Hydrolysis-resistant and stress-buffering bifunctional polyurethane adhesive for durable dental composite restoration

Jiahui Zhang, Xiaowei Guo, Xiaomeng Zhang, Huimin Wang, Jiufu Zhu, Zuosen Shi, Song Zhu and Zhanchen Cui

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2nd revised submission: 5 June 2020
Final acceptance: 12 June 2020

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History
RSOS-200457.R0 (Original submission)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
Yes
Recommendation?
Major revision is needed (please make suggestions in comments)

Comments to the Author(s)
The paper is in general well written and describes a clinically relevant topic. However, several aspects needs to be answered.

Abstract.
It’s necessary that all methodologies performed are specified.
The conclusion does not answer the objective of the paper.

Fig.1 and 3 are unnecessary. Graphic abstract is more suitable.

Some methodologies used are insufficient described, as contact angle (how the calculation was performed; kind of liquid; device information; software used); thermal stability characterization; failure pattern in SEM.
Which light-curing unit was used?
Only PU4 was used as experimental group for several methodologies. Clarify.

Statistical test of normality and homoscedasticity distribution is required.
Only two-way ANOVA is presented for Microtensile bond strength with uncertainty post-hoc test (Tukey or Dunnet?). It is necessary to present all statistical analyzes for all the methodologies developed.
In the results topic, p-values are presented (p<0.05). It is required to present the specific p-value, when p is statistically significant.
Letters indicating statistical difference in alphabetical order relating lower to higher means are preferable.
Was microleakage qualitatively evaluated?

Z350XT is a conventional resin composite used as oblique and incremental technique. However, the author used this material as bulk fill, with 4-5 increment layer. This technique directly influences the adhesive performance, compromising the results and discussion.
I suggest that it be redone correctly (without research bias) or removed.

The thickness of resin composite directly influences on behavior of composite material, as well as the interface bonding. Therefore, it can be observed that fig. 9C presents a greater thickness compared to other images, influencing marginal microleakage in the back wall of the cavity (bias). Clarify.

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
No

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No
Have you any concerns about statistical analyses in this paper?
Yes

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Major revision is needed (please make suggestions in comments)

Comments to the Author(s)
Journal: Royal Society Open Science
Manuscript ID: RSOS-200457
Title: Hydrolysis-resistant and stress-buffering bifunctional polyurethane adhesive for durable dental composite restoration.
In this manuscript a new elastic polyurethane (PU) adhesive is reported. After its synthesis by the solution polymerization method, it was evaluated in different endpoints: water sorption, water solubility, contact angle, thermal stability, mechanical properties, and in-vitro biocompatibility. This new material has better stability and durability of the dental adhesion interface in comparison to other commercial.

Comments:

1) Table 1 should be simplified. For example, the manufacturer's name could be removed, and the batch number could go as a table foot.

2) Figure 6A and 6B show the mechanical properties of the synthesized PUs. However, these properties of commercial PUs are not shown. These data are also not included in the discussion of the results.

3) Why are the conversion data for samples PU1, PU2, PU3, PU5 and PU6 not included?

4) The microtensile bond strength can be related to tensile strength?. Explain it. By other hand, both microtensile and tensile are measured in the same scale (MPa)?

5) In the TGA curve there is a small inflection in the slope, if the curve was derived, it could detect PUA and PUB ?. Could you show DSC of PUA, PUB and PU4?.

6) Figure 8F shows that SBU aging can also penetrate the dentinal tubules, and Figure 6G shows that the microtensile bond strength of SBU is greater than PU4 ?. Could you say that by this measure that PU4 is not better than SBU?.

7) In Figure 10A, the viability values are not shown in percentages as indicated (i.e., 1.0= 100%). Setting viability at 70% is arbitrary (the reader can suppose a 30% death of fibroblasts in the mouth?). What is the variation in your controls?. Even a 20% decrease in viability, this is significant in a cell population?. You can perform an ANOVA followed by a Tukey test to set significance to 5%.

8) The light microscopy images in Figure 10B must be improved, or show SEM images, in order to visualize the morphology of the cells. With the viability shown in Figure 10A, some dead (red) cells should be observed. Fibroblasts grow extended (in spreading), however the fluorescence image gives the appearance of cluster or aggregates. Can you explain it?
We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don't hesitate to let us know at the email address below.

Dear Dr Zhang:

Title: Hydrolysis-resistant and stress-buffering bifunctional polyurethane adhesive for durable dental composite restoration
Manuscript ID: RSOS-200457

Thank you for your submission to Royal Society Open Science. The chemistry content of Royal Society Open Science is published in collaboration with the Royal Society of Chemistry.

The editor assigned to your manuscript has now received comments from reviewers. We would like you to revise your paper in accordance with the referee and Subject Editor suggestions which can be found below (not including confidential reports to the Editor). Please note this decision does not guarantee eventual acceptance.

Please submit your revised paper before 22-May-2020. Please note that the revision deadline will expire at 00.00am on this date. If we do not hear from you within this time then it will be assumed that the paper has been withdrawn. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office in advance. We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Editors, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers.

To revise your manuscript, log into http://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. Revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you must respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload." Please use this to document how you have responded to the comments, and the adjustments you have made. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response.

Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Yours sincerely,
Dr Laura Smith
Publishing Editor, Journals

Royal Society of Chemistry
Thomas Graham House
Science Park, Milton Road
Cambridge, CB4 0WF
Royal Society Open Science - Chemistry Editorial Office
On behalf of the Subject Editor Professor Anthony Stace and the Associate Editor Dr Ya-Wen Wang.

******************************************************

RSC Associate Editor:
Comments to the Author:
(There are no comments.)

RSC Subject Editor:
Comments to the Author:
(There are no comments.)

******************************************************

Reviewers' Comments to Author:
Reviewer: 1

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Reviewer: 2

Comments to the Author(s)
Journal: Royal Society Open Science
Manuscript ID: RSOS-200457
Title: Hydrolysis-resistant and stress-buffering bifunctional polyurethane adhesive for durable dental composite restoration.

In this manuscript a new elastic polyurethane (PU) adhesive is reported. After its synthesis by the solution polymerization method, it was evaluated in different endpoints: water sorption, water solubility, contact angle, thermal stability, mechanical properties, and in-vitro biocompatibility. This new material has better stability and durability of the dental adhesion interface in comparison to other commercial.

Comments:

1) Table 1 should be simplified. For example, the manufacturer's name could be removed, and the batch number could go as a table foot.

2) Figure 6A and 6B show the mechanical properties of the synthesized PUs. However, these properties of commercial PUs are not shown. These data are also not included in the discussion of the results.

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Author's Response to Decision Letter for (RSOS-200457.R0)

See Appendix A.
RSOS-200457.R1 (Revision)

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
Many of the responses to the reviewer are information that the reader needs to understand the manuscript, especially the parts marked with yellow (see Appendix B). Authors have to include and/or adapt these parts in the paragraphs of their main text for better clarity of the paper.

Decision letter (RSOS-200457.R1)

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don’t hesitate to let us know at the email address below.

Dear Dr Zhang:

Title: Hydrolysis-resistant and stress-buffering bifunctional polyurethane adhesive for durable dental composite restoration
Manuscript ID: RSOS-200457.R1

Thank you for submitting the above manuscript to Royal Society Open Science. On behalf of the Editors and the Royal Society of Chemistry, I am pleased to inform you that your manuscript will be accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the reviewers' comments at the end of this email.

The reviewers and handling editors have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.

Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript before 13-Jun-2020. Please note that the revision deadline
will expire at 00.00am on this date. If you do not think you will be able to meet this date please let me know immediately.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions". Under "Actions," click on "Create a Revision." You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referees.

When uploading your revised files please make sure that you have:

1) A text file of the manuscript (tex, txt, rtf, docx or doc), references, tables (including captions) and figure captions. Do not upload a PDF as your "Main Document".
2) A separate electronic file of each figure (EPS or print-quality PDF preferred (either format should be produced directly from original creation package), or original software format)
3) Included a 100 word media summary of your paper when requested at submission. Please ensure you have entered correct contact details (email, institution and telephone) in your user account
4) Included the raw data to support the claims made in your paper. You can either include your data as electronic supplementary material or upload to a repository and include the relevant doi within your manuscript
5) All supplementary materials accompanying an accepted article will be treated as in their final form. Note that the Royal Society will neither edit nor typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details where possible (authors, article title, journal name).

Supplementary files will be published alongside the paper on the journal website and posted on the online figshare repository (https://figshare.com). The heading and legend provided for each supplementary file during the submission process will be used to create the figshare page, so please ensure these are accurate and informative so that your files can be found in searches. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Once again, thank you for submitting your manuscript to Royal Society Open Science. The chemistry content of Royal Society Open Science is published in collaboration with the Royal Society of Chemistry. I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Kind regards,

Dr Laura Smith
Publishing Editor, Journals

Royal Society of Chemistry
Thomas Graham House
Science Park, Milton Road
Cambridge, CB4 0WF
Royal Society Open Science - Chemistry Editorial Office
On behalf of the Subject Editor Professor Anthony Stace and the Associate Editor Dr Ya-Wen Wang.

*************************************

RSC Associate Editor:
Comments to the Author:
(There are no comments.)

RSC Subject Editor:
Comments to the Author:
(There are no comments.)

*************************************

Reviewer comments to Author:
Reviewer: 2

Comments to the Author(s)
Many of the responses to the reviewer are information that the reader needs to understand the manuscript, especially the parts marked with yellow (see attach file). Authors have to include and/or adapt these parts in the paragraphs of their main text for better clarity of the paper.

Author's Response to Decision Letter for (RSOS-200457.R1)

See Appendix C.

Decision letter (RSOS-200457.R2)

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don't hesitate to let us know at the email address below.

Dear Dr Zhang:

Title: Hydrolysis-resistant and stress-buffering bifunctional polyurethane adhesive for durable dental composite restoration
Manuscript ID: RSOS-200457.R2

It is a pleasure to accept your manuscript in its current form for publication in Royal Society Open Science. The chemistry content of Royal Society Open Science is published in collaboration with the Royal Society of Chemistry.

The comments of the reviewer(s) who reviewed your manuscript are included at the end of this email.
Thank you for your fine contribution. On behalf of the Editors of Royal Society Open Science and the Royal Society of Chemistry, I look forward to your continued contributions to the Journal.

Yours sincerely,
Dr Laura Smith
Publishing Editor, Journals

Royal Society of Chemistry
Thomas Graham House
Science Park, Milton Road
Cambridge, CB4 0WF

On behalf of the Subject Editor Professor Anthony Stace and the Associate Editor Dr Ya-Wen Wang.

*******

RSC Associate Editor
Comments to the Author:
(There are no comments.)

*******

Reviewer(s)' Comments to Author:
Dear Editor and Reviewers,

We hope you are keeping well at this difficult time.

Thanks very much for taking your time to review this manuscript. I really appreciate all these precious comments and suggestions. Please find my itemized responses in below and my revisions in the resubmitted files.

Thanks again.

