

AUS DEM LEHRSTUHL FÜR ZAHNERHALTUNG UND
PARODONTOLOGIE
PROF. DR. WOLFGANG BUCHALLA
DER FAKULTÄT FÜR MEDIZIN
DER UNIVERSITÄT REGENSBURG

Randdichtigkeit von Klasse-II-Komposit-Restaurationen
nach selektiver und vollständiger Kariesexkavation *in vitro*

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Zahnmedizin

der Fakultät für Medizin
der Universität Regensburg

vorgelegt von
Michael Hinderberger

2020

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Bei der vorliegenden Promotionsschrift handelt es sich um eine Publikationspromotion, die wie folgt veröffentlicht wurde (siehe Anhang):

Konstantin J. Scholz*, Michael Hinderberger*, Matthias Widbiller, Marianne Federlin, Karl-Anton Hiller, Wolfgang Buchalla:

Influence of selective caries excavation on marginal penetration of class II composite restorations in vitro.

Eur J Oral Sci 2020: 2020 Aug 4. doi: 10.1111/eos.12726. Online ahead of print.

(Impact Factor 2019: 2.22)

(* = gleichberechtigte Erstautorenschaft)

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Tag der mündlichen Prüfung: 25.01.2021

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1. Einführung

Lange Zeit galt die komplette Exkavation als die Methode der Wahl zur Behandlung kariöser Läsionen vor der direkten Füllungstherapie (1). Um mehr Zahnhartsubstanz zu erhalten, wurden Behandlungsalternativen zur vollständigen Exkavation eingeführt, wie die Versiegelung, die Infiltration kariöser Läsionen sowie die selektive Kariesentfernung im pulpanahen Bereich (2). Bei letzterer wird auf die komplette Entfernung des kariösen Dentins zugunsten einer Vermeidung der Eröffnung der Pulpa verzichtet (3,4). Klinische Studien zeigen vielversprechende Ergebnisse zur teilweisen Kariesentfernung sowie einer ultrakonservativen Therapie, bei der kein Dentin exkaviert und lediglich eine Schmelzanschrägung vor der Restauration durchgeführt wurde (5–7).

Ein möglicher Nachteil der selektiven Kariesentfernung ist der Umstand, dass mit dem Zurücklassen von weichem Dentin schlechtere mechanische Eigenschaften des adhäsiven Verbundes und damit auch der Restauration einhergehen könnten (8,9). Isolan et al. zeigten in einer Meta-Analyse, dass kariös verändertes Dentin im Vergleich zu gesundem Dentin allgemein niedrigere Haftwerte – unabhängig vom adhäsiven Ansatz – aufweist (10). Inwieweit dies einen relevanten Einfluss auf den Gesamtverbund einer adhäsiven Restauration hat, ist bislang allerdings unklar. Ebenso ist nicht bekannt, ob das Belassen erweichten Dentins die Randsichtigkeit adhäsiver Restaurationen beeinflusst. Weiterhin stellen größere Mengen zurückgelassener pathogener Mikroorganismen ein Risiko für eine Infektion der Pulpa dar. Gleichzeitig begünstigen sie auch das Fortschreiten einer kariösen Läsion, falls diese nicht erfolgreich versiegelt wird (8,9,11). In einer randomisierten klinischen Studie wiesen Kavitäten nach selektiver Exkavation im Vergleich zur kompletten Exkavation eine höhere initiale Bakterienlast auf. Nach drei Monaten jedoch zeigte sich zum einen ein Rückgang der Anzahl an lebenden Mikroorganismen im Allgemeinen als auch von Streptokokken und Laktobazillen im Speziellen. Zum anderen konnte kein signifikanter Unterschied der Anzahl kultivierbarer anaerober Bakterien, Streptokokken-Spezies und Laktobazillen-Spezies zwischen den beiden Exkavationsstrategien mehr festgestellt werden (12). Die Arretierung der Karies durch eine dichte Verbindung zwischen Zahn und Restauration ist ein zentrales Element der selektiven Kariesexkavation. Eine *in vivo* Studie wies drei bis vier Monate nach Anwendung dieser Behandlungsstrategie Dentin nach, welches sowohl dunkler als auch härter als direkt nach der Exkavation war (13).

Das Ziel dieser *in vitro* Studie war daher, die Auswirkung der kompletten und selektiven Exkavation von künstlich erzeugter Karies auf die Stabilität adhäsiver Kompositfüllungen zu

vergleichen. Untersucht wurde dabei der Einfluss der Exkavationsstrategie auf die proximale und okklusale Randdichtigkeit von Kompositfüllungen in Klasse-II-Kavitäten in extrahierten humanen Oberkiefermolaren.

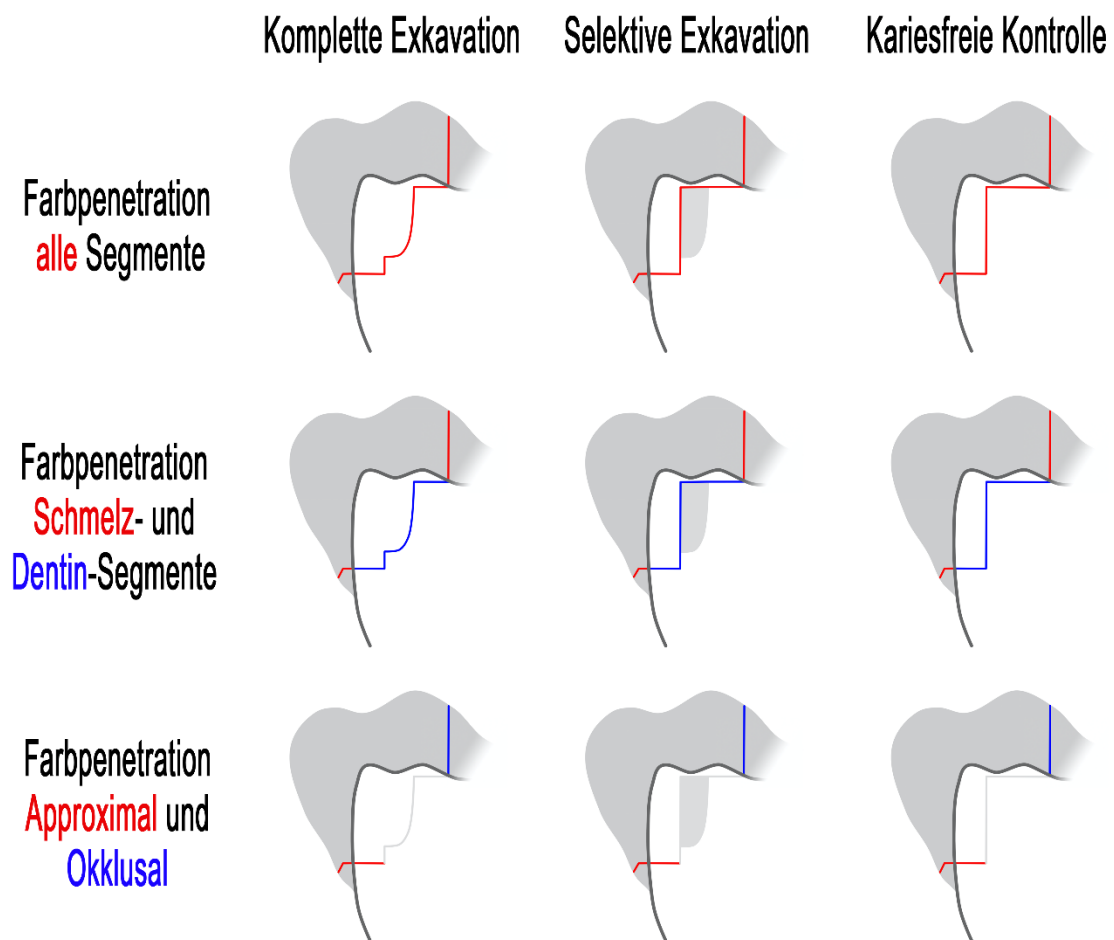
Die Hypothese dieser Studie war, dass zurückgelassenes demineralisiertes Dentin keinen Einfluss auf die marginale Integrität und damit den Erfolg von Kompositrestaurationen hat.

2. Material und Methoden

Im Rahmen dieser Studie wurden in 40 extrahierten menschlichen Oberkiefermolaren mithilfe einer EDTA-Lösung (0,5M; pH 7,0) in einem standardisierten Demineralisationsprotokoll approximale Läsionen erzeugt. Nach normierter Präparation schmelzbegrenzter Kavitäten wurden die Läsionen unter Verwendung eines Mikro-Computertomographen (phoenix v|tome|x^s 240/180 research edition, General Electric Measurement & Control, Wunstorf, Germany) vermessen und die Kavitäten anhand der Exkavationsstrategie in drei Gruppen eingeteilt. Bei der kompletten Exkavation wurden die demineralisierten Bereiche mit Hilfe eines selbstlimitierenden Polymerrosenbohrers vollständig exkaviert. Bei der selektiven Exkavation wurden die nach standardisierter Präparation verbliebenen demineralisierten Bereiche belassen. Die kariesfreie Kontrolle stellte eine Kontrollgruppe dar, deren Kavitätengeometrie der der selektiven Exkavation entsprach. In jedem Zahn wurden Kavitäten von zwei unterschiedlichen Gruppen angelegt. Nach adhäsiver Restauration (selektive Schmelzätzung, Self-Etch-Adhäsiv und Nanofüller-Komposit) wurde die Hälfte der Zähne einer thermomechanischen Wechselbelastung ausgesetzt (TCML; 5.000 Zyklen zu je 30 Sekunden bei 5 - 55°C und 500.000 Zyklen bei 72,5 N, 1,6Hz) (14,15). Die Randdichtigkeit wurde nach einer Silbernitrat-Farbpenetrationsanalyse in mesio-distaler Schnittrichtung bewertet (6 – 14 Schnittbilder pro Zahn, Median 9 Schnittbilder; s. Abbildung 1). Dabei wurden die Grenzflächen in Segmente eingeteilt und innerhalb eines Zahnes der größte Farbpenetrationswert je Segment als Auswertungskriterium herangezogen. Die erhaltenen Daten wurden nicht-parametrisch analysiert. Der Mann-Whitney U-Test wurde für paarweise Vergleiche verwendet (Signifikanzniveau $\alpha = 0,05$). Um den Einfluss von TCML zu beurteilen, wurde das Signifikanzniveau auf $\alpha * (k) = 1 - (1 - \alpha)^{1/k}$ mithilfe der Error Rates Methode angepasst ($k =$ Anzahl der durchgeführten gepaarten Tests).

