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Comparison of Human Dental Tissue RNA Extraction Methods for RNA sequencing

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Abstract

Objective: The purpose of this study was to identify an efficient RNA extraction method for periodontal ligament (PDL) and dental pulp (DP) tissues to be used in RNA sequencing studies, given the increased use of these techniques in dental research and the lack of standard protocols.

Design: PDL and DP were harvested from extracted third molars. Total RNA was extracted with four RNA extraction kits. RNA concentration, purity and integrity were assessed by means of NanoDrop and Bioanalyzer and statistically compared.

Results: RNA from PDL was more likely to be degraded than that of DP. The TRIzol method yielded the highest RNA concentration from both tissues. All methods harvested RNA with A260/A280 close to 2.0 and with A260/A230 above 1.5, except for the A260/A230 from PDL obtained with the RNeasy Mini kit. For RNA integrity, the RNeasy Fibrous Tissue Mini kit yielded the highest RIN values and 28S/18S from PDL, while the RNeasy Mini kit obtained relatively high RIN values with an appropriate 28S/18S for DP.

Conclusion: Significantly different results were obtained for PDL and DP when using the RNeasy Mini kit. The RNeasy Mini kit provided the highest RNA yields and quality for DP, while the RNeasy Fibrous Tissue Mini kit obtained the highest quality RNA from PDL.

Keywords: periodontal ligament; dental pulp; RNA; RNA-seq; dental research

Introduction

In recent years, RNA sequencing (RNA-seq) and transcriptome profiling have proven to be valuable tools to investigate the underlying mechanisms of gene regulation and signal transduction, aiming to provide an overview of multi-gene interaction (Stark et al., 2019). The rapid development and simplification of these technologies has led to an increase of their use in dental research, since they offer a great opportunity to study the complex biological processes involving several dental tissues in a variety of scenarios, such as periodontitis, pulpitis or root resorption. (Kim et al., 2020; Lu et al., 2019; Mohanakumar et al., 2021; Rathinam et al., 2021; Spitz et al., 2021).

The periodontal ligament (PDL) and the dental pulp (DP) are two of the most relevant tissues in dental research (Gong et al., 2017; Matichescu et al., 2020). PDL tissues surround the root surfaces and connect the tooth to the alveolar bone, providing the teeth with support, proprioception and physical protection. DP tissues, located inside of the tooth chamber, form secondary dentin and provide the tooth with innervation and blood supply. Examples of research involving these tissues are pulp regeneration (Schmalz et al., 2020; Xie et al., 2021), periodontal research (Guo et al., 2015; Pei et al., 2017) or the study of tissue reactions to masticatory or orthodontic force (Li et al., 2018; Vansant et al., 2018).

However, the execution and success of RNA-seq is highly dependent on the quality and quantity of RNA. Low RNA content samples can lead to sequencing bias caused by library preparation and RNA degradation can lead to inaccurate gene expression results through misinterpretation of sequencing reads yield (Gallego Romero et al., 2014; L. Wang et al., 2016). In addition, the isolation of intact RNA from PDL and DP tissue can be challenging due to their inherent chemical composition, which is more prone to hydrolysis. RNA from these tissues also presents a high sensitivity to enzymatic degradation by ribonucleases (RNases), since RNases are very active, widespread, stable and require no cofactors (Gayral et al., 2011; Riesgo et al., 2012; Vasilenko, 2019). The different steps of RNA extraction, such as tissue disruption and RNases activity avoidance, can also have an impact on the RNA yield, purity and integrity (Locy et al., 2021).

Although genetic research conducted on human PDL and DP tissue is still scarce, a trend can be observed towards an increasing use of these techniques. Most available studies use RNeasy Fibrous Tissue Mini kit and TRIzol reagent for RNA extraction (Gong et al., 2017; Kim et al., 2020; Lee et al., 2013; Mohanakumar et al., 2021; Song et al., 2013; Spitz et al., 2021), but no

standard, optimized protocols for RNA extraction are available for PDL nor for DP tissue. Because of this, the present study aims to identify an efficient RNA extraction method for PDL and DP tissues to be used in dental research involving RNA-seq.