Reviewer: 1

1) Abstract.

It’s necessary that all methodologies performed are specified.
The conclusion does not answer the objective of the paper.

We are grateful for the suggestion.

We have revised the abstract according to the suggestion that all methodologies performed are specified and the conclusion answers the objective of the paper. The revised text is as following. “A new elastic polyurethane (PU) adhesive was reported in this study to improve the stability and durability of the dental adhesion interface. A polyurethane oligomer was synthesized by the solution polymerization method, and a diluent and solvent were added to prepare PU adhesives. The water sorption, water solubility, contact angle, thermal stability, degree of conversion and mechanical properties of the PU adhesives were evaluated. Experimental applications for tooth restoration (microtensile bond strength and microleakage) were also performed. And cytotoxicity test was carried out. The water sorption and solubility of the PU adhesives were significantly lower than those of three commercial adhesives. The microtensile bond strength of the PU adhesives was improved after thermocycling test, and the extent of microleakage was diminished when compared with that of commercial adhesives. Biocompatibility testing demonstrated that the PU adhesive was nontoxic to L929 fibroblasts. This study shows the ability of PU adhesive to improve the stability and durability of the dental adhesion interface and may refocus the attention of scientists from rigid bonding to flexible bonding for dental adhesion, and it sheds light on a new strategy for the stable and durable bonding interface of dentin adhesives.”
2) Fig.1 and 3 are unnecessary. Graphic abstract is more suitable.

We are grateful for the suggestion.

Based on the comments, Fig.1 and 3 were removed in the article and graphic abstract was added.

3) Some methodologies used are insufficient described, as contact angle (how the calculation was performed; kind of liquid; device information; software used); thermal stability characterization; failure pattern in SEM.

We are grateful for the suggestion.

More detailed information was added to supplement the insufficient methodologies according to the comment to make it more clearly. The revised text is as following.

contact angle

“Contact angles were obtained using the sessile drop method with a Dataphysics contact angle analyzer (OCA-20, DataPhysics Co., German). This instrument consists of a CCD video camera with a resolution of 768 × 576 pixel and up to 50 images per second and multiple microsyringe units. A drop of 6 µl of deionized water was gently dropped onto the surface of the adhesive to take a digital photo. The digital drop image was processed by a specialized software SCA 20, which calculated both the left and right contact angles from the shape of the drop.” The representative digital drop images are shown in the following picture.

thermal stability characterization;

“Thermogravimetric analysis measurement of PU4 adhesive was performed using a TGA thermal analyzer (Q500, TA Instruments, USA). Initial sample masse is around 5 mg. The heating rate is 10 °C/min. The experiments were performed in an inert atmosphere with a continuous flow of nitrogen at the rate of 150 ml/min and heated from room temperature to 800 °C.”
failure pattern in SEM.

The SEM in this study is the intact bonding surface of the PU4 adhesive and three commercial adhesives. We are sorry that failure pattern in SEM was not conducted in this study. In the following study, we will supplement this experiment according to the suggestion.

Which light-curing unit was used?

The light-curing unit was a LED light (SLC-VIIIA, China) which was used in clinical practice. The LED light had an output light intensity of approximately 900 mW/cm², which was monitored by a radiometer to ensure light intensity.

Only PU4 was used as experimental group for several methodologies. Clarify.

In this study, we prepared seven kinds of PU adhesives. Then, we conduct several experiments (tensile strength, elongation at break, water solubility, water sorption and contact angle) to evaluate them. Finally, among these seven adhesives, PU4 possesses the highest tensile strength and relatively higher elongation at break. Considering the water absorption/solubility and contact angle of seven kinds of PU adhesives comprehensively, PU4 was considered to have the best comprehensive performance and chosen as the final experimental adhesive formulation for the following tests (degree of conversion, microtensile bond strength, microleakage and biocompatibility) to compare with three commercial adhesives. So, only PU4 was used as experimental group for the following methodologies.

4) Statistical test of normality and homoscedasticity distribution is required. Only two-way ANOVA is presented for Microtensile bond strength with uncertainty post-hoc test (Tukey or Dunnet?). It is necessary to present all statistical analyzes for all the methodologies developed.

We are grateful for the suggestion.

Statistical test of normality and homoscedasticity distribution has been conducted. All statistical analyzes for all the methodologies developed have been presented in the section of statistical analysis. We have added this section in the text of statistical analysis to make it more clearly. The revised text is as following.

"Data were expressed as the mean ± standard deviation. The data were consistent with normality and homoscedasticity distribution. Data for microtensile bond strength were analyzed using two-
way ANOVA, and the data of tensile strength, elongation at break, water solubility, water sorption, contact angle and degree of conversion were submitted to one-way ANOVA using SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA). Multiple comparison analysis was conducted using the Tukey test. The significance level was set at $p = 0.05$ for this study.

In the results topic, p-values are presented ($p<0.05$). It is required to present the specific p-value, when $p$ is statistically significant.

The specific p-values have been presented when $p$ is statistically significant in the manuscript. However, there are several summary statements with more than one P value. Therefore, we have listed them in the following tables to ensure the conciseness of the article.

(1) “Compared with these methacrylic resin adhesives, the solubility of PU adhesives was significantly reduced ($p < 0.05$), as shown in Fig. 6 (C).”

|       | SB2     | SPB     | SBU     |
|-------|---------|---------|---------|
| PU1   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU2   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU3   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU4   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU5   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU6   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU7   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |

(2) “Fig. 6 (D) shows that the water absorption values of the seven PU adhesives are significantly lower than those of the three commercial adhesives ($p < 0.05$).”

|       | SB2     | SPB     | SBU     |
|-------|---------|---------|---------|
| PU1   | $P < 0.0001$ | $P = 0.0039$ | $P < 0.0001$ |
| PU2   | $P < 0.0001$ | $P = 0.0015$ | $P < 0.0001$ |
 PU3  P < 0.0001  P = 0.0009  P < 0.0001  
 PU4  P < 0.0001  P < 0.0001  P < 0.0001  
 PU5  P < 0.0001  P < 0.0001  P < 0.0001  
 PU6  P < 0.0001  P = 0.0013  P < 0.0001  
 PU7  P < 0.0001  P = 0.0003  P < 0.0001  

(3) “The contact angles of the PU adhesives are all greater than 83°, significantly larger than that of the commercial adhesive (p < 0.05).”

Table. 3 The specific p-values of Tukey’s multiple comparisons in contact angle test.

|          | SB2     | SPB     | SBU     |
|----------|---------|---------|---------|
| PU1      | P < 0.0001 | P = 0.0081 | P = 0.0042 |
| PU2      | P < 0.0001 | P = 0.0003 | P = 0.0001 |
| PU3      | P < 0.0001 | P = 0.0007 | P = 0.0003 |
| PU4      | P < 0.0001 | P = 0.03  | P = 0.0158 |
| PU5      | P < 0.0001 | P = 0.0033 | P = 0.0013 |
| PU6      | P < 0.0001 | P = 0.0001 | P < 0.0001 |
| PU7      | P < 0.0001 | P = 0.0428 | P = 0.0254 |

(4) “Fig. 6 (A) showed that there was no significant difference among PU2, PU3, and PU4, but their tensile strength was significantly higher than that of other groups (p < 0.05).”

Table. 4 The specific p-values of Tukey’s multiple comparisons in tensile strength test.

|          | PU1     | PU5     | PU6     | PU7     |
|----------|---------|---------|---------|---------|
| PU2      | /       | P = 0.0011 | P < 0.0001 | P < 0.0001 |
| PU3      | /       | P = 0.0003 | P < 0.0001 | P < 0.0001 |
| PU4      | P = 0.0274 | P < 0.0001 | P < 0.0001 | P < 0.0001 |
In terms of elongation at break, as shown in Fig. 6 (B), there was no significant difference between PU4 and PU5, and their elongation was obviously higher than that of the other groups (p < 0.05).

Table. 5 The specific p-values of Tukey's multiple comparisons in elongation at break test.

|        | PU1  | PU2  | PU3  | PU6  | PU7  |
|--------|------|------|------|------|------|
| PU4    | P < 0.0001 | P < 0.0001 | P < 0.0001 | /    | P < 0.0001 |
| PU5    | P < 0.0001 | P < 0.0001 | P < 0.0001 | P = 0.0056 | P < 0.0001 |

Letters indicating statistical difference in alphabetical order relating lower to higher means are preferable.

Letters indicating statistical difference in alphabetical order have been adjusted. And figures have been changed correspondingly.

Was microleakage qualitatively evaluated?

Microleakage was qualitatively evaluated as following. The microleakage of group SB2, group SPB and group SBU reached the axial surface with depth of 1.75 ± 0.08 mm, 1.79 ± 0.10 mm, and 1.70 ± 0.12 mm respectively. The leakage of group PU4 exceeded half of the depth of the hole, reaching 0.98 ± 0.16 mm. There is no significant difference among group SB2, group SPB and group SBU. However, the microleakage of commercial adhesive is significant higher than that of PU4 adhesive (P < 0.0001).

5) Z350XT is a conventional resin composite used as oblique and incremental technique. However, the author used this material as bulk fill, with 4–5 increment layer. This technique directly influences the adhesive performance, compromising the results and discussion. I suggest that it be redone correctly (without research bias) or removed.

We are grateful for the suggestion.

In the manuscript, we described the using method of Z350XT as follows “The commercial composite resin Z350XT was placed on the surface of the treated dentin layer by layer approximately 4-5 mm in height with light curing for 40 s.” The describing phrase “layer by layer” means layered filling technique. We are sorry about the confusing writing, so we have modified the
manuscript describing this part to make it more clearly. The revised text is as following. “Three 1.5-mm-thick layers of commercial composite resin Z350XT were placed over the surface of the treated dentin. Each resin composite was light cured for 40 s using a light-curing unit.”

6) The thickness of resin composite directly influences on behavior of composite material, as well as the interface bonding. Therefore, it can be observed that fig. 9C presents a greater thickness compared to other images, influencing marginal microleakage in the back wall of the cavity (bias). Clarify.

We are grateful for the suggestion.

We are sorry about using the misleading picture. Now, fig. 9C was replaced with a new picture as following. The thickness of resin composite in four different groups was almost the same to ensure the reliability of the experiment.
Reviewer: 2

Comments:

1) Table 1 should be simplified. For example, the manufacturer's name could be removed, and the batch number could go as a table foot.

We are grateful for the suggestion.

Table 1 has already been simplified to make it more clearly. The manufacturer's name was removed, and the batch number went as a table foot.

| Material                  | Code | Category | Formulation                                                                                                                                 |
|---------------------------|------|----------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Single bond 2             | SB2  | 2-step etch-and-rinse | bis-GMA, HEMA, dimethacrylates, silica nanofiller, polyalkenoic acid copolymer, initiators, water, ethanol                                      |
| Spectrum bond             | SPB  | 2-step etch-and-rinse | UDMA, trimethacrylate, PENTA, highly dispersed silicon dioxide, camphorquinone, BHT, cetylamine hydrofluoride, acetone                   |
| Single bond universal     | SBU  | Universal adhesive | MDP phosphate monomer, bis-GMA, dimethacrylate resins, HEMA, Vitrebond copolymer, fillers, ethanol, water, initiators, silane          |

* bis-GMA: bisphenol a diglycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, UDMA: urethane dimethacrylate, PENTA: phosphoric acid modified acrylate resin, BHT: butylhydroxytoluene, MDP: methacryloyloxydecyl dihydrogenphosphate. Lot number: SB2 (N912223); SPB (1801000919); SBU (4330297).

