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High Throughput Multi Parameter TEM Chemical Processing Protocol Development with the mPrep-s Capsule System: *Schmidtea mediterranea*

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It is widely accepted that chemical processing protocols for transmission electron microscopy (TEM) must be optimized for each different organism or tissue type for a particular research need. While processing conditions have been established for many tissues, there are some organisms and tissue types that are not well preserved with standard protocols and aren't represented in current literature for a particular research need. This study demonstrates a high throughput strategy for multi-parameter protocol testing in one processing run using the mPrep capsule and micropipettor-based system from Microscopy Innovations [1], and a quick comparison method using a silicone micro-well insert from Ibidi [2].

Freshwater planarian worm *Schmidtea mediterranea* were processed with 16 parameter variations based on a published protocol [3] which included four molarities of sodium cacodylate buffer (25 mM, 50 mM, 100 mM, 200 mM), secondary fixation in buffered osmium tetroxide (OsO_4) with and without the additive potassium ferricyanide (PFC) for membrane enhancement, and embedding in either Araldite (502) or Spurr's resin. Reagents were loaded into either 96 or 24 well plates for processing using a color coded paper template created in Excel and attached to each plate as a guide (Fig. 1). Planarian specimens were loaded into mPrep-s capsules and attached to a color coded 12 channel pipette (Fig. 1), which was used to pipet reagents from the well plates into the corresponding mPrep capsule for incubations. One piece of tissue from each of the eight conditions was embedded together in one micro-well of an Ibidi silicone insert for Araldite resin, and one micro-well for Spurr's resin. The resulting block faces produced sections that fit within the 2X1 mm opening in a slot grid (Fig. 2). Sections with a silver interference color were cut with a Diatome Histo diamond knife on a Leica UTC and placed on a formvar/carbon coated slot grid for comparison in the TEM (Fig. 2).

The 25 mM and 50 mM buffer flame cells showed defined membranes and clear ultrastructural detail (Fig. 2). However, some of the 25 mM flame cells showed swelling artifacts and some of the 50 mM flame cells showed a more electron dense cytoplasm that obscured ultrastructural details as compared to the 25 mM. Both the 100 mM and 200 mM buffer samples showed significant shrinking and electron dense cytoplasm. Spurr's resin produced more fully infiltrated samples than Araldite. While the use of PFC did not have a discernable effect on the Spurr's samples, the 50 mM and 100 mM PFC Araldite samples showed significantly more electron dense cytoplasm than the same buffer molarities without PFC.

The mPrep system facilitated the controlled and consistent testing of many conditions, which greatly increased the chances of obtaining optimized specimen preservation in one processing run. Embedding eight different samples in one block substantially reduced microtomy and imaging time. Using this approach for whole planarian worms created a set of embedded test samples which can be examined for preservation of any organ system or cell population without having to perform several test runs [4].

References:

[1] mPrep/s capsules (Catalog #S0812), Microscopy Innovations LLC, Marshfield, WI, USA.
[2] 4 well micro-inserts (Catalog # 90406), Ibidi LLC, Munich, Germany and Verona, WI, USA.

[3] James A McKanna, Fine Structure of the Protonephridial System in Planaria II. Ductules, Collecting Ducts, and Osmoregulatory Cells, Zeitschrift für Zellforschung **92** (1968), p. 524-535.

[4] Thanks to Hanh Vu for providing *S. mediterranea* samples and a starting protocol, Steven Goodman and Tom Strader from Microscopy Innovations for product support, and the Stowers Institute for Medical Research.

