

FE-SEM and VP-SEM imaging of human calcified aortic valves: conventional vs Ionic Liquid innovative techniques

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Conventional FE-SEM protocol for calcified aortic valves (CAVs) consist of following steps: glutaraldehyde fixation, OsO₄ post-fixation, dehydration in alcohol series, critical point drying and finally sputter coating. CAVs can be observed in their native state (fixed in glutaraldehyde with and without post-fixation in OsO₄) by Variable Pressure-SEM (range 6- 650 Pa). Gas presence allows an inferior resolution (low signal to noise ratio), however there is the possibility to perform EDS elemental analysis without background noise due to sputter coating. Recently Ionic liquids (IL, salts in the liquid state at room temperature) were used as suppliers of electronic conductivity with insulating properties, so we have tested their ability to replace sputter coating on CAVs in high vacuum condition. Samples fixed in glutaraldehyde 2,5% in PBS with and without OsO₄ post-fixation treated with ionic liquid (Hitachi HILEM® IL 1000) were compared with samples treated with conventional FE-SEM procedures. Several IL concentration (range from 5% to 20%) were tested, different operating voltages (range from 3 to 20Kv) were used. This novel technology requires a high degree of customization for each sample type. In our opinion fixation in glutaraldehyde with OsO₄ post-fixation is recommended to preserve finest details, moreover residual liquid elimination is important to increase resolution and avoid beam interference as linear markings. Setting of a proper accelerating voltage allows to correctly visualize the surface topography. Processing CAVs with IL with respect to conventional FE-SEM is useful for several reasons. Mainly this method is time saving (and cost saving), secondary the same sample can be processed for transmission electron microscopy after SEM observations (allowing correlative microscopy), finally EDS can be performed without background noise due to sputter coating. Perhaps now this technique can not completely replaces the conventional SEM in terms of resolution but in our opinion rapid technical improvement can further reduce this gap.

Keywords

FE-SEM, VP-SEM, EDS analysis, ionic liquid, aortic valve