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Use of Laser Capture Microdissection with Pressure Cycle Sample Preparation to Analyze the Proteome of Retinal Sub-Structures

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Abstract:

The combination of laser capture microdissection (LCM) and sample preparation based on pressure cycle technology (PCT) provides means to study proteins that are specific for cell subpopulations or anatomic regions. This proof-of-concept study examined whether this approach can achieve separation of retinal sub-structures, e.g. outer segments of photoreceptors, from the surrounding tissue and detect proteins characteristic to these specialized structures. Eyes of wild-type mice were removed and fixed with cross-linking (10% buffered formalin) or denaturing (methanol or acetone) fixatives. We examined the ability of these commonly used fixative regimens to preserve both cell morphology and protein quality. LCM was used to isolate minute amounts of outer segments (~200,000 mm3). Catapulted tissue patches were either processed directly or subjected to PCT, in order to facilitate bottom-up proteomic analysis. Methanol was the best fixative for tissue preservation, while acetone fixation increased the number of identified proteins substantially (PFA: 771 vs. MeOH: 681 vs. acetone: 1154). Nevertheless, tissue morphology was inadequate for proper isolation of outer segments in this latter case. Formalin fixation gave best overall results. More than 300 proteins of the outer segments were identified without PCT, whereas PCT not only abridged processing, but vastly improved protein identification to greater than 700 identifications, while elevating the average sequence coverage 1.8-fold. The results presented here suggest that the combined use of LCM and PCT during proteomic sample preparation represents a promising tool to be effective in detecting protein profiles within the eye specific to a cell type or to certain pathologies.