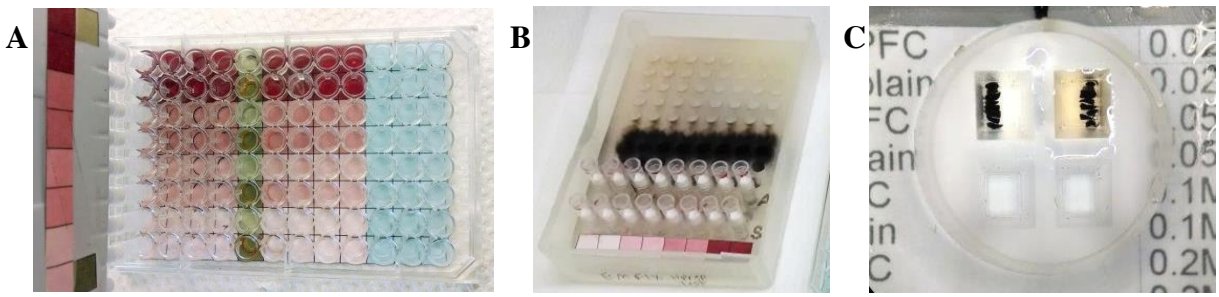

Figure 1. (A) Samples in capsules attached to a color coded micropipettor and lined up with the corresponding colored well on the 96 well plate for reagent changes. **(B)** Capsules in silicone bench for incubation. (C) Ibidi cell culture micro-well insert with samples embedded in Spurr's and Araldite resin.

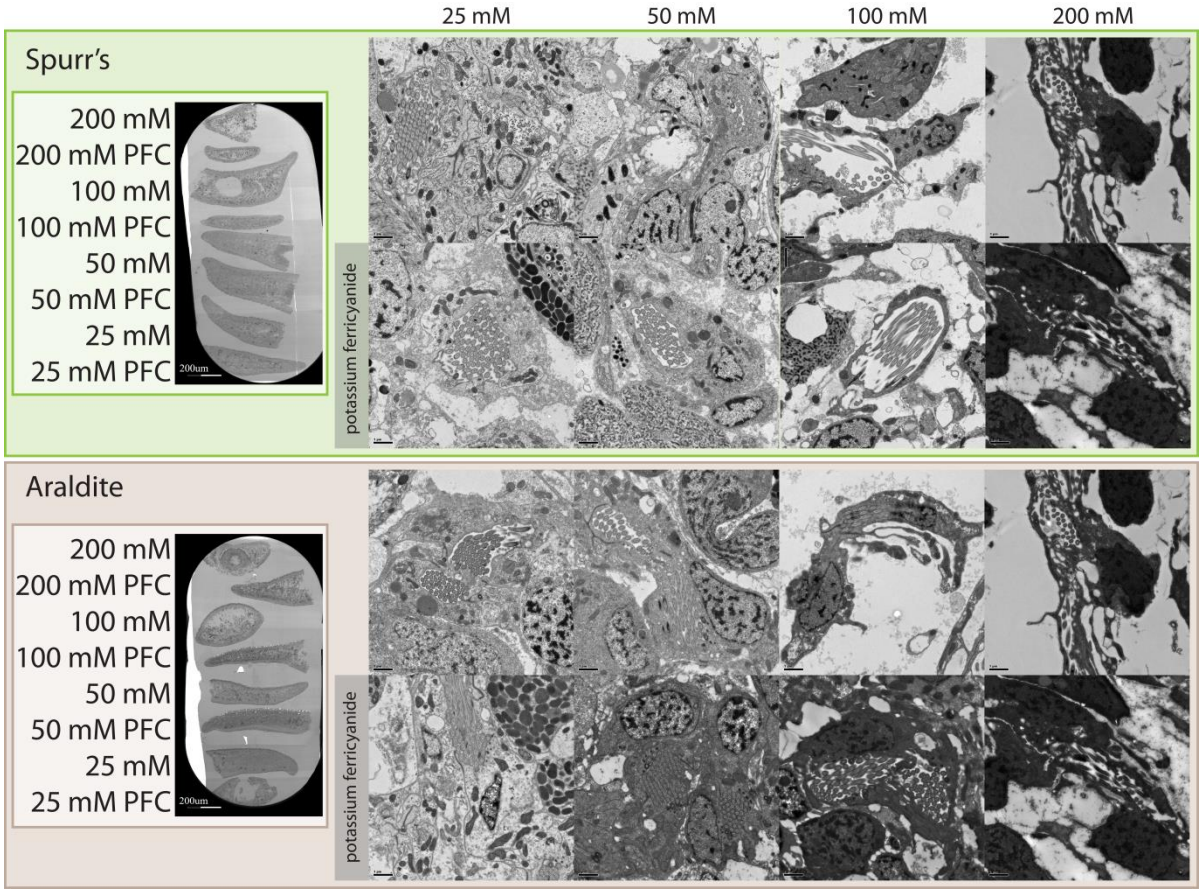

Figure 2. (Left) TEM image of a section from each block face mounted on a slot grid showing eight samples in each section, labeled with buffer molarity and potassium ferricyanide (PFC). Scale 200nm. (Right) TEM images of a representative flame cell from each condition, scale 1um.

