Platinum blue staining mitigates charging and enhances cell membrane visualization in oyster hemocytes

Andrew D. McQuiston[1], Vera B.S. Chan[1], Andrew S. Mount[1]

[1] Department of Biological Sciences, Clemson University, Clemson, SC

Abstract

Hemocyte cells from the Eastern oyster, *Crassostrea virginica*, were either sputter coated with platinum or stained with platinum blue (Pt-blue), a heavy metal stain, and imaged using scanning electron microscopy (SEM). Some of the cells from each of these treatment groups were further incubated with osmium tetroxide (OsO_4). The membranes of cells treated with Pt-blue were more clearly delineated than those of the sputter coated cells, but static electric charging was more prevalent with the Pt-blue stained cells. However, charging effects appeared to be alleviated with longer Pt-blue staining duration concomitant with OsO_4 treatment. Overall, Pt-blue demonstrated its utility as a plasma membrane stain, especially when used in combination with OsO_4.

Introduction

In the Eastern oyster, *Crassostrea virginica*, hemocytes have been shown to play a role in shell repair by depositing calcium crystals at the mineralization front (1). Although the mechanism by which these crystals are deposited is unclear, investigations into mineralization of human cardiovascular plaques has demonstrated the participation of membrane-derived exosomes (2-3). Furthermore, there is support for the hypothesis that macrophages are the source of calcifying exosomes in human atherosclerosis (4). Thus, successfully identifying hemocyte-derived exosomes in oysters has the potential to illuminate a large piece of the biomineralization puzzle, with applications in material and biomedical sciences.

Methods

After notching the shell outer margin with a circle saw (left), oyster hemolymph was drawn from the adductor muscle with a hypodermic needle and allocated onto 1 cm^2 stainless steel foils. Half of the cells were stained with OsO_4, and then the OsO_4+ and OsO_4- groups underwent identical SEM preparations as outlined in the table below.

	Control	Platinum blue			Sputter coat		
OsO_4	X	0.5 h	1.0 h	2.0 h	40 s	60 s	120 s
No OsO_4	X	0.5 h	1.0 h	2.0 h	40 s	60 s	120 s

Table 1. Overview of experimental design illustrating different treatments and durations.

Results

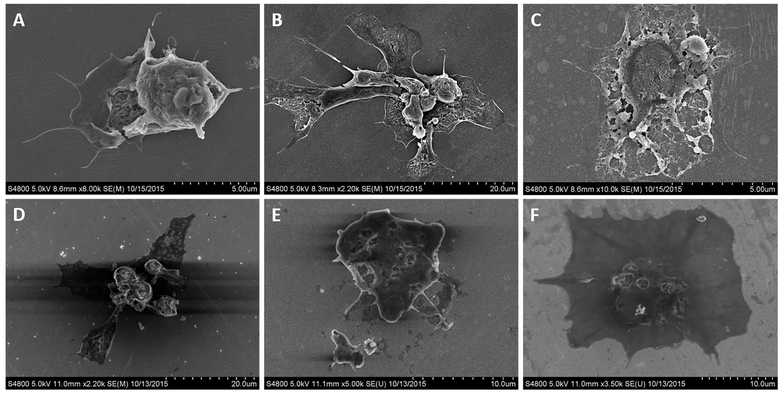

Figure 1. Oyster hemocytes treated with OsO_4 and platinum sputter coated for 40 s (a), 60 s (b) and 120 s (c), or incubated with Pt-blue for 0.5 h (d), 1.0 h (e) and 2.0 h (f).

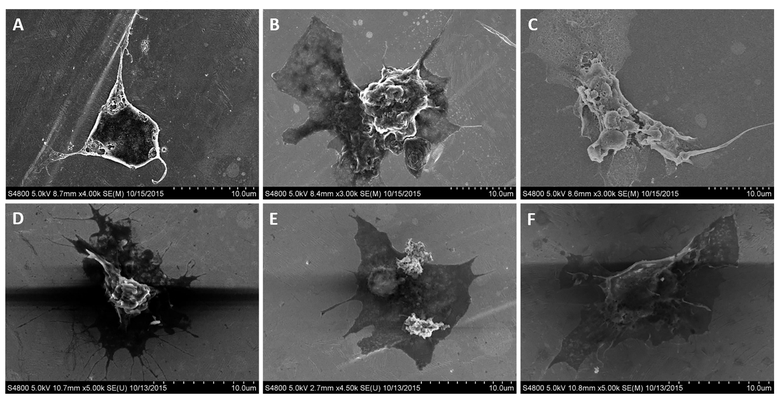

Figure 2. Oyster hemocytes without OsO_4 treatment; sputter coated for 40 s (a), 60 s (b) and 120 s (c), or incubated with Pt-blue for 0.5 h (d), 1.0 h (e) and 2.0 h (f).

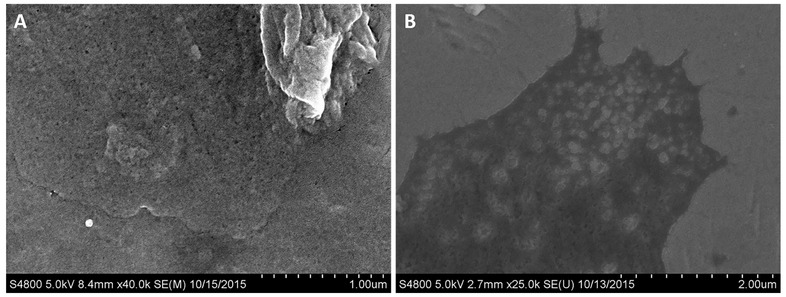

Figure 3. Higher magnification comparison of a sputter coated cell membrane (a, no OsO_4, 60 s) and a Pt-blue stained membrane (b, no OsO_4, 1.0 h).

Conclusions

- Cell membrane contrast was significantly higher in Pt-blue stained cells compared to sputter coated cells.
- Longer duration of Pt-blue incubation decreased degree of charging
- OsO_4 further reduces charging when used in combination with Pt-blue

References

1. Mount, A.S., A.P. Wheeler, R.P. Paradkar, D. Snider. 2004. Hemocyte-mediated shell mineralization in the eastern oyster. Science. 304(5668):297-300.
2. Chen, N.X., K.D. O'Neill, X. Chen, S.M. Moe. 2008. Annexin-mediated matrix vesicle calcification in vascular smooth muscle cells. J Bone MinerRes. 23:1798-1805. Kapustin, A.N., J.D. Davies, J.L. Reynolds, R. McNair, G.T. Jones, A. Sidibe, 3. L.J. Schurgers, J.N. Skepper, D. Proudfoot, M. Mayr, C.M. Shanahan. 2011. Calcium regulates key components of vascular smooth muscle cell-derived matrix vesicles to enhance mineralization. Circ Res. 109:el-12.
4. New, S.E., C. Goettsch, M. Aikawa, J.F. Marchini, M. Shibasaki, K. Yabusaki, P. Libby, C.M. Shanahan, K. Croce, E. Aikawa. 2013. Macrophage-derived matrix vesicles: an alternative novel mechanism for microcalcification in atherosclerotic plaques. Circ Res. 113(1):72-77.

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