2) Figure 6A and 6B show the mechanical properties of the synthesized PUs. However, these properties of commercial PUs are not shown. These data are also not included in the discussion of the results.

We are grateful for the suggestion.

The tensile strength and elongation at break is mainly used for testing materials with elasticity, such as the synthesized PU adhesive in this study. The composition of the commercial adhesive
system is different from that of the experimental PU adhesive. The commercial adhesive is mainly composed of the organic resin matrix and inorganic filler which is a rigid structure with almost no tensile deformation. Therefore, commercial adhesives were not tested. In this experiment, we make use of the tensile strength and elongation at break test to evaluate seven kinds of synthetic elastic PU adhesives. And combined with other tests (water absorption/solubility and contact angle), PU4 was considered to possess the best performance. And PU4 was chosen as the final experimental group to compare with three commercial adhesives in the follow-up tests.

3) Why are the conversion data for samples PU1, PU2, PU3, PU5 and PU6 not included?

We are grateful for the suggestion.

In this study, we prepared seven kinds of PU adhesives. Then, we conduct several experiments (tensile strength, elongation at break, water solubility, water sorption and contact angle) to evaluate them. Finally, among these seven adhesives, PU4 possesses the highest tensile strength and relatively higher elongation at break. Considering the water absorption/solubility and contact angle of seven kinds of PU adhesives comprehensively, PU4 was considered to have the best performance and chosen as the final experimental adhesive formulation for the following tests (degree of conversion, microtensile bond strength, microleakage and biocompatibility) to compare with three commercial adhesives. So, the conversion data for samples PU1, PU2, PU3, PU5 and PU6 was not included.

4) The microtensile bond strength can be related to tensile strength?. Explain it. By other hand, both microtensile and tensile are measured in the same scale (MPa)?

We are grateful for the suggestion.

Tensile strength is the maximum stress that a material can withstand while being stretched or pulled before breaking. In brittle materials the tensile strength is close to the yield point, whereas in ductile materials the tensile strength can be higher. The tensile strength is usually found by performing a tensile test. In this study, the dumbbell-shaped specimens were prepared in accordance with the standard ASTM-D638-2003 to evaluate the elastic property of PU adhesives. The prepared specimen is shown in Fig. 5 (H).
Microtensile bond strength test is currently considered as a versatile and standard bond strength testing method. It was introduced in 1994. Since then, it has been utilized profoundly across many bond strength testing laboratories, making it currently one of the most standard and versatile bond strength test.

In general, tensile strength is the elastic property of the material itself, however, microtensile bond strength is the adhesion ability of adhesive with tooth. There is no direct relationship between them.

And both microtensile bond strength and tensile strength are measured in the same scale (MPa). Microtensile bond strength is determined by dividing the loading force at break by the cross-sectional area of the sticks. Tensile strength is calculated by dividing the maximum stress at fracture by the cross-sectional area of the sample at the breaking point. So, they are measured in the same scale (MPa).

5) In the TGA curve there is a small inflection in the slope, if the curve was derived, it could detect PUA and PUB ?. Could you show DSC of PUA, PUB and PU4?.

Thank you very much for your comments. We are grateful for the suggestion. Your comments are very valuable for us to improve the thermal stability of the PU adhesive material. The TGA curve is the result of final formulation of PU4. TGA of separate PUA and PUB need to be tested. Due to COVID-19, the school has been closed. We contacted the school laboratory and it was uncertainty when it would be available. Therefore, the supplement results of TGA and DSC may need waiting a long time. The TGA of PU4 tested in this study is not enough for evaluating the thermal stability of the material which is a limitation of the study, but it can also show some information about the PU4 adhesive. The initial degradation temperature of 5% weight loss was observed at 269.00 °C. The maximum tolerant temperature of the oral mucosa is approximately 60 °C. Therefore, PU4 adhesive may be applied in the oral environment. We searched the relevant literature, and the following two articles can be used for reference. Our laboratory will continue to
study in this area. In the subsequent research, we will conduct more detailed and complete experiments of the thermal stability of the material.

1. Solís-Correa R, Vargas-Coronado R, Aguilar-Vega M, Cauich-Rodriguez J, San Romn J, Marco A. Synthesis of HMDI-based segmented polyurethanes and their use in the manufacture of elastomeric composites for cardiovascular applications. J Biomater Sci Polym Ed. 2007;18(5):561-78.

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6) Figure 8F shows that SBU aging can also penetrate the dentinal tubules, and Figure 6G shows that the microtensile bond strength of SBU is greater than PU4?. Could you say that by this measure that PU4 is not better than SBU?

We are grateful for the suggestion.

Although SBU aging can also penetrate the dentinal tubules, its adhesive layer fractured more severely. In the aging group, microtensile bond strength of SBU is greater than that of PU4, but there is no statistical significance. The microtensile bond strength of SBU decreased obviously after aging, while the microtensile bond strength of PU4 increased. It is indicated that longer-term aging test may be needed in the future study. In term of the microtensile experiment, SBU is better than PU4, but water absorption and water solubility of PU4 are much lower than that of SBU which may contribute to reducing hydrolytic degradation of the bonding interface. And microleakage of PU4 has also been improved when compared with SBU. Its elastic properties can buffer various stresses during long-term use, improving the stability and durability of the adhesive bonding interface. Therefore, the comprehensive performance of PU4 is still better than that of SBU.

7) In Figure 10A, the viability values are not shown in percentages as indicated (i.e., 1.0= 100%). Setting viability at 70% is arbitrary (the reader can suppose a 30% death of fibroblasts in the mouth?). What is the variation in your controls?. Even a 20% decrease in viability, this is significant in a cell population?. You can perform an ANOVA followed by a Tukey test to set significance to 5%.

We are grateful for the suggestion.

In Figure 10A, the viability values have been shown in percentages in the manuscript.
According to GB/T 16886.5-2003 (ISO 10993-5:1999), samples with cell viability larger than 75\% of blank group can be considered as non-cytotoxicity\[1-3\]. We are sorry that 70\% is not accurate. It has been revised in the text. In this study, cell viability of PU4 adhesive was all greater than 80\% which demonstrated good cell viability and biocompatibility, meeting the clinical biosafety requirements.

The variation of controls has already been added.

We have performed an ANOVA followed by a Tukey test. In the group of 48 h and 72 h, there is significant difference between control group and the other groups (SB2, SPB, SBU, PU4). However, no statistical difference exists between PU4 adhesive and three commercial adhesives.

It is important to mention that the commercial adhesive system may show some toxicity to a certain extent. Adhesive systems components [e.g. Bis-GMA (bisphenol A diglycidyl methacrylate), HEMA (2-hydroxyethyl methacrylate), TEGDMA (triethylene glycol dimethacrylate), and UDMA (urethane dimethacrylate), camphorquinone] are cytotoxic. Despite its cytotoxic produced by direct contact on pulp cells, these may be clinically attenuated or neutralized, as adhesive systems are applied to dentin, which acts as a physical barrier depending on its thickness.

In this study, there is no significant difference between commercial adhesives which is used in clinical practice and PU adhesives in MTT test which indicates that PU4 adhesive can meet the clinical biosafety requirements.

1. Cao D, Zhang Y, Li Y, Shi X, Gong H, Feng D, Guo X, Shi Z, Zhu S, Cui Z. Fabrication of superhydrophobic coating for preventing microleakage in a dental composite restoration. Mater Sci Eng C Mater Biol Appl. 2017;78:333-40.
2. Yang X, Yang K, Yu F, Chen X, Wu S, Zhu Z. Preparation of novel bilayer hydrogels by combination of irradiation and freeze-thawing and their physical and biological properties. Polym Int. 2009; 58:1291-8.
3. Sudarsan S, Franklin D S, Sakthivel M, Guhanathan S. Non toxic, antibacterial, biodegradable hydrogels with pH-stimuli sensitivity: Investigation of swelling parameters. Carbohyd Polym. 2016; 148:206-15.

8) The light microscopy images in Figure 10B must be improved, or show SEM images, in order to visualize the morphology of the cells. With the viability shown in Figure 10A, some dead (red) cells should be observed. Fibroblasts grow extended (in spreading), however the fluorescence image gives the appearance of cluster or aggregates. Can you explain it?

We are grateful for the suggestion.

The light microscopy images in Figure 10B have been improved to make it more clearly. And we adjusted the contrast of the fluorescence images, and the dead cells (red) could be seen more clearly.
L929 fibroblasts should grow extended (in spreading), however the fluorescence image gives the appearance of cluster or aggregates. In this study, the live/dead cell staining kit includes two
parts that calcein-AM is used for living cell staining and propidium iodide is used for dead cell staining. The staining solution is slightly toxic to the cells. If the staining time is too long, it may cause poor cell morphology. And the osmotic pressure of the staining solution may not be exactly the same with the cytoplasm which can also lead to the cell deformation.
# Appendix B

## ROYAL SOCIETY OPEN SCIENCE

**Hydrolysis-resistant and stress-buffering bifunctional polyurethane adhesive for durable dental composite restoration**

| Journal: | *Royal Society Open Science* |
| --- | --- |
| Manuscript ID | RSOS-200457.R1 |
| Article Type: | Research |
| Date Submitted by the Author: | 14-May-2020 |
| Complete List of Authors: | zhang, Jiahui; Jilin University, Guo, Xiaowei; Jilin University, Zhang, Xiaomeng; Jilin University, Wang, Huimin; Jilin University, Zhu, Jiuju; Jilin University, Shi, Zuosen; Jilin University, Zhu, Song; Jilin University, Cui, Zhanchen; Jilin University |
| Subject: | Materials science < CHEMISTRY, biomaterials < CROSS-DISCIPLINARY SCIENCES |
| Keywords: | polyurethane adhesive, dental restoration, hydrolysis-resistant, stress-buffering |
| Subject Category: | Chemistry |

[https://mc.manuscriptcentral.com/rsos](https://mc.manuscriptcentral.com/rsos)
Author-supplied statements

Relevant information will appear here if provided.

Ethics

Does your article include research that required ethical approval or permits?:
Yes

Statement (if applicable):
All relevant ethical applications (permit no. KT202003081) in this experiment were approved by the Ethics Committee of School and Hospital of Stomatology of Jilin University. Informed consent was obtained from all donors.

Data

It is a condition of publication that data, code and materials supporting your paper are made publicly available. Does your paper present new data?:
Yes

Statement (if applicable):
The data is provided as electronic supplementary materials.

Conflict of interest

I/We declare we have no competing interests

Statement (if applicable):
CUST_STATE_CONFLICT :No data available.

