Advancing Brain Tissue and CNS Sample Handling and Distribution

Preserving Analytical Profiles of Central Nervous System (CNS) Samples for Neurodegenerative Disease Research using Frozen Aliquotting Technology

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ABSTRACT

As life expectancy continues to grow, and the average age of most developed countries' populations increases, it is widely expected that neurodegenerative disease will become a major healthcare burden, significantly impacting economies. Investment in research on Alzheimer's, Parkinson's and other neurodegenerative diseases is essential to development of improved diagnostic and treatment options. Access to high quality annotated central nervous system (CNS) disease tissue is a significant bottleneck to progress in this area. Additionally, it is evident that in brain banking there is no one globally accepted standard protocol for tissue collection and handling.

Analysis of CNS samples can be challenging as both cerebrospinal fluid (CSF) and frozen brain tissue are prone to incurring damage with repeated freeze-thaw cycles, which negatively impacts analytical outcomes. Currently, sample acquisition for analysis can be as crude as using a hammer and chisel on partially thawed sections of brain. The use of frozen aliquotting technology to acquire normalized amounts of tissue and frozen biofluids such as CSF can help reduce the impact of freeze-thaw damage to stored CNS biospecimens. The CXT350 Frozen Sample Aliquotter was used to generate frozen aliquots of both frozen human brain tissue and frozen CSF samples. Quantitative bioanalytical assays were performed to measure the presence of myelin basic protein (MBP) and apolipoprotein E (ApoE) in frozen brain tissue cores and CSF cores respectively. In both cases, the assays were observed to work as expected when analyzing frozen cores of CSF and brain tissue, demonstrating the potential for the integration of frozen aliquotting in modern neurobiological research.

INTRODUCTION

Analyzing stored biospecimens for CNS disease research presents some significant challenges as many specimen types, including brain tissue and CSF, can potentially suffer significant damage and degradation of the molecular content with even limited exposure to temperatures above -80^{0}C. Nucleic acid extraction and analysis of proteomic and lipid moieties of fresh frozen and formalin-fixed paraffin-embedded (FFPE) brain tissues is essential to the retrospective investigation of neurological and neurodegenerative diseases. FFPE specimens often have low DNA & RNA yields (Wang, JH et al), and produce poor degraded nucleic acid templates, particularly for RNA (Chow, ML et al) in part due to extensive formalin crosslinking. Consequently, fresh frozen tissues and biofluids are often the preferred source for molecular analysis of neurological disease patterns. However, preservation and safe access to these samples can be particularly difficult, especially for brain tissue as the frozen tissue is very brittle. Techniques to obtain samples of brain tissue for analysis are frequently crude in nature including the use of band saws (incurring significant loss of material), hammer and chisels, heated scalpel blades, hot filament cutters, and laser dissection. This may involve warming the tissue sample to temperatures above -80^{0}C (even with partial thawing), which frequently results in damage to the tissue content and imprecise amounts of material being obtained. Furthermore, cerebrospinal fluid has been shown to display unstable protein and molecular profiles if not maintained in a frozen state at temperatures of -80^{0}C or below (Ranganathan, S et al; Grossman, MH, et al).

Frozen aliquotting technology provides a simple and effective method for acquire more precise amounts of tissues and frozen biofluids such as CSF, while simultaneously maintaining the samples at ultra-cold temperatures (-80^{0}C to -150^{0}C) and thus preserving the analytical profiles of the samples.

ENRICHMENT OF WHITE AND GRAY MATTER USING FROZEN ALIQUOTTING

The ability to target white matter and gray matter has the potential for enabling differential omic-analysis of the neuron body (gray matter) versus the axonal compartment (white matter) for a variety of neurophysiological related research efforts. For example, the ability to elucidate differences and changes in lipid and protein profiles in the neural and axonal compartments may lead to critical insights regarding progression and treatment in diseases such as Alzheimer's and other neurodegenerative conditions. As gray matter is mainly comprised of neuron bodies and white matter is predominately comprised of myelinated axon bodies, myelin basic protein (MBP, a protein found in myelin) may be utilized as a biomarker to differentiate white from gray. The evaluating laboratory's primary goal was to investigate if frozen aliquotting could be utilized to generate frozen cores enriched in either white or gray matter, using MBP as a biomarker to gauge the level of enrichment achieved.