Materials and Methods

Sample collection and tissue processing

The present project was approved prior to the start by the Commission for Medical Ethics of KU Leuven (file number S-60530).

Intact third molars were collected immediately after extraction from 15 healthy patients. From these, 24 teeth were used in this experiment. In the operation room, immediately after extraction, the teeth were placed in 50 ml centrifuge tubes with sterile Phosphate Buffered Saline (PBS; Sigma Aldrich, St. Louis, MO, USA) and were transported to the lab within 5 mins. All teeth were rinsed in 70% ethanol (Hydral 70, VWR, Leuven, Belgium) for 1 min, then placed in RNase-free PBS until further processing. PBS and ethanol were pre-cooled at 4°C before use. The PDL tissue was carefully obtained from the middle third of the root with a sterile scalpel blade (Swann Morton, Sheffield, UK). The teeth were then immediately covered by RNase-free gauze and gloves and split with a hammer to reveal the pulp chamber, enabling the gentle collection of DP tissue using sterile tweezers. All the materials used were RNase-free. The extracted PDL and DP samples were then immediately frozen and stored in liquid nitrogen. The tissue from each tooth was stored as one sample. Tissue collection and RNA extraction were processed by the same investigator.

RNA extraction methods

The RNA extraction methods used in this study are as follows. Additionally, the cost of per sample and the processing time of each method was noted.

RNeasy Mini kit (QIAGEN, Hilden-Germany)

For the extraction of total RNA, following the manufacturer's instructions, 20-30 mg of dental tissue was disrupted and lysed in 600 µl of the Buffer RLT (containing β-mercaptoethanol 10 µl/ml of Buffer RLT) by using a hand-held disposable pellet homogenizer. The lysate was centrifuged for 3 minutes at maximum speed and the supernatant was transferred to a new RNase-free tube, followed by a mixture of the same volume of 70% ethanol. RNA was bind to the RNeasy spin column by transferring the sample to the column and centrifuging at 8000×g for 15 seconds (Eppendorf Centrifuge 5425, Germany). In order to remove the residual buffer, 350 µl Buffer RW1 was added and centrifuged at 8000×g for 15 seconds and the flow-through was discarded. For on-column DNase digestion, 80 µl of DNase mixture (10µL DNase stock solution mix with 70 µl Buffer RDD) was added to the center of the membrane and incubated for 15 minutes at room temperature. For the RNA wash step, 350 µl of Buffer RW1 was added to the column and flow-through was discarded after centrifuging at 8000×g for 15 seconds. 500 µl of Buffer RPE was added for a second wash, followed by 15 seconds of centrifugation. This process was repeated again with 2 minutes of centrifugation. After that, the column was subjected to

additional centrifugation for 1 minute at maximum speed to dry the membrane. For the RNA elution step, the column was inserted to a new 1.5 ml RNase-free microtube, in which 30 µl of RNase-free water was added and RNA was harvested after 1 minute of centrifugation at 8000×g. Then, the obtained RNA was stored at -80℃.

RNeasy Fibrous Tissue Mini kit (QIAGEN, Hilden-Germany)

The tissue (20-30mg) was homogenized and lysed using the same protocol described for the RNeasy Mini Kit. The sample was then incubated at 55 ℃ for 10 minutes after mixture with 590 µl RNase-free water and 10 µl proteinase K. Then, the sample was centrifuged at 10,000×g for 3 minutes and the supernatant was transferred to a new tube. Half volumes of absolute ethanol were added and mixed completely. Later on, the lysate was followed by the same RNA binding, washing, on-column DNase digestion and elution steps as performed for the RNeasy Mini kit.