Authors’ contributions

This paper has multiple authors and our individual contributions were as below

Statement (if applicable):
Authors’ major contribution:
Jiahui Zhang: conducted the statistical analysis and wrote this manuscript;
Xiaowei Guo and Xiaomeng Zhang: finish the acquisition of data;
Huimin Wang, Jiufu Zhu and Zuosen Shi: conduct the experiments and repeated them;
Corresponding author: Song Zhu and Zhanchen Cui: designed and oversaw the study, and reviewed and revised this manuscript.
Dear Editor and Reviewers,

We hope you are keeping well at this difficult time.

Thanks very much for taking your time to review this manuscript. I really appreciate all these precious comments and suggestions. Please find my itemized responses in below and my revisions in the resubmitted files.

Thanks again.

Reviewer: 1

1) Abstract.

It’s necessary that all methodologies performed are specified.

The conclusion does not answer the objective of the paper.

We are grateful for the suggestion.

We have revised the abstract according to the suggestion that all methodologies performed are specified and the conclusion answers the objective of the paper. The revised text is as following. “A new elastic polyurethane (PU) adhesive was reported in this study to improve the stability and durability of the dental adhesion interface. A polyurethane oligomer was synthesized by the solution polymerization method, and a diluent and solvent were added to prepare PU adhesives. The water sorption, water solubility, contact angle, thermal stability, degree of conversion and mechanical properties of the PU adhesives were evaluated. Experimental applications for tooth restoration (microtensile bond strength and microleakage) were also performed. And cytotoxicity test was carried out. The water sorption and solubility of the PU adhesives were significantly lower than those of three commercial adhesives. The microtensile bond strength of the PU adhesives was improved after thermocycling test, and the extent of microleakage was diminished when compared with that of commercial adhesives. Biocompatibility testing demonstrated that the PU adhesive was nontoxic to L929 fibroblasts. This study shows the ability of PU adhesive to improve the stability and durability of the dental adhesion interface and may refocus the attention of scientists from rigid bonding to flexible bonding for dental adhesion, and it sheds light on a new strategy for the stable and durable bonding interface of dentin adhesives.”
2) Fig.1 and 3 are unnecessary. Graphic abstract is more suitable.

We are grateful for the suggestion.

Based on the comments, Fig.1 and 3 were removed in the article and graphic abstract was added.

3) Some methodologies used are insufficient described, as contact angle (how the calculation was performed; kind of liquid; device information; software used); thermal stability characterization; failure pattern in SEM.

We are grateful for the suggestion.

More detailed information was added to supplement the insufficient methodologies according to the comment to make it more clearly. The revised text is as following.

contact angle

“Contact angles were obtained using the sessile drop method with a Dataphysics contact angle analyzer (OCA-20, DataPhysics Co., German). This instrument consists of a CCD video camera with a resolution of 768 × 576 pixel and up to 50 images per second and multiple microsyringe units. A drop of 6 µl of deionized water was gently dropped onto the surface of the adhesive to take a digital photo. The digital drop image was processed by a specialized software SCA 20, which calculated both the left and right contact angles from the shape of the drop.”

The representative digital drop images are shown in the following picture.

thermal stability characterization;

“Thermogravimetric analysis measurement of PU4 adhesive was performed using a TGA thermal analyzer (Q500, TA Instruments, USA). Initial sample masse is around 5 mg. The heating rate is 10 °C/min. The experiments were performed in an inert atmosphere with a continuous flow of nitrogen at the rate of 150 ml/min and heated from room temperature to 800 °C.”

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failure pattern in SEM.

The SEM in this study is the intact bonding surface of the PU4 adhesive and three commercial adhesives. We are sorry that failure pattern in SEM was not conducted in this study. In the following study, we will supplement this experiment according to the suggestion.

Which light-curing unit was used?

The light-curing unit was a LED light (SLC-VIIIA, China) which was used in clinical practice. The LED light had an output light intensity of approximately 900 mW/cm², which was monitored by a radiometer to ensure light intensity.

Only PU4 was used as experimental group for several methodologies. Clarify.

In this study, we prepared seven kinds of PU adhesives. Then, we conduct several experiments (tensile strength, elongation at break, water solubility, water sorption and contact angle) to evaluate them. Finally, among these seven adhesives, PU4 possesses the highest tensile strength and relatively higher elongation at break. Considering the water absorption/solubility and contact angle of seven kinds of PU adhesives comprehensively, PU4 was considered to have the best comprehensive performance and chosen as the final experimental adhesive formulation for the following tests (degree of conversion, microtensile bond strength, microleakage and biocompatibility) to compare with three commercial adhesives. So, only PU4 was used as experimental group for the following methodologies.

4) Statistical test of normality and homoscedasticity distribution is required. Only two-way ANOVA is presented for Microtensile bond strength with uncertainty post-hoc test (Tukey or Dunnet?). It is necessary to present all statistical analyzes for all the methodologies developed.

We are grateful for the suggestion.

Statistical test of normality and homoscedasticity distribution has been conducted. All statistical analyzes for all the methodologies developed have been presented in the section of statistical analysis. We have added this section in the text of statistical analysis to make it more clearly. The revised text is as following.

“Data were expressed as the mean ± standard deviation. The data were consistent with normality and homoscedasticity distribution. Data for microtensile bond strength were analyzed
using two-way ANOVA, and the data of tensile strength, elongation at break, water solubility, water sorption, contact angle and degree of conversion were submitted to one-way ANOVA using SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA). Multiple comparison analysis was conducted using the Tukey test. The significance level was set at $p = 0.05$ for this study.

In the results topic, p-values are presented ($p<0.05$). It is required to present the specific p-value, when $p$ is statistically significant.

The specific p-values have been presented when $p$ is statistically significant in the manuscript. However, there are several summary statements with more than one $P$ value. Therefore, we have listed them in the following tables to ensure the conciseness of the article.

(1) “Compared with these methacrylic resin adhesives, the solubility of PU adhesives was significantly reduced ($p < 0.05$), as shown in Fig. 6 (C).”

Table. 1 The specific p-values of Tukey's multiple comparisons in water solubility test.

|       | SB2       | SPB       | SBU       |
|-------|-----------|-----------|-----------|
| PU1   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU2   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU3   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU4   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU5   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU6   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |
| PU7   | $P < 0.0001$ | $P < 0.0001$ | $P < 0.0001$ |

(2) “Fig. 6 (D) shows that the water absorption values of the seven PU adhesives are significantly lower than those of the three commercial adhesives ($p < 0.05$).”

Table. 2 The specific p-values of Tukey's multiple comparisons in water sorption test.

|       | SB2       | SPB       | SBU       |
|-------|-----------|-----------|-----------|
| PU1   | $P < 0.0001$ | $P = 0.0039$ | $P < 0.0001$ |
| PU2   | $P < 0.0001$ | $P = 0.0015$ | $P < 0.0001$ |
PU3    P < 0.0001     P = 0.0009     P < 0.0001
PU4    P < 0.0001     P < 0.0001     P < 0.0001
PU5    P < 0.0001     P < 0.0001     P < 0.0001
PU6    P < 0.0001     P = 0.0013     P < 0.0001
PU7    P < 0.0001     P = 0.0003     P < 0.0001

(3) “The contact angles of the PU adhesives are all greater than 83°, significantly larger than that of the commercial adhesive (p < 0.05).”

Table 3 The specific p-values of Tukey’s multiple comparisons in contact angle test.

|     | SB2    | SPB    | SBU    |
|-----|--------|--------|--------|
| PU1 | P < 0.0001 | P = 0.0081 | P = 0.0042 |
| PU2 | P < 0.0001 | P = 0.0003 | P = 0.0001 |
| PU3 | P < 0.0001 | P = 0.0007 | P = 0.0003 |
| PU4 | P < 0.0001 | P = 0.03  | P = 0.0158 |
| PU5 | P < 0.0001 | P = 0.0033 | P = 0.0013 |
| PU6 | P < 0.0001 | P = 0.0001 | P < 0.0001 |
| PU7 | P < 0.0001 | P = 0.0428 | P = 0.0254 |

(4) “Fig. 6 (A) showed that there was no significant difference among PU2, PU3, and PU4, but their tensile strength was significantly higher than that of other groups (p < 0.05).”

Table 4 The specific p-values of Tukey’s multiple comparisons in tensile strength test.

|     | PU1    | PU5    | PU6    | PU7    |
|-----|--------|--------|--------|--------|
| PU2 | /      | P = 0.0011 | P < 0.0001 | P < 0.0001 |
| PU3 | /      | P = 0.0003 | P < 0.0001 | P < 0.0001 |
| PU4 | P = 0.0274 | P < 0.0001 | P < 0.0001 | P < 0.0001 |
(5) “In terms of elongation at break, as shown in Fig. 6 (B), there was no significant difference between PU4 and PU5, and their elongation was obviously higher than that of the other groups (p < 0.05).”

Table. 5 The specific p-values of Tukey's multiple comparisons in elongation at break test.

|       | PU1    | PU2    | PU3    | PU6    | PU7    |
|-------|--------|--------|--------|--------|--------|
| PU4   | P < 0.0001 | P < 0.0001 | P < 0.0001 | /      | P < 0.0001 |
| PU5   | P < 0.0001 | P < 0.0001 | P < 0.0001 | P = 0.0056 | P < 0.0001 |

Letters indicating statistical difference in alphabetical order relating lower to higher means are preferable.

Letters indicating statistical difference in alphabetical order have been adjusted. And figures have been changed correspondingly.

Was microleakage qualitatively evaluated?

Microleakage was qualitatively evaluated as following. The microleakage of group SB2, group SPB and group SBU reached the axial surface with depth of 1.75 ± 0.08 mm, 1.79 ± 0.10 mm, and 1.70 ± 0.12 mm respectively. The leakage of group PU4 exceeded half of the depth of the hole, reaching 0.98 ± 0.16 mm. There is no significant difference among group SB2, group SPB and group SBU. However, the microleakage of commercial adhesive is significant higher than that of PU4 adhesive (P < 0.0001).

5) Z350XT is a conventional resin composite used as oblique and incremental technique. However, the author used this material as bulk fill, with 4-5 increment layer. This technique directly influences the adhesive performance, compromising the results and discussion. I suggest that it be redone correctly (without research bias) or removed.

We are grateful for the suggestion.

In the manuscript, we described the using method of Z350XT as follows “The commercial composite resin Z350XT was placed on the surface of the treated dentin layer by layer approximately 4-5 mm in height with light curing for 40 s.” The describing phrase “layer by layer” means layered filling technique. We are sorry about the confusing writing, so we have modified the
manuscript describing this part to make it more clearly. The revised text is as following. “Three 1.5-mm-thick layers of commercial composite resin Z350XT were placed over the surface of the treated dentin. Each resin composite was light cured for 40 s using a light-curing unit.”

6) The thickness of resin composite directly influences on behavior of composite material, as well as the interface bonding. Therefore, it can be observed that fig. 9C presents a greater thickness compared to other images, influencing marginal microleakage in the back wall of the cavity (bias). Clarify.

