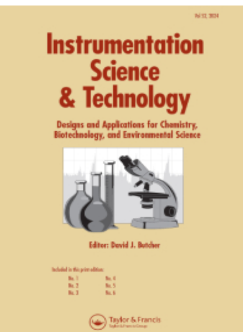

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Comparison of two mechanical disaggregation methods of fresh lung tissues for extraction of high-quality RNA

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ABSTRACT

Gene expression studies are widely used in medical, biological, and pharmaceutical research. Obtaining high-quality RNA from tissues is a prerequisite for high-quality data that should accurately represent gene expression levels in-vivo. The main source of technical bias, which could affect the results from transcriptomic studies, is variation in RNA quality. In this regard, tissue preparation is critical: different disruption techniques can affect RNA quality, influencing further applications. Mechanical disaggregation is a common, inexpensive, and simple method to obtain a high cell yield, demonstrated to efficiently disrupt the extracellular matrix and release single cells. However, its efficacy is operator-dependent, leading to poorly reproducible results. A fast, reproducible, and standardized technique could undoubtedly overcome this problem, avoiding wasting time and resources. In this study, our goal was to evaluate the impact of two mechanical tissue disruption techniques on the purity and quality of RNA extracted from fresh lung biopsies. The samples were processed in parallel using manual mechanical disaggregation or an automated mechanical device. The results showed that samples processed with the automated device had a higher integrity compared to those processed manually with a median Fragmentation Index of 0.86 and 0.71 respectively. This difference is statistically significant ($p = 0.0084$). Overall, our results indicated that the use of automatic mechanical disaggregation could undoubtedly help to overcome the technical biases related to fresh tissues processing.

KEYWORDS
Tissue mechanical disaggregation; RNA extraction; RNA fragmentation; RNA quality

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Introduction

Solid tissue disaggregation protocols that can generate vital cellular suspensions were first described in the 1970s.[1,2] Over the years, several protocols have been developed which usually include multistep procedures involving one or a combination of enzymatic, mechanical or chemical methods.[3,4] Enzymatic digestion commonly involves collagenase, hyaluronidase, dispase, trypsin or DNase while mechanical tissue dissociation methods rely on aspiration, scraping or tissue pressure.[5–7] However, these methods can selectively damage some types of cells.[8] In addition, because manual mechanical methods depend on the operator, the results are not highly reproducible and require more investment in term of time and cost.[9]

In recent years, the use of transcriptomic analysis has increased exponentially due to its potential to provide large amounts of information from small samples, for research purposes and clinical needs, such as diagnosis and prognosis.[10,11] Despite the intrinsic importance of these studies, a universal protocol for obtaining reliable analyses has not yet been fully defined. For transcriptomic studies, the extraction of RNA, compared to DNA, is more challenging due to its intrinsic characteristics of instability and fast degradation rate.[12] Obtaining RNA with high purity, quality, and reproducibility, is the starting point for downstream analyses and can affect the whole process.[13]

Proper tissue storage after acquisition from the patient is the first obstacle: to preserve the quality, samples should be transported on ice, and frozen or processed as soon as possible to avoid degradation.[14] Transcriptomic studies from FFPE specimens undoubtedly have the great advantage of relying on a large number of archival samples. However, pre-analytical processing often affects the quality of nucleic acids. Therefore, the use of fresh or frozen tissues should be preferred, to preserve transcriptional differences representative of the original tissue.

RNA extraction from fresh biopsies requires a preliminary step for disaggregation. Enzymatic digestion and mechanical disruption are the most common. Both techniques have been demonstrated to break down the extracellular matrix and release single cells.[15]

Enzymatic disaggregation can be carried out using a mixture of two or more enzymes such as trypsin, collagenase, and hyaluronidase. It is mainly used when high recovery of cells is required and has the potential to isolate a good cell suspension.[16] However, it is an expensive and difficult to standardize procedure that may affect the phenotype and function of the isolated cells.[17] Additionally, for regenerative medicine applications, the FDA considers the enzymatic disaggregation method "more than minimally

manipulated", compared to mechanical procedure considered to be "minimally manipulated", with consequent restrictions on their use to these purposes.

