10.1016/j.jdent.2019.07.004>.