Abbildung 1 Auswertung der Grenzfläche zwischen Restauration und Zahn

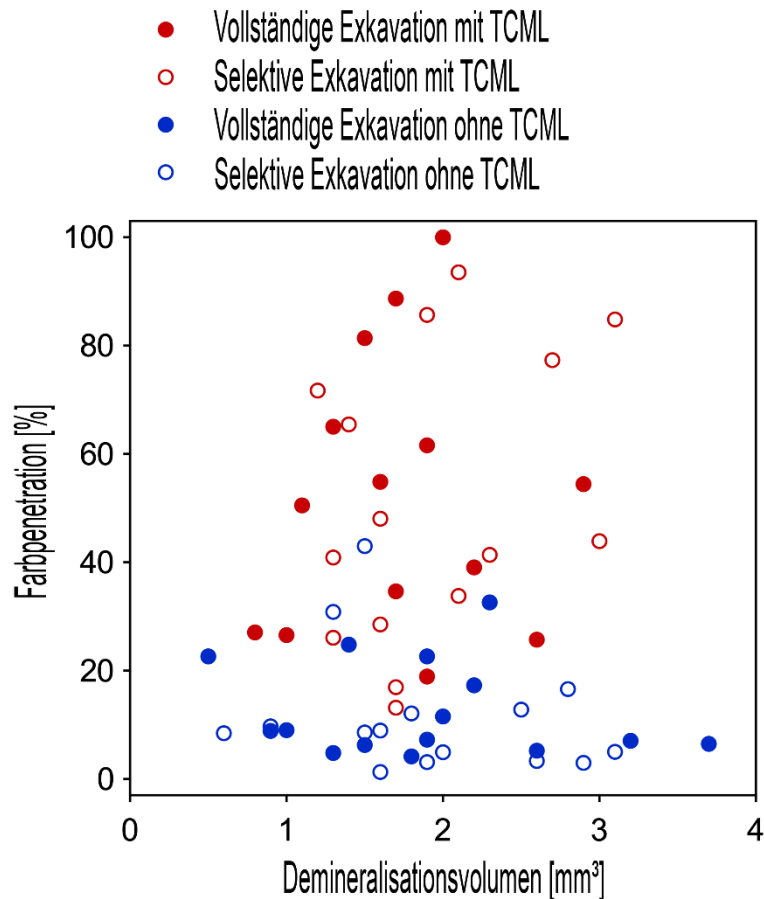
Die obere Reihe zeigt die Betrachtung aller Segmente (Ergebnisse in Abbildung 3), die mittlere die Einteilung nach Zahnschubstanz (Schmelzsegmente rot, Dentinsegmente blau; Auswertung in Abbildung 4) und die untere nach Lokalisierung des Restaurationsrandes (okklusal (Schmelz) blau, approximal (Schmelz und Dentin) rot); Auswertung in Abbildung 5. Bei der selektiven Exkavationsstrategie wurde zudem die Grenzfläche zwischen demineralisiertem und gesundem Dentin im pulpoaxialen Bereich evaluiert. In jedem Schnittbild wurde jede identifizierbare Grenze ausgewertet.



3. Ergebnisse

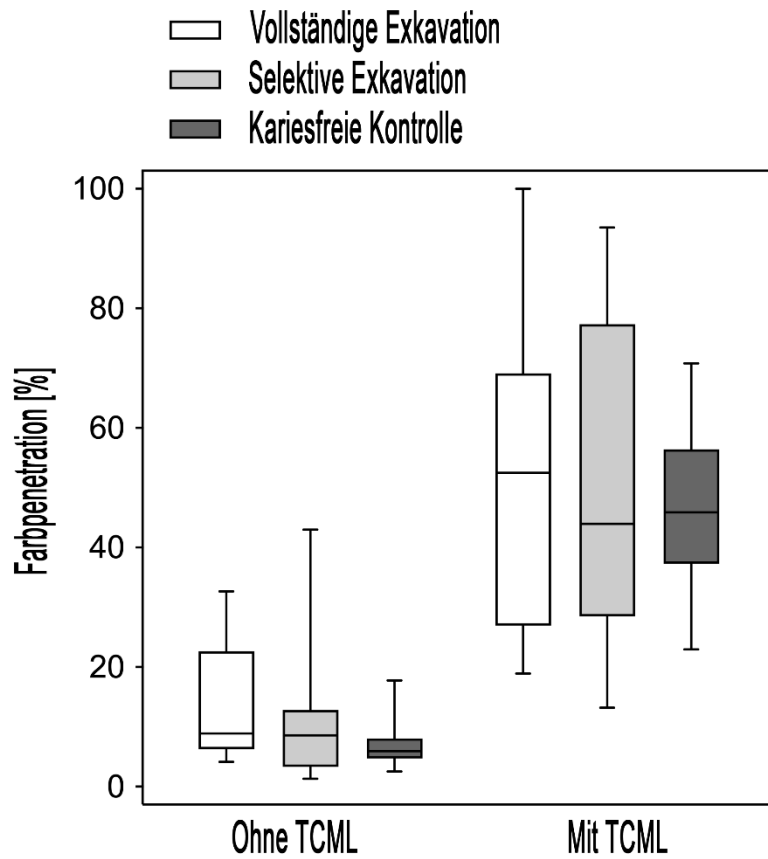
Der Median des Volumens (25% - 75% Perzentil) der künstlichen Demineralisation betrug 1,8 (1,4 - 2,3) mm³. Lineare Regressionskoeffizienten in einem Bereich zwischen 0,004 bis 0,11 zeigten keine Korrelation zwischen dem Volumen der demineralisierten Zahnhartsubstanz und dem Ausmaß der Farbpenetration aller Segmente (Abbildung 2). Der Median der Ausdehnung (25% - 75% Perzentile) der präparierten Kavitäten in mesio-distaler Richtung war für die komplette Exkavation 2,8 (2,7 - 3,1) mm, für die selektive Exkavation 2,6 (2,2 - 3,0) mm und für die kariesfreie Kontrolle 2,7 (2,5 - 3,0) mm.

Abbildung 2 Farbpenetration aller Segmente (in Prozent; komplette und selektive Exkavation, mit und ohne TCML) aufgetragen gegen das Volumen des demineralisierten Dentins nach Mikro-CT Untersuchung. Jeder Punkt stellt eine Kavität dar.



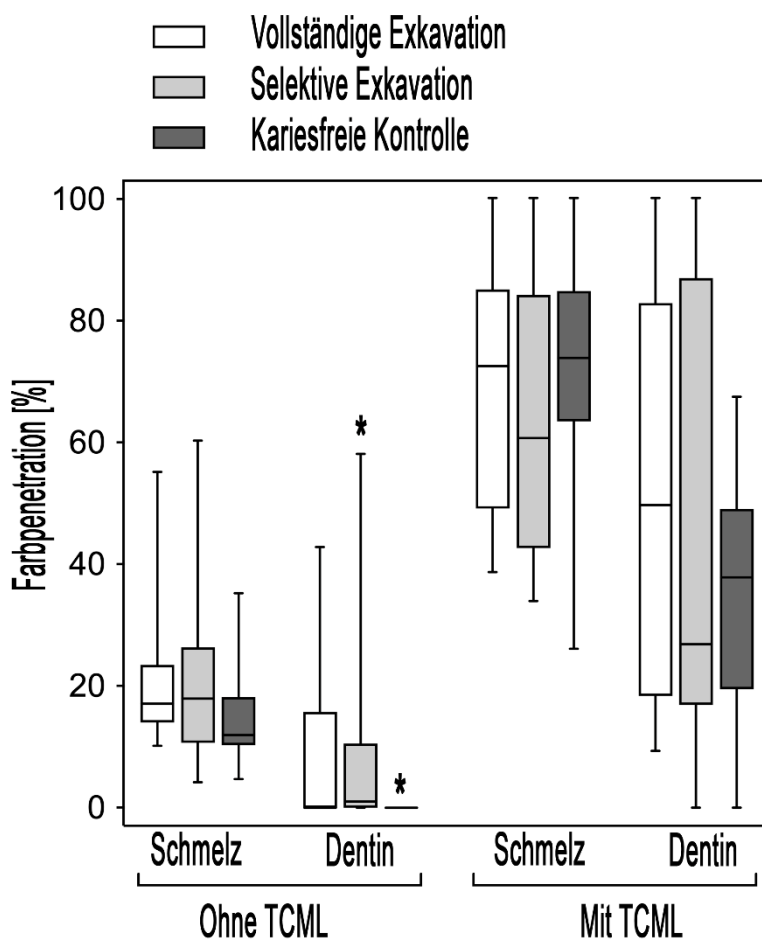
Die Farbpenetration ohne TCML über alle Strecken betrug $\leq 8,9\%$ in allen Gruppen und bewegte sich mit TCML im Median zwischen 43,9% und 52,5% (Abbildung 3). Es zeigte sich kein statistisch signifikanter Unterschied zwischen den Gruppen.