PureLink™ RNA Micro Scale Kit (Invitrogen, Carlsbad-CA–USA)

Dental tissue (20-30mg) was homogenized in 600 µl of the Lysis Buffer (containing β-mercaptoethanol 10 µl/ml of Lysis Buffer). Then, a centrifugation step followed at 12,000×g for 2 minutes at room temperature. The supernatant was transferred to a new RNase-free tube and an equivalent volume of 70% ethanol was added and mixed thoroughly. Then, RNA binding, washing and elution steps were performed. The sample was then transferred to the column for RNA binding. The column was centrifuged at 12,000×g for 1 minute and the flow-through was discarded. Subsequently, 350 µl of Wash Buffer (WB) I was added to column and centrifuged at 12,000 ×g for 1 minute to remove the residual buffer. For on-column DNase digestion, 20 µl DNase mixture was added to the center of the column and incubated at room temperature for 15 minutes. 350 µl of WB I was added to the column again and the flow-through was discarded after centrifuging at 12,000 ×g for 15 seconds. 500 µl of WB II was used for a second wash, followed by 15 seconds of centrifugation. This process was repeated again with 2 minutes of centrifugation. After that, the column was subjected to additional centrifugation for 1 minute to dry the membrane. For the RNA elution step, the column was placed in a new 1.5 ml RNase-free microtube, together with 30 µl of RNase-free water. RNA was harvested after 1 minute of incubation and centrifugation at 12,000×g. Then, the obtained RNA was stored at -80℃.

TRIzol method (Invitrogen, Carlsbad-CA–USA)

TRIzol reagent (1ml) was added to the dental tissue (20-30 mg) of each sample. The tissue was then homogenized with the same homogenizer. The entire sample with TRIzol reagent was incubated for 5 minutes at room temperature before 200 µl of chloroform (Sigma-Aldrich, St. Louis-MO–USA) was added to the sample. After vigorously shaking the tube by hand for 15 seconds, the tube was incubated for 3 minutes at room temperature. This was then followed by a centrifugation step at 12,000×g for 15 min at 4℃. The colorless upper layer was transferred to a new RNase-free tube. For RNA precipitation, an equal volume of 70% ethanol (Sigma-Aldrich, St. Louis- MO–USA) was added to the sample, which was vortex to mix well. For the RNA binding, washing, Dnase and elution steps, the same procedures described for the PureLink™ RNA Micro Scale Kit were followed.

Measurement of RNA yields, purity and integrity

RNA purity and concentration were assessed by determination of RNA absorbance in RNase-free water at 230, 260, and 280 nm using a NanoDrop ND-8000 spectrophotometer (Thermo Scientific, USA). The optical density (OD) A260/A280 and A260/A230 ratio was used to evaluate RNA purity. RNA integrity was measured with Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). The total RNA integrity was assessed according to the RIN and 28S/18S ratio. RIN values were calculated by using 2100 Expert software (Agilent Technologies, USA). The 28S/18S bands were examined by agarose gel electrophoresis.

Statistical analysis

Each of the RNA extraction methods was performed at least in triplicate. The concentration, purity and integrity of the RNA obtained with the four methods were statistically compared for PDL and DP tissue. The statistical analyses were performed by using IBM SPSS Statistics software (version 24.0, IBM Corporation, NY). Normality of data was analyzed by Shapiro-Wilk test. The paired t-test was applied to compare the results of both tissues. Within the same tissue, comparisons among the four methods were analyzed with one-way ANOVA and Kruskal-Wallis test. Tukey's and Dunn's multiple comparison tests were applied to compare the difference between each group. All data were reported as mean ± standard deviation (SD). Significant difference was defined as $p < 0.05$.

Results

Concentration and quality of RNA

The concentration and quality of the total RNA solutions extracted from both tissues are shown in Table 1 and Figure 1. The cost of per sample and the processing time of each method is presented in Table 2. For PDL, the TRIzol yielded the highest RNA concentration (231.98±119.85ng/ul) followed by the RNeasy Fibrous Tissue Mini kit (129.83±57.90ng/ul). All methods yielded RNA with A260/A280 close to 2.0 without significant differences. However, the ratios of the A260/A230 for all methods were higher than 1.5 except with the RNeasy Mini kit (0.31±0.13). For DP, the TRIzol also yielded the highest RNA concentration (190.84±85.46ng/ul) followed by RNeasy Mini kit (133.85±7.77ng/ul). All methods yielded high-quality RNA with A260/A280 close to 2.0 and with A260/A230 above 1.5. The RNA concentration measured by Nanodrop was compared with that measured with Bioanalyzer (Table 1), and there were no significant differences between the two measurements from both tissues except for the RNeasy Mini Kit on DP tissue.

