The Use of Poly-L-lysine as an Adhesive in Scanning Electron Microscopy

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Poly-L-lysine of a medium molecular weight, 30,000 to 70,000 Daltons (Sigma–Aldrich), is currently used in our facility as a poly-cationic adhesive on 5 mm square silicon chips to secure individual anionic cells and particles for ease of processing and viewing in the scanning electron microscope. In this technique, a solution of 1 mg/ml of poly-L-lysine is dissolved in distilled water. Drops of the solution are placed onto clean 5 mm square silicon chips and allowed to sit for one to several hours before being wicked away. The prepared chips are used immediately. Suspended cells are then applied to the chips and allowed to settle and adhere, after which they are washed and fixed. Alternatively, fixed suspended cells or particles are applied to these silicon chips. The cells and particles secured to the silicon chips are easily handled through processing steps such as dehydration, critical point drying, and metal sputter coating for viewing in the scanning electron microscope. Presented herein is a brief history of the evolution of the technique of using poly-L-lysine as an adhesive for SEM.

The use of a cationic polymer to make a surface "sticky" to an anionic cell was employed by Steinhardt, Lundin, and Mazia in 1971 (1) when they needed to secure echinoderm (sea urchin and sand dollar) eggs in order to stick them with multiple electrodes and measure the bioelectric response to fertilization. The eggs are round, about 0.1 mm in diameter, and tended to roll away from the electrodes. The adhesive in this case was 1% protamine sulfate, an arginine rich, positively-charged protein of a molecular weight ranging from 5,000 to 10,000 Daltons (Sigma-Aldrich). The authors described the eggs as each being a "giant anion" and took advantage of this fact. They covered the bottom of plastic Petri dishes with 1% protamine sulfate, let it sit for a while and washed it away. The echinoderm eggs, suspended in whichever of the several media that were used in the experiments, attached strongly to the treated surface.

Poly-L-lysine was perhaps first used to secure single living cells to a substrate in 1972 when A. Macieira-Coelho and S. Avrameas (2) tested it with several other polymers and monomers for the purpose of preparing surfaces for culturing cell monolayers. They tried positively and negatively charged polymers and found that those that left a positively charged surface worked best. The poly-L-lysine used was reported to have a molecular weight of 130,000 Daltons and used at a dilution of 1 mg/ml. Concentrations of 0.1 mg/ml and 0.01 mg/ml poly-L-lysine resulted in a lower concentration of cells. Though there were some differences modulating the behavior of the cells in culture, the polymers of different molecular weights gave similar results. The exception was that the negatively charged polymers did not work very well. The amino acid monomers tested did not bind as well at even a ten-fold increase in concentration of the relative polymer, in that the monomers would not have had the many multiple cationic sites to both bond to the substrate and to the cell or particle.

D. Mazia, G. Schatten, and W. Sale, in 1975 reported using poly-L-lysine as an adhesive for use in securing cells to substrates for eventual viewing in the SEM (3). That work was apparently built on the earlier work by Mazia with the echinoderm [illegible] on Macieira-Coelho and Avrameas' work modulating cell behavior on different substrates. Working with a 0.1% solution (1mg/ml) of poly-L-lysine of molecular weight of 80,000 to 100,000 Daltons, small squares of clean glass and Formvar coated grids were treated briefly to the poly-L-lysine and thoroughly washed with distilled water. Suspensions of living cells applied to the surface of the treated glass strongly attached. Some cells spread out on the prepared surface. The cells were so strongly attached to the poly-L-lysine that when blasted with a stream of water or buffer, the cells broke off, leaving the base stuck to the substrate. Sea urchin sperm secured to treated grids and broken apart with Triton-X, left attached only the outer doublets and central microtubules.

Further work in 1975 by M. Clark, G. Schatten, D. Mazia, and J. A. Spudich (4) explored this strong attachment using poly-L-lysine and glass slides. The authors reported using a poly-L-lysine of a molecular weight and concentration as before: a 0.1% solution (1mg/ml) of poly-L-lysine of molecular weight of 80,000 to 100,000 Daltons. The secured cells were sheared from their bases, exposing the cytoplasmic face. The prepared glass surface was washed with the cell suspension buffer. The cell suspension was applied and allowed to settle for about 1 minute before being washed again with buffer. The cells were either fixed, or disrupted and fixed, followed by post fixation, dehydration, and critical point drying.

The authors also prepared disrupted cells for TEM, securing them to carbon-stabilized Parlodion film covered grids *via* 1mg/ml poly-L-lysine adhesion. The disrupted cells were negatively stained with 1% Uranyl Acetate. Some preparations of disrupted cells were treated to myosin subfragment, which bound to exposed filaments confirming them to be actin. It might be possible to disrupt the secured cells in this same way and label them *via* immuno-gold for viewing in the TEM.