Abbildung 3 Farbpenetrationswerte aller Segmente mit und ohne TCML (Maximum, Minimum, Median, 25% - 75% Perzentil)



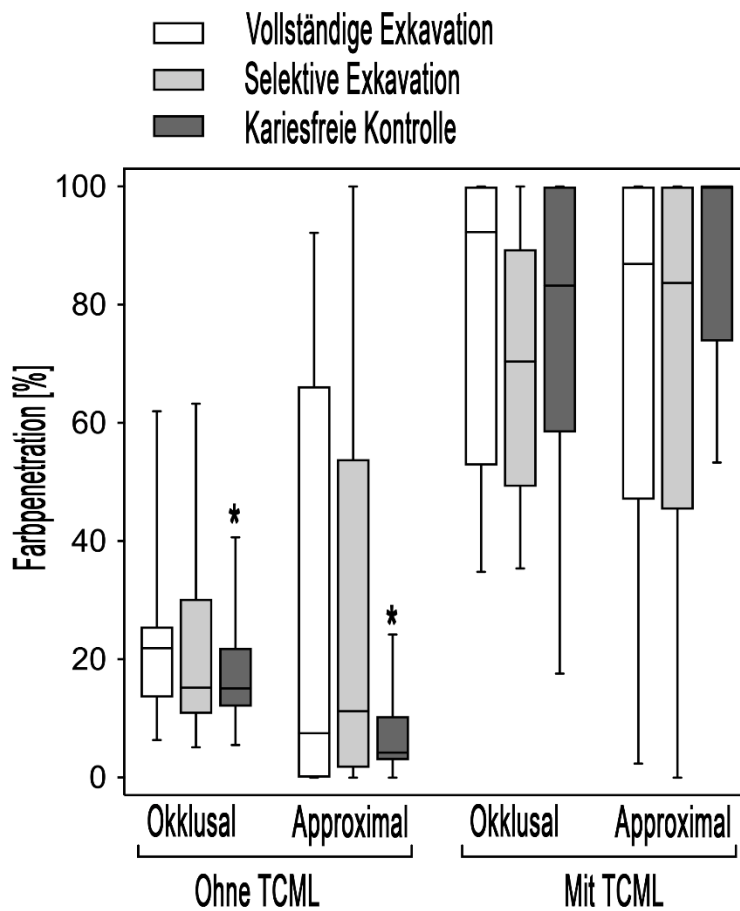
Bei den Schmelzsegmenten war der Median der Farbpenetration ohne TCML $\leq 17,9\%$ in allen Gruppen. Mit TCML betrug die Farbpenetration im Median zwischen $60,7\%$ und $73,9\%$ (Abbildung 4, Tabelle 1). Für die Dentinsegmente war die Farbpenetration ohne TCML $\leq 1\%$ in allen Gruppen und bewegte sich mit TCML im Median zwischen $26,9\%$ und $49,7\%$. Ohne TCML war die Farbpenetration im Dentin bei der selektiven Exkavation signifikant erhöht im Vergleich zur kariesfreien Kontrolle ($p = 0,026$).

Abbildung 4 Farbpenetrationswerte der Schmelz- und der Dentinsegmente innerhalb einer Exkavationsstrategie mit und ohne TCML (Maximum, Minimum, Median, 25% - 75% Perzentil; * = statistisch signifikanter Unterschied, $p = 0,026$)



Die Farbpenetration der approximalen Segmente bewegte sich ohne TCML im Median zwischen 4,3% und 11,3% und mit TCML zwischen 83,7% und 100,0% (Abbildung 5). Bei den okklusalen Segmenten bewegte sie sich im Median ohne TCML zwischen 15,1% und 21,9%, mit TCML zwischen 70,4% und 92,3%. Lediglich innerhalb der Gruppe C ohne TCML stellte sich okklusal eine signifikant höhere Farbpenetration als approximal dar ($p = 0,007$).

Abbildung 5 Farbpenetrationswerte der Segmente der approximalen und der okklusalen Restaurationsränder innerhalb einer Exkavationsstrategie (Maximum, Minimum, Median, 25% - 75% Perzentil; * = statistisch signifikanter Unterschied, $p = 0,007$)



Mit TCML war die Farbpenetration über alle Segmentgruppen hinweg signifikant erhöht im Vergleich zur Farbpenetration ohne TCML ($0 \leq p < 0,011$).

Tabelle 1 Mediane Farbpenetration (25% - 75% Perzentil) in Prozent für alle Gruppen und Segmente mit und ohne TCML

Segment-Gruppe	Ohne TCML [% Farbpenetration]			Mit TCML [% Farbpenetration]		
	Komplette Exkavation (n = 15)	Selektive Exkavation (n = 15)	Kariesfreie Kontrolle (n = 10)	Komplette Exkavation (n = 14)	Selektive Exkavation (n = 15)	Kariesfreie Kontrolle (n = 10)
Alle Segmente	8,9 (6,3 – 22,6)	8,6 (3,3 – 12,8)	6,0 (4,7 – 8,1)	52,5 (27,0 – 69,2)	43,9 (28,5 – 77,3)	45,9 (37,3 – 56,4)
Schmelz	17,1 (14,0 – 23,5)	17,9 (10,6 – 26,3)	12,0 (10,3 – 26,3)	72,6 (49,2 – 85,2)	60,7 (42,6 – 84,3)	73,9 (63,5 – 84,9)
Dentin	0,0 (0,0 – 15,7)	1,0 (0,0 – 10,5)	0,0 (0,0 – 0,0)	49,7 (18,4 – 82,9)	26,9 (16,9 – 87,0)	37,8 (19,5 – 49,1)
Approximal	7,5 (0,0 - 66,2)	11,3 (1,7 - 53,9)	4,3 (2,9 – 10,4)	86,9 (47,0 – 100,0)	83,7 (45,3 – 100,0)	100,0 (73,8 – 100,0)
Okklusal	21,9 (13,5 – 25,5)	15,2 (10,8 – 30,3)	15,1 (12,0 – 21,9)	92,3 (52,8 – 100,0)	70,4 (49,2 – 89,4)	83,3 (58,4 – 100,0)

4. Diskussion

Kariös verändertes Dentin stellt für die Anfertigung adhäsiver Restaurationen aufgrund verschiedener Gesichtspunkte - insbesondere hinsichtlich des eingeschränkten adhäsiven Haftvermögens - eine Herausforderung dar (10,16). Zudem gibt es bei der selektiven Kariesentfernung Bedenken, dass demineralisiertes Dentin die mechanischen Eigenschaften und die Randdichtigkeit der späteren Restauration negativ beeinflusst (8,9). Allerdings bleiben die tatsächlichen klinischen Auswirkungen unklar, da *in vitro* Studien mit widersprüchlichen Ergebnissen vorgelegt wurden, die den Einfluss von zurückgelassenem kariösen Dentin auf die mechanischen Eigenschaften sowie die Frakturresistenz beschreiben (9,17).

Restaurationen sind in der Mundhöhle ständigen Temperaturschwankungen sowie intermittierenden Kaubelastungen ausgesetzt. Diese führen über die Zeit zu einer Verschlechterung der Randdichtigkeit der Füllungen (18,19). Aus diesem Grund untersuchten wir die Farbpenetration sowohl ohne künstlichen Alterungsprozess im Anschluss an die Anfertigung der Restauration als auch nach Anwendung eines etablierten TCML-Protokolls (19–22). Zusätzlich werteten wir diese an so vielen Schnitten wie möglich (6 – 14 Schnittbilder pro Zahn, Median 9 Schnittbilder) aus. In unserem standardisierten *in vitro* Modell untersuchten wir den Einfluss von zurückgelassenem demineralisiertem Dentin auf die Qualität der adhäsiven Restauration. Dabei wurde die Randdichtigkeit - gemessen durch marginale Farbpenetration - als Bewertungskriterium gewählt, da sie einen wichtigen Faktor für den klinischen Erfolg darstellt.

Um den Einfluss der Kavitätenausdehnung gesondert betrachten zu können, entschieden wir uns für die Implementierung einer Kontrollgruppe. Dieser Aspekt wurde in früheren Studien zu diesem Thema nicht immer berücksichtigt (22,23). Bei der kariesfreien Kontrolle wurde die Zahnhartsubstanz nicht demineralisiert. Die Geometrie und Ausdehnung der Kavitäten entsprach jedoch denen der Untersuchungsgruppe der selektiven Exkavation. Um den möglichen Einfluss interindividueller Unterschiede der einzelnen Zähne gering zu halten, platzierten wir in diesen jeweils mesial und distal unterschiedliche Untersuchungsgruppen. In unpublizierten Versuchen zur Auswahl des Demineralisationsprotokolls zeigte sich, dass selbst bei identischem Demineralisationsprotokoll das Volumen der erzeugten Läsion variiert. Die Bedeutung der Läsionsgröße wurde zwar in einer früheren Studie als möglicher Einflussfaktor erwähnt, dabei wurde allerdings das demineralisierte Volumen nicht quantifiziert (22). Anhand eines zweidimensionalen Röntgenbildes gelingt bestenfalls eine grobe Abschätzung, was sich für unsere Fragestellung als ungeeignet darstellt. Stattdessen wurden die Zähne unter

Verwendung eines Mikro-CT vermessen. Anhand der Ergebnisse konnten daraufhin die Untersuchungsobjekte der Läsionsgröße nach geordnet und so stratifiziert auf die Untersuchungsgruppen verteilt werden. Im Nachhinein war jedoch keinerlei Zusammenhang zwischen der gemessenen Läsionsgröße und unseren Untersuchungsergebnissen festzustellen. (Abbildung 2)

Zur Auswertung und zum Vergleich der verschiedenen Exkavationsstrategien wurde das Verfahren der Farbpenetration gewählt. Auch wenn die Aussagekraft der Ergebnisse - wie bei allen *in vitro* Verfahren - auf den tatsächlichen Erfolg klinischer Restaurationen mit Vorsicht interpretiert werden muss (24), erlaubt sie einen schnellen, präzisen und einfachen Vergleich der verschiedenen Behandlungsstrategien (19,25,26). Aufgrund seiner im Vergleich zu kariogenen Bakterien deutlich geringeren Partikelgröße von 0,059 nm (27,28) wird Silbernitrat als überkritisch angesehen. Für eine Versiegelung der kariösen Läsion ist jedoch auch das Abschneiden der Mikroorganismen von der Nährstoffzufuhr entscheidend. Die Silbernitratfarbpenetration könnte ein geeignetes Modell sein, um zu untersuchen, inwieweit beispielsweise Saccharose entlang der Restaurationsgrenze vordringen kann. Eine frühere *in vitro* Studie zeigte ähnliche Penetrationswerte für verschiedene Farbstoffe trotz variierender Partikelgröße (29).