We are grateful for the suggestion.

We are sorry about using the misleading picture. Now, fig. 9C was replaced with a new picture as following. The thickness of resin composite in four different groups was almost the same to ensure the reliability of the experiment.
Reviewer: 2

Comments:

1) Table 1 should be simplified. For example, the manufacturer's name could be removed, and the batch number could go as a table foot.

We are grateful for the suggestion.

Table 1 has already been simplified to make it more clearly. The manufacturer's name was removed, and the batch number went as a table foot.

Table 1. Commercial adhesive for this study

| Material            | Code | Category       | Formulation                                                                                                                                 |
|---------------------|------|----------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Single bond 2       | SB2  | 2-step etch-   | bis-GMA, HEMA, dimethacrylates, silica nanofiller, polyalkenoic acid copolymer, initiators, water, ethanol                                  |
|                     |      | and-rinse      |                                                                                                                                             |
| Spectrum bond       | SPB  | 2-step etch-   | UDMA, trimethacrylate, PENTA, highly dispersed silicon dioxide, camphorquinone, BHT, cetyamine hydrofluoride, acetone                      |
|                     |      | and-rinse      |                                                                                                                                             |
| Single bond universal | SBU | Universal      | MDP phosphate monomer, bis-GMA, dimethacrylate resins, HEMA, VITREBOND copolymer, fillers, ethanol, water, initiators, silane                   |
|                     |      | adhesive       |                                                                                                                                             |

* bis-GMA: bisphenol a diglycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, UDMA: urethane dimethacrylate, PENTA: phosphoric acid modified acrylate resin, BHT: butylhydroxytoluene, MDP: methacryloyloxydecyl dihydrogenphosphate. Lot number: SB2 (N912223); SPB (1801000919); SBU (4330297).

2) Figure 6A and 6B show the mechanical properties of the synthesized PUs. However, these properties of commercial PUs are not shown. These data are also not included in the discussion of the results.

We are grateful for the suggestion.

The tensile strength and elongation at break is mainly used for testing materials with elasticity, such as the synthesized PU adhesive in this study. The composition of the commercial adhesive
system is different from that of the experimental PU adhesive. The commercial adhesive is mainly composed of the organic resin matrix and inorganic filler which is a rigid structure with almost no tensile deformation. Therefore, commercial adhesives were not tested. In this experiment, we make use of the tensile strength and elongation at break test to evaluate seven kinds of synthetic elastic PU adhesives. And combined with other tests (water absorption/solubility and contact angle), PU4 was considered to possess the best performance. And PU4 was chosen as the final experimental group to compare with three commercial adhesives in the follow-up tests.

3) Why are the conversion data for samples PU1, PU2, PU3, PU5 and PU6 not included?

We are grateful for the suggestion.

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Microtensile bond strength test is currently considered as a versatile and standard bond strength testing method. It was introduced in 1994. Since then, it has been utilized profoundly across many bond strength testing laboratories, making it currently one of the most standard and versatile bond strength test.

In general, tensile strength is the elastic property of the material itself, however, microtensile bond strength is the adhesion ability of adhesive with tooth. There is no direct relationship between them.

And both microtensile bond strength and tensile strength are measured in the same scale (MPa). Microtensile bond strength is determined by dividing the loading force at break by the cross-sectional area of the sticks. Tensile strength is calculated by dividing the maximum stress at fracture by the cross-sectional area of the sample at the breaking point. So, they are measured in the same scale (MPa).

5) In the TGA curve there is a small inflection in the slope, if the curve was derived, it could detect PUA and PUB ?. Could you show DSC of PUA, PUB and PU4?.

Thank you very much for your comments. We are grateful for the suggestion. Your comments are very valuable for us to improve the thermal stability of the PU adhesive material. The TGA curve is the result of final formulation of PU4. TGA of separate PUA and PUB need to be tested. Due to COVID-19, the school has been closed. We contacted the school laboratory and it was uncertainty when it would be available. Therefore, the supplement results of TGA and DSC may need waiting a long time. The TGA of PU4 tested in this study is not enough for evaluating the thermal stability of the material which is a limitation of the study, but it can also show some information about the PU4 adhesive. The initial degradation temperature of 5% weight loss was observed at 269.00 °C. The maximum tolerant temperature of the oral mucosa is approximately 60 °C. Therefore, PU4 adhesive may be applied in the oral environment. We searched the relevant literature, and the following two articles can be used for reference. Our laboratory will continue to
study in this area. In the subsequent research, we will conduct more detailed and complete experiments of the thermal stability of the material.

1. Solís-Correa R, Vargas-Coronado R, Aguilar-Vega M, Cauich-Rodríguez J, San Román J, Marco A. Synthesis of HMDI-based segmented polyurethanes and their use in the manufacture of elastomeric composites for cardiovascular applications. J Biomater Sci Polym Ed. 2007;18(5):561-78.

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We are grateful for the suggestion.

Although SBU aging can also penetrate the dentinal tubules, its adhesive layer fractured more severely. In the aging group, microtensile bond strength of SBU is greater than that of PU4, but there is no statistical significance. The microtensile bond strength of SBU decreased obviously after aging, while the microtensile bond strength of PU4 increased. It is indicated that longer-term aging test may be needed in the future study. In term of the microtensile experiment, SBU is better than PU4, but water absorption and water solubility of PU4 are much lower than that of SBU which may contribute to reducing hydrolytic degradation of the bonding interface. And microleakage of PU4 has also been improved when compared with SBU. Its elastic properties can buffer various stresses during long-term use, improving the stability and durability of the adhesive bonding interface. Therefore, the comprehensive performance of PU4 is still better than that of SBU.

7) In Figure 10A, the viability values are not shown in percentages as indicated (i.e., 1.0= 100%). Setting viability at 70% is arbitrary (the reader can suppose a 30% death of fibroblasts in the mouth?). What is the variation in your controls?. Even a 20% decrease in viability, this is significant in a cell population?. You can perform an ANOVA followed by a Tukey test to set significance to 5%.

We are grateful for the suggestion.

In Figure 10A, the viability values have been shown in percentages in the manuscript.
According to GB/T 16886.5-2003 (ISO 10993-5:1999), samples with cell viability larger than 75% of blank group can be considered as non-cytotoxic[1-3]. We are sorry that 70% is not accurate. It has been revised in the text. In this study, cell viability of PU4 adhesive was all greater than 80% which demonstrated good cell viability and biocompatibility, meeting the clinical biosafety requirements.

The variation of controls has already been added.

![Cell viability graph]

We have performed an ANOVA followed by a Tukey test. In the group of 48 h and 72 h, there is significant difference between control group and the other groups (SB2, SPB, SBU, PU4). However, no statistical difference exists between PU4 adhesive and three commercial adhesives.

It is important to mention that the commercial adhesive system may show some toxicity to a certain extent. Adhesive systems components [e.g. Bis-GMA (bisphenol A diglycidyl methacrylate), HEMA (2-hydroxyethyl methacrylate), TEGDMA (tri-ethylene glycol dimethacrylate), and UDMA (urethane dimethacrylate), camphorquinone] are cytotoxic. Despite its cytotoxic produced by direct contact on pulp cells, these may be clinically attenuated or neutralized, as adhesive systems are applied to dentin, which acts as a physical barrier depending on its thickness.

In this study, there is no significant difference between commercial adhesives which is used in clinical practice and PU adhesives in MTT test which indicates that PU4 adhesive can meet the clinical biosafety requirements.

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8) The light microscopy images in Figure 10B must be improved, or show SEM images, in order to visualize the morphology of the cells. With the viability shown in Figure 10A, some dead (red) cells should be observed. Fibroblasts grow extended (in spreading), however the fluorescence image gives the appearance of cluster or aggregates. Can you explain it?

We are grateful for the suggestion.

The light microscopy images in Figure 10B have been improved to make it more clearly. And we adjusted the contrast of the fluorescence images, and the dead cells (red) could be seen more clearly.
L929 fibroblasts should grow extended (in spreading), however the fluorescence image gives the appearance of cluster or aggregates. In this study, the live/dead cell staining kit includes two
parts that calcein-AM is used for living cell staining and propidium iodide is used for dead cell staining. The staining solution is slightly toxic to the cells. If the staining time is too long, it may cause poor cell morphology. And the osmotic pressure of the staining solution may not be exactly the same with the cytoplasm which can also lead to the cell deformation.
Hydrolysis-resistant and stress-buffering bifunctional polyurethane adhesive for durable dental composite restoration

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Abstract
A new elastic polyurethane (PU) adhesive was reported in this study to improve the
stability and durability of the dental adhesion interface. A polyurethane oligomer was
synthesized by the solution polymerization method, and a diluent and solvent were
added to prepare PU adhesives. The water sorption, water solubility, contact angle,
thermal stability, degree of conversion and mechanical properties of the PU adhesives
were evaluated. Experimental applications for tooth restoration (microtensile bond
strength and microleakage) were also performed. And cytotoxicity test was carried out.
The water sorption and solubility of the PU adhesives were significantly lower than
those of three commercial adhesives. The microtensile bond strength of the PU
adhesives was improved after thermocycling test, and the extent of microleakage was
diminished when compared with that of commercial adhesives. Biocompatibility
testing demonstrated that the PU adhesive was nontoxic to L929 fibroblasts. This study
shows the ability of PU adhesive to improve the stability and durability of the dental
adhesion interface and may refocus the attention of scientists from rigid bonding to
flexible bonding for dental adhesion, and it sheds light on a new strategy for the stable
and durable bonding interface of dentin adhesives.

Keywords: polyurethane adhesive; dental restoration; hydrolysis-resistant; stress-
buffering

1 Introduction
Composite resin has been widely used in dental restoration for more than 60 years due
to its aesthetic advantages, excellent mechanical properties, ease of use and acceptable
price(1-4). The success of composite resin restoration relies on bonding techniques that
can bond these plastic materials to the tissue of teeth. Therefore, strong and durable
bonding properties are necessary for successful composite resin restoration(5-8).

The failure of restorations is mainly due to defects in the bonding interface, which
are caused by the polymerization stress when the composite resin is polymerized using
a curing light(9). Scientists have made many efforts to reduce the polymerization
shrinkage of composite resins, for example, by using low-shrinkage composite resin. It
has been reported that the volume of polymerization shrinkage of the composite resin
can be reduced to less than 1%, and the generation of gaps between the tooth and the
composite resin can be temporarily avoided(10, 11). However, there will be continuous
mechanical chewing stress in the mouth after dental restoration. Moreover, studies have shown that the thermal expansion coefficient of the composite resin is usually $2.0 \times 10^{-3} \%/{\degree}C$, which is larger than that of dentin (approximately $1.1 \times 10^{-3} \%/{\degree}C$)(12). During temperature changes, different volume expansion and contraction will occur repetitively between dentin and composite resins, which may cause stress within the adhesive layer and eventually lead to the occurrence of gaps in the bonding interface(13). Bacteria and their acidic by-products, bacterial enzymes, liquids in the mouth and nutrients can penetrate into the interfacial gap, causing microleakage and eventually leading to demineralization of the teeth and secondary caries (14). Therefore, we need to explore a new method to solve the problem of microleakage.