Integrity of RNA

RNA integrity was evaluated by assessing the RIN values and the ratios of 28S/18S, shown in Table 1 and Figure 1. When comparing the RNA from PDL tissue, RNeasy Fibrous Tissue Mini kit yielded significantly higher RIN (5.75±0.58) compared to the other three methods. RNeasy Fibrous Tissue Mini kit also yielded the highest 28S/18S (3.00±0.47). For RNA from DP tissue, the RNA samples extracted by RNeasy Mini kit and RNeasy Fibrous Tissue Mini kit showed relatively high RIN (8.35±0.98 and 8.18±0.40, respectively). While RNeasy Mini kit yielded an appropriate 28S/18S (1.40±0.42). Representative electropherograms and agarose gel electrophoresis of the total RNA extracted from both tissues by the four methods are shown in

Figures 2 and 3, respectively.

Comparison of RNA extraction methods between PDL and DP tissue

The effectiveness of every single RNA extraction method was compared between PDL and DP tissues (Figure 4) . RNeasy Mini Kit and RNeasy Fibrous Tissue Mini Kit yielded significantly different RNA concentration of both studied tissues. No significant differences were found between PDL and DP regarding RNA purity, except the ODA260/A230 extracted by RNeasy Mini kit. The RIN values between PDL and DP tissues extracted by RNeasy mini kit, RNeasy Fibrous Tissue Mini kit and PureLink™ RNA Micro Scale Kit were significantly different, while differences between PDL and DP tissues were only detected regarding the 28S/18S ratio with RNeasy Mini kit . This kit yielded largely different results from these two dental tissues.

Discussion

RNA-seq is increasingly being used in dental research, due to its potential to both map and quantify transcriptomes from human PDL and DP tissues under different physiological and pathological conditions (Ozsolak & Milos, 2011; Z. Wang et al., 2009). However, harvesting high quality and quantity of RNA is crucial for downstream gene expression studies using RNA-seq, which can be challenging for several reasons. First, harvesting enough RNA from a clinical sample depends on obtaining sufficient tissue. In contrast to the DP tissue, which has an average weight of 16.7 mg in the third molar (Guerrero-Jiménez et al., 2019), the PDL tissue has an average width of 0.25mm. PDL can only be harvested partially during tooth extraction, after breaking the PDL fibers with surgical instruments and often leaving remnants in the socket (de Jong et al., 2017). In addition, to avoid contamination of gingival and pulpal cells, only the middle third of the PDL is normally scrapped from the dental root, which reduces the amount of tissue even more (Mohanakumar et al., 2021). Secondly, the histology of the dental tissues can also affect RNA yield. PDL fibers are constituted by 90% collagen, mainly type I with a minor contribution of type III (de Jong et al., 2017). DP tissue presents 32% collagen, much less than PDL tissue, and is mainly composed of collagen type III. Total RNA extraction from fibrous tissues can be challenging due to the abundance of contractile proteins such as collagen, which are extremely difficult to homogenize (Reimann et al., 2019).

Aside from quantity, the quality of RNA is also important, which is normally defined by concentration, purity and integrity. The concentration of extracted RNA can be measured by NanoDrop and Bioanalyzer. Research shows that NanoDrop can also measure oligo nucleotides, while Bioanalyzer yields a negative result when evaluating nucleic acids (Rodríguez et al., 2020). RNA concentration values are normally higher with NanoDrop, but this doesn't mean this method is more precise. Sensitivity testing of Nanodrop and Bioanalyzer by dilution series of dsDNA oligos, has shown the RNA concentrations detected by Bioanalyzer to be the closest to those given by the oligo supplier, whereas NanoDrop overestimates RNA concentration (Hussing et al., 2018). The relative values obtained from PDL and DP tissue by the four RNA extraction methods analyzed in this study are consistent with these statements. The apparent contradiction of some of our results could be explained by the limited samples and different tissues. In our study, while the

RNA concentration obtained by RNeasy Fibrous Tissue Mini kit and PureLink™ RNA Micro Scale Kit was enough for downstream application, the TRIzol method obtained the highest RNA concentration from both PDL and DP tissue, measured both by Nanodrop and Bioanalyzer. The RNeasy Mini Kit only yielded enough RNA concentration to perform RNA-seq on DP tissue.