Aufgrund großer interindividueller Unterschiede bei kariösen Läsionen in nicht erhaltungswürdigen extrahierten Zähnen stellt sich die *in vitro* Erzeugung künstlicher Karies als sinnvolle Alternative dar. Gebräuchlich dafür sind organische Säuren, Chelatoren oder bakterielle Biofilme, wobei sich die verschiedenen Modelle insbesondere bezüglich Läsionstiefe, mechanischen Eigenschaften und Zeitaufwand unterscheiden (30–36). Obgleich ein solches Modell den Anforderungen an eine exakte Nachbildung nicht in allen Aspekten komplett gerecht werden kann, so vereinfacht es die Realisierbarkeit der erforderlichen Anzahl möglichst übereinstimmender Versuchsobjekte (37). Eine *in vitro* Studie verglich verschiedene Demineralisationsprotokolle zur Simulation von Restkaries unter Verwendung der transversalen Mikroradiographie an Dentinproben. Unter Verwendung einer EDTA-Lösung erzeugte Läsionen wiesen zwar im Unterschied zu natürlichen Läsionen einen vollständigen Mineralverlust und keine Oberflächenschicht auf, allerdings konnten innerhalb kurzer Zeit (96 Stunden) zuverlässig Läsionstiefen von $655 \pm 68 \mu\text{m}$ erzeugt werden (36). In unserer Studie konnten jedoch in allen demineralisierten Kavitäten stabile demineralisierte Volumina nachgewiesen werden (Abbildung 2).

Alle Kavitäten waren zirkulär von Schmelz begrenzt und wurden unter Anwendung desselben klinisch etablierten Protokolls restauriert. Dabei wurde nach Schmelzanschrägung und selektiver Schmelzätzung ein 10-MDP-haltiges Adhäsivsystem appliziert und ein Nanohybrid-Komposit verwendet (22,38–42). Im Einklang mit früheren Studien (19,43) war die Farbpenetration mit TCML in allen Untersuchungsgruppen signifikant höher als ohne TCML. Im Rahmen unserer Studie zeigte sich, dass die Restaurationen der Gruppen der kompletten und selektiven Exkavation, die im Vergleich zur kariesfreien Kontrolle in Richtung Pulpa eine größere Extension aufwiesen, die Farbpenetrationswerte nicht signifikant erhöhten.

In verschiedenen *in vitro* Studien stellte sich der adhäsive Verbund zum Schmelz mit deutlich höheren Haftwerten als zum Dentin dar (19,22,40). Obwohl der proximale Kavitätenrand zu einem höheren Anteil von Dentin gebildet wird als der okklusale Kavitätenrand, ergab sich kein signifikanter Unterschied zwischen den Farbpenetrationswerten der beiden Bereiche. Dies könnte darauf schließen lassen, dass selbst ein schmal ausgeprägter Schmelzrand bereits Randintegrität gewährleisten kann.

Unsere *in vitro* Studie konnte keinen statistisch signifikanten Unterschied der Randdichtigkeit zwischen den verschiedenen Exkavationsstrategien – selektiver und kompletter Kariesexkavation – sowie der nicht demineralisierten Kontrollgruppe feststellen. Die Nullhypothese konnte daher unabhängig von der Anwendung von TCML nicht widerlegt werden. TCML führte zu einem signifikanten Anstieg der Farbpenetration entlang der Grenzen von Kompositrestaurationen. In Klasse-II-Kavitäten mit zirkulärer Schmelzbegrenzung müssen daher keine höheren Penetrationswerte durch die Anwendung der selektiven Kariesexkavation im Vergleich zur kompletten Exkavation befürchtet werden. Weitere *in vitro* Untersuchungen, beispielsweise zur Vorbehandlung kariöser Oberflächen oder zu unterschiedlichen direkten Restaurationstechniken und –materialien, sind daher angezeigt, um ein besseres Verständnis über die verschiedenen Einflussfaktoren erhalten zu können.

5. Ausblick

EDTA stellt eine einfache und schnelle Methode zur Erzeugung von künstlich kariösen Läsionen dar. Die verschiedenen De- und Remineralisationsvorgänge im Entstehungsprozess natürlich kariöser Läsionen können jedoch nicht vollständig abgebildet werden (36).

Säurebasierte Demineralisationskonzepte haben jedoch gegenüber Modellen, die auf der Züchtung von bakteriellen Biofilmen beruhen, den Nachteil, dass mit ihnen die Auswirkungen von bakteriellen Stoffwechselprodukten und Proteasen auf Kollagenfasern ebenfalls nicht nachgebildet werden können. Ein weiterer Schritt wäre daher die Verwendung von Biofilmbasierten Konzepten zur künstlichen Karieserzeugung.

Das in dieser Studie verwendete Modell ist auf schmelzbegrenzte Klasse-II-Restaurationen in Oberkiefermolaren beschränkt. Naheliegender wäre die Untersuchung anderer Kavitätenklassen beziehungsweise Zahntypen oder approximal dentinbegrenzter Restaurationen. Eine Erweiterung durch die Verwendung von zusätzlichen Unterfüllungs- und Überkappungsmaterialien, anderer Restaurationsmaterialien oder Restaurationstechniken ist denkbar.

Die Ergebnisse dieser *in vitro* Studie deuten darauf hin, dass sich die selektive Kariesentfernung nicht negativ auf die Randdichtigkeit von schmelzbegrenzten Klasse-II-Kavitäten auswirkt. In einer klinischen Situation kann durch die selektive Kariesexkavation eine Eröffnung der Pulpa vermieden werden.

Allerdings zeigte sich in dieser Studie auch, dass sich der marginale adhäsive Verbund unter Belastung (simuliert durch TCML) signifikant verschlechterte. Dies kann dahingehend interpretiert werden, dass jede adhäsive Restauration - unabhängig vom Vorhandensein zurückgelassener kariöser Läsionen - mit der Zeit an Adhäsion einbüßt. Dies würde darauf hindeuten, dass jeder Restaurationsrand auf die Dauer durchlässig für Saccharose und andere kariogene Nährstoffe wird. Ob dies tatsächlich so ist und dazu führt, dass zurückgelassene Mikroorganismen wieder stoffwechselaktiv werden oder gar proliferieren können, sollte weiter untersucht werden. Ebenso ist der daraus resultierende Einfluss auf das pulpare Gewebe zu untersuchen.

6. Publikation

Konstantin J. Scholz*, Michael Hinderberger*, Matthias Widbiller, Marianne Federlin, Karl-Anton Hiller, Wolfgang Buchalla:

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Scholz KJ, Hinderberger M, Widbiller M, Federlin M, Hiller K-A, Buchalla W. Influence of selective caries excavation on marginal penetration of class II composite restorations in vitro.

Eur J Oral Sci 2020; 00: 1–10. © 2020 The Authors. Eur J Oral Sci published by John Wiley & Sons Ltd

Selective caries excavation may support pulp preservation. This in vitro study investigated the influence of selective removal of demineralized dentin on marginal integrity of composite restorations as determined by dye penetration. Dentinal caries-like lesions were produced in the approximal surfaces of 40 extracted human molars (ethylenediaminetetraacetate, 0.5 M, 96 h). The following test procedures were established: complete excavation, selective excavation, and caries-free control. Two class II cavities with enamel at the cervical margins were prepared per tooth and demineralization volume was determined by micro-computed tomography for the purpose of a stratified distribution to receive complete excavation or selective excavation. After complete or selective excavation (30 cavities each), adhesive composite restorations were placed. Cavities without demineralized dentin (20 cavities) served as control. The marginal integrity of restorations was evaluated by dye penetration with and without thermocycling or mechanical loading. Results were analyzed by non-parametrical statistical tests (Mann-Whitney *U* Test) with an $\alpha = 0.05$ level of significance. Dye penetration did not differ significantly among completely excavated, selectively excavated, or undemineralized teeth, but was increased by thermocycling and mechanical loading in all experimental groups. Selective caries removal did not increase marginal penetration in class II restorations. The presence of remaining demineralized dentin surrounded by sound dentin did not impair marginal integrity of restorations with margins placed in sound enamel.

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Key words: dental bonding; dental caries; dental cavity preparation; permanent dental restorations

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Accepted for publication June 2020

Complete excavation of caries, as it was traditionally performed in the course of restorative therapy, has been widely superseded by less invasive techniques, such as infiltration technique, atraumatic restorative treatment, or selective caries excavation (1). While atraumatic restorative treatment might have its limitations (2), clinical studies show promising success rates for selective excavation (3,4).