Further proof of the efficacy of poly-L-lysine as an adhesive for SEM preparations was demonstrated by S. K. Sanders, E. L. Alexander, and R. C. Braylan in 1975 (5). These authors sought to secure suspensions of fixed cells rather than to work with living cells as live suspended cells are reactive to the substrate and may alter their shape and surface morphology when adhering to a solid surface. Macieira-Coelho and Avrameas actually exploited this in their work above. Therefore, to view cells cultured in suspension in their natural shape, they were first fixed in suspension and then allowed to secure to the poly-L-lysine treated surface, which they did nicely as shown by S. K. Sanders, E. L. Alexander, and R. C. Braylan. These authors fixed the human lymphocyte and monocyte cells in 1% glutaraldehyde in Sorensen's buffer, pH 7.3, for 24 to 48 hours after which the cells were rinsed in Sorensen's buffer then brought to a final known concentration and placed on prepared glass coverslips. A Finder Grid was glued to the backside of the coverslip in order to count cells in selected areas before and after the full procedure of critical point drying. The count was made using a light microscope. After critical point drying, the cell count was 96 to 98% of the count prior to critical point drying. The authors noted therefore that selective cell loss due to differences in cell surface charge was unlikely; all cell types in the population had adhered well.

Up to this point, all the solid substrates to which the poly-L-lysine has been applied have been glass or plastic. In 1978, P. M. Male and D. Biemesderfer presented doped silicon wafers as a sup-

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port for biological macromolecules for SEM (6). They used a low molecular weight (3,400 Daltons) poly-L-lysine as an adhesive to hold native ferritin onto the surface of the silicon wafer. The doped silicon wafers were considered an improvement over glass as they were more electrically conductive, allowed a lighter metal coating, and resulted in reduced charging artifacts in the SEM, especially at higher magnifications and higher accelerating voltages. The silicon wafers were doped with antimony or boron.

Poly-L-lysine is available in a broad range of molecular weights and as suggested by the work done by these various authors, one may select a size molecule appropriate to the cell or particle of interest. The poly-L-lysine molecule has to be large enough to hold the particle or cell but not so large as to obscure it. Sigma-Aldrich (7) offers poly-L-lysine in molecular weights ranging from 500 up to greater than 300,000 Daltons, so one has a broad range of molecular weights with which to work. In all these examples, the poly-L-lysine was applied to the substrate, not to the cells.

To recap the evolution of this method; it started out with the need to secure a relatively small thing, an echinoderm egg, to a substrate without the adhesive greatly changing the egg itself. From there it progressed to using poly-L-lysine specifically, and it was noticed that in the very act of settling, the living cell did experience some changes; the cells tended to flatten and fibers inside rearranged at the site of contact. This response was halted by fixation. Fixing the cells prior to adhesion maintained the cell shape and the cells stuck just fine. Finally, we come to the use of poly-L-lysine to secure cells, living or fixed, onto poly-L-lysine treated silicon chips, which is what we do in our core facility. ■

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Microscopy Society of America Local Affiliate Society
New England Society for Microscopy

The New England Society for Microscopy was founded (as the New England Society for Electron Microscopy) in 1967, and has marked its 40th anniversary during 2007 with several special events. Most notable was the "Presidents' Evening" during the Spring Symposium in May, when a number of former presidents offered reflections on their times in the Society, and which culminated in the honoring of the Society's first (and ninth and twenty-fourth) president, Dr. Francis Heckman, with an Emeritus membership award and certificate. Dr. Heckman is one of very few charter members of the society who remain as active members, though two or three others are known to be active professionally in other parts of the country. The society was delighted to welcome his wife and daughter, who accompanied him as he was clearly delighted to accept the award. All attendees at the meeting were given a commemorative 10x triplet magnifying loupe with the NESM logo.

The society changed its name, dropping the "Electron" in 1992, at the same time that the Electron Microscopy Society of America made a similar change.

The society, which is a local affiliate of both the Microscopy Society of America and of the Microbeam Analysis Society, draws a preponderance of its membership from the Eastern Massachusetts conurbation, though there are active members as far away as Burlington, VT and Bar Harbor, ME. There are presently over 100 active members, and 20 corporate sponsors. There are four technical meetings each year, and there are three different groups of members active with Project Micro kits, working with elementary/middle school students in their respective communities.

The largest of the meetings is the three-day Spring symposium, held the first weekend of May at the Marine Biological Laboratory in Woods Hole. Typically, the first day consists of a workshop on some topic related to microscopy, while the Friday and Saturday are taken up with platform and poster presentations, a banquet and after-dinner talk, and a time to mingle with the corporate sponsors.

The Fall symposium consists of a scientific and annual business meeting, ending with a dinner, and taking an afternoon and evening in early December, while the two remaining meetings are typically evening meetings held at the facilities of a corporate sponsor or a local college, though the Board is always willing to try different formats.

All NESM meetings are planned to include both Biological and Physical Sciences topics of general interest to all microscopists. Students are encouraged to attend and receive substantial discounts on membership dues and meeting registration fees. The meetings are sometimes co-sponsored with other groups, such as the Connecticut Microscopy Society (CMS).

NESM has hosted the MSA Traveling Speakers as well as the Traveling Poster Collections. We have invited speakers from all over the country to present their work to our members. Recently, these speakers have come from facilities such as Oak Ridge National Laboratories, Yale, Harvard, MIT, and many other internationally recognized facilities. We include topics from academia, industry, and health sciences.

Political economic initiatives like support for bio and nano tech projects in Massachusetts will continue to drive the microscopy community in New England. The concentration of high tech industry and educational institutions in New England all make the microscopy community very valuable to the area and to science in general.

Please visit our website to check out our next meetings, newsletters, and other information. We welcome new members and encourage other local organizations to come to New England. Web Site: http://nesm.cims.harvard.edu. ■