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Validation of Vacuum-Based Refrigerated System for Biobanking Tissue Preservation: Analysis of Cellular Morphology, Protein Stability, and RNA Quality

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Biobanks of fresh, unfixed human normal and malignant tissues represent a valuable source for gene expression analysis in translational cancer research and molecular pathology. However, the success of molecular and cellular analysis in both clinical and translational research is strongly dependent on the collection, handling, storage, and quality control of fresh human tissue samples. The aim of this study was to evaluate an innovative vacuum-based refrigerated system, as a logistically feasible technology to increase the collection of tissue specimens, preserving the integrity of cellular and molecular components. We tested randomly-selected tissues stored under vacuum at 4°C by using endpoints important for research and diagnosis, including tissue morphology, epitope stability, and RNA integrity. Gene expression was evaluated by qualitative and quantitative RT analysis of selected housekeeping and tissue-specific genes. Tissue morphology and overall protein stability were generally well preserved, being compromised only in gallbladder tissue. By contrast, phosphoprotein and RNA analysis demonstrated a time-dependent degree of degradation, with progressive loss of stability from 24 to 72 hours. However, this reduction in RNA quality did not represent a limitation for successful expression analysis of selected genes. Indeed, a comparative qualitative and quantitative RT-PCR analysis showed that RNA extracted from tissues stored under vacuum is suitable for gene expression profiling, but requires highly sensitive technologies, such as quantitative RT-PCR. These data suggest that the refrigerated vacuum-based system represents a suitable and feasible technology for routine transport of fresh specimens from surgery to biobanks, thus increasing the opportunity to collect biospecimens.

Introduction

Recent progress in health research toward realizing the goal of personalized medicine,[1] together with advances in new technological platforms, and the "omics" revolution,[2] have opened new opportunities to derive important information about disease mechanisms directly from tissue samples.[3] This has especially been the case with research focused on human tumors, for which there are relatively few good animal models,[4] and this has increased the efforts to establish biobanks of fresh tissues.[5]

The purpose of tissue banks is to enhance the quality and the speed of both basic and translational research,[2] providing unique resources to study molecular changes in the *in situ* environment of cancer. Thus, storage of tissues with intact morphology, protein, DNA, and RNA for research or diagnosis is the main goal of human tissue biobanks.[6] Biospecimen quality is vital for tissue biobanks, being dependent on standardized and established handling processes.[7–9] Samples are generally obtained immediately after excision and prior to fixation, to ensure optimal preservation of protein and nucleic acids.[2] Even though formalin-fixed, paraffin-embedded tissues are adequate for some morphological procedures, the analysis of frozen tissue samples is required for most molecular diagnostic and research applications, which need intact genomic DNA, RNA, or proteins.[5,10,11] A major objective of molecular and proteomic profiling is to represent the *in vivo* state rather than a modified state induced by preanalytical variables.[12] Indeed, after resection, tissue is still alive, and this favors detection of dramatic changes in gene expression and protein profiles, especially phosphoproteins, due to ischemia, absence of vascular perfusion, hypoxia, or temperature changes.[13,14] Thus, at present, cryopreservation is considered

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