Based on a meta-analysis, ISOLAN *et al.* (5) concluded that caries-affected dentin in general leads to a lower bond strength than sound dentin, irrespective of the adhesive approach used (5). Dissolution of minerals, degradation of collagen, increasing porosities, and changes in elemental composition of carious dentin might be factors leading to a decrease in bond strength (6–8). Concerns about the use of selective caries excavation have been raised with respect to the possible progression of carious lesions and compromised mechanical properties due to the softness of the carious

dentin serving as the foundation for the restoration (9). A soft base underneath a restoration may trigger marginal gap formation, which itself may lead to recurrent or secondary caries (10,11). Besides the influence on mechanical performance, residual caries in dentin might pose a risk for the dental pulp. In a randomized clinical trial, cavities showed a higher bacterial load after selective excavation but before restoration compared to cavities that were completely excavated (12). In the same study, a decrease of total viable microorganisms in general, as well as streptococcus species and lactobacillus species specifically, was observed after three months for both excavation strategies and no significant differences between selective and complete excavation remained. In another in vivo study, 3–4 months after selective excavation harder and darker dentin was found compared to the date of restoration, irrespective of the restorative material (13). Furthermore, selective caries excavation can be biologically advantageous because pulp exposure

is avoided. Indirect pulp capping using bioactive materials may reduce the concentrations of bacterial lipopolysaccharides and lipoteichoic acid and it can provide a barrier against the potentially cytotoxic effects of adhesive system monomers (14-16). Apart from the biological advantages of selective excavation, little is known about the influence of residual carious dentin on the mechanical properties of restorations (4,17). The marginal seal of a restoration is crucial when selective excavation is performed in order to ensure long term arrest of microbial activity in the remaining carious dentin through a tight seal (17). Avoiding marginal leakage is a critical factor for restoration success, especially the exclusion of carbohydrates from reaching residual carious dentin. In vitro, dye penetration has been frequently applied as a method for measuring marginal leakage of dental restorations (18-20).

The aim of this in vitro study was to investigate the influence of complete or selective excavation of artificial caries lesions in human molars on the marginal integrity of subsequently placed class II composite restorations. Since it is difficult to control lesion size and interindividual factors in clinical studies, this in vitro study used micro-computed tomography (micro-CT) to ensure teeth with similar demineralization volumes were stratified into two groups. A controlled in vitro design was chosen to examine the microleakage as determined by dye penetration on similar sized restorations with the complete margins in enamel. All groups were investigated without and with thermocycling and mechanical loading on multiple sections. The null hypothesis was that leaving demineralized dentin in a cavity before placing class II composite restorations in human molars leads to a similar amount of marginal dye penetration compared to complete excavation in vitro.

Material and methods

Sample preparation

Forty caries-free human third molars were collected and placed in 0.5% chloramine solution directly after extraction and stored for a maximum of 6 months. The University of Regensburg Ethics Committee (Reference: 19-1327-101) approved the use of extracted teeth on the condition that informed consent was obtained from each patient.

After removal of soft tissue remnants, the roots of the sample teeth were embedded in acrylic resin (Paladur, Kulzer, Hanau, Germany) 2 mm apical the cemento-enamel junction. During the entire experimental period, the teeth were stored in demineralized water.

Caries model

In order to allow standardized dentin demineralization in proximity to the pulp chamber, an approximal cylindrical pit (1.3 mm diameter, 2 mm depth) was prepared perpendicularly to the tooth axis with a diamond bur (Meisinger, Neuss, Germany) at 200,000 r.p.m. and with extensive water-cooling. This demineralization pit was located

3.5 mm above the cemento-enamel junction and directed towards the pulp chamber, all the way through the enamel into dentin. The pit was closed with a customized gutta-percha place holder and the whole crown was encased in polyether impression material, leaving the ends of the gutta-percha place holder protruding from the impression material (Impregum, 3M Espe, St. Paul, USA). After the polyether had set, the gutta-percha was removed, providing direct access to dentin.

Ethylendiaminetetraacetate (EDTA Disodium Salt 2-hydrate; AppliChem, Darmstadt, Germany) was used as demineralization solution (0.5 M, pH 7). Initially, the demineralization agent was injected into the pits using needles designed to prevent vapor lock (BD Microlance 20G; Becton Dickinson, Franklin Lakes, NJ, USA). Subsequently, each tooth was placed in 400 ml EDTA, which was stirred continuously (300 r.p.m.) for 96 h at room temperature. Immediately afterwards, the teeth were removed from EDTA, the polyether impression material was taken off, and the teeth were extensively rinsed with demineralized water.

A mesial and a distal approximal adhesive class II cavity was prepared (200,000 rpm, water-cooling) with cylindrical diamond burs (Reference number 806 314 111 524, diameter 1.4 mm; Meisinger). Cavities had an oro-vestibular dimension of 4 mm with a cervical margin above the cemento-enamel junction and were prepared in a standardized way to create occlusal and approximal boxes, leaving a demineralized area at the pulpal dentin (Fig. 1). Standard dental radiographs were taken to identify irregularities (e.g., subclinical carious lesions, insufficient demineralization) during the experimental procedure. Occlusally, the two cavities in each tooth were separated by at least 1 mm of sound enamel supported by underlying dentin. Cavities had a minimum extension of 2 mm in mesio-distal direction. Sharp edges on approximal cavity margins were minimally beveled 45° to remove loose enamel prisms and slightly enlarge the adhesive surface in enamel.

The volume of demineralized dentin was determined by micro-CT. Therefore, each tooth was kept wet wrapped in a parafilm (Parafilm M; Merck, Darmstadt, Germany) and scanned (phoenix v|tome|xS 240/180 research edition; General Electric Measurement & Control, Wunstorf, Germany) with the following parameters: duration = 170 min, Images = 3,000, Averaging = 15, Skip = 2, tube voltage = 80 kV, beam current = 500 μ A, voxel size = 35 μ m. Demineralized dentin was defined as tissue showing an absorption coefficient between 0.028 and 0.063 (Fig. 1).

Sample allocation

As Fig. 2 illustrates, 20 teeth (Category 1) were demineralized on one approximal surface, whereas the opposite approximal surface was not demineralized. Furthermore, 20 teeth (Category 2) were artificially demineralized on both approximal surfaces. For Category 1, teeth were sorted in ascending order of demineralization volume as determined by micro-CT. Teeth with similar demineralization volumes were distributed stratified into two groups: one cavity with complete excavation or one cavity with selective excavation. The endpoint of caries removal was defined by hardness using polymer burs for excavation (PolyBur, Gebr. Brasseler, Lemgo, Germany). In the selectively excavated cavities, demineralized dental hard tissue was removed from the outermost 0.5 mm next to all outer

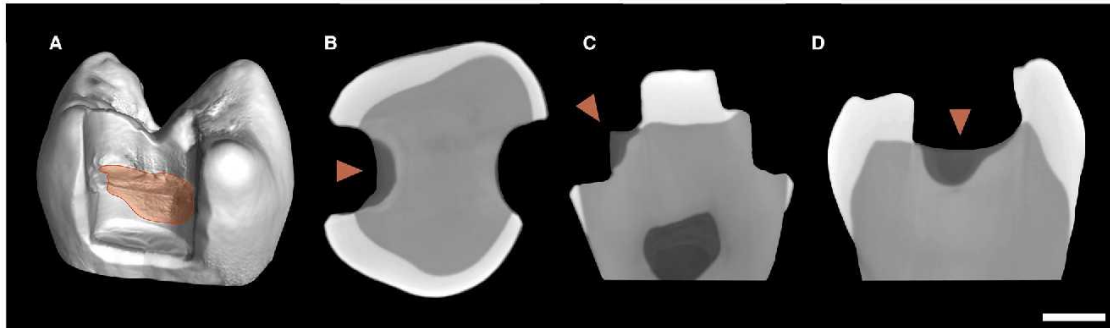


Fig. 1. Example micro-CT images. Micro-CT images of a tooth with demineralized dentin on one side only (Category 1) before excavation. (A) 3-D reconstruction of a tooth with demineralized dentin (absorption coefficient: 0.028–0.063), which is highlighted in orange. (B) Horizontal sectional view with demineralized dentin on left side (arrow). (C) Mesio-distal vertical section with demineralized dentin on left side (arrow). (D) Oro-vestibular vertical section with centrally located demineralized dentin (arrow). Scale bar: 2 mm.

cavity margins. The demineralized dentin left centrally remained untouched. The central demineralized dentin showed stable but tactile softened consistency, as described in the literature (17). Opposing approximal surfaces, which were not demineralized, were also prepared for adhesive class II restorations of equal size as the control. For Category 2, one demineralized surface of each tooth was randomly allocated by coin toss to complete excavation and the other demineralized surface to selective excavation (Fig. 2).

Adhesive restoration

Teeth were cleaned with demineralized water in an ultrasonic bath (Bransonic 221; Branson, Danbury, CT, USA) for 5 min. A metal matrix band was placed over the

cavities in each tooth (Hawe Tofflemire Matrices; Kerr, Bioggio, Switzerland). Enamel was selectively etched for 20 s using 37% H₃PO₄ (Total Etch; Ivoclar Vivadent, Schaan, Liechtenstein), rinsed with water for 20 s and gently air-dried. A two-bottle self-etch adhesive system (Clearfil SE; Kuraray, Chiyoda, Japan) was used on all surfaces with a saturated applicator tip (Flocked Applicator Tips; Dentsply I, York, PA, USA). First, the primer was rubbed into the dentin and then applied to the enamel for 20 s and dried by mild air-flow. Subsequently, the bonding agent was applied for 10 s across dentin and enamel, distributed evenly with mild air-flow and light-cured for 10 s (Satelec Mini LED; Acteon Group, Mérignac Cedex, France; 1,250 mW cm⁻²). Cavities were restored with three increments of a nano-hybrid resin composite material (Tetric Evo Ceram XL Bleach; Ivoclar Vivadent) creating a flat occlusal surface. The first and second increments were