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Introduction

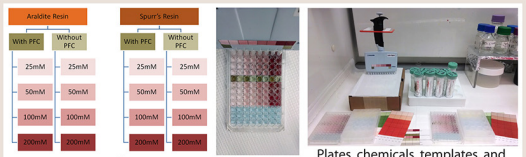
Chemical processing protocols for transmission electron microscopy (TEM) have been established for many different organisms and tissue types for many research needs. However, when published protocols fail to produce adequate results, protocol development is required. This study demonstrates a high throughput strategy for multi-parameter protocol testing in one processing run using the mPrep capsule and micropipettor-based system from Microscopy Innovations, and a quick comparison method using a silicone micro-well insert from Ibidi.

Problem

The planarian worm Schmidtea mediterranea is a model organism with the ability to regenerate an entire worm from only a small cutting. The published protocols for processing this organism for secretory system studies for TEM weren't providing adequate ultrastructural detail, and a method was needed to compare different protocol variations efficiently while minimizing errors. While the secretory system was the main focus in evaluating results for this study, other organ systems were also compared.

Materials and Methods

16 chemical parameter variations were chosen using 4 buffer molarities, 2 resins, and the presence or absence of the additive potassium ferricyanide (PFC) in the osmium secondary fixation step. Reagents were loaded into either 96 or 24 well plates for processing using a color coded paper template created in Microsoft Excel as a guide. Planarian specimens were loaded into mPrep/s capsules and attached to a color coded 12 channel pipette which was used to pull reagents from the well plates into the corresponding mPrep capsule for incubations.

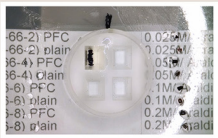


Parameter Chart

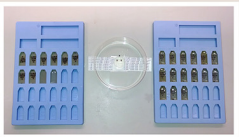
Color Coding

Plates, chemicals, templates, and pipettor with stand

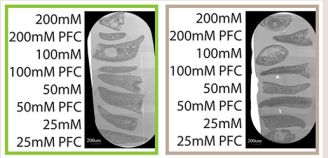
One sample from each of the eight conditions was embedded together in one micro-well of an Ibidi silicone insert for Araldite (502) resin, and one micro-well for Spurr's resin. One section per grid was cut from each of these block faces with a Diatome Histo diamond knife and picked up on 1X2mm formvar carbon copper slot grids. Images were taken at 80kV on a FEI Technai BioTwin TEM.



Ibidi mold with samples placed in label order for later identification



All samples embedded



Whole grid images of sections from Ibidi mold. Spurr's (left) and Araldite (right)

Results

In comparing resins, the Spurr's resin infiltrated samples completely, while the Araldite samples had infiltration problems mostly in the gut (Fig. 3A) and pharynx, but also in some nuclei (*) throughout the animal. The 25mM and 50mM buffer flame cells showed defined membranes and clear ultrastructural detail (Fig. 1), however the 25mM flame cells showed swelling artifacts which were also present throughout most of the animal (Fig. 2A). Both the 100mM and 200mM buffer samples showed significant shrinking and electron dense cytoplasm which obscured ultrastructural details (Fig. 1). While the use of PFC did not have a discernible effect on the Spurr's samples, the 50mM and 100mM PFC Araldite samples showed significantly more electron dense cytoplasm than the same buffer molarities without PFC.