RNA purity is one of the most important requirements for RNA-seq and is evaluated by measuring the ratio of absorbance readings at 260 nm (specific for nucleic acids), 230 nm (possible contaminations for carbohydrates and phenol) and 280 nm (specific for proteins). A260/A280 ratios between 1.80-2.10 are usually considered as indication of no significant RNA contamination (Manchester, 1996), while the ratios of A260/A230, used as a secondary measure of nucleic acid purity, are expected within the range of 2.0-2.2 (Ahlberg et al., 2021). In our study, the values of A260/A280 were all around 2, which proves the efficiency of all analyzed methods in protein contamination prevention. Regarding A260/A230, only PureLink™ RNA Micro Scale Kit showed values between 2.0-2.2 for PDL, while all other samples were above 1.5. The A260/A230 of the PDL sample extracted with RNeasy Mini Kit were extremely low and cannot be used for RNA-seq. This is directly linked with the very low RNA collected from PDL with this method.

Lastly, RNA degradation is one of the major sources of variation in gene expression results when using RNA-seq (L. Wang et al., 2016). Integrity of RNA is expressed in RIN values, which range from 1 to 10, with values over 7 being considered sufficient for most downstream techniques (Fleige & Pfaffl, 2006; Ibberson et al., 2009). Also, values of 28S/18S over 2 and below 1 are indicative of contamination with genomic DNA and RNA degradation respectively, while between 1-2 is considered optimal (Fleige & Pfaffl, 2006). In our study, RNeasy Fibrous Tissue Mini kit obtained the highest RIN value for PDL tissue, but also a high 28S/18S (3.0±0.47). The other three methods yielded samples with low RIN values, but both TRIzol and PureLink™ RNA Micro Scale Kit met the requirement for 28S/18S. However, as the main index of RNA degradation, the RIN value is more important than the ratio 28S/18S. For DP tissue, all methods yielded low RNA degradation, with the RNeasy Fibrous Tissue Mini kit also obtaining 28S/18S over 2. This may be related to the tissue-specific anatomy and histology discussed above. Additionally, the use of a homogenizer might raise the temperature of the RNA samples due to the high frequency vibration, which could eventually lead to sample degradation. However, this degradation was only limited to PDL tissue, and neglectable degradation was observed on DP tissue. Furthermore, PDL tissues have been subjected to RNA degradation by ambient RNases throughout the whole process since being taken from the patient, while DP tissue would have been at least partially insulated from interaction with external RNases, which might explain the outcome. Another limitation of the present study is the number of kits evaluated. Testing additional kits and increasing sample replications could help better understand the efficacy of all commercial RNA extraction kits on human dental tissues obtained from clinical samples.

Different RNA-seq techniques can be used for transcriptome analysis, such as whole transcript RNA-Seq or 3’ RNA-Seq. The classic method (e.g. TruSeq Stranded RNA) shears longer transcripts into more fragments and then reverse-transcribes them into cDNAs (Hrdlickova et al., 2017), providing a combination of both mRNA and whole-transcriptome (Palomares et al., 2019). With the 3’ RNA-Seq method (e.g. QuantSeq (Moll et al., 2014)), cDNAs are only

reverse-transcribed from the 3′ end of the mRNAs without previously fragmenting the mRNA. The whole transcript RNA-Seq has higher requirements of RNA compared to the 3' RNA-Seq (minimum of 200 ng total RNA in 10 ul sample, 1.5 for both A260/A280 and A260/A230 and RIN ≥ 8, according to the Genomics Core Leuven (https://www.genomicscore.be/). For the 3' RNA-Seq, 100 ng total RNA in 5 ul sample is needed, without any specific requirement of RNA integrity. Based on our results, degradation of RNA is inevitable to some extent on PDL tissue. In this respect, the 3' RNA-Seq could be a better choice for gene expression analysis of PDL tissue, since both RNA-seq methods had similarly high reproducibility between replicates (Ma et al., 2019). For DP tissue, both RNA-seq methods could be used.