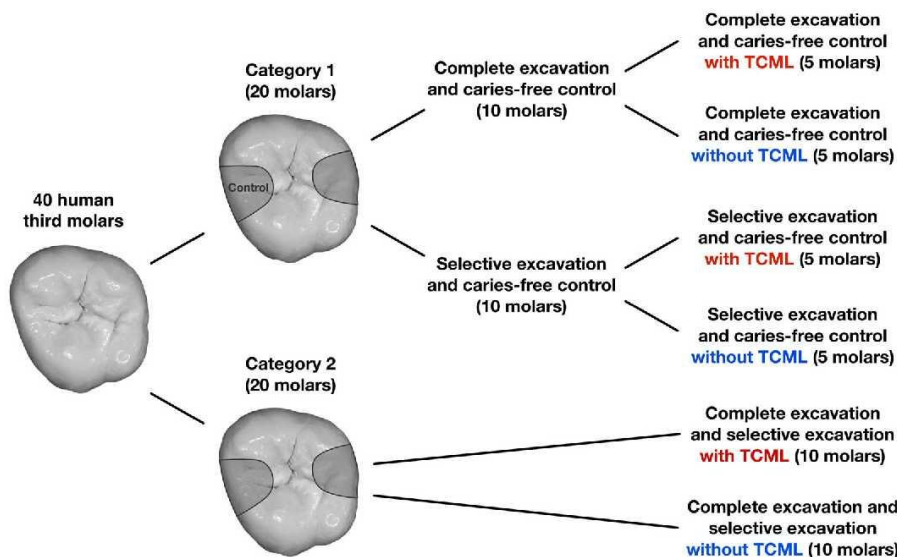


Fig. 2. Group allocation. Allocation to Categories 1 and 2, treatment groups complete excavation, selective excavation and caries-free control and application of thermocycling and mechanical loading. In total, 80 restorations in 40 human molars were investigated. Positions of restorations on occlusal and proximal surfaces are symbolized by grey fields.

light-cured for 20 s each; the third increment was light-cured for 40 s. After removing the matrix band, the entire restoration was light-cured for 20 s and polished (Busch diamond polisher #9588 and #9578; Busch, Engelskirchen, Germany). The teeth were then stored in demineralized water for five days at room temperature.

Thermocycling and mechanical loading

Tooth pairs with similar demineralization volumes were selected from all combinations of treatment groups (complete excavation, selective excavation, and caries-free control). As shown in Fig. 2, one tooth per pair was randomly assigned by coin toss to thermocycling and mechanical loading (TCML: 5,000 cycles of 30 s at 5–55°C and 500,000 cycles at 72.5 N, 1.6 Hz), and the other tooth was stored (4°C) in demineralized water for the same time (19,21). Thus, the same numbers of cavities with similar demineralization volumes were evaluated without and with thermocycling and mechanical loading. Central mechanical loading was performed upon a metal stop representing the opposing cusp. This metal stop was placed in the middle of the occlusal surface not in contact with the adhesive restorations.

Dye penetration

After thermocycling and mechanical loading or storage, nail varnish was applied to the surface of the teeth. The restoration, the cemento-enamel junction, and 1 mm of tooth surface around the restoration margin remained uncovered. The teeth were immersed in 50 wt% AgNO₃ solution (S-6506; Sigma-Aldrich Chemie, Steinheim, Germany) for 120 min in the dark. Afterwards, the teeth were rinsed with demineralized water, immersed in a photographic developing solution (Tetenal Ultrafin Plus; Tetenal, Norderstedt, Germany), and exposed to fluorescent light (Philips Master PL-S 840/2P, 11 W = 900 Lumen, WD 100 mm) for 6 h. After copious rinsing with demineralized water, samples were stored in a humidity chamber prior to sectioning.

Teeth were cut longitudinally in mesio-distal direction using a water-cooled rotating diamond saw with blade thickness of 300 µm (Leitz 1600; Leica Microsystems, Wetzlar, Germany) to obtain as many sections of 300 µm thickness as possible (depending on tooth-size). Standardized images were taken from both sides of the sections using a photomicroscope (Makroskop M420; Wild, Heerbrugg, Germany, magnification x3.15; and Axiocam 105 color; Carl Zeiss, Oberkochen, Germany; 2,560 × 1,920 pixels). Images were analyzed using Optimas 6.51 software (Bioscan, Washington DC, USA) and a standardized scheme adapted to the cavities of complete excavation group, selective excavation group, and caries-free control (Fig. 3). The entire interface between tooth and restoration was subdivided into enamel segments, dentin segments, and demineralized segments. Penetration between demineralized dentin and sound dentin was recorded in selectively excavated cavities as well. For further analysis, the occlusal margin was defined as segment and the approximal margin was defined as the sum of the approximal enamel segment and the horizontal dentin segment next to it.

Data treatment and statistical analysis

Dye penetration depth was calculated by measuring penetration depth per segment or segments combined as percent of total length of the respective segment(s). Length

was measured using the Optimas software's line morphometry tool. Dye penetration in all segments (sum of all segments of a tooth), in enamel segments, in dentin segments, for approximal margins, and occlusal margins was calculated separately (Fig. 3).

Additionally, the maximum dye penetration (%) from all sections of every tooth was used as the representative value for that tooth. Maxima, minima, medians, 25%- and 75%- percentiles were calculated for each group.

Data were analyzed nonparametrically and Mann-Whitney *U*-Test was used to test for statistically significant differences between groups (SPSS version 25.0, IBM, Armonk, NY, USA). The level of significance was set to $\alpha = 0.05$. To evaluate the impact of thermocycling and mechanical loading, the level of significance α was adjusted to $\alpha^*(k) = 1 - (1 - \alpha)^{1/k}$ by the Error Rates Method (k = number of paired tests performed). Regression analysis was performed for dye penetration and volume of demineralized dentin.

Results

The median volume (25–75% percentile) of the demineralized dentin was 1.8 mm³ (1.4–2.3 mm³). Linear regression coefficients ranging from 0.004 to 0.11 for all segments indicate that there is no correlation between demineralization volume and dye penetration for all segments (Fig. 4). The measured median (25–75% percentile) extension of prepared cavities in mesio-distal direction was for complete excavation 2.8 (2.7–3.1) mm, for selective excavation 2.6 (2.2–3.0) mm, and for caries-free control 2.7 (2.5–3.0) mm.

Two-hundred and sixty-six images were evaluated for complete excavation: 285 for selective excavation and 176 for the caries-free control cavities. The median number of images from sections per tooth was nine in every group. The number of images per tooth ranged from 6 to 14.

The median dye penetration for all segments was ≤8.9% in all groups with no thermocycling or mechanical loading, and it ranged between 43.9% and 52.5% in all groups with thermocycling and mechanical loading (Table 1, Fig. 5). There was no significant difference between complete excavation, selective excavation, or caries-free control for all segments.

Within enamel, dye penetration in all groups was ≤17.9% when no thermocycling and mechanical loading had taken place, and it ranged between 60.7% and 73.9% after thermocycling and mechanical loading (Table 1, Fig. 6). There was no significant difference between complete excavation, selective excavation, or caries-free control for enamel. Dye penetration within dentin was ≤1% with no thermocycling or mechanical loading and 26.9–49.7% after thermocycling and mechanical loading for all groups. With no thermocycling or mechanical loading, there was significantly more dye penetration in dentin following selective caries excavation compared to the caries-free control ($P = 0.026$).

Dye penetration for all groups from the approximal margins ranged between 4.3% and 11.3% without

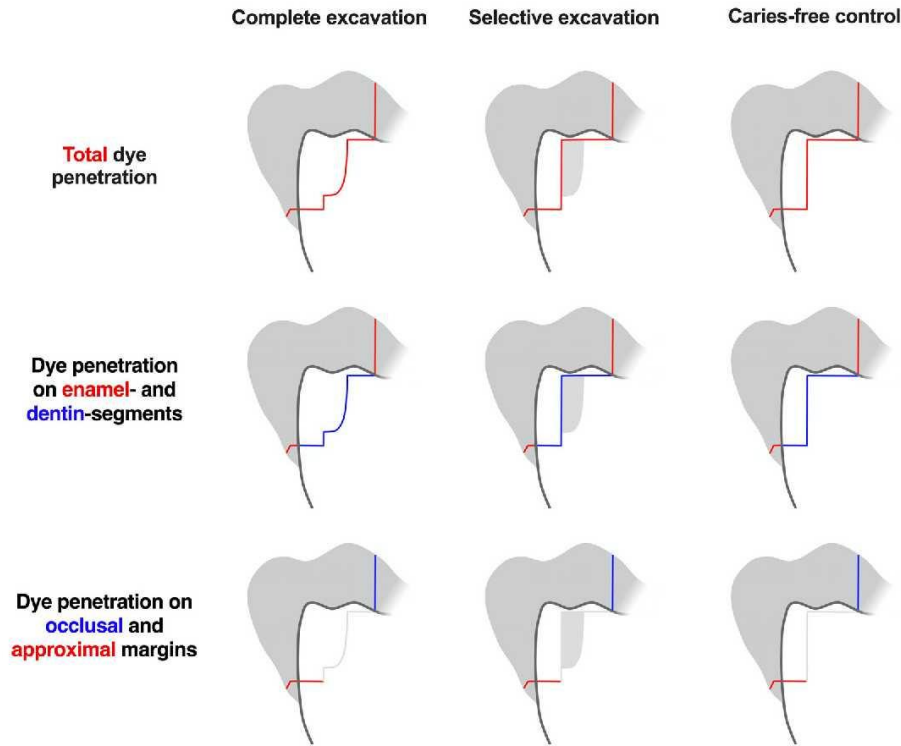


Fig. 3. Evaluation-schemes for marginal dye penetration. Complete excavation, selective excavation and caries-free (undemineralized) control. Marginal dye penetration was evaluated for all segments (upper row, results shown in Fig. 5), by tooth substance (middle row, red = enamel, blue = dentin, results shown in Fig. 6) and margins (lower row, blue = occlusal, red = approximal, results shown in Fig. 7). The border between demineralized and sound dentin on the pulpo-axial wall was also evaluated in selectively excavated teeth. Every identifiable segment was evaluated in each section.

thermocycling or mechanical loading and between 83.7% and 100.0% in the presence of thermocycling and mechanical loading (Table 1, Fig. 7). Dye penetration at the occlusal margin ranged from 15.1% to 21.9% without thermocycling or mechanical loading and 70.4%–92.3% in the presence of thermocycling and mechanical loading for all groups. When occlusal and approximal dye penetration were compared, the only statistically significant difference was for the caries-free control without TCML, which showed more dye penetration from the occlusal surface ($P = 0.007$) than from the approximal.