Frozen cores were generated from two distinct brain structures, the corpus callosum and occipital lobe, with an attempt to enrich for white matter or gray matter by frozen aliquotting in the latter. Corpus callosum was used as a positive control for MBP expression as it is primarily made up of white matter. Frozen samples of occipital lobe were obtained from two distinct cases. The frozen tissues were mounted using Tissue-Tek O.C.T. Compound and standard CXT350 tissue mounting accessories. Frozen cores were generated with 3mm diameter single-use coring probes and stored at -80^{0}C prior to further downstream processing and analysis. Detection and quantification of MBP was carried out using Western blot and chemiluminescence detection. MBP levels were expressed as relative pixel counts and normalized against a standard curve to allow gel-to-gel comparisons.

Overall, frozen aliquotting showed promise for targeting and enrichment of white and gray matter in frozen brain tissue. White matter enriched cores exhibited significantly higher levels of MBP in comparison to cores enriched with gray matter, as expected. Especially encouraging is that this dynamic was further demonstrated in occipital lobe samples from a single case (see Figure 2). Visual inspection of the cores did reveal the inclusion of gray matter to some extent in white matter cores, but the transition of white to gray matter on the cores was well defined and may offer an opportunity to refine the technique to achieve a higher degree of enrichment of one over the other.

FROZEN ALIQUOTTING OF CEREBROSPINAL FLUID (CSF)

CSF is known to be sensitive and unstable over multiple freeze-thaw cycles. A second experiment was performed to explore the suitability of frozen aliquotting frozen CSF samples and the impact on downstream detection and quantification of Apolipoprotein E (ApoE), an important biomarker in Alzheimer's disease. The ability to deploy frozen aliquotting for such a workflow would allow for the increased utilization of a given vial of frozen CSF without incrementally impacting the sample's integrity over successive freeze-thaw cycles.

Two individual vials of frozen CSF were frozen aliquotted on the CXT350, generating four cores per vial. Concentrations of ApoE were determined by ELISA. Each core was measured in triplicate at a standard dilution indicated by the ELISA kit manufacturer. The average concentration, standard deviation (SD), and coefficient of variation (CV) for each vial was calculated (see Figure 3). The coefficient of variation was observed to be less than 15% across the four cores measured from each vial of frozen CSF.

