World Academy of Science, Engineering and Technology
International Journal of Medical and Health Sciences
Vol:17, No:4, 2023

Effects of Chlorhexidine in Application to Hybrid Layers

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***Abstract*—**The hybrid layer (HL), the way it is created and how it is protected against degradation over time, is the key to the clinical success of a composite restoration. The composite supports the dentinal structure exactly with the realized surface of micro-retention. Thus, this surface is in direct proportion to its size versus the duration of clinical use of composite dental restoration. Micro-retention occurs between dentin or acidified enamel and adhesive resin extensions versus pre-prepared spaces, such as hollow dentinal tubules. The way the adhesive resin binds to the acidified dentinal structure depends on the physical or chemical factors of this interrelationship between two structures with very different characteristics. During the acidification process, a precursor to the placement of the adhesive resin layer, activation of metalloproteinases of dental origin occurs, enzymes which are responsible for the degradation of the HL. These enzymes have expressed activity depending on the presence of Zn^{2+} or Ca^{2+} ions. There are several ways to inhibit these enzymes, and consequently, there are several ways to inhibit the degradation process of the HL. The study aim is to evaluate chlorhexidine (CHX) as a solution element, inhibitor of dentin activated metalloproteinases, as a result of the application of acidification. This study aims to look at this solution in advantage or contraindication theories, already published in the literature.

***Keywords*—**Hybrid layer, chlorhexidine, degradation, smear layer.

I. Background

THE composition of dentin from the fusion of hydroxyapatite nanocrystals with the collagen matrix, is actually a combination that has biological viability. This composition of biological nature also has a well-distinguishable microscopic structure, which with stands attacks of chemical or physical nature of the elements causing degradation.

Degradation of HL is performed by enzymes located in the dentinal matrix, which from the same elements of physical or chemical nature, are stimulated to activate the present enzyme [1]-[7].

The structure of dentin is based on dentinal tubules with Thomson fibers that fluctuate in the aquatic environment where they are immersed. The number of these fibers per dentine surface unit is different, depending on whether it is clinical crown dentin, or of the tooth cervix [7]-[10].

The organic composition of dentin is higher than the organic composition of cement and enamel. This element makes dentin more sensitive to the decomposition of essential content that has stability based on the biological balance of the constituent elements. It is more sensitive, as part of the HL, to degradation enzymes of a dissociative nature, in cases where these enzymes undergo intentional activation or not [11]-[13]. The HL is not just a combination or physical union of two elements together, but an adhesion of two substances that manage to create a micro-tensioned physical fixation of resin extensions in liquid form and open dentinal tubules through the acidification process. After polymerization, it is the resin that hardens, thus inserted inside the empty spaces of the dentinal tubules, to remain fixed there and with the primary purpose, to withstand the mechanical forces of the composite fillings [14]-[20].

The hybrid layer (HL) is described as the "Achilles heel" for restorative dentistry using the composite as a filler [1], [8]. The HL is composed of that part of the dentin prepared for the cavity that undergoes acidification according to predetermined acidification protocols. Recall that we acidify not only the prepared dentinal walls, but also the dentinal chips left over from the preparation, which form the smear layer before the application of acidification [21]-[25]. Wetting of these areas with bonding resin is performed according to bond application protocols; this always depends on the type of bond applied to carry out the restoration. And if degradation of the HL occurs, the stimulus comes from the activation of endogenous metalloproteinases, inside the dentinal structure, which destroys the microretension of the filling realized with the composite. The larger the surface to be acidified or bonded, the larger the surface area of the HL created and the higher the micro-stress created by the filling [22], [23]-[29].

The study focuses on collecting already published data on the effect of CHX on inhibiting HL degradation. Application of this solution either simple in composition, or as part of a primer, draws attention to the fact that as CHX solution is used in different concentrations and for different treatment purposes. This is the element that attracts attention with the ups and downs in the appearance of the advantages and disadvantages of applying this solution [1], [3], [30]-[38].