Dye penetration was significantly ($P < 0.011$) higher when thermocycling and mechanical loading had been performed than without thermocycling or mechanical loading for all locations and excavation procedures (Fig. 8). No restorations with fractures or loss of retention were observed.

Discussion

Caries-affected dentin is a challenging substrate for restorations for several reasons (5). The in vitro bond

strength of composite materials to caries-affected dentin, in general, is reported to be lower than that to sound dentin (5,22). However, the clinical consequences remain unclear, as in vitro studies on the influence of the remaining carious dentin on the mechanical properties and fracture resistance have disagreed (10,23,24). The clinically derived hypothesis of this study was that remaining demineralized dentin does not affect the marginal integrity and, thus, the success of composite restorations.

To investigate the influence of restoration geometry in our model, the dimensions of the cavities in the caries-free control teeth matched those of the selective excavation group but without any artificial caries formation. This kind of control is not always considered in the literature (25,26). Two cavities of equal extension were prepared in each tooth and specimens were paired according to the volume of the artificial demineralization, as measured by using a micro-CT.

Despite the general limitations of dye penetration tests to predict clinical success (27), this test allows a reliable, fast, and commonly accepted preclinical comparison of marginal integrity (18,20,28). Silver ions with a particle size of 0.059 nm are much smaller than

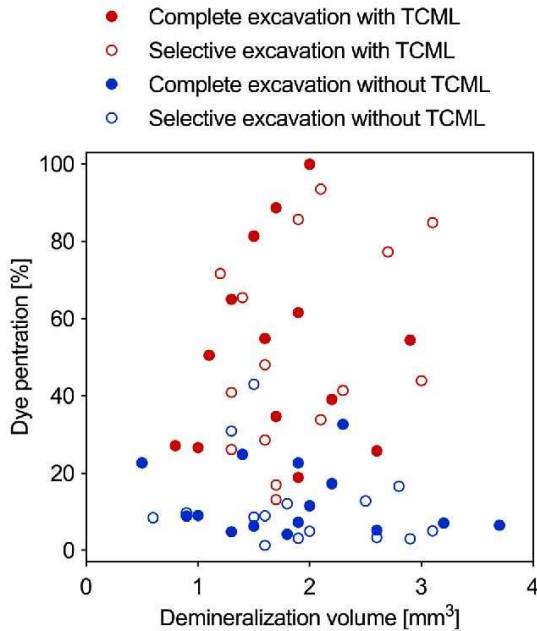


Fig. 4. Dye penetration depending on demineralization volume. Dye penetration (%) of restorations after complete or selective excavation, without and with TCML of all segments plotted against volume of demineralized dentin before caries excavation. Every circle represents one cavity. TCML: thermocycling and mechanical loading.

cariogenic bacteria (0.5–1 μm), so AgNO₃-penetration is a strict in vitro test parameter (29). However, because nutritional substrates, such as sugars, may penetrate marginal gaps and feed bacteria in the residual caries lesion, dye penetration using AgNO₃ may be a reasonably good model in order to determine whether sucrose is able to penetrate the margins of restorations. In our study, as many sections as possible were evaluated to accurately identify the weakest spot in every restoration. Optical coherence tomography (OCT) is a

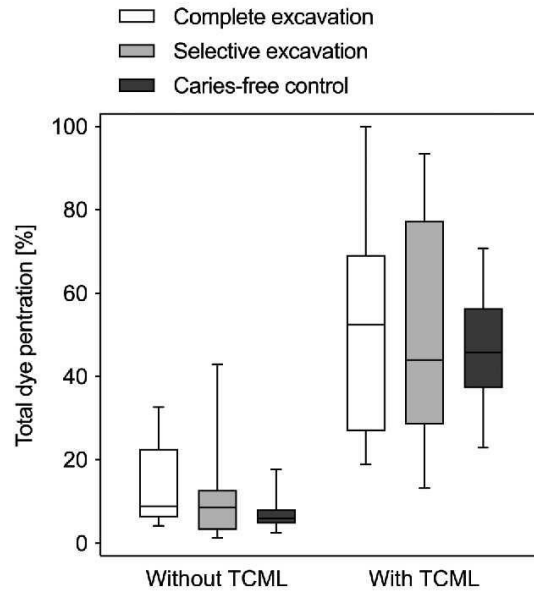


Fig. 5. Total dye penetration. Results of dye penetration for all segments within each tooth under the influence of TCML (Maximum, minimum, median, 25%–75% percentile). TCML: thermocycling and mechanical loading.

promising and clinically applicable alternative analytical method to the dye penetration test used in this study (30,31), as it allows repeated non-invasive measurements on the same specimen. With the OCT-method, restorations with a maximum thickness of 2.5 mm may be analyzed (32). As our restorations mostly had a thickness above 2.5 mm, OCT would be limited only to the outer margins. Moreover, all OCT-systems available today for use with extracted teeth still suffer from low resolution. Both aspects favor dye penetration for this in vitro investigation. High resolution scanning electron microscopy (SEM) studies analyzing marginal integrity using replicas and SEM-images of

Table 1

Dye penetration (%) for all three treatment protocols treated with or without thermocycling or mechanical loading

Segment groups	Without TCML [% dye penetration]			With TCML [% dye penetration]		
	Complete excavation (n = 15)	Selective excavation (n = 15)	Caries-free control (n = 10)	Complete excavation (n = 14)*	Selective excavation (n = 15)	Caries-free control (n = 10)
All segments	8.9 (6.3–22.6)	8.6 (3.3–12.8)	6.0 (4.7–8.1)	52.5 (27.0–69.2)	43.9 (28.5–77.3)	45.9 (37.3–56.4)
Enamel	17.1 (14.0–23.5)	17.9 (10.6–26.3)	12.0 (10.3–26.3)	72.6 (49.2–85.2)	60.7 (42.6–84.3)	73.9 (63.5–84.9)
Dentin	0.0 (0.0–15.7)	1.0 (0.0–10.5)	0.0 (0.0–0.0)	49.7 (18.4–82.9)	26.9 (16.9–87.0)	37.8 (19.5–49.1)
Approximal	7.5 (0.0–66.2)	11.3 (1.7–53.9)	4.3 (2.9–10.4)	86.9 (47.0–100.0)	83.7 (45.3–100.0)	100.0 (73.8–100.0)
Occlusal	21.9 (13.5–25.5)	15.2 (10.8–30.3)	15.1 (12.0–21.9)	92.3 (52.8–100.0)	70.4 (49.2–89.4)	83.3 (58.4–100.0)

Penetration data in the form of median (25%–75% percentile) is given for all segments, and limited to margins in enamel or dentin, or to penetration into approximal and occlusal margins (See Fig. 3). TCML: thermocycling and mechanical loading.

*One restoration was excluded due to remaining demineralized dentin detectable by microscopy.

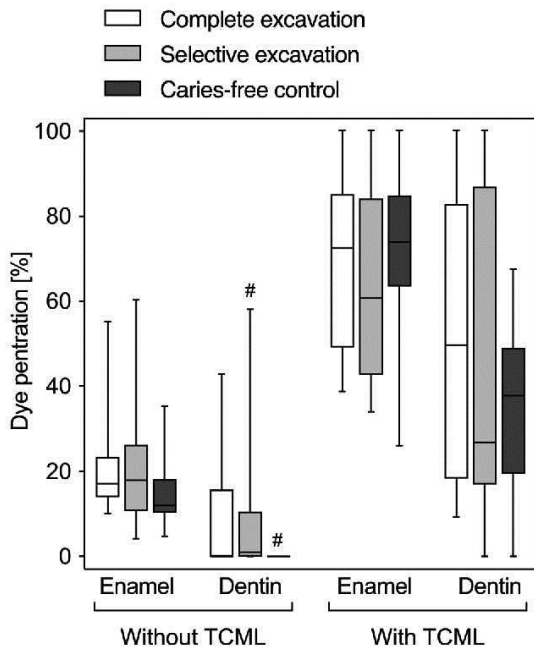


Fig. 6. Dye penetration on enamel and dentin segments. Results of dye penetration for enamel segments and dentin segments for treatment groups complete excavation, selective excavation and caries-free control under the influence of TCML (Maximum, minimum, median, 25%–75% percentile; # = statistically significant difference, $P = 0.026$). TCML: thermocycling and mechanical loading.

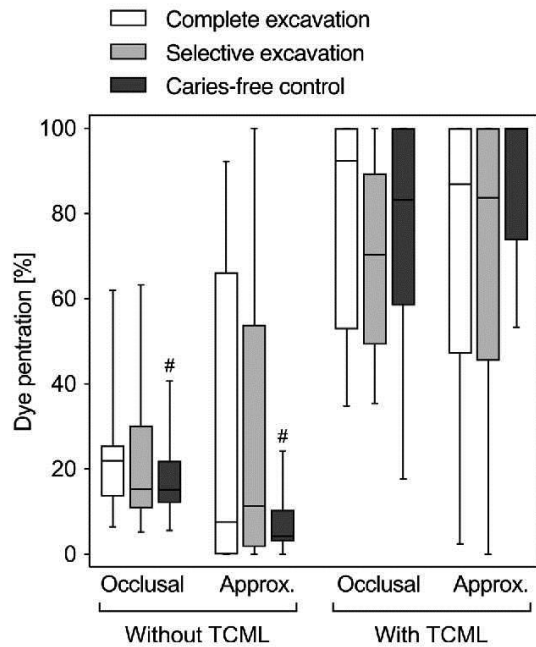


Fig. 7. Dye penetration on occlusal and approximal margins. Dye penetration for all groups subdivided in penetration from occlusal and approximal direction under the influence of TCML. (Maximum, minimum, median, 25%–75% percentile; # = statistically significant difference, $P = 0.007$). TCML: thermocycling and mechanical loading.

the adhesive interface allow marginal gap analysis at the outermost part of the tooth-restoration interface, but do not provide information on the depth of defects without cutting of the specimens (33). In the past, we observed that the marginal quality became so good with modern adhesive techniques that direct restoration margins were either no longer identifiable or interpretation of the marginal quality became difficult. Direct SEM-visualization without replicas allows marginal analysis at high resolution (30), but it is compromised by artifacts at the adhesive interface due to vacuum exposure, which is not the case using dye penetration.