Previous studies performing RNA-seq on human PDL in dental research (Kim et al., 2020; Spitz et al., 2021) used RNeasy Fibrous Tissue Mini kit and TRIzol to extract RNA. According to our results, although both methods are able to harvest enough RNA concentration and purity, RNeasy Fibrous Tissue Mini kit performs better. RNA integrity, which is crucial for sequencing, was not evaluated in these studies (Kim et al., 2020; Spitz et al., 2021). As a reference for future research and according to our results, the RNeasy mini kit yields the best results for DP, although these are really close to the TRIzol results, which also performs good on PDL tissue. The cost per sample and the processing time are also worth taking into consideration in order to make a global assessment of which method to choose. The TRIzol method was found to be the cheapest one (Table 2), followed by the RNeasy Mini kit and RNeasy Fibrous Tissue Mini kit. The processing time was not very different between methods, except for TRIzol, which needs 30 mins more compared to the other three methods, which is not a considerable difference. Therefore, TRIzol could be an acceptable strategy for both tissues which can potentially reduce costs and enable the set-up of one single RNA extraction pipeline in the lab instead of two, although the lower RIN values obtained with this method can jeopardize RNA-seq results. Previous studies using the TRIzol method on PDL tissue did not report degradation, (George et al., 2020; Gong et al., 2017; Kim et al., 2020; Mohanakumar et al., 2021) but also failed to report RIN values, which makes comparisons with our results difficult. The low RIN values of TRIzol in our study may have been due to the small sample size (n=10). In this sense, it would be interesting to include more samples to the comparison in future studies.

Conclusions

The RNeasy mini kit was found to be the most efficient method to provide the highest RNA yields and quality for DP tissue, while the RNeasy Fibrous Tissue Mini kit obtained the highest RNA quality from PDL tissue. Additionally, it was demonstrated that RNA from PDL samples are more likely to be degraded compared to those from DP tissue. Given the increased use of RNA-seq techniques in dental research, these aspects should be taken into consideration in future studies.

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Declaration of competing interests

The authors declare that they do not have conflicts of interest with the contents of this article.

Data Availability: Data will be made available on request

Reference:

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Tables and Figures

Table 1. RNA concentration, purity and integrity of PDL and DP tissue obtained with the four different RNA extraction methods.

		Sample (n)	RNA Concentration (ng/µl)		*p*-value	RNA Purity		RNA integrity	
			Nanodrop	Bioanalyzer		A260/280	A260/230	RIN value	28S/18S
PDL	RNeasy Mini Kit	3	5.53±1.59	0.73±0.45	0.055	2.06±0.13	0.31±0.13	2.57±0.06	0
	RNeasy Fibrous Tissue Mini kit	4	129.83±57.90	119.50±44.83	0.631	2.03±0.05	1.54±0.38	5.75±0.58	3.00±0.47
	PureLink™ RNA Micro Scale Kit	3	54.80±15.50	63.33±18.18	0.367	2.03±0.17	2.19±0.09	2.53±0.15	1.93±3.35
	TRIzol method	9	231.98±119.85	253.67±135.14	0.064	2.06±0.01	1.70±0.55	3.62±0.86	1.36±0.64
DP	RNeasy Mini Kit	4	133.85±7.77	117.25±7.37	0.046*	2.07±0.03	1.85±0.20	8.35±0.98	1.40±0.42
	RNeasy Fibrous Tissue Mini kit	5	59.46±20.77	57.40±21.48	0.836	2.05±0.05	1.53±0.35	8.18±0.40	3.84±2.28
	PureLink™ RNA Micro Scale Kit	5	49.44±9.31	56.60±9.81	0.096	2.04±0.03	1.65±0.45	7.04±0.63	1.02±0.23
	TRIzol method	10	190.84±85.46	202.60±93.58	0.251	2.06±0.02	1.76±0.42	6.25±0.63	2.17±1.36

* ($p < 0.05$)

Table 2. Cost per sample and processing time of the four different RNA extraction methods.