Since John introduced wet bonding technology into the field of dentin adhesive to prevent the collapse of demineralized collagen fibers of dentin, manufacturers have increased the concentration of hydrophilic monomers. For example, hydroxyethyl methacrylate (HEMA) can act as a solvent for mixing hydrophobic monomers to avoid phase separation and help the adhesive monomers to better penetrate into the dentinal tubule to form resin tags, forming a micromechanical interlocking(15-17). However, hydrophilic resin monomers easily absorb water and are easily hydrolyzed due to the presence of ester bond linkages. Therefore, increasing the content of the hydrophilic monomer in the adhesive will increase the water absorption of the polymer network, resulting in degradation of the adhesive layer, lowering the mechanical properties and ultimately leading to failure of the restoration(8, 18-20).

Therefore, the study of adhesives with low water sorption and microleakage has attracted the attention of scientists striving to significantly improve the quality of the adhesive and reduce the failure of the restoration. Our research team has reported some methods. Cao et al. prepared a coating of a superhydrophobic polyurethane coating to reduce microleakage(21). Zhang and colleagues introduced a polyurethane elastic layer between the adhesive and the composite resin to buffer the stress generated by the restoration during use (13). Gong and coworkers synthesized a dual-curing polyurethane adhesive with carbon-carbon double bonds for conventional photocuring and the NCO group for continuously reacting with water molecules to improve bonding strength and durability(22). However, the NCO group is unstable in the air and is not easily stored, and the NCO group reacts with water to generate CO$_2$, which will generate bubbles in the adhesive layer, forming a weak point and jeopardizing the strength of
the adhesive layer. Therefore, the adhesives synthesized in this study are all terminated with C=C for traditional photocuring. A polyurethane oligomer was synthesized by solution polymerization method using methylene-bis(4-cyclohexylisocyanate) and poly(tetrahydrofuran)1000/2000. The two types of synthetic polyurethane oligomers, the diluent and the solvent, were mixed in different proportions to prepare the adhesives, and the thermal stability, mechanical properties and biocompatibility were evaluated. It is desirable to obtain a polyurethane adhesive with a lower water absorption and solubility and elastic property that can buffer the stress within the adhesive, improving the stability and durability of the adhesive interface. A schematic model for this elastic PU adhesive used for tooth dentin bonding is illustrated in Fig. 1.

Fig. 1 Schematic model for elastic PU adhesive using for tooth dentin bonding.

2 Experimental section

2.1. Materials

Spectrum Bond (Dentsply DeTrey GmbH, De-Trey-Strasse1, 78467 Konstanz, Germany), Single Bond Universal (3M ESPE, St. Paul, MN, USA), Adper Single Bond 2 (3M ESPE, St. Paul, MN, USA), and Filtek Z350 XT (3M ESPE, St. Paul, MN, USA) were used. Information on these three common commercial adhesives is shown in Table 1. L929 cells were obtained from the School of Life Science, Jilin University. 3- (4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from
Sigma Aldrich. Methylene-bis(4-cyclohexylisocyanate) (HMDI), 2-hydroxyethyl
methacrylate (HEMA), triethylene glycol dimethacrylate (TEGDMA), dibutyltin
dilaurate (DBTDL), camphorquinone (CQ), ethyl-4-dimethylaminobenzoate (4-
EDMAB) and methylene blue, acetone, tetrahydrofuran (THF),
poly(tetrahydrofuran)1000/2000 (PTMEG1000/2000) were of analytical grade and
were provided by Aladdin.

Table 1. Commercial adhesive for this study

| Material                  | Code | Category             | Formulation                                                                 |
|---------------------------|------|----------------------|------------------------------------------------------------------------------|
| Single bond 2             | SB2  | 2-step etch-         | bis-GMA, HEMA, dimethacrylates, silica nanofiller, polyalkenoic acid copolymer, initiators, water, ethanol |
| Spectrum bond             | SPB  | 2-step etch-         | UDMA, trimethacrylate, PENTA, highly dispersed silicon dioxide, camphorquinone, BHT, cetylamine hydrofluoride, acetone |
| Single bond universal     | SBU  | Universal adhesive  | MDP phosphate monomer, bis-GMA, dimethacrylate resins, HEMA, Vitrebond copolymer, fillers, ethanol, water, initiators, silane |

* bis-GMA: bisphenol a diglycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, UDMA: urethane dimethacrylate, PENTA: phosphoric acid modified acrylate resin, BHT: butylhydroxytoluene, MDP: methacryloyloxydecyl dihydrogenphosphate. Lot number: SB2 (N912223); SPB (1801000919); SBU (4330297).

### 2.2. Preparation and Characterization of polyurethane (PU) adhesive matrix

The synthesis process of polyurethane oligomer A (PUA) is shown in Fig. 2. First, H-
MDI (13.1175 g, 0.05 mol), DBTDL (0.09936 g, 3‰) and anhydrous THF were added
into a three-necked round bottom flask with a water-cooled condenser in a water bath
of 70 °C. PTMEG 1000 (20 g, 0.02 mol) was then added into the reaction with
mechanical stirring (500 rpm) for 4 h with continuous N₂. When the hydroxyl groups
disappeared, as monitored by infrared spectroscopy, HEMA (8.2 g) was added into the
flask with continuous stirring for 3 h. The reaction was completed when the NCO group
could not be detected by infrared spectroscopy. The liquid was precipitated with
petroleum ether 3 times to give a white solid. The final products were dried in a vacuum
oven to remove the remaining petroleum ether.
The synthesis process of polyurethane oligomer B (PUB) is shown in Fig. 2. First, H-MDI (6.55875 g, 0.05 mol), DBTDL (0.07968 g, 3‰) and anhydrous tetrahydrofuran (THF) were added into a three-necked round bottom flask with a water-cooled condenser in a water bath of 70 °C. PTMEG 2000 (20 g, 0.01 mol) was then added into the reaction with mechanical stirring (500 rpm) for 4 h with continuous N₂. When hydroxyl groups disappeared, as monitored by infrared spectroscopy, HEMA (4.2 g) was added into the flask with continuous stirring for 3 h. The reaction was completed until the NCO group could not be detected by infrared spectroscopy. The liquid was precipitated with petroleum ether 3 times to give a white solid. The final products were dried in a vacuum oven to remove the remaining petroleum ether.

![Fig. 2 Illustration of the fabrication process of the PU oligomer.](image)

Two types of polyurethane oligomers (PUA/PUB) are mixed as a matrix of adhesive at different mass ratios (12:0/9:3/8:4/6:6/4:8/3:9/0:12). The formulation is shown in Table 2.
### Table 2. Formulation of 7 kinds of PU adhesives

|   | PUA  | PUB  | HEMA | TEGDMA | Acetone | CQ   | 4-EDMAB |
|---|------|------|------|--------|---------|------|---------|
| PU1 | 12g  | 0g   | 1.71g| 1.71g  | 1.54g   | 0.05g | 0.12g   |
| PU2 | 9g   | 3g   | 1.71g| 1.71g  | 1.54g   | 0.05g | 0.12g   |
| PU3 | 8g   | 4g   | 1.71g| 1.71g  | 1.54g   | 0.05g | 0.12g   |
| PU4 | 6g   | 6g   | 1.71g| 1.71g  | 1.54g   | 0.05g | 0.12g   |
| PU5 | 4g   | 8g   | 1.71g| 1.71g  | 1.54g   | 0.05g | 0.12g   |
| PU6 | 3g   | 9g   | 1.71g| 1.71g  | 1.54g   | 0.05g | 0.12g   |
| PU7 | 0g   | 12g  | 1.71g| 1.71g  | 1.54g   | 0.05g | 0.12g   |

PU adhesive = 70% PU (PUA + PUB) + 10% HEMA + 10% TEGDMA + 9% acetone + 0.3% CQ + 0.7% 4-EDMAB

The structure of the polyurethane oligomer is characterized by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (1H NMR spectrum). FTIR was measured by a BRUKER VERTEX 80 V infrared spectrometer in the range of 4000-500 cm\(^{-1}\). The 1H NMR spectrum was measured by a Bruker AVANCE 500 MHz type III nuclear magnetic resonance spectrometer using deuterated chloroform as a solvent.

#### 2.3 Water sorption (W\(_{SP}\)) and water solubility (W\(_{SL}\))

Water absorption and water solubility were determined according to ISO 4049:2009. Disk-shaped samples (d = 15.0 mm, h = 1.0 mm, n = 5) were prepared. The polyurethane adhesive was poured into the mold, covered with a piece of polyester film, and cured with a light intensity of 900 mW/cm\(^2\) for 10 s. The curing light unit was monitored by a radiometer to ensure light intensity. All the samples prepared were placed in a desiccator with silica gel at 37 ± 2 °C for 24 h. The samples were then transferred into another desiccator for 2 h at 23 ± 1 °C and weighed. This process was repeated until a constant mass (M\(_1\)) was obtained. The diameter and thickness of each sample were measured by electronic digital caliper to calculate the volume (V; mm\(^3\)) of the sample. Each sample was then immersed in a sealed glass vial containing 15 ml of deionized water and soaked for 7 days at 37 ± 1 °C. The samples were rinsed with running deionized water, and the surface water was dried with filter paper. Then, the samples were weighed to obtain mass M\(_2\). The samples were redried in a 37 ± 1 °C
desiccator, as described above, until a stable mass M3 was obtained. The calculation formula for water absorption and solubility of the sample is as follows:

\[ W_{SP} = \frac{(M2 - M3)}{V} \]
\[ W_{SL} = \frac{(M1 - M3)}{V} \]

2.4 Contact angle measurements

Contact angles were obtained using the sessile drop method with a Dataphysics contact angle analyzer (OCA-20, DataPhysics Co., German). This instrument consists of a CCD video camera with a resolution of 768 × 576 pixel and up to 50 images per second and multiple microsyringe units. A drop of 6 µl of deionized water was gently dropped onto the surface of the adhesive to take a digital photo. The digital drop image was processed by a specialized software SCA 20, which calculated both the left and right contact angles from the shape of the drop.

2.5 Tensile strength and elongation at break of PU adhesives

The dumbbell-shaped specimens (n=5) were prepared in accordance with the standard ASTM-D638-2003. The prepared specimen is shown in Fig. 5 (H). The specimen was tested using a universal testing machine (AG-X plus, Shimadzu Corporation, Japan) with a crosshead speed of 10 mm/min until it was broken.

2.6 Thermal stability characterization

Thermogravimetric analysis measurement of PU4 adhesive was performed using a TGA thermal analyzer (Q500, TA Instruments, USA). Initial sample masse is around 5 mg. The heating rate is 10 °C/min. The experiments were performed in an inert atmosphere with a continuous flow of nitrogen at the rate of 150 ml/min and heated from room temperature to 800 °C.