The *in vitro* demineralization models commonly use organic acids, chelators, or bacterial biofilms to mimic carious lesions (34–36). Although *in vitro* caries models never reproduce all aspects of the clinical situation, sample acquisition is simple and specimens can be treated in a standardized and reproducible way (37). For example, EDTA is claimed to lead to nearly complete mineral loss within the lesion body (38). Micro-CT measurements in our study, however, revealed a demineralized volume in every cavity before complete excavation and selective excavation, which was depictable in micro-CT and, thus, not completely depleted from calcium and phosphate (Fig. 4). This stable demineralization might be due to the complex geometry of our model with long diffusion paths, which is more similar

to the way demineralization takes place clinically than the complete demineralization often used in *in vitro* studies.

It is widely accepted that color and stainability of dentin are not reliable parameters in the determination of the endpoint of caries excavation (39,40). Furthermore, bacteria-based detection techniques, such as fluorescence aided caries excavation (FACE), could not be used in our study, since we did not use a bacterial demineralization protocol (41–44). Therefore, polymer burs were chosen to provide an objective endpoint for complete excavation in the present study (45,46).

In the present study, enamel was slightly beveled and etched selectively before applying the self-etch adhesive system, as these procedures facilitate reduced marginal discoloration and increased retention (47). The self-etch adhesive system used allowed a chemical interaction with dentin by 10-methacryloyloxy-decyl-dihydrogen-phosphate (10-MDP) monomers without exposure of vulnerable collagen (48). Because our study aimed to evaluate the influence of complete or selective excavation, and not to test other restorative procedure parameters, all cavities had complete margins in enamel and were restored according to one clinically accepted protocol (25,47,49).

Leakage at the margin of restorations increases over time due to thermal and mechanical stress (28,50). Corroborating previous *in vitro* studies (28,51), we found

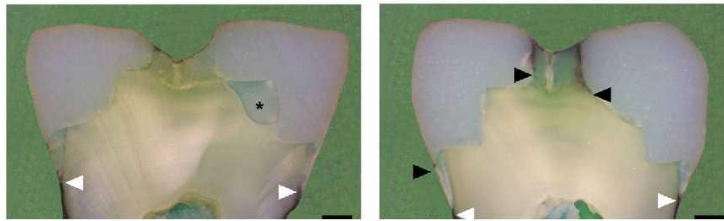


Fig. 8. Example tooth-sections for evaluation. Left image: Section of a tooth (Category 2 – carious lesions produced on both, mesial and distal, surfaces of the tooth) without TCML. Composite restoration after complete excavation on left side, composite restoration after selective excavation with residual demineralized dentin (*) on right side. Restoration margins are located above the cemento-enamel-junction (white arrow heads). No dye penetration is visible at the restoration-tooth interfaces. Scale bar: 1 mm. Right image: Section of a tooth (Category 1 – carious lesion produced on one, mesial or distal, surface of the tooth) with TCML. The composite restoration of caries-free control is located on the left side, the composite restoration after complete excavation on the right side. Restoration margins are located above the cemento-enamel-junction (white arrow heads). Occlusal dye penetration (black arrow heads) appeared on both occlusal margins reaching dentin at the restoration on the right side. Little approximal dye penetration (black arrowhead) could be detected for the left restoration. Scale bar: 1 mm. TCML: thermocycling and mechanical loading.

marginal dye penetration to be significantly higher after thermocycling and mechanical loading than without thermocycling and mechanical loading, where dye penetration was seen mostly in enamel segments. Unlike other authors, we investigated dye penetration both initially *and* under the influence of thermocycling and mechanical loading (25,52). To stay in line with previous studies, we aimed not to exceed physiological forces in our *in vitro* model by using a mechanical load of 72.5 N (10,28,53). A significantly decreased marginal integrity after thermocycling and mechanical loading was observed in all experimental groups, highlighting the validity of the chosen model of artificial aging (i.e. thermocycling and mechanical loading). Furthermore, the marginal integrity of the caries-free controls with less extension in pulpal direction did not differ from the marginal integrity observed in the other groups. The greater extension of restorations after complete excavation or selective excavation did not reduce the marginal integrity of the restorations having enamel margins compared to the restorations made in caries-free control teeth. Some authors have speculated that the volume of the caries lesion may have an effect on the marginal quality of restorations (25). Our findings do not support this speculation, because in our study demineralization volume did not correlate with dye penetration, irrespective of whether the samples were exposed to thermocycling and mechanical loading or not.

As is typical clinically, the length of approximal enamel segments after preparation was much shorter than at the occlusal margin. Composites have been shown to provide better bond strength to enamel than to dentin in several *in vitro* studies (25,28,54). Even though the approximal margin consists of a higher proportion of dentin, dye penetration between the occlusal and approximal margin was not significantly different in our experiments. This might indicate that even a short enamel segment at approximal margins yields a tight seal and protects the dentin from penetration. The main finding of this study is that there is no effect on the marginal seal of adhesive composite restorations,

irrespective of whether there were no caries at all, or whether caries were removed completely or selectively prior to placing the restoration. A conservative approach, using selective caries excavation in case of well-established dentin caries lesions, can therefore be recommended.

However, the second finding of this study is that marginal seals deteriorate with wear (tested here using thermocycling and mechanical loading). This can be interpreted to mean that every adhesive restoration, despite the presence or absence of residual caries, loses its tight seal with time. Hence, with time, every restoration margin will become permeable for sucrose and other nutritive molecules. Whether this might enable residual bacteria to reestablish metabolic activity, or even proliferate, needs to be further evaluated (55). Likewise, the impact thereof on pulp hemostasis and biology and its reaction patterns is highly interesting and warrants further investigation.

Within the limitations of our *in vitro* study, we could not find any significant differences in the marginal integrity of restorations made in teeth exposed to complete excavation, selective excavation, or caries-free control lesions. Thus, the null hypothesis could not be rejected for each situation with and without thermocycling and mechanical loading. However, aging, as simulated by thermocycling and mechanical loading, significantly increased the dye penetration of composite restorations. The presence of demineralized dentin close to the pulp following selective excavation compared to complete excavation does not compromise marginal integrity in class II cavities with enamel margins.

Acknowledgements – Helga Ebensberger and Gerlinde Ferstl (Department of Conservative Dentistry and Periodontology, University Medical Center Regensburg, Germany) are gratefully acknowledged for their great technical support in all stages of this study.

The authors are grateful to Dr. Birgit Striegl (Regensburg Center of Biomedical Engineering, OTH and University of Regensburg, Germany) for the technical assistance with micro-CT measurements. The micro-CT (phoenix v|tome|x^s 240/180 research edition,

General Electric Measurement & Control, Wunstorf, Germany) was funded by Deutsche Forschungsgemeinschaft (DFG-Nr. INST102/11-1 FUGG). Dr. Henrik Fleiner is acknowledged for his support in specimen collection.

The authors thank PD Dr. Á.M. Lennon (Department of Conservative Dentistry and Periodontology, University Medical Center Regensburg, Germany) for linguistic support with the manuscript. Konstantin J. Scholz received funding from the Medical Faculty of the University of Regensburg (ReForM A program).

The experiments were conceived and designed by W. Buchalla, K.J. Scholz, M. Widbiller, K.-A. Hiller, M. Hinderberger, and M. Federlin. M. Hinderberger, and K.J. Scholz performed the experiments. K.-A. Hiller, M. Hinderberger, and K.J. Scholz analyzed the data. The paper was written by K.J. Scholz, M. Hinderberger, M. Widbiller, K.-A. Hiller, M. Federlin, and W. Buchalla. Open access funding was enabled and organized by Projekt DEAL.

Conflicts of interest – The authors have no conflicts of interest to declare.

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Danksagung

Zuallererst danke ich vielmals Herrn Prof. Wolfgang Buchalla für die Überlassung des Themas, die Anleitung zu wissenschaftlichem Arbeiten, die großzügige und fortwährende Unterstützung, sowie die Erstellung des Erstgutachtens. Die ausgezeichneten Bedingungen der Poliklinik für Zahnerhaltung und Parodontologie trugen maßgeblich zum Erfolg der Arbeit bei.

Bei Frau Prof. Carola Kolbeck möchte ich mich für die Erstellung des Zweitgutachtens bedanken.

Ganz besonders danke ich Herrn Dr. Konstantin Scholz für die hervorragende Betreuung. Seine unermüdliche Unterstützung in allen Bereichen war eine große Hilfe und hat wesentlich zum Gelingen dieser Arbeit beigetragen.

Ebenso herzlich bedanken möchte ich bei Frau Helga Ebensberger und Frau Gerlinde Ferstl für ihre tatkräftige Unterstützung bei der Durchführung und Auswertung der Studie.

Herrn Priv. Doz. Matthias Widbiller danke ich für die Unterstützung bei der Themenfindung und die wertvollen Hinweise bei der Erstellung der Arbeit.

Herrn Dr. Karl-Anton Hiller gilt mein Dank für die Hilfe bei der Planung und Umsetzung des Versuchs sowie der statistischen Auswertung der Ergebnisse.

Frau Prof. Marianne Federlin danke ich für die Hilfe bei der Literaturrecherche sowie der Planung und Umsetzung des Versuchs.