Figure 1: Myelin Basic Protein Levels Detected in Frozen Aliquots

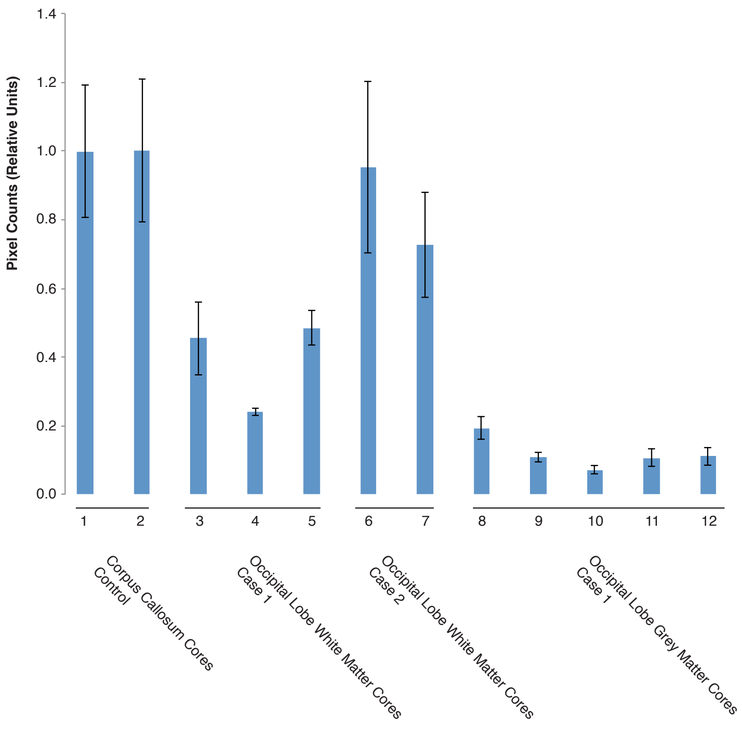


Figure 2: Comparison of Myelin Basic Protein in White Matter vs. Grey Matter

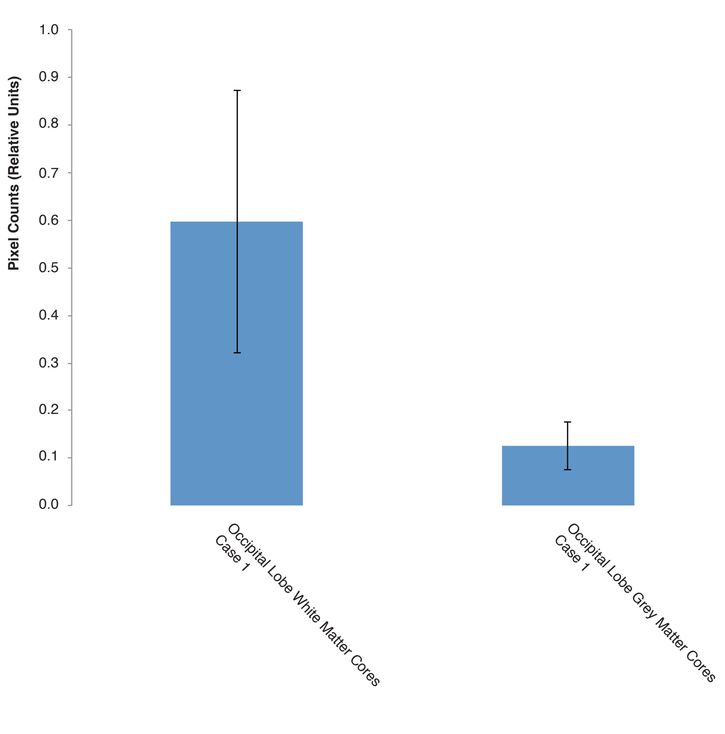
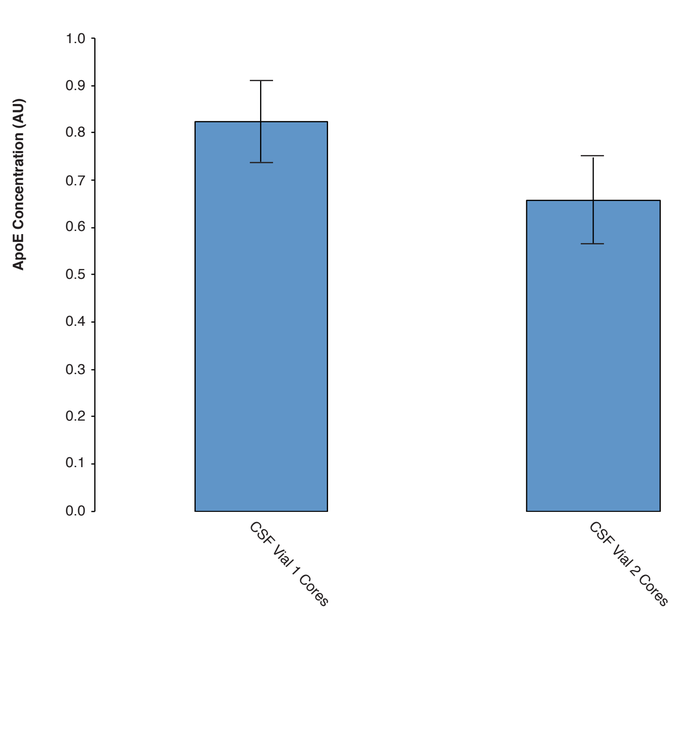


Figure 3: Apolipoprotein E Levels Detected in Frozen Vials of Cerebrospinal Fluid



CONCLUSIONS

These experimental proof-of-concept results demonstrate the potential for frozen aliquotting to be used in biomarker detection and quantification workflows involving frozen brain tissue and CSF. Moreover, there is potential for the CXT350 instrument to enable a novel method for the targeting and enrichment of white and gray matter within frozen brain tissue samples, thus, increasing the resolution of data in areas of research such as Alzheimer's Disease and other neurodegenerative conditions. Frozen aliquotting technology promises to enable safer, more accurate and consistent sampling of frozen neurological biospecimens for neurodegenerative and CNS disease research.

REFERENCES

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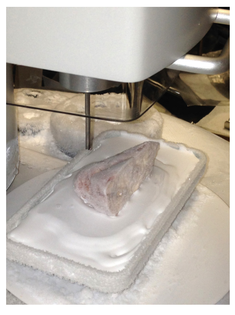
Ranganathan, S et al., "Assessment of protein stability in cerebrospinal fluid using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry protein profiling", Clinical Proteomics, 1 March 2006, Volume 2, pp 91–101.

Grossman, MH et al., "Stability of GABA levels in CSF under various conditions of storage", Brain Research, 20 January 1980, Volume 182, Issue 1, pp 99–106.

Frozen human occipital lobe OCT mounted and processed by frozen aliquotting.



Large section of frozen brain tissue OCT mounted on modified tissue fixture.

Frozen coronal slice OCT mounted on modified tissue fixture.

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