The collection of information should be oriented around finding the trend of scientific research in this regard, towards how these data are required through the results according to studies of the review type, or studies based on experiments performed *in vitro* or *in vivo* on permanent teeth during composite restorations [38]-[43]. Electronic research was done at PubMed using several keywords with the aim of finding articles about this topic [44]-[94].

II. Methods

Combination of keywords done with several stages is how

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the electronic research was done within time period of 15 years. First stage included the finding of key terms, derived from articles that talked about the dentin HL and the reducing ability of collagen degradation, with an indication for treatment protocol. These terms, in the MESH database on the PubMed site, were:

1. Dental hybrid layer (940)
2. Adaptation (60)
3. Smear layer (7).

Second stage: When we tried to talk specifically about the interconnection of acidification and the process of application of adhesive materials, with a tendency towards knowledge of the morphology of the adhesive interface, it was noticed that the number of items involved was reduced, even with coverage and repetition, specifically divided as follows:

1. Acidification (813)
2. Morphology of the adhesion interface (65)
3. Smear layer (7).

After the electronic search of this stage, the number 5 of the selected articles was reached (1 article with coverage from the first stage).

Stage Three: Specific terms about HL degradation prevention strategies were applied at this stage, with the aim of orienting around dentin biomodification agents. The key words for this stage were:

1. Dental adhesive layer (1332)
2. Application protocol (37)
3. Solution (6).

From the electronic search was reached the selection of 6 items.

Stage Four: At this stage specific terms were applied to HL degradation prevention strategies, with the aim of orienting around dental biomodification agents. These keywords, in the MESH database on the PubMed site, were:

1. Dental hybrid layer (940)
2. Collagen Degradation (104)
3. Dental biomodification (4)

At this stage, 4 items were collected.

Phase Five: In this phase we tried to overlap the fields containing the keywords of the previous stages, to mechanically find any possible correlation between the two processes of HL adaptation and that of the same layer degradation, the fact previously published. The keywords for this inclusion were as follows:

1. Hybrid layer (15276)
2. Collagen Degradation (272)
3. Dental solutions (13).

After the electronic control, 13 items were collected, two of which were included in the other phases of the control.

In the end, 44 articles were selected for the discussion part of the study. Table II presents all the articles collected in order to see the trend of scientific research over the years about CHX as an inhibitory solution of MMPs.

TABLE I
COLLECTION OF DATA ON REVIEW TYPE ARTICLES ABOUT THE TECHNIQUE OF APPLICATION OF CHX TO THE HL, SEEN FROM THE POINT OF VIEW OF MACRO AND MICROSCOPIC CHANGES THAT IT PRESENTS

Chlorhexidine	Macroscopic changes	Microscopic changes
Instant clinical effect	Changes in mechanical properties of protein and non- protein structure [53] Complete resin infiltration [59] Needed a refined clinical protocol [56] Incomplete removal of water after bonding [53]	Degradation of collagen fibrils [59]
0.2-2% of CHX	CHX 2% increases bond strength [58] There is no relationship between CHX concentration and bond strength [66], [62]	
Self-acidification (acid primer) Acid-phosphoric acid rinse 37%	Both methods activate MMP - matrix metalloproteinase [67] CHX 2% and phosphoric acid need long-term clinical results [58], [59]	
Bond strength	Incomplete resin impregnation [82], [67], [53] CHX application provides long-term bond strength [62]	MMP activation-collagen integrity [63], [67] Destruction of collagen fibers [58] 37% Phosphoric Acid and Acid Bonding Components [59]
6-12 months 0.5-5 years	Retention loss after 6-36 months [54] CHX and phosphoric acid 0.5-5 years [59] After 6-12months the bond strength decreases 30% to 40% [82] Bond strength is lost for 0.5-5 years [59] Hypersensitivity, secondary caries [59]	

