Techniques for Preserving Endothelial Glycocalyx when Using Electron Microscopy

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Supplemental Video

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Background:
During hypoxia-inducing events such as hemorrhagic shock, damage to the endothelial glycocalyx occurs. Injury to this luminal carbohydrate-protein matrix has been linked to downstream dysfunction in coagulation mechanisms. Prevention of this coagulopathy via glycocalyx preservation is especially of interest in the field of trauma. The study of glycocalyx shedding requires various methods of quantification. Damage can be inferred by increases in glycocalyx constituents in plasma such as syndecan-1, hyaluronic acid, or heparan sulfate. Immunofluorescent staining is frequently used to quantify changes in signal before and after glycocalyx injury. Intravital microscopy can assess damage in-vivo, but this practice is expensive and has anatomical limitations. Interestingly, electron microscopy (EM) has been shown to both successfully quantify glycocalyx damage and highlight endothelial microstructure. However, there is concern that fixation/staining techniques used in many EM experiments may damage the glycocalyx. In this study, methodology will be discussed for successful in-vivo glycocalyx EM imaging of rat pulmonary arteries. We hypothesized that staining the glycocalyx in-vivo prior to fixation would better preserve glycocalyx for EM imaging when compared to staining after organ harvesting and submersion fixation.

Methods:
Rats underwent a “sham” hemorrhagic shock and resuscitation protocol by cannulating the femoral artery and jugular vein without inducing blood loss or fluid resuscitation. Following exposure to these conditions, experimental rats intravenously received a dose of EM dye. Organs were harvested, fixed via submersion using glutaraldehyde, then treated with osmium tetroxide. Control rats were exposed to similar conditions of sham shock but whole tissue was stained via submersion following organ harvest and fixation. All tissues were embedded in Spurr resin for EM sectioning and imaging. Glycocalyx preservation was measured using ImageJ by analyzing the total surface area of a cross-sectional EM image.

Results:
When compared to control, glycocalyx microstructure was found to be significantly preserved using in-vivo staining prior to fixation.

Conclusion:
We conclude that intravenous staining prior to organ harvest and submersion fixation is superior for preservation of glycocalyx microstructure when compared to staining after fixation. Future experimentation aims to confirm that EM imaging using these methods is appropriately sensitive to measure differences in sham versus hemorrhaged rats.

Learning Objectives
Discuss what the glycocalyx is and how its injury relates to the field of trauma.
Identify various methods of glycocalyx analysis and their aims and limitations.
Describe how in-vivo staining can lead to superior electron microscopy imaging of the glycocalyx.

References and Resources
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