2.7 Degree of conversion

The degree of conversion (DC) of PU4 adhesives and three commercial adhesives were measured. The DC was determined by a Fourier transform infrared spectrometer equipped with an attenuated total reflectance device for 5 samples per group (n=5). The FTIR of uncured adhesive was obtained as a control. The adhesive was cured for 10 s, and the polymerized adhesive was immediately subjected to FTIR. After light-curing,
the area of infrared absorption peak of methacrylate double bonds (C = C, peak at 1637 cm\(^{-1}\)) decreased, and the carbonyl group (peak at 1720 cm\(^{-1}\)) was used as the internal standard. The calculation of the DC used the following equation:

\[
DC\% = \left[ 1 - \frac{A_{1636}}{A_{1720}} \right] \times 100\%
\]

2.8 Microtensile bond strength test (µ-TBS)
The extracted teeth were stored in 1% chloramine T solution, placed at 4 °C, and used within one month. The tooth was cut perpendicular to the long axis with slow-speed saw under water cooling in the middle section of the tooth to expose the dentin surface. Then, the dentin was sanded with 600-grit SiC paper to produce a uniform smear layer and was ultrasonically cleaned for 5 min. The prepared teeth were randomly divided into four groups (PU4 adhesive, SB2, SPB, and SBU groups). The specimen was etched with 37% phosphoric acid gel for 15 s, rinsed for 30 s, and air-blown for 5 s. The adhesive was applied to the dentin surface using a microbrush, air-thinned for 5 s, and light-cured for 10 s. Three 1.5-mm-thick layers of commercial composite resin Z350XT were placed over the surface of the treated dentin. Each resin composite was light cured for 40 s using a light-curing unit. The specimens were soaked in deionized water at 37 °C for 24 h. After immersion, the specimens were longitudinally cut into sticks of approximately 1.0 mm in width using a slow speed saw. The dentin-resin stick was fixed to a microtensile mold using isocyanate glue. Then, it was carried out on a universal testing machine with a crosshead speed of 1 mm/min. Microtensile strength was determined by dividing the loading force at break (N) by the cross-sectional area of the sticks (mm\(^2\)).

2.9 Scanning electron microscopy
The bonding surface of the PU4 adhesive and three commercial adhesives was detected by scanning electron microscopy (SEM; S4800, Hitachi Ltd., Tokyo, Japan). The specimen was sequentially grounded with 600 grit, 800 grit, 1200 grit, 2000 grit SiC paper under running water and ultrasonically cleaned for 10 min. Then, the specimen was etched with 37% phosphoric acid gel for 15 s and treated with 5.25% NaClO for 15 min followed by immersion in 50%, 70%, 90%, 100% ethanol for 15 min in
sequence. Finally, the specimen was sprayed with platinum and observed by scanning electron microscopy.

2.10 Microleakage in composite restoration
Two standard class V cavities were prepared on the opposite surface of a molar (4 mm wide, 2.0 mm deep, and 3.0 mm high) while a 45-degree edge bevel was prepared. The dentin was etched with 35% phosphoric acid gel for 15 s, rinsed for 15 s, and continuously air-blown with condensed air. One side was randomly selected for the PU4 adhesive, and the other side received the commercial adhesive. The adhesive was directly applied for 20 s, lightly air-blown for 5 s and cured for 10 s. The cavity was filled with Filtek Z350XT composite resin layer by layer and cured for 40 s. After polishing with sandpaper, the sample was stored in deionized water for 24 h. Artificial aging was performed using a thermocycling instrument (PTC2c, Proto-tech, USA) for 5000 cycles between 5 °C and 55 °C baths with a dwell time of 30 s. After thermocycling was completed, the root apex was sealed with wax. The entire surface of the tooth was coated with transparent nail polish twice, except for the area within 1 mm of the tooth restoration interface. A microleakage test was conducted using a standard dye-leakage method. The prepared sample was placed in 1% methylene blue dye for 4 h at 37 °C. The tooth surface was rinsed with deionized water and dried with filter paper. The crown portion was cut into a 1 mm sheet along the tooth long axis under running water cooling using a slow-speed diamond saw. The evaluation of microleakage was determined by evaluating the depth of dye into the tooth-restoration interface using a stereomicroscope. The depth of leakage of the dye was evaluated by the following criteria(21):

0. no obvious dye leakage;
1. The dye gets to the interface to half the depth of the cavity;
2. Leakage exceeds half of the depth of the hole but does not involve the axial surface;
3. Leakage involves the axial surface, but not the pulp;
4. Leakage involves the pulp.

2.11 Cytotoxicity test
The extracted solution was prepared by immersing the cured adhesive specimen in Dulbecco’s modified Eagle’s medium cell culture medium containing 10% fetal calf
serum and 1% penicillin and streptomycin at a ratio of 3 cm²/mL (the surface area of the specimen to the extracted solution volume) for 24 h, 48 h, 72 h.

The L929 cells were cultured for an MTT assay. The cells were seeded in a 96-well plate at a density of 1.5 × 10⁴ cells/mL and incubated at 37 °C in 5% CO₂ and 95% relative humidity for 24 h until the monolayer cells were spread over the bottom of the well. The original culture solution was replaced with 24 h, 48 h, 72 h extracted solutions, and the control group was added to the cell culture medium with the surrounding wells sealed with phosphate buffer saline buffer. Then, the cells were continuously incubated for 24 h and removed from the incubator. The morphology of the cells was observed under an inverted microscope. Mitochondrial dehydrogenase in living cells enabled the MTT to become insoluble formazan particles that can dissolve in dimethyl sulfoxide (DMSO). Next, 20 µL of MTT solution (5 mg/mL) was added to each well, and the incubation was terminated after 4 h. Then, 150 µL of DMSO was added to each well, and the 96-well plate was shaken at a low speed for 10 min to fully dissolve the crystal formazan particles. The absorbance was read at a wavelength of 490 nm by a microplate reader (RT-6000, Lei Du Life Science and Technology Co., Shenzhen, China). The control group was regarded as the 100% cell proliferation rate, and the relative growth rate of each group was calculated.

The effect of PU4 adhesive on the activity of L929 cells was also evaluated using a live/dead cell staining kit. The cells were seeded at a density of 5 × 10⁴ cells/ml in a 6-well plate for 24 h. After incubation with the 24 h extracted solution, the cells were stained with a live/dead cell staining kit according to the manufacturer's instructions. Live cells were stained green, and dead cells were stained red. The 6-well plate was observed under a fluorescence microscope.

2.12 Statistical analysis
Data were expressed as the mean ± standard deviation. The data were consistent with normality and homoscedasticity distribution. Data for microtensile bond strength were analyzed using two-way ANOVA, and the data of tensile strength, elongation at break, water solubility, water sorption, contact angle and degree of conversion were submitted to one-way ANOVA using SPSS software (version 19.0, SPSS Inc., Chicago, IL, USA). Multiple comparison analysis was conducted using the Tukey test. The significance level was set at p = 0.05 for this study.
2.13 Ethics statement

All relevant ethical applications in this experiment were approved by the Ethics Committee of School and Hospital of Stomatology of Jilin University. Informed consent was obtained from all donors.

3 Results and discussion

3.1 Characterization of polyurethane oligomer

Figure 3 (A) shows the $^1$H NMR spectrum of PUA. The peak at 7.29 ppm was attributed to the newly formed urethane group (-NCOO-) after the reaction between HMDI and PTMEG. The protons belonging to the H of methylene (CH$_2$) from HEMA were clearly shown at 6.11 ppm. The resonance peak at 1.59 ppm was assigned to the signal of polytetrahydrofuran1000. The signal of methylene (CH$_2$) from HMDI appeared at 1.08 ppm. Fig. 3 (B) shows the $^1$H NMR spectrum of PUB, which was almost the same as that of PUA.
The PU oligomer (PUA/PUB) is synthesized by a conventional solution polymerization method. As shown in Fig. 4 (A) of the infrared spectrum of the NCO-terminated PU prepolymer, the N-H stretching vibration at a wavenumber of 3340 cm\(^{-1}\) and the C=O stretching vibration peak observed at 1725 cm\(^{-1}\) were derived from the urethane group (-NHCOO-), which was attributed to the reaction of the NCO group with the OH group. The infrared stretching vibration peak of the NCO group can be seen at 2260 cm\(^{-1}\). After the addition of HEMA, as shown in Fig. 4 (B), a C=C stretching vibration peak at 1636 cm\(^{-1}\) was observed. The absorption peak of the NCO group at 2260 cm\(^{-1}\) disappeared completely, indicating that all of the PU prepolymer reacted completely with HEMA. FTIR and \(^1\)H NMR spectra indicated that the PU oligomer (PUA/PUB) was successfully synthesized.
Fig. 4 (A) FTIR spectra of the NCO-terminated PU prepolymer. (B) FTIR spectra of the PU oligomer.

3.2 Water sorption and water solubility and contact angle measurements

The hydrophilic nature of a polymer depends largely on the chemical structure of the monomers and the linkage of the polymer. The most commonly used monomers in the dentin commercial adhesive system (HEMA, BPDM, MDP, bis-GMA) are hydrophilic monomers (23). Moreover, an ester bond that is easily hydrolyzed exists in the polymer formed by these hydrophilic monomers (23, 24). Therefore, it is more apt to absorb water. Since water molecules have a small molecular size and a high molar concentration, they can penetrate into the nanometer-size free volume space between polymer chains (25, 26) or form clusters around functional groups (hydrophilic and
ionic regions) to generate a hydrogen bond with the functional group(27). These molecules are called bound water, which will break the hydrogen bond between the polymer chains, change the molecular structure and increase the mobility of partial polymer segment, leading to the swelling of the polymer(28), which plays a decisive role in the plasticization of the polymer(29, 30). Water absorption can lower the glass transition temperature, reduce thermal stability, and deteriorate the mechanical properties of the polymer. It can be predicted that the strength of the adhesive connecting the dentin and the composite resin will decrease due to the absorption of water, which may affect the dispersion of the interfacial stress and eventually lead to interfacial damage after repeated loadings.

Fig. 5 (D) shows that the water absorption values of the seven PU adhesives are significantly lower than those of the three commercial adhesives (p < 0.05). PU adhesives are mainly composed of oligomers, containing urethane groups that are relatively hydrophobic and may form weaker hydrogen bonds with water molecules when compared to hydroxyl groups, reflected by lower cohesive energy density (urethane group: 1425 J/cm$^3$; OH group: 2980 J/cm$^3$)(31). This is consistent with the results of the static contact angle, as shown in Fig. 5 (E). The contact angles of the PU adhesives are all greater than 83°, significantly larger than that of the commercial adhesive (p < 0.05), indicating that the PU adhesive is more hydrophobic than the commercial adhesive.