RNA extraction method	Cost/Sample	Processing time
RNeasy Mini Kit	€7.31	Less than 1 hour
RNeasy Fibrous Tissue Mini kit	€9.21	Less than 1 hour
PureLink™ RNA Micro Scale Kit	€10.1	Less than 1 hour
TRIzol method	€1.58	1 - 1.5 hour

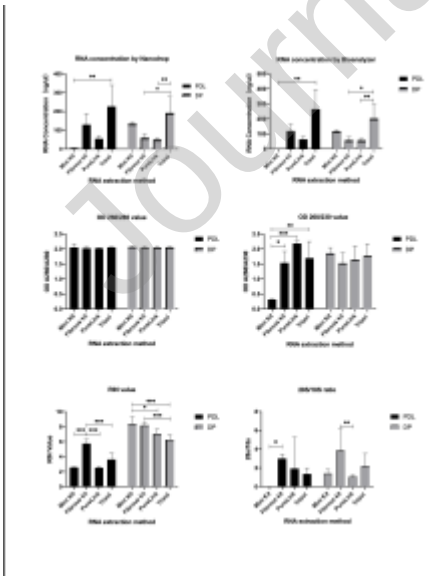


Figure 1. Comparison of RNA concentration, purity and integrity among the four RNA extraction methods in both tissues. The error bars represent the standard deviations. Statistically significant differences are marked with * ($p < 0.05$), ** ($p < 0.01$) or *** ($p \leq 0.001$).

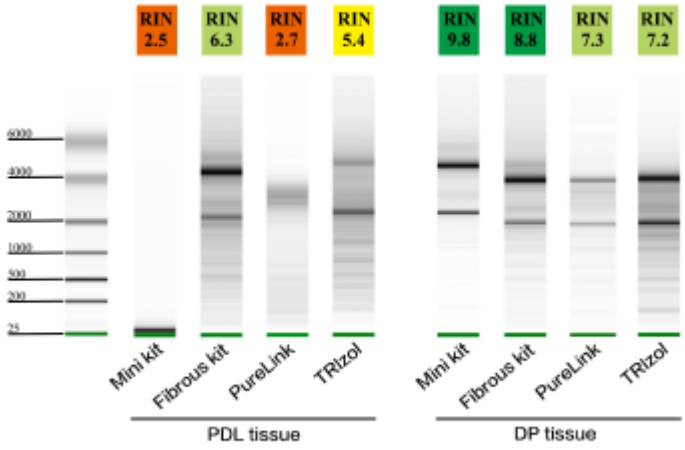


Figure 2. Representative bioanalyzer electropherograms of RNA. A: total RNA extracted from PDL tissue with each of the methods. B: total RNA extracted from DP tissue by using the four methods. FU: fluorescence units, nt: nucleotides.

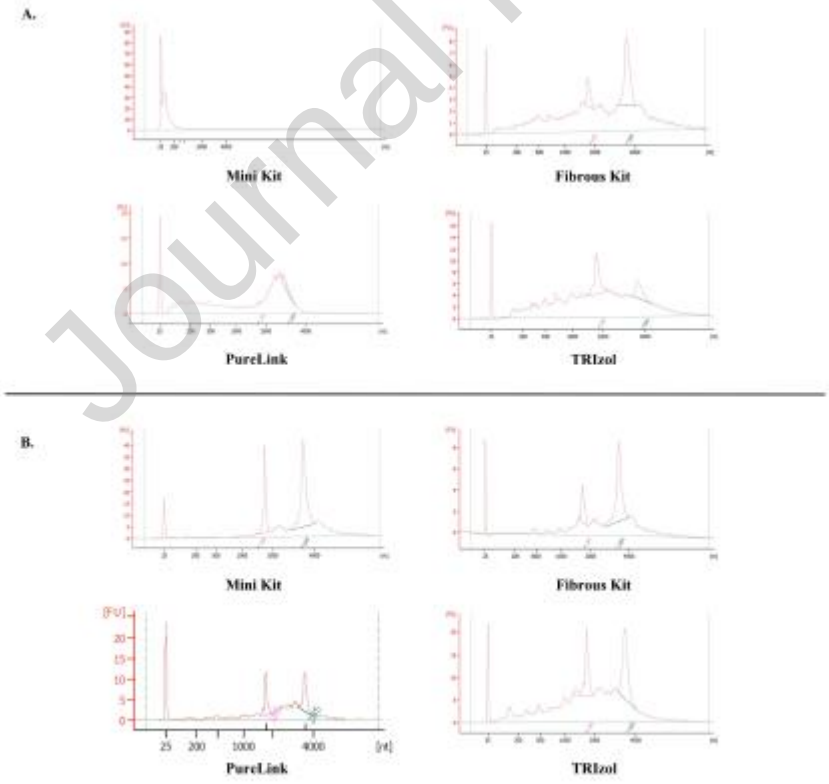


Figure 3. Representative bioanalyzer agarose gel electrophoresis of total RNA. RIN values were calculated by bioanalyzer.

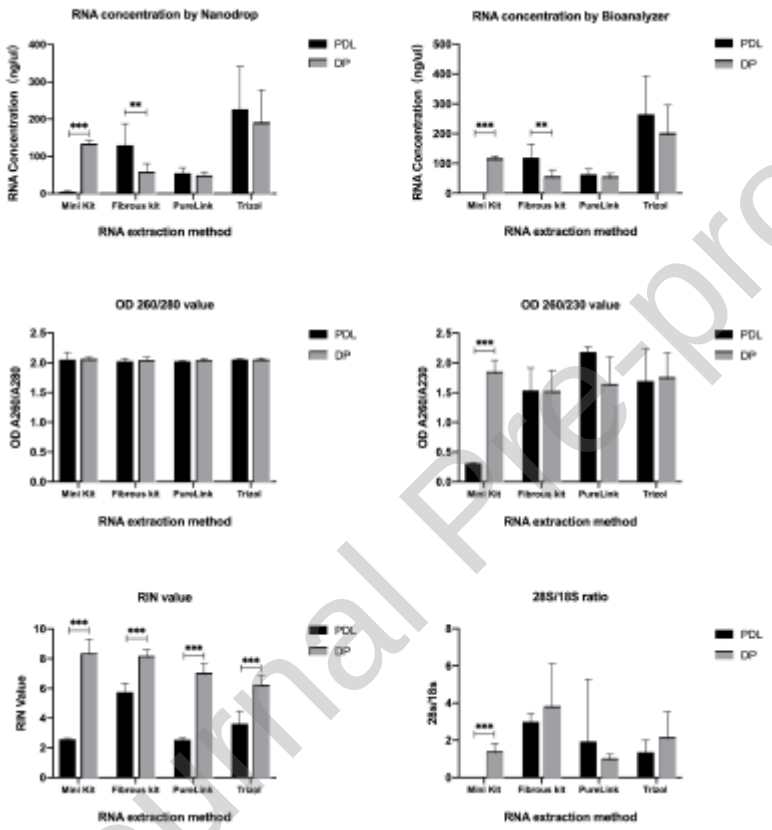
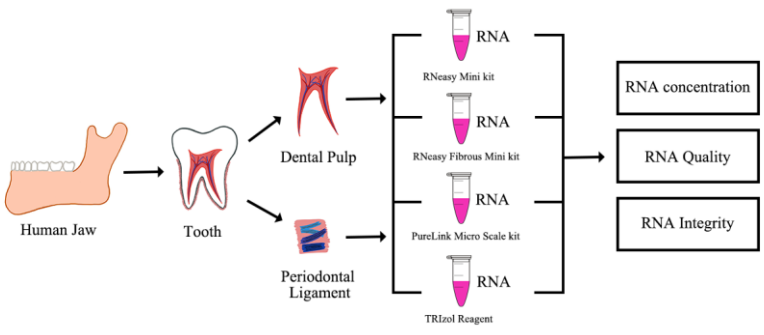


Figure 4. Comparison of RNA concentration, purity and integrity between PDL and DP tissues. The error bars represent the standard deviations. Statistically significant differences are marked with * ($p < 0.05$) , ** ($p < 0.01$) or *** ($p \leq 0.001$).

Graphical abstract



Highlights:

- Identify efficient dental tissue RNA extraction kit is crucial for RNA sequencing
- RNeasy Fibrous Mini kit obtained the highest quality RNA from periodontal ligament
- RNeasy Mini kit provided the highest RNA yields and quality for dental pulp tissue
- RNA from periodontal ligament is more likely to be degraded than dental pulp