Manual mechanical disaggregation, such as scraping and mincing with a scalpel or with mortar and pestle, has the additional advantage of being a cheap and simple method of obtaining high cell yield'[18] and debris reduction. However, its efficacy in highly dependent on the experience of the performer, leading to poorly reproducible results and tends to generate a suspension of damaged cells.[15] The comparison of different mechanical disaggregation techniques has been demonstrated to affect RNA quality.[18]

Rigenera technology is an innovative method based on the mechanical disaggregation of human solid tissues to obtain a suspension of calibrated micrografts able to preserve a high cell viability and an optimal regenerative potential. Rigenera technology is based on the use of Rigeneracons, an automated, portable, safe, standardized, and easy-to-handle device able to mechanically disaggregate small tissue's biopsies without use of additives or enzymes, allowing fast processing, reproducible results, and an increase in the disaggregation rate.[19] This device is routinely used to obtain autologous and homologous micrografts from human tissues which in turn can be used to promote tissue regeneration in clinical procedures of regenerative medicine. Rigenera technology is successfully applied in oral-maxillofacial surgery, dermatology, orthopedic and wound care applications.[13]

Based on the properties of this device and the growing demand for high quality transcriptomic data, the goal of this study is to compare the mechanical technique with mince and scraping, with the automatic disaggregation system Rigeneracons to improve both the quality of disaggregation process and increase the extraction of high-quality RNA that can be used for subsequent analysis. The effects of these procedures on the integrity of RNA have been evaluated by calculating the degree of fragmentation of RNA using the Myriapod NGS Cancer panel RNA kit, routinely used in medical laboratories to perform real-time polymerase chain reaction (PCR) and next-generation sequencing (NGS).

Materials and methods

Samples

The samples were collected in collaboration with the Pathology Section and Thoracic Surgery Units of Siena Hospital "Le Scotte", Italy. Lung surgical specimens were obtained from 37 patients, who underwent lobectomy. The Pathology Unit diagnosed lung adenocarcinoma (LUAD) in 32 patients, lung squamous cell carcinoma (LUSC) in 4 patients, and neuroendocrine

Table 1. Lung specimens histotypes analyzed for RNA purity and integrity. LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; LCNE: neuroendocrine lung cancer. n = number of samples.

LUAD	$n = 41$
LUSC	$n = 3$
LCNE	$n = 1$
LUSC adjacent parenchyma	$n = 7$
LUAD adjacent parenchyma	$n = 53$
LCNE adjacent parenchyma	$n = 1$

lung cancer (LCNE) in 1 patient. Non-cancerous adjacent parenchyma was also collected during surgery.

Only the samples for which sufficient material was available (tumor or parenchyma tissue) after diagnostic sampling, were processed. A total of 106 specimens were analyzed (66 processed with Rigeneracons, 40 processed manually). Each sample was divided into two equal parts which were simultaneously disaggregated using these methods. In order to standardize the two protocols, tissue disaggregation, extraction, purity and RNA quality evaluation were performed on 106 samples by 5 different operators. The histotypes are summarized in Table 1. Lung surgical specimens were transported to the laboratory for samples processing within 30′ from collection.

Informed consent was obtained from all subjects involved in the study. The investigations were conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of Regione Toscana-Area Vasta Sud Est, Italy (protocol code 19317 #Mi-PP, and date of approval: 2021, April 24th). This study is conformed to the standards of the Declaration of Helsinki.

Tissue processing

40 lung surgical specimens (16 tumors and 24 parenchyma tissues) (Table 2) were split in two and processed with Rigeneracons or manually, in equal volumes (1,4 ml) of RLT RNeasy Lysis buffer (QIAGEN RNeasy Mini Kit, Cat #74106) to allow both cell lysis and RNase inhibition.

The samples processed with Rigenera technology were inserted into the Rigeneracons device and disrupted by activating the machine for 1 min (Supplementary Video 1).

The samples processed manually were placed in a Petri dish, minced and scraped with a scalpel. The procedure lasted for a maximum of 3 min (Supplementary Video 2).

26 additional samples (13 tumors and 13 parenchyma; Table 3) were processed using only Rigeneracons.

The lysates obtained from both procedures were transferred to a 15 mL tube for RNA extraction. To avoid bias, all the analyzed samples were randomly assigned to five different operators for processing.

Table 2. Histology of 40 specimens processed manually and with Rigeneracons. n = number of samples.

ADK adjacent parenchyma	$n = 21$
LUSC adjacent parenchyma	$n = 3$
ADK	$n = 15$
LUSC	$n = 1$

Table 3. Histology of 26 specimens processed only with Rigeneracons. n = number of samples.

ADK Adjacent Parenchyma	$n = 11$
LUSC Adjacent Parenchyma	$n = 1$
LCNE Adjacent Parenchyma	$n = 1$
ADK	$n = 11$
LUSC	$n = 1$
LCNE	$n = 1$

RNA extraction and quantification

RNA was extracted using the QIAGEN RNeasy Mini Kit, according to the manufacturer's instructions, and then stored at −80 °C. Briefly, after disaggregation the samples were transferred to RNeasy spin columns for separation. RNA concentration was measured using a NanoDrop Spectrophotometer (ND-1000, Thermo Fisher).

Assessment of RNA purity by 260/280 ratio

The ultraviolet (UV) absorbance ratio of 260/280 nm was used to estimate RNA contamination with protein or other materials.[20] An A260/280 ratio of approximately 1.8 was considered as indicative of low contamination; $\geq$ 2.0 and $\leq$ 2.2 indicate high purity; values higher than 2.2 ratio are indicative of high contamination.

Assessment RNA quality

RNA quality was evaluated by Real Time PCR (RT-PCR) using the Myriapod NGS Cancer panel RNA kit according to the manufacturer's instructions. The assay allows the detection of two highly conserved regions of 105 bp and 175 bp, using respectively the FAM probe, which labels the major amplicon length, and the HEX probe, which labels the shorter amplicon length. The specific easyPGX analysis software calculates RNA fragmentation as the ratio between the concentration (ng/µl) of the long fragment (FAM channel) and the concentration of short fragments (HEX channel) of RNA.

A fragmentation index (FI) $\geq$ 0.7 corresponds to a low degree of fragmentation, between 0.7–0.05 indicates a medium degree of fragmentation, and $\leq$0.05 a high RNA fragmentation.

Table 4. $A_{260/280}$ ratio in 80 RNA samples. An $A_{260/280}$ ratio of approximately 1.9 was considered as indicative of low contamination; $\geq$2.0 and $\leq$2.2 indicate high purity; values higher than 2.2 ratio are indicative of high contamination. n = number of samples.

Ratio $A_{260/280}$ ($n = 40$)	>1.9	$\geq 2 - \leq 2.2$	>2
Rigeneracons	$n = 3$	$n = 34$	$n = 3$
Manual processing	$n = 2$	$n = 30$	$n = 8$

Statistical analysis

Statistical analyses were performed using the GraphPad Prism Software, version 5.01 for Windows. To compare two unmatched groups, which included samples processed with and without Rigeneracons, we used the two-sided unpaired Student's t test. $p < 0.05$ was considered statistically significant.

Results

Comparison of RNA purity in 40 samples processed manually or with Rigeneracons by A260/280 ratio measurement

RNA extracted from 40 specimens processed manually and 40 processed with Rigeneracons were analyzed for purity by measuring the absorbance at 260 nm and 280 nm. All samples processed with the two procedures derived from the same patients. The results are shown in Table 4. As expected, the difference between the two groups (M and R) was not significative ($p = 0.3891$) meaning that the RNA extraction procedure was correctly performed for all the samples. The median A260/280 ratios were 2.10 and 2.15 for the R and M groups, respectively. The purity percentages of the samples obtained using these two methods are shown in Figure 1.

Comparison of RNA integrity in 40 samples processed manually or with Rigeneracons by RT-PCR

To verify the quality of RNA extracted from the tissues, the 80 samples (40 processed manually and 40 processed with Rigeneracons were analyzed by RT-PCR using the Myriapod NGS Cancer panel RNA kit. The results obtained demonstrated that samples processed with Rigeneracons had a higher integrity compared to those processed manually with a median FI of 0.86 and 0.71 respectively (Table 5 and Figure 2). The difference is statistically significant ($p = 0.0084$).

Assessment of RNA purity and quality in 26 specimens processed with Rigeneracons

An additional 26 samples were provided by the Thoracic Surgery Unit for which there was insufficient material to perform processing with both

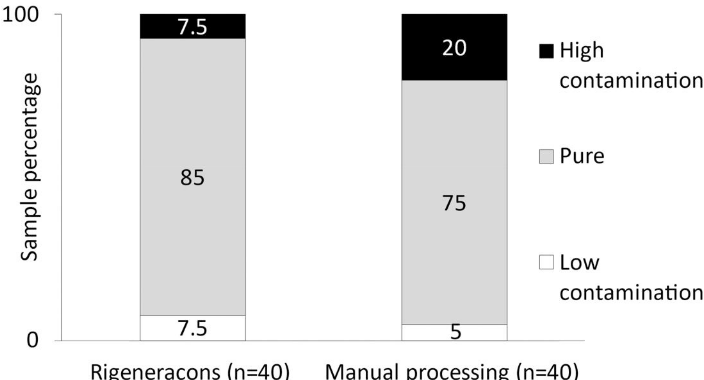

Figure 1. Percentage of contamination in RNA samples extracted using the two procedures. RNA purity was estimated by measuring the absorbance at 260 and 280 nm using a Nanodrop ND-1000 spectrophotometer. Statistical differences between subsets were evaluated using two-sided unpaired Student's *t*-test. n = number of samples.

Table 5. Fragmentation index (FI) of RNA extracted from 40 samples processed with Rigeneracons and 40 manually. FI ≥ 0.7 corresponds to a low degree of fragmentation, between 0.7–0.05 indicates a medium degree of fragmentation, and ≤0.05 a high RNA fragmentation. n = number of samples.

FI	≥ 0.7	<0.7 ≥ 0.05	< 0.05
Rigeneracons	$n = 30$	$n = 9$	$n = 1$
Manual processing	$n = 21$	$n = 13$	$n = 6$

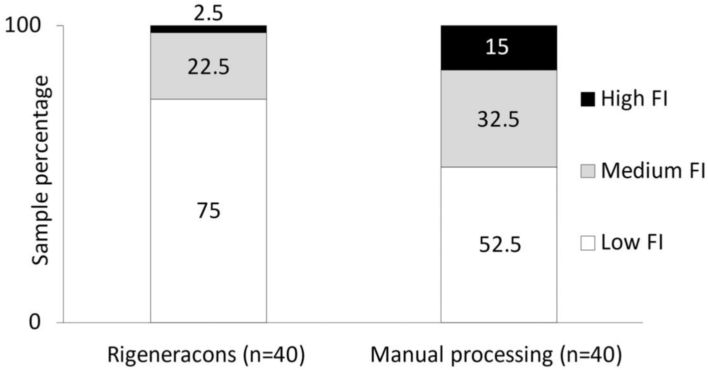

Figure 2. Percentage of RNA fragmentation in the samples extracted using the two procedures. RNA integrity was estimated by RT-PCR using a Myriapod NGS Cancer panel RNA kit. The samples processed with Rigeneracons had higher integrity than those processed manually. Statistical significance between subsets was evaluated using a two-sided unpaired Student's t-test (**$p < 0{,}05$). A Fragmentation Index (FI) ≥0.7 corresponds to a low degree of fragmentation, between 0.7 and 0.05 indicates a medium degree of fragmentation, and ≤0.05 a high RNA fragmentation. n = number of samples.

Table 6. (A) $A_{260/280}$ ratio and (B) Fragmentation Index (FI) of RNA extracted from 26 samples processed by using Rigeneracons. An $A_{260/280}$ ratio of approximately 1.9 was considered as indicative of low contamination; $\geq$ 2.0 and $\leq$ 2.2 indicate high purity; values higher than 2.2 ratio are indicative of high contamination. A FI $\geq$ 0.7 corresponds to a low degree of fragmentation, between 0.7–0.05 indicates a medium degree of fragmentation, and $\leq$0.05 a high RNA fragmentation. n = number of samples.

A	Ratio A260/280 ($n = 26$)		
	>1.9 $n = 0$	≥ 2 – ≤ 2.2 $n = 26$	>2 $n = 0$
B	**FI ($n = 26$)**		
	≥ 0.7 $n = 21$	<0.7 – ≥ 0.05 $n = 4$	<0.05 $n = 1$

procedures. Based on the results previously obtained, we have chosen to extract RNA with Rigeneracons to haven high quality RNA for further ongoing experiments.

All samples were analyzed for both purity and integrity. The A260/280 ratio was between 2 and 2.2 for all the samples indicating a high RNA purity, with a median A260/280 ratio of 2.05. The analysis of FI indicated a low fragmentation in 80.8% of the samples, a medium fragmentation in 15.4% and a high FI in 3.8% with a median of 0.84.

The results are summarized in Table 6 and Figure 3. Overall, 66 samples (40 + 26) were processed with Rigeneracons. The results of purity and integrity are summarized in Table 7 and Figure 4.

Discussion

The quality of any scientific data depends on the quality of the initial sample to be analyzed. Genome expression profiling studies aim to address clinical needs such as the discovery of new targeted drugs and new biomarkers for diagnosis. It becomes clear that the analysis of low-quality samples may not represent gene expression levels *in vivo*, resulting in misleading conclusions.[21–26] The purity, quality, and reproducibility of the extracted RNA depend on the entire workflow which, starting from tissue excision, involves multidisciplinary skills, including surgeons, pathologists, and molecular biologists. Therefore, the speed of sample handling and processing should be maximal as it may hamper downstream application.[25]

In transcriptomic studies, the main source of technical bias is variation in RNA quality. In this regard, tissue preparation is critical because different disruption techniques can affect RNA quality, influencing further applications.[26] The most used methods to homogenize tissues and preserve cells integrity are based on mechanical disaggregation. Manual mechanical processing is an inexpensive and simple method for achieving high cell

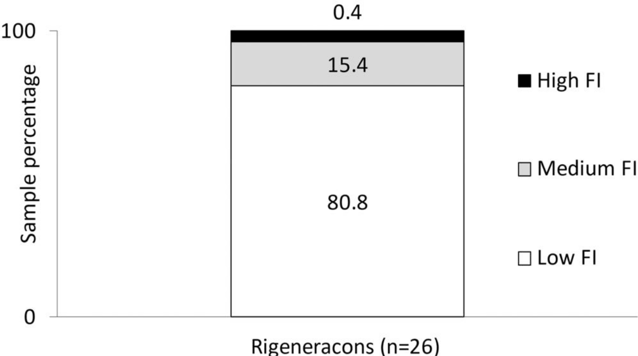

Figure 3. RNA fragmentation (%) in 26 samples extracted only with Rigeneracons. 80.8% of the samples had a high integrity. n = number of samples; FI = Fragmentation Index.

Table 7. (A) $A_{260/280}$ ratio and (B) Fragmentation Index (FI) of RNA extracted from all 66 samples processed by Rigeneracons. n = number of samples.

A	Ratio $A_{260/280}$ ($n = 66$)		
	>1.9 $n = 3$	$\geq 2 - \leq 2.2$ $n = 60$	>2 $n = 3$
B	FI ($n = 66$)		
	≥ 0.7 $n = 51$	$<0.7 - \geq 0.05$ $n = 13$	<0.05 $n = 2$

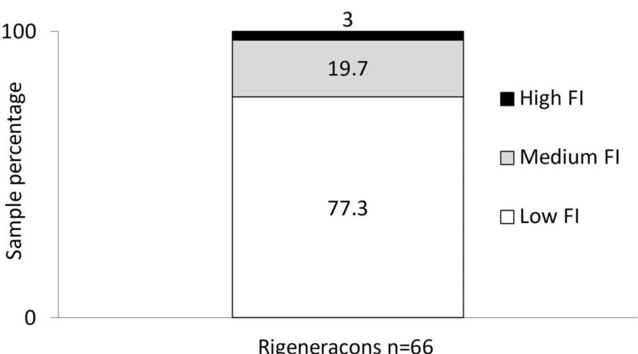

Figure 4. Percentage of RNA fragmentation in all samples ($n = 66$) processed with Rigeneracons. n = number of samples. FI = Fragmentation Index.

yield. However, it tends to generate a suspension of damaged cells and is operator-dependent, limiting the reproducibility of the results.[15] A fast, reproducible, and standardized technique could undoubtedly overcome this problem, avoiding wasting time and resources.

In this study, our goal was to evaluate the impact of two mechanical tissue disruption techniques on the purity and quality of RNA extracted from

fresh lung biopsies. Our approach involved manual disaggregation of 80 samples using two different methods. We compared the classical manual mechanical disruption by scraping and mincing 40 fresh tissues using a scalpel, with the automated mechanical disruption device Rigeneracons, routinely used into clinical practice to disaggregate autologous tissues and obtain a suspension of micrografts immediately ready to use for different applications in oral-maxillofacial surgery'[27] wound care, dermatology, and orthopedics.[20]

With both procedures, we obtained high RNA purity,[28] with A260/280 ratios of ≈2 in 85% (R group) and 75% (M group) of the samples ($p = 0.3891$). Conversely, the RNA quality was higher in the samples processed with Rigeneracons, with 1 highly fragmented sample (2.8%) in the R group and 6 highly fragmented samples in the M group (11.1%). The difference in FI between the two techniques was statistically significant ($p = 0{,}0084$).

Overall, our results indicate that using the Rigeneracons device the time of tissue disaggregation is reduced compared with enzymatic or other mechanical procedures. In addition, the process is more standardized, safe and fast. In addition, in samples automatically processed with Rigeneracons, the quality of RNA is higher than that extracted with the manual method. Therefore, samples disaggregated with Rigeneracons are highly suitable for transcriptomic profiling of fresh biopsies, helping to overcome technical distortions related to fresh tissue disaggregation. Additionally, from a biological point of view the mechanical disaggregation with Rigeneracons devices allow to select a calibrated population of about 80-micron size opening at the perspective to select specific tumoral cells population to better characterize the pathogenesis of tumor.

Conclusions

To conclude, this study highlights the possibility of introducing in clinical practice the method of automated mechanical tissue disaggregation operated by the Rigeneracons device to have more advantages on the study of cancer tissue. This aspect could be important to deepen the study of tumor heterogeneity and its role in medicine precision oncology.

Author contributions

Conceptualization, M.B.[1]; writing-original draft preparation; M.B.[1]; writing—review and editing, M.B.[2]; study design, M.B. and C.B.; formal analysis, M.B.[1], M.B.[2], MP; investigation, M.P. M.B.[2], C.T., G.I., S.B., F.A.; resources, P.P., C.B., A.G.; supervision, M.B.[2], C.B., A.G.; funding acquisition, A.G.[1], A.G.[2].

All authors have read and agreed to the published version of the manuscript.

Disclosure statement

M.B.,1 C.B., M.B.,2 M.P., C.T., G.I., S.B., P.P., A.G[1] declare no conflict of interest.

A.G.2 is also CEO of Human Brain Wave, the company manufacturing Rigeneracons devices and funding this study.

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References

[1] Hamburger, A. W.; Salmon, S. E. Primary Bioassay of Human Tumor Stem Cells. *Science* **1977**, *197*, 461–463. DOI: 10.1126/science.560061.

[2] Rasey, J. S.; Nelson, N. J. Effect of Tumor Disaggregation on Results of in Vitro Cell Survival Assay after in Vivo Treatment of the EMT-6 Tumor: X-Rays, Cyclophosphamide, and Bleomycin. *In Vitro.* **1980**, *16*, 547–553. DOI: 10.1007/BF02618377.

[3] Cerra, R.; Zarbo, R. J.; Crissman, J. D. Chapter 1 Dissociation of Cells from Solid Tumors. *Methods Cell Biol.* **1990**, *33*, 1–12. DOI: 10.1016/S0091-679X(08)60506-3.

[4] Cunningham, R. E. Tissue Disaggregation. *Methods Mol. Biol.* **2010**, *588*, 327–330. DOI: 10.1007/978-1-59745-324-0_32.

[5] Cornacchiari, A.; Grigolato, P. G.; Facchetti, F.; Morassi, M. L.; Cadei, M.; Alpi, F.; Battocchio, S.; Chirico, E. Usefulness of the Scraping Method for DNA Flow Cytometry in Breast Tumors. *Cytometry* **1995**, *19*, 263–266. DOI: 10.1002/cyto.990190310.

[6] Ottesen, G. L.; Christensen, J.; Larsen, J. K.; Hansen, B.; Andersen, J. A. Tissue Disaggregation for Flow Cytometric DNA Analysis: Comparison of Fine-Needle Aspiration and an Automated Mechanical Procedure. *Commun. Clin. Cytom.* **1996**, *26*, 65–68. DOI: 10.1002/(SICI)1097-0320(19960315)26:1<65::AID-CYTO10>3.0.CO;2-Q.

[7] Singh, N. P. A Rapid Method for the Preparation of Single-Cell Suspensions From Solid Tissues. *Cytometry* **1998**, *31*, 229–232. DOI: 10.1002/(SICI)1097-0320(19980301)31:3<229::AID-CYTO10>3.0.CO;2-T.

[8] Meistrich, M. L. Separation of Spermatogenic Cells and Nuclei from Rodent Testes. *Methods Cell Biol.* **1977**, *15*, 15–54. DOI: 10.1016/s0091-679x(08)60207-1.

[9] Rodríguez-Casuriaga, R.; Geisinger, A.; López-Carro, B.; Porro, V.; Wettstein, R.; Folle, G. A. Ultra-Fast and Optimized Method for the Preparation of Rodent Testicular Cells for Flow Cytometric Analysis. *Biol. Proced. Online.* **2009**, *11*, 184–195. DOI: 10.1007/s12575-009-9003-2.

[10] Supplitt, S.; Karpinski, P.; Sasiadek, M.; Laczmanska, I. Current Achievements and Applications of Transcriptomics in Personalized Cancer Medicine. *Int. J. Mol. Sci.* **2021**, *22*, 1422. DOI: 10.3390/ijms22031422.

[11] Byron, S. A.; Van Keuren-Jensen, K. R.; Engelthaler, D. M.; Carpten, J. D.; Craig, D. W. Translating RNA Sequencing into Clinical Diagnostics: Opportunities and Challenges. *Nat. Rev. Genet.* **2016**, *17*, 257–271. DOI: 10.1038/nrg2016.10.

[12] Gallego Romero, I.; Pai, A. A.; Tung, J.; Gilad, Y. RNA-Seq: Impact of RNA Degradation on Transcript Quantification. *BMC Biol.* **2014**, *12*, 42. DOI: 10.1186/1741-7007-12-42.

[13] Astarita, C.; Arora, C. L.; Trovato, L. Tissue Regeneration: An Overview from Stem Cells to Micrografts. *J. Int. Med. Res.* **2020**, *48*, 30006052091479. DOI: 10.1177/0300060520914794.

[14] Kap, M.; Sieuwerts, A. M.; Kubista, M.; Oomen, M.; Arshad, S.; Riegman, P. The Influence of Tissue Procurement Procedures on RNA Integrity, Gene Expression, and Morphology in Porcine and Human Liver Tissue. *Biopreserv. Biobank.* **2015**, *13*, 200–206. DOI: 10.1089/bio.2014.0076.

[15] Quatromoni, J. G.; Singhal, S.; Bhojnagarwala, P.; Hancock, W. W.; Albelda, S. M.; Eruslanov, E. An Optimized Disaggregation Method for Human Lung Tumors That Preserves the Phenotype and Function of the Immune Cells. *J. Leukoc. Biol.* **2015**, *97*, 201–209. DOI: 10.1189/jlb.5TA0814-373.

[16] Russell, S. W.; Doe, W. F.; Hoskins, R. G.; Cochrane, C. G. Inflammatory Cells in Solid Murine Neoplasms. I. TUMOR Disaggregation and Identification of Constituent Inflammatory Cells. *Int. J. Cancer.* **1976**, *18*, 322–330. DOI: 10.1002/ijc.2910180309.

[17] Trivisonno, A.; Alexander, R. W.; Baldari, S.; Cohen, S. R.; Di Rocco, G.; Gentile, P.; Magalon, G.; Magalon, J.; Miller, R. B.; Womack, H.; Toietta, G. Intraoperative Strategies for Minimal Manipulation of Autologous Adipose Tissue for Cell- and Tissue-Based Therapies: Concise Review. *Stem Cells Transl. Med.* **2019**, *8*, 1265–1271. DOI: 10.1002/sctm.19-0166.

[18] Torres, F. X.; Mackowiak, P. G.; Brown, R. D.; Linden, M. D.; Zarbo, R. J. Comparison of Two Methods of Mechanical Disaggregation of Scirrhous Breast Adenocarcinomas for DNA Flow Cytometric Analysis of Whole Cells. *Am. J. Clin. Pathol.* **1995**, *103*, 8–13. DOI: 10.1093/ajcp/103.1.8.

[19] Trovato, L.; Monti, M.; Del Fante, C.; Cervio, M.; Lampinen, M.; Ambrosio, L.; Redi, C. A.; Perotti, C.; Kankuri, E.; Ambrosio, G.; et al. A New Medical Device Rigeneracons Allows to Obtain Viable Micro-Grafts From Mechanical Disaggregation of Human Tissues. *J. Cell. Physiol.* **2015**, *230*, 2299–2303. DOI: 10.1002/jcp.24973.

[20] Manchester, K. L. Value of A260/A280 Ratios for Measurement of Purity of Nucleic Acids. *Biotechniques* **1995**, *19*, 208–210.

[21] Atz, M.; Walsh, D.; Cartagena, P.; Li, J.; Evans, S.; Choudary, P.; Overman, K.; Stein, R.; Tomita, H.; Potkin, S. et al. Methodological Considerations for Gene Expression Profiling of Human Brain. *J. Neurosci. Methods.* **2007**, *163*, 295–309. DOI: 10.1016/j.jneumeth.2007.03.022.

[22] Muyal, J. P.; Muyal, V.; Kaistha, B. P.; Seifart, C.; Fehrenbach, H. Systematic Comparison of RNA Extraction Techniques from Frozen and Fresh Lung Tissues: Checkpoint towards Gene Expression Studies. *Diagn. Pathol.* **2009**, *4*, 9. DOI: 10.1186/1746-1596-4-9.

[23] Copois, V.; Bibeau, F.; Bascoul-Mollevi, C.; Salvetat, N.; Chalbos, P.; Bareil, C.; Candeil, L.; Fraslon, C.; Conseiller, E.; Granci, V.; et al. Impact of RNA Degradation on Gene Expression Profiles: Assessment of Different Methods to Reliably Determine RNA Quality. *J. Biotechnol.* **2007**, *127*, 549–559. DOI: 10.1016/j.jbiotec.2006.07.032.

[24] Lu, W.; Zhou, Q.; Chen, Y. Impact of RNA Degradation on Next-Generation Sequencing Transcriptome Data. *Genomics* **2022**, *114*, 110429. DOI: 10.1016/j.ygeno.2022.110429.

[25] Shen, Y.; Li, R.; Tian, F.; Chen, Z.; Lu, N.; Bai, Y.; Ge, Q.; Lu, Z. Impact of RNA Integrity and Blood Sample Storage Conditions on the Gene Expression Analysis. *Onco. Targets Ther.* **2018**, *11*, 3573–3581. DOI: 10.2147/OTT.S158868.

[26] Fasold, M.; Binder, H. Variation of RNA Quality and Quantity Are Major Sources of Batch Effects in Microarray Expression Data. *Microarrays (Basel)* **2014**, *3*, 322–339. DOI: 10.3390/microarrays3040322.

[27] Mummolo, S.; Mancini, L.; Quinzi, V.; D'Aquino, R.; Marzo, G.; Marchetti, E. Rigenera® Autologous Micrografts in Oral Regeneration: Clinical, Histological, and Radiographical Evaluations. *Appl. Sci.* **2020**, *10*, 5084–5096. DOI: 10.3390/app10155084.

[28] Manchester, K. L. Use of UV Methods for Measurement of Protein and Nucleic Acid Concentrations. *Biotechniques.* **1996**, *20*, 968–970. DOI: 10.2144/96206bm05.