After the polymer absorbs moisture, the polymer network is softened by swelling itself and reducing the friction between the polymer chains(32). When too much moisture is absorbed, the macromolecular polymer chains undergo a relaxation process. Meanwhile, the residual monomer in the polymer is released to the surrounding environment at a rate that is related to the swelling and relaxation ability of the polymer. A more hydrophilic polymer network, such as commercial adhesives, with better relaxation capacity allows for faster release of residual monomers through the nanovoids in the material(33, 34), resulting in a decreasing quality under short-term water soaking. At the same time, the released residual monomers, such as TEGDMA and HEMA, enter the dentinal tubules, causing harmful inflammatory responses (35, 36). The components dissolved from the adhesives have a potential negative effect on the stability of their own structures, ultimately resulting in degradation of the resin-dentin bonding interface. Compared with these methacrylic resin adhesives, the
The solubility of PU adhesives was significantly reduced (p < 0.05), as shown in Fig. 5 (C). The soft segment of the synthetic PU adhesives (polytetrahydrofuran diol) imparts elasticity and hydrolysis resistance to the adhesives. The amount of released residual monomers is also dependent on the degree of conversion of the monomers (31). The PU adhesive with a higher degree of conversion showed a lower amount of residual monomer release, improving the bonding durability.
3.3 Tensile strength and elongation at break

Fig. 5 (A) showed that there was no significant difference among PU2, PU3, and PU4, but their tensile strength was significantly higher than that of other groups \((p < 0.05)\). In terms of elongation at break, as shown in Fig. 5 (B), there was no significant difference between PU4 and PU5, and their elongation was obviously higher than that of the other groups \((p < 0.05)\). The mechanical properties of PU adhesives depend on the soft segment and hard segment(37). The soft segment used in this study is polytetrahydrofuran1000/2000, which endows synthesized PU adhesives with good mechanical properties, flexibility and hydrolysis resistance(38). In PU1-PU3, PUA synthesized by PTMG1000 accounts for a larger proportion, leading to a relatively high amount of hard segments in PU adhesives. Therefore, PU1-PU3 has better mechanical properties. The PUB synthesized by PTMEG2000 occupies a larger percentage in PU5-PU7, resulting in a relatively large amount of soft segments in PU adhesives. Therefore, PU5-PU7 has better elastic properties and greater elongation at break, but after reaching a certain extent, the elongation at break is lowered due to the decreasing mechanical properties. Among these seven adhesives, PU4 possesses the highest tensile strength and relatively higher elongation at break. Considering the water absorption/solubility and contact angle of 7 kinds of PU adhesives comprehensively, PU4 was chosen as the final experimental adhesive formulation for the follow-up dental restoration tests.

During the crosslinking of the polymer initiated by light irradiation, radical polymerization occurs, and the polymer chain becomes denser, resulting in a decrease in volume (average 1% to 3%)(39). Polymerization shrinkage causes internal stress, leading to pain, microleakage and secondary caries, and ultimately failure of the restoration(40, 41). The PU elastic adhesive can buffer stress coming from
polymerization shrinkage, inconsistent thermal expansion coefficients between dentin and composite resin and occlusal force by deformation. Elongation at the break of PU4 reached 95.74%, according to Zhang's report, this can meet the requirements(13). Therefore, the PU4 adhesive can reduce the interfacial stress, decrease the occurrence of microleakage and secondary caries, and improve the stability and durability of the adhesive.

3.4 Thermal stability characterization
Assessment of the thermal properties of the PU4 adhesive is important to assess its applicability in the oral environment. TGA was carried out to investigate the thermal performance of the PU4 adhesive. Fig. 6 shows the TGA thermogram results of the PU4 adhesive. The initial degradation temperature of 5% weight loss was observed at 269.00 °C. The maximum tolerant temperature of the oral mucosa is approximately 60 °C. Therefore, PU4 adhesive can be applied to the oral environment.

![Figure 6 TGA spectra of PU4 adhesive.](https://mc.manuscriptcentral.com/rsos)

3.5 Degree of conversion (DC)
Adequate polymerization of the adhesive layer is necessary to ensure its physical, chemical, and mechanical strength. The DC of the dental adhesive is closely related to the structure of the monomers, the polymerization conditions and the photoinitiator
concentration(42). PU4 adhesive is mainly composed of oligomers. As shown in Fig. 5 (F), the DC of PU4 reached 68.86 ± 6.72%, which was higher than that of the commercial adhesive SB2 (p = 0.0004). A higher DC of the adhesive is advantageous for the improvement of the bonding quality because it may enhance the strength of the adhesive layer, thus improving the bonding durability.

3.6 Microtensile bond strength test

Fig. 5 (G) shows the microtensile strength of PU4 adhesive and three commercial adhesives before and after 5000 thermocycles. In the immediate group, the microtensile strength of the PU4 adhesive was lower than that of the three commercial adhesives. This is due to the elastic properties of the PU4 adhesive and its cohesive energy is lower than that of the rigid commercial adhesives. Although the immediate microtensile strength of PU4 is low, it is also greater than 20 MPa, which can meet clinical requirements(13). After aging, the microtensile strength of the three commercial adhesives decreased, and the SBU group showed the most obvious decline. However, the microtensile strength of the PU4 adhesive increased. On the one hand, PU4 adhesive can buffer the stress during 5000 thermocyclings. On the other hand, the urethane group (-NHCOO-) contained in the PU4 adhesive has a strong polarity, and hydrogen bonds can be formed between the adhesive molecules and between the adhesive and the dentin, thereby enhancing the molecular cohesion and improving the bonding strength and durability.

The mechanism of dentin bonding allows the adhesive monomer to penetrate into the demineralized dentin collagen fiber matrix to form a hybrid layer (HL), resulting in micromechanical interlocking(4, 6). The bonding interface of the adhesive was detected by scanning electron microscopy, as shown in Fig. 7. A good bonding interface can be observed in all the immediate groups. PU4 adhesive can also penetrate into the dentinal tubules, some of which even reach 50 μm in depth. After 5000 thermocyclings, significant cracks can be seen in the bonding interface of SB2 and SBU. However, the hybrid layer of the PU4 adhesive remains intact.
Fig. 7 SEM of the bonding surface of commercial adhesives and PU4 adhesive before and after 5000 thermocyclings. (A) SB2, immediate (B) SB2, aging (C) SPB, immediate (D) SPB, aging (E) SBU, immediate (F) SBU, aging (G) PU4 immediate (H)
PU4, aging (I/J) PU4, immediate. PU4 adhesive can penetrate into the dentinal tubules, some of which even reach 50 μm. *Dentin(d), Composite resin(c), Adhesive layer(a).

3.7 Microleakage in composite restoration

The microleakage of the PU4 adhesive is significantly smaller than that of the other three commercial adhesives, and the microleakage exceeds half of the depth of the hole but does not involve the axial surface and only reaches grade 2. However, the microleakage of three commercial adhesives has been found to involve the axial surface or even the pulp, reaching grades 3 and 4, as shown in Fig. 8. Thermocycling is often used in dental adhesive experiments to simulate temperature changes in the oral cavity. Due to the difference in thermal expansion coefficient between the composite resin and the tooth, stress will be generated after repeated temperature changes, which eventually accelerates the formation of microleakage(43). In this study, PU4 adhesive can significantly reduce the occurrence of microleakage, mainly due to its elastic properties, which change the rigid connection to elastic bonding between composite resin and dentin. The stress generated by the inconsistent thermal expansion coefficient can be buffered through deformation of the PU4 adhesive, reducing the probability of fracture and secondary caries and improving the stability and durability of the adhesive. The microleakage of the small molecule dye is qualitative, so more evaluation is needed in the future study.
Fig. 8 Microleakage between dentin and composite resins after 5000 thermocycling. A (SB2); B (SPB); C (SBU); D (PU4). Bar = 500 μm.

3.8 Biocompatibility of PU adhesive

Cytotoxicity was evaluated by examining the effect of PU4 adhesive on cell activity and morphological changes in L929 fibroblasts. As shown in Fig. 9 (A), there was no significant difference between the experimental groups and the blank control group in cell proliferation activity, regardless of the time (24 h, 48 h, 72 h) of extraction. Cell viability greater than 75% is considered to be noncytotoxic(21). In this experiment, the cell viability was all greater than 80%.

L929 cells were cultured for 24 h using the 24 h extraction, and their cell viability was quantified by a live/dead staining experiment. As shown in Fig. 9 (B), the proportion of living cells was calculated using ImageJ software, and the result is consistent with that of the MTT experiment. The morphology of L929 cells was directly observed with an inverted microscope after culture with the 24 h extraction, as shown in Fig. 9 (B). L929 cells were lengthened and became spindle-shaped, similar to the morphology of typical fibroblasts in the control group. The cell culture was evenly distributed, and the intercellular spaces did not change significantly. This indicates that PU4 adhesive can meet the clinical biosafety demands.
A

Cell viability (%)

B

Control

PU4

SB2

SPB

SBU

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Fig. 9 (A) Cytotoxicity of the PU4 adhesive and commercial adhesives SB2, SPB, and SBU was determined by MTT assay. A cell relative survival rate greater than 70% can be considered non-cytotoxic. (B) Optical microscopy images (left) and live/dead fluorescence cell staining (right) after L929 cells were cultured for 24 h in culture medium (control), extracted liquid of the PU4 adhesive and commercial dental adhesives SB2, SPB, SBU. Living cells were stained with calcium—AM (green), and dead cells were stained with PI (red). Scale bars=100 μm

4 Conclusion
In summary, an elastic PU adhesive was prepared and evaluated using comprehensive methods. The lower water sorption/solubility and decreased microleakage of PU adhesive indicates that it can prevent water from permeating into the bonding interface and enhance marginal sealing. The PU adhesive can also buffer the stress coming from volumetric polymerization shrinkage, temperature changes and repeated chewing force by deformation due to its elastic property. Furthermore, it is biosafe for L929 cells. This study lays the foundation for the application of PU adhesive in clinical practice to produce stable and long-lasting adhesion.

Conflicts of interest
There are no conflicts to declare.

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Authors' Contributions

The authors meet all of the following criteria:

1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data;
2) drafting the article or revising it critically for important intellectual content;
3) final approval of the version to be published; and
4) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Authors’ major contribution:

Jiahui Zhang: conducted the statistical analysis and wrote this manuscript;
Xiaowei Guo and Xiaomeng Zhang: finish the acquisition of data;
Huimin Wang, Jiufu Zhu and Zuosen Shi: conduct the experiments and repeated them;

Corresponding author: Song Zhu and Zhanchen Cui: designed and oversaw the study, and reviewed and revised this manuscript.
PU elastic dental adhesive

composite resin

PU adhesive

185x107mm (300 x 300 DPI)
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We hope you are keeping well at this difficult time.

Thanks very much for taking your time to review this manuscript. I really appreciate all these precious comments and suggestions. Please find my itemized responses in below and my revisions in the resubmitted files.

Thanks again.

Reviewer comments to Author:

Reviewer: 2

Comments to the Author(s)

Many of the responses to the reviewer are information that the reader needs to understand the manuscript, especially the parts marked with yellow (see attach file). Authors have to include and/or adapt these parts in the paragraphs of their main text for better clarity of the paper.

We are grateful for the suggestion.

We have adapted relevant parts in the paragraphs of the main text according to the suggestion for better clarity of the paper. The revised section of the manuscript was marked in red.