SCANNING ELECTRON MICROSCOPIC SCREENING OF HEART VALVE ALLOGRAFT SPECIMENS PROCESSED BY SIMPLE AND RAPID DRYING METHOD.

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Aortic and pulmonary allograft heart valves (AHV) have been used for surgical valve replacement for 40 years. The most important factors of their long term clinical performance are methods of their handling and cryopreservation. Present protocols of AHV processing and storage differ among AHV Banks. Therefore we decided to evaluate the impact of our own protocol on valve tissue morphology. Scanning electron microscope (SEM) is especially suitable for detection of fine changes on surfaces of cardiac valves. In order to broaden the scope of our research, we have developed our own modified method for desiccation of biological samples for SEM. Control “fresh” aortic and pulmonary AHV samples, harvested from “heart-beating” cadaveric donors, were compared with following four experimental groups: (1) tissue from AHV obtained from non heart-beating donors, (2) samples stored in 4 °C saline for 24 h, (3) antibiotic-treated tissue for 24 h at 37 °C and finally (4) cryopreserved valves, stored in liquid nitrogen (-196 °C) for 6–38 months. All samples were dissected, fixed in 4% formaldehyde overnight, then washed in distilled water for 5 min, and dehydrated in a graded ethanol series (70, 85, 95, and 100%) for 5 min at each level. The tissue samples were then immersed in 100% HMDS (hexamethyl-disