



Review Article

Tissues under-vacuum to overcome suboptimal preservation

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ARTICLE INFO

Keywords:

Under-vacuum
Tissue preservation
Pre-Analytics
DNA
RNA
Proteins

ABSTRACT

The accuracy of histopathological diagnosis is strictly reliant on adequate tissue preservation, which is completely dependent on pre-analytical variables. Among these variables, the time interval between the end of surgical excision to the onset of fixation (the cold ischemia time) may adversely affect preservation of tissue morphology, influencing the interpretation and reproducibility of diagnosis. During this time interval, the activation of enzymes may produce autolysis and degradation of antigens and nucleic acids, thus potentially affecting immunocytochemical and molecular results. Several studies have described under-vacuum at 4 °C storage of fresh surgical specimens as a safe and reliable method to control cold ischemia and preserve fresh tissues, as well as to standardize fixation times and implement tissue-banking. This review article gives a systematic overview of the advantages and drawbacks of the use of under-vacuum tissue preservation and cooling in surgical pathology, highlighting the impact this procedure may have on diagnostic and experimental pathology. It also documents our experience acquired within daily practice and national and international projects.

Introduction

A lack of standardized procedures and poor control of the pre-analytical phase for management of fresh surgical specimens accounts for suboptimal tissue preservation, which in turn affects diagnosis and downstream results of immunocytochemical and molecular tests. During the so called “cold ischemia time” (i.e. the time interval from the end of surgical excision to the onset of fixation), fresh surgical specimens are highly vulnerable and can undergo autolysis due to enzyme activation. For this reason, it is recommended to monitor the cold ischemia time [1] and to send fresh specimens immediately to the pathology laboratory to proceed with grossing procedures and rapid fixation [2]. Nevertheless, it is important to note that operating theatres may not always be located in the proximity of the pathology laboratory. In addition, surgical specimens are commonly immersed in formalin during transport in the attempt to block autolysis. This procedure does not effectively protect the central part of large specimens from autolysis, because of the low penetration of formalin at room temperature, which is 1 mm per hour for the first hour, then 1 mm in the subsequent 3 h.

Recent evidence suggests that tissue vacuum sealing followed by

immediate preservation at 4 °C reduces the possibility of contamination by removing air and inhibiting the growth of microorganisms [3] as well as avoiding the use of formalin in the surgical premises [4,5]. Vacuum sealing has been gradually implemented in hospitals as a method of fresh tissue transportation to pathology laboratories, and allows control over the time of fixation, leading to optimal histology [3,5]. In addition, under-vacuum preserved fresh tissues at low temperature are ideal for tissue-banking and enable reproducible immunohistochemical and molecular analyses [4,5] (Fig. 1).

SPIDIA, a European project tackling standardization and improvement of pre-analytical procedures for *in vitro* diagnostics, developed a comprehensive portfolio of pan-European pre-analytical CEN/TS and ISO/IS documents, which are currently being pursued by the SPIDIA4P consortium (<http://www.spidia.eu>). Among those related to molecular *in vitro* diagnostic examinations, vacuum packaging at low temperature is considered as an alternative for fresh tissue preservation and transport in order to preserve DNA, RNA and proteins.

The under-vacuum sealing procedure: origins and advantages

In 2006, the declaration of the International Agency for Research on

Abbreviations: ASCO/CAP, American Society of Clinical Oncology/College of American Pathologists; CEN/TS, European Committee for Standardization/Technical Specification; FFPE, formalin fixed paraffin embedded; HTA, Health Technology Assessment; IARC, International Agency for Research on Cancer; ISO/IS, International Organization for Standardization/International Standard; RIN, RNA Integrity Number; SELDI-TOF/MS, surface-enhanced laser desorption/ionization-time of flight/mass spectrometry; TQI, Tissue Quality Index

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<https://doi.org/10.1016/j.nbt.2019.05.007>

Available online 28 May 2019

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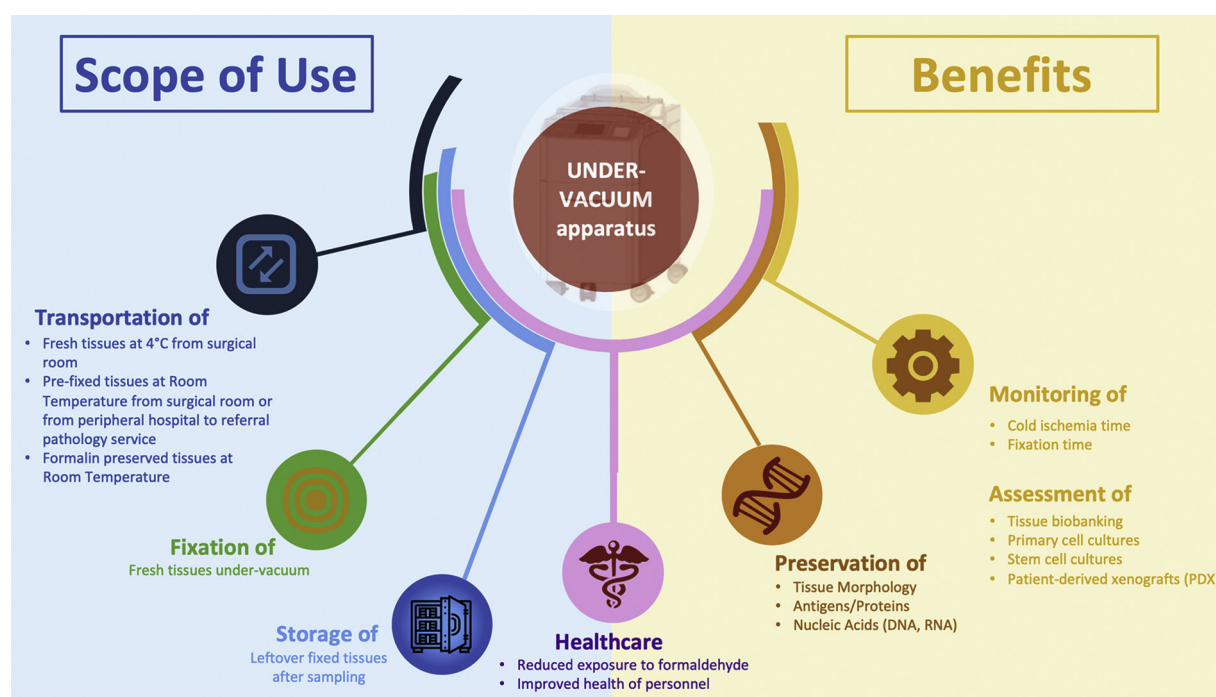


Fig. 1. Applications and benefits of under-vacuum procedure. The figure summarizes the possible uses of the under-vacuum procedure and the benefits that can be drawn from it (copyright credit is given to PresentationGO.com for the creation of the template used [41]).

Cancer (IARC) on the Evaluation of Carcinogenic Risk to humans, classified formaldehyde as a class 1 carcinogen, thus prompting the need to reduce exposure [6]. In the attempt to overcome this issue in pathology laboratories and most importantly in surgical operating theatres, Bussolati et al. proposed transfer of tissue specimens based on under-vacuum sealing as a safe alternative to conventional transportation in buckets filled with formalin [5]. The under-vacuum procedure followed a very simple workflow: at the operating theatre, immediately following excision, surgical specimens were under-vacuum sealed in sterilized plastic bags using a semi-professional machine (Model VAC 10, by Milestone, Bergamo, Italy) and kept at 4 °C until transfer to the pathology laboratory. The process was demonstrated to preserve morphology, immunohistochemical reactivity and quality of nucleic acids from tissue specimens preserved as such for up to 72 h [5]. Since then, several studies have tested and consolidated the under-vacuum technique, as detailed below (see also Table 1), and professional machines for under-vacuum set up were systematically manufactured [7]. Notably, although vacuum sealing does not protect from ischemia, nevertheless the procedure itself allows monitoring of the duration of ischemia in tissue samples. In addition, it is the rapid cooling to 4 °C of specimens that is crucial for tissue preservation [8,9] and the elimination of air around tissues with the under-vacuum system enable faster cooling at 4 °C, decreasing autolytic processes. Finally, the vacuum sealing in sterilized bags helps prevent bacterial and fungal contamination [9].

By using a Tissue Quality Index (TQI), an intrinsic control for measurement of effects of pre-analytical variables on formalin fixed paraffin embedded (FFPE) tissues, it was shown that samples preserved under-vacuum at 4 °C before fixation performed better than samples immersed in formalin during transportation. Indeed, the TQI score defined by combinations of measurements of cytokeratin, pERK1/2 and pHSP-27 was positive in 87% of under-vacuum sealed cases [10].

Recently, Saliceti et al. [7] published a comprehensive Health Technology Assessment (HTA) report to support healthcare decision makers in purchasing, replacing or disposing of the under-vacuum system for tissue preservation. The analysis considered four areas. The first was related to the vacuum technologies - which pertain to the field

of *in vitro* diagnostic medical devices (Directive 98/79/CE) - and they considered (i) the vacuum instruments, (ii) the clinical validity of under-vacuum tissue preservation, and (iii) solutions found in the market to monitor and maintain the temperature preservation of histological materials at constant values of about 4 °C. The second area was related to organization of the workflow within and outside the pathology laboratories and traceability of biological materials. The third area, concerning the safety of the operators, analysed the chemical hazard related to the use of fixatives. The fourth and last was related to the economic assessment and compared the traditional management of biological materials with the vacuum-based management contextualized in the New Prato Hospital (“Nuovo Ospedale di Prato”, run by the Local Sanitary Unit 4 of Prato, Italy) framework. The authors concluded that “*The HTA reported no significant drawbacks related to the use of the technology being examined. Nonetheless, the workflow for managing the transfer of biological materials from the operating room to the anatomic pathology department needs to be redefined - in terms of handling, processing, storage and disposal. Other elements concerned the monitoring of storage temperature, fresh tissue handling and especially fixative amount reduction, which positively impacts on the operators’ safety with regard to chemical hazards*”. This highlights the need to adopt a procedure for tissue transportation and preservation that can monitor several parameters in terms of tissue quality as well as personnel safety.

Under-vacuum allows tissue-banking

Current guidelines for tissue biobanking recommend collection of specimens immediately after surgical excision to guarantee optimal preservation of biomolecules. However, studies are required to clarify the impact of transport logistics on global gene expression [11]. Vacuum package at 4 °C of surgical specimens enables transportation of fresh tissue (Fig. 1). We are conducting a study to evaluate whether prolonged under-vacuum storage at 4 °C affects the analysis of RNA using next generation sequencing. Briefly, a tissue sample is directly taken in surgical room and snap-frozen in isopentane from specimens excised for breast cancer. The residual tissue is stored under-vacuum at 4 °C for different times (17–24 hours and 72–73 hours), after which a

Table 1
Main literature on the use of under-vacuum procedure.

Title	Year Pub.	Topic	Analyses	Ref.
Tissue transfer to pathology labs: under vacuum is the safe alternative to formalin	2008	Comparison of tissues under-vacuum stored at 4 °C with snap-frozen immediately after the surgical removal	Morphological preservation; immunohistochemical reactivity; RNA preservation assessed by run on 1% denaturing agarose gel and by RT-PCR; satisfaction of personnel of the surgical theatre	[5]
Vacuum-based preservation of surgical specimens: an environmentally-safe step towards a formalin-free hospital	2010	Under-vacuum storage at 4 °C as a method of transport and storage	Series of questionnaires on staff satisfaction, technical feasibility, quality of tissue preservation	[3]
Nuclear shape in papillary thyroid carcinoma: a role for lamin B receptor?	2010	Study of nuclei of specimens preserved under-vacuum at 4 °C	Assessment of immunohistochemical reactions; RNA extraction and RT-PCR analysis; western blot analysis	[25]
Role of the surgical pathology laboratory in the pre-analytical approach of molecular biology techniques	2010	Optimization of pre-analytical phase within the surgical pathology laboratory and use of under-vacuum at 4 °C		[26]
Vacuum sealing and cooling as methods to preserve surgical specimens	2011	Under-vacuum storage at room temperature and at 4 °C compared with no vacuum sealing and storage at room temperature and at 4 °C	Morphological preservation; immunohistochemical reactivity; RNA preservation assessed by RT-qPCR	[8]
Formalin fixation at low temperature better preserves nucleic acid integrity	2011	Use of cold formalin to fix specimens preserved under-vacuum at 4 °C	Set up of a new fixation procedure; histological and immunohistochemical assessment; DNA and RNA preservation; microarray-based gene expression profiles	[22]
Preparing pathology for personalized medicine: possibilities for improvement of the pre-analytical phase	2011	Transport of surgical specimens to the pathology laboratory using under-vacuum		[27]
Critical steps in tissue processing in histopathology	2012	Under-vacuum sealing: evaluation of different temperatures and different time intervals	RNA integrity assessed by Agilent 2100 Bioanalyzer (RIN values)	[23]
The mandate for a proper preservation in histopathological tissues	2012	Review on the sequence of technical steps to guarantee a proper preservation of tissues		[28]
Efficient stem cell isolation from under vacuum preserved tissue samples	2012	Isolation of stem cells from kidney specimen preserved under-vacuum at 4 °C	Cells isolation; phenotypic characterization; cells <i>in vitro</i> and <i>in vivo</i> differentiation	[12]
Occupational hazards of hospital personnel: assessment of a safe alternative to formaldehyde	2012	To quantify in hospital workers the probability of showing respiratory symptoms with respect to under-vacuum sealing as a benchmark	Interview of staff	[29]
A collection of primary tissue cultures of tumors from vacuum packed and cooled surgical specimens: a feasibility study	2013	Use of under-vacuum storage at 4 °C to preserve specimens for isolation of cells for <i>in vitro</i> cultures	Assessment of specimen temperature; assessment of short-term primary cell cultures; evaluation of cell viability by trypan blue dye-exclusion assay; evaluation of morphological and immunophenotypical features of cultured cells; effect of surgical and under-vacuum times on cell viability	[9]
Nucleic acid extraction methods from fixed and paraffin-embedded tissues in cancer diagnostics	2013	Review on variables affecting extraction of nucleic acids (RNA) from formalin-fixed paraffin-embedded tissues. Transport of surgical specimens under-vacuum storage at 4 °C to avoid over-fixation		[30]
Histopathology and cytology of supposedly benign tumors of the ovary	2013	Under-vacuum procedure at 4 °C considered for transport specimens to the pathology laboratory and to preserve tissues and nucleic acids		[31]
Role of the surgical pathologist for tissue management in oncology	2013	Review on strategies for an optimal tissue management in an oncology-pathology laboratory to get an accreditation according the ISO15189 norm		[32]
Validation of vacuum-based refrigerated system for biobanking tissue preservation: analysis of cellular morphology, protein stability, and RNA quality	2014	Under-vacuum storage at 4 °C. Comparison with tissues snap-frozen immediately after the surgical removal	Morphological preservation; immunohistochemical reactivity; assessment of protein/phosphoprotein stability by immunoblot analysis; RNA preservation assessed by run on 1% agarose gel; evaluation of RNA suitability for gene expression analysis by RT-PCR and RT-qPCR	[4]
A tissue quality index: an intrinsic control for measurement of effects of preanalytical variables on FFPE tissue	2014	To construct a tissue quality index (TQI) or an intrinsic control that would allow a global assessment of protein status based on quantitative measurement of a small number of selected, informative epitopes	Validation of TQI defined by combinations of measurements of cytokeratin, pERK1/2 and pHSP-27 and their relationship to cold ischemic time; evaluation of effect of cold ischemia on breast cancer cases stored under-vacuum at 4 °C or non-vacuum preserved	[10]
Guidance for laboratories performing molecular pathology for cancer patients	2014	To provide minimum requirements for the management of molecular pathology laboratories	Under-vacuum procedure is considered as a possible procedure for transport of fresh specimens to the pathology laboratory	[1]
Pre-analytical stage for biomarker assessment in breast cancer: 2014 update of the GEPICCS guidelines in France	2014	Analysis of pre-analytical steps that are critical for the quality of immunohistochemical and <i>in situ</i> hybridization procedures, whatever the biomarker analysed	Under-vacuum storage at 4 °C considered for tissue preservation	[33]
Histologic validation of vacuum sealed, formalin-free tissue preservation, and transport system	2015	Five validation trials of under-vacuum storage-Comparison with tissues immediately formalin fixed	Validation Trial #1: defining the parameters of temperature and time. Validation Trial #2: defining the transport system from community hospital. Validation Trial #3: evaluating the histology of wider variety	[20]

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Table 1 (continued)

Title	Year Pub.	Topic	Analyses	Ref.
Critical roles of specimen type and temperature before and during fixation in the detection of phosphoproteins in breast cancer tissues	2015	To evaluate the influence of pre-analytical parameters (cold ischemia time, temperature before and during tissue fixation, and sample type) on the levels of proteins and phosphoproteins in breast cancer tissues, focusing on the PI3 kinase/AKT pathway	of large specimens. Validation Trial #4: evaluating the histology of needle biopsies. Validation Trial #5: evaluating reduced formalin for tissue storage Use of a cohort of breast cancer surgical specimens stored under-vacuum at 4 °C	[18]
The pre-analytical phase in surgical pathology	2015	Review on pre-analytical handling of surgical specimens from resection in the surgical theatre to paraffin-embedding and storage	Under-vacuum storage at 4 °C considered for tissue preservation	[34]
RNA quality in fresh-frozen gastrointestinal tumour specimens-experiences from the tumour and healthy tissue bank TU Dresden	2015	To determine RNA quality by assessing the RIN values of specimens from different organs and to assess the influence of vacuum preservation	Use of a cohort of surgical specimens stored under-vacuum at room temperature. RNA integrity assessed by the Agilent 2100 Bioanalyzer (RIN values)	[35]
Applicability of Under Vacuum Fresh Tissue Sealing and Cooling to Omics Analysis of Tumor Tissues	2016	To test under-vacuum storage capability to preserve tissues during the time between surgery and storage for "omics" analyses	Comparison with tissues snap-frozen in liquid nitrogen within 20 minutes from surgical removal; morphological preservation; immunohistochemical reactivity; RNA integrity assessed by the Agilent 2100 Bioanalyzer (RIN values); evaluation of RNA suitability for transcriptomic examination (HumanHT-12-v3 expression BeadChip, Illumina); evaluation of protein suitability for proteomic examination by western blot, two-dimensional gel electrophoresis (2-DGE), surface-enhanced laser desorption/ionization-time of flight/mass spectrometry (SELDI-TOF/MS); assessment of metabolic profiles by nuclear magnetic resonance spectroscopy	[24]
Diagnostic procedures for non-small-cell lung cancer (NSCLC): recommendations of the European Expert Group	2016	Consensus manuscript on the essential steps recommended to optimise tissue-based molecular testing for NSCLC	Under-vacuum storage at 4 °C considered for tissue preservation	[36]
The Influence of Tissue Ischemia Time on RNA Integrity and Patient-Derived Xenografts (PDX) Engraftment Rate in a Non-Small Cell Lung Cancer (NSCLC) Biobank	2016	To define the impact of cancer tissue ischemia time on RNA and DNA quality, and for the generation of Patient-Derived Xenografts (PDXs)	Use of a cohort of surgical specimens stored under-vacuum at 4 °C. RNA integrity assessed by the Agilent 2100 Bioanalyzer (RIN values) and by RT-qPCR; assessment of DNA quality by multiplex PCR; assessment of Patient-Derived Xenograft engraftment rate	[13]
Towards a formalin-free hospital. Levels of 15-F2t-isoprostane and malondialdehyde to monitor exposure to formaldehyde in nurses from operating theatres	2016	Cross-sectional study to evaluate the conditions favouring the risk of exposure to formaldehyde and the effect of measures to prevent it	94 female workers enrolled as being potentially exposed to formaldehyde. From each nurse were collected: personal air-formaldehyde by a personal dosimeter (8 hours); a standardized questionnaire; a urine sample to test 15-F2t-isoprostane, malondialdehyde, cotinine. The use of the under-vacuum sealing technique is associated with a significant reduction of the exposure to air-formaldehyde and redox status	[37]
Translational Research in Pediatrics IV: Solid Tissue Collection and Processing	2016	Review on ethical standards for solid tissue collection from children and the various factors affecting tissue integrity and on newer methods for determining tissue quality	Under-vacuum procedure at 4 °C suggested for transferring specimens to the pathology laboratory and to preserve morphology, nucleic acids, proteins and cell viability	[38]
Safe transportation of formalin-fixed liquid-free pathology specimens	2018	Under-vacuum preservation in liquid-free plastic bags of already formalin fixed tissues. Comparison with corresponding standard samples	Morphological preservation; immunohistochemical reactivity; assessment of DNA quality by PCR and gene mutation analysis; RNA integrity assessed by the Agilent 2100 Bioanalyzer (DV200 values)	[21]
Coping with formalin banning in pathology: under vacuum long-term tissue storage with no added formalin	2019	To compare histology specimens stored by formalin immersion and specimens stored after fixation with under-vacuum sealing technique with no additional formalin, at different time periods	Under-vacuum preservation of tissues after fixation, with no additional formalin, up to 12 months. Comparison with histological specimens stored by formalin immersion. Morphological preservation; immunohistochemical reactivity; assessment of DNA quality by the Agilent 2200 TapeStation (DIN values)	[39]
Biopathology of ovarian carcinomas early and advanced-stages: Article drafted from the French guidelines in oncology entitled "Initial management of patients with epithelial ovarian cancer" developed by FRANCOGYN, CNGOF, SFOG, GINECO-ARCA under the aegis of CNGOF and endorsed by INCa	2019	Guidelines	Under-vacuum procedure at 4 °C considered for transferring specimens to the pathology laboratory and to preserve morphology and RNA	[40]

sample is taken and snap-frozen in isopentane. RNA is extracted and analysed by RNA-seq for different clinically validated gene signatures. Our preliminary data indicate that classification of under-vacuum sealed samples is concordant with those of snap-frozen fresh tissues (manuscript in preparation). Such studies are crucial to validating under-vacuum package at 4 °C as a method to preserve tissue for biobanking.

Under-vacuum preserves cell viability

Primary cell cultures, organoids and xenopatient (i.e. patient-derived xenografts, tumour tissue sampled from a lesion of a patient and implanted into mice) represent an invaluable tool in research. Essential rules to produce primary cell cultures are: (i) acquisition of fresh specimens as soon as possible after surgical excision and (ii) avoidance of bacterial and fungal contamination. When dealing with cell cultures and xenograft implantations, it is ideal to collect the sample for experiments directly in the surgery room in order to keep the cold ischemia time as short as possible. However, this process may not meet the pathologist's expectations, since correct assessment of size of lesions and status of surgical margins are paramount for tumour staging and may be hampered by prior manipulation of surgical specimens [9].

We have demonstrated that the storage of fresh surgical specimens under-vacuum at 4 °C may represent a reliable source for setting up primary cell cultures, while maintaining at the same time proper preservation of surgical specimens for gross evaluation (Fig. 1) [9]. Primary cell cultures were successfully obtained from surgical specimens preserved under-vacuum (success rate of 85%). Upon correlation between the percentage of viable cells and the duration of surgery (i.e. warm ischemia time), it was shown that for warm ischemia times ≤ 1.5 h, the proportion of viable cells was $\geq 84\%$, while a progressive increase of surgical time dramatically decreased cell viability [9]. Viability was also correlated with cold ischemia produced in tissues stored under-vacuum at 4 °C (i.e. the vacuum interval). As expected, the longer the specimen was kept under-vacuum the lower was the percentage of viable cells: under-vacuum storage extended up to 24, 24–48 and 48–72 h resulted in 89.3, 84.2 and 60% of viable cells respectively [9]. Others have shown that storage of surgical tissues under-vacuum at 4 °C allows stem/progenitor cell isolation (Fig. 1) [12]. It was assumed that the hypoxic conditions present in the tissue after under-vacuum storage could select for stem cells, which are generally more resistant to hypoxia [12]. Finally, evidence has been provided that tissues stored under-vacuum at 4 °C can be used to successfully establish patient-derived xenografts [13].

Under-vacuum to standardize fixation

Although formalin has been classified as carcinogenic [6], it still represents the fixative of choice in clinical practice. Importantly, under or over-fixation in formalin can have detrimental effects on tissue specimen immunophenotyping. The ASCO/CAP guidelines for immunohistochemical testing of estrogen and progesterone receptors in breast cancer state that specimens should be rejected if fixation time is < 6 h or > 72 h [2]. The panel specified that breast cancer samples sliced at 5-mm intervals after appropriate gross inspection should be placed in sufficient volume of formalin and suggested monitoring the length of tissue fixation by recording the time of fixation [2]. It has been reported that prolonged formalin fixation significantly reduces the nuclear staining of the proliferation marker Ki67 [14]. Thus, monitoring and standardizing the length of tissue fixation is becoming mandatory to guarantee optimal results in biomarker analysis. The impact of fixation time becomes even more apparent when considering the assessment of phosphoproteins. Indeed, numerous phosphobiomarkers, including pAKT, pEGFR and pERK, are profoundly affected by fixation conditions or cold ischemia [15–17] and some reports suggest alternative fixation protocols to preserve these analytes [17,18].

Vacuum packing and cooling at 4 °C as a method of transport of fresh tissues can optimise and standardize the time of fixation (Fig. 1) [3]. At the pathology laboratory, the specimen must be kept under-vacuum at 4 °C up to grossing and immersion in formalin for fixation. This allows recording and monitoring of the fixation time.

Another issue to be considered is the disposal of formalin after use. Using traditional fixation procedures by immersion, a 1:10 ratio of tissue volume to formalin volume is recommended. However, recent studies show that proper tissue fixation can also be achieved using the ratio of tissue weight to formalin volume of 1:1–1:2 [19,20]. Commercial systems for formalin under-vacuum fixation allow determination of weight of the tissue specimen placed in a plastic bag, dispense the fixative into the bag while under a closed and ventilated cavity/chamber and then seal the specimen under-vacuum [20]. These systems reduce the quantity of formalin used for fixation (Fig. 1). In addition, dry sealing under-vacuum of previously and adequately formalin-fixed tissues can be adopted to transfer biopsies and surgical specimens from remote areas to the referral pathology services [21].

Under-vacuum to preserve tissue specimens for transcriptomic and proteomic examinations

As mentioned above, an important advantage of the under-vacuum procedure is the preservation of nucleic acids, thus enabling molecular analyses (Fig. 1). It has been found that even tissues kept under-vacuum at 4 °C for 72 h provided nucleic acids of acceptable quality, in agreement with reports on the stability of RNA in non-fixed surgical specimens kept on ice [5,11]. Subsequently, other studies confirmed that good quality DNA and RNA isolation is feasible from tissues preserved under-vacuum at 4 °C (Table 1) [4,22,23]. In terms of RNA integrity, a consecutive series of 129 breast cancer specimens, stored under-vacuum at 4 °C for different time intervals (1–4, 5, 6–23, 24–48, and 60–72 h), was sampled by a punch apparatus and collected into RNARetain [23]. Before molecular analysis, specimens were tested for RNA integrity (RIN value, Agilent Bioanalyzer), and satisfactory values (i.e. > 6) were obtained from all specimens [23]. This cohort was subjected to MammaPrint® test [23]. As a result of the optimization of tissue preservation using the under-vacuum procedure, it was possible to perform the test even on specimens sampled 72 h after surgical excision, even though the manufacturer's recommendations suggest MammaPrint® analysis to be feasible only with samples taken within 1 h from surgical excision [23].

It was recently reported that under-vacuum storage has no impact on RNA and protein integrity or on specific phosphorylation sites on mTOR and STAT3 [24], but that storage time did affect the preservation of some metabolites. Analysis of vacuum sealed tissues by surface-enhanced laser desorption/ionization-time of flight/mass spectrometry (SELDI-TOF/MS) showed that the under-vacuum procedure preserved diagnostic peptide features, whereas measurement of metabolites revealed pronounced changes after 1 h of storage [24].

Conclusion

Ten years after the introduction of under-vacuum tissue package and cooling, in the light of the data presented here and supported by extensive literature, we can conclude that under-vacuum is a simple procedure, easily implementable in routine diagnostic practice and for research purposes. Indeed, fresh tissues are properly preserved by combining vacuum with preservation at 4 °C, which blocks enzyme activity and prevents tissue autolysis. The optimization of the pre-analytical phase is ensured through the monitoring of cold ischemia time. In addition, dry vacuum packaging allows fixation times in the pathology laboratory to be standardized, avoiding under or over-fixation. Under-vacuum procedures and preservation at 4 °C enable the collection of fresh tissue for biobanking, allows the assessment of cell culture and xenografting and preserves nucleic acids and proteins,

making the tissue suitable for “omics” analyses. Last but not least, under-vacuum packaging represents a valuable option to prevent the risk of exposure to formaldehyde, achieving a much higher level of security for operators.

New applications are emerging. The implementation of under-vacuum bags for tissue fixation and storage of the leftover fixed specimens reduces the volume of formalin used in the pathology laboratories and prevents exposure to formalin fumes. Finally, the use of this procedure will facilitate and improve transport of fixed tissues from remote areas to reference laboratories, ensuring optimal and accurate histopathological diagnosis.

Acknowledgments

The authors are funded by the H2020 project SPIDIA4P (contract no. 733112). AS is funded by FPRC 5xmille 2014 Ministry of Health and by AIRC 5xmille Metastatic disease: the key unmet need in oncology (Project ID 21091). CM is funded in part by the Italian Ministry of Education, University and Research (MIUR ex-60%-2017 and 2015HAJH8E) and by FPRC 5xmille 2015 MIUR (“Progetto Futuro”). LA is funded by Fondazione Umberto Veronesi (Post-Doctoral Fellowships 2018 and 2019). MIUR project “Dipartimenti di Eccellenza 2018-2022” (Project no. D15D18000410001).

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