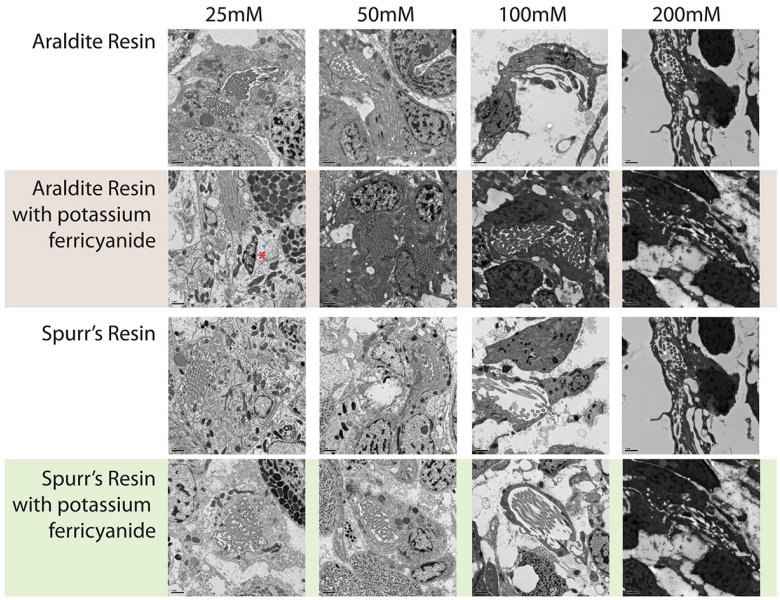


Fig. 1 (Left) A comparison of the flame cells of the secretory system for all conditions. Lower buffer molarities show clear ultrastructural detail while higher molarities show dense cytoplasm.

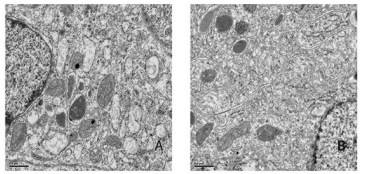


Fig. 2 Comparison of molarities. A. 25mM Spurr's resin Planarian gut shows swelling artifacts. B. 50mM Planarian gut shows less swelling without the shrinking artifacts seen in the 100mM.

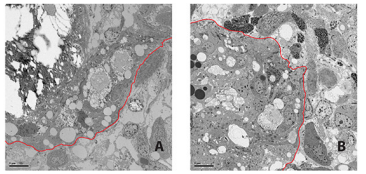


Fig. 3 Comparison of resins, gut outlined in red. A. 50mM Planarian gut shows incomplete infiltration in Araldite resin. B. 50mM Planarian gut shows complete infiltration in Spurr's resin.

Conclusions

The mPrep system facilitated the controlled and consistent testing of many conditions. This greatly increased the chances of obtaining optimized specimen preservation in one processing run. For S. mediterranea, using the mPrep system produced a greatly improved protocol in two weeks processing time.

The main advantages of the mPrep system for this application are:

- All organization is done before processing begins, so there is less chance of mix-ups while trying to keep samples and chemicals straight during processing.
- All fluid exchanges are done simultaneously, so a fluid exchange on up to 12 samples is as fast as 1 sample.

	mPrep Sample Prep	Conventional Sample Prep
Processing Time	1 run (2-3 weeks)	4 runs (8-12 weeks)
Orientation and Embedding Time	~3.5 hours	~1.5 hours

Using the Ibidi silicone insert to embed eight different samples in one block greatly reduced microtomy and imaging time and amount of consumables used. While it took additional time to embed samples together in the mold in a way that made it easy to tell them apart, this time was far less than the microtomy and imaging time saved.

	Ibidi Mold	Conventional Molds
Ultramicrotomy	~1 hour 1 slide 4 grids	~5 hours 4 slides 32 grids
TEM Imaging	2 grid exchanges	16 grid exchanges

Using this approach for whole planarian worms created a set of embedded test samples which can be examined for preservation of any organ system or cell population without having to perform several test runs.

Resources

mPrep/s capsules (Catalog #S0812)
Booth 929, Microscopy Innovations LLC, Marshfield, WI, USA.

4 well micro-inserts (Catalog # 90406)
Booth 1105, Ibidi LLC, Munich, Germany and Verona, WI, USA.

Acknowledgements

Thanks to Hanh Vu for providing S. mediterranea samples and a starting protocol, Steven Goodman and Tom Strader from Microscopy Innovations for product support, and the Stowers Institute for Medical Research.

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