TABLE II
ITEMS SELECTED FOR INCLUSION IN THIS STUDY, DEPENDING ON THE TYPE OF PAPER PRESENTED BY THEM

Year of publication \ Article type	Review		*in vitro*		*in vivo*	
	Chlorhexidine	Control group	Chlorhexidine	Control group	Chlorhexidine	Control group
2005-2009	1	-	1	1	2	2
2010-2014	4	1	1	10	-	-
2015-2019	1	2	2	6	-	1
2020-2021	1	-	-	2	-	1
Total	7	3	4	19	2	4

The Local Ethics Committee ruled that no formal ethics approval was required in this particular case. This study was submitted to and approved by Albanian University Institutional Ethics Committee, date 07.07.2022, Tirana, Albania, according to national regulations.

Zhang et al. [67] in the meta-analysis on the application of

CHX throw as an idea that CHX is the most potential way of inhibiting collagen degradation assessed on bond strength.

Ease of application or adding application stages is another element that according to Tjäderhane et al. in 2013 calls it as advantage of CHX [56].

For different percentages of CHX at application time, 2% was the right concentration, which is refuted by Collares et al. [62]; they stated that CHX % and bond strength do not correlate linearly. Long-term evaluation is up to 5 years after CHX application.

Table II lists all the articles on *in vitro* experiments on the application of CHX and its effect on HL degradation.

If we analyze the data of Table II we will come to some summaries of opinions and conclusions that somehow confront the data collected from the review type articles.

Some of the conclusions are listed below:

- Application protocol: acid rinse CHX 0.2% gluconate as conditioner solution or CHX 2% for 30 seconds: HLs are indisputable conditioners and application conditions referred to by the manufacturing plants of these materials.
- CHX increases the hardness of the resin that penetrates deeper and more easily into the dentinal tubules.
- CHX reduces nanofluids that other MMP-inhibitors do not have, or have to a lesser extent.
- Among MMP inhibitors, CHX both 2% and 0.2% is effective in inhibiting HL degradation, but no more effective with increasing %.
- Application of chlorhexidine is better in case when its application is done as part of controlled release systems after acidification as a primer, or as an integral part of the adhesive resin.
- CHX when applied in different percentages, stays in the hybrid layer even after 10 years in HL.

MMPs are the enzymes depended from Zn and Ca ions. In carious dentin MMP activity is 10 times higher than in intact dentin.

During *in vivo* studies, the clinical evaluation of microtensile hardness of bond concludes that 2% CHX digluconate after acidification with 37% phosphoric acid and before the adhesive, is an effective treatment according to controls from 1 week to 36 months post-treatment.

2% CHX digluconate initially was applied *in vivo* at cases of caries media, Class I, and then to cervical fillings with composite, during a clinical case part of a study dating in 2017. Clinical data of 2005-2009 showed that 2% CHX digluconate is more effective clinically as it maintains the integrity of the HL. But this opinion changes, in cases of the application of 2% CHX digluconate at cervical fillings. This application does not bring clinical advantages.

III. Conclusions

From the analysis of the collected information, it is clear that the way of application of CHX has a predetermined working protocol, which includes the elements of what precursor phase is indicated or contraindicated in cases of application of this solution. Already, there is well-documented data that classify this solution as more advantageous than another metalloproteinase inhibitor.

Although there are contradictory attitudes within the evolution of adhesive resins, but these attitudes have never reduced or rejected the importance of CHX as a solution that prevents the degradation of the HL.

Declarations

Ethics Approval and Consent to Participate

Not Applicable.

Consent for Publication

Not Applicable.

Availability of Data and Materials

The datasets analyzed during the current study are available from the corresponding author.

Competing Interests

The authors declare that they have no competing interests.

Funding

No funding was obtained for this study.

Author Contributions

I.R. collected the scientific data and wrote the manuscript. S.H. revised and edited the manuscript. Literature research was conducted by S.H. and I.R. E.H. and V.O. collected the scientific data. All authors read and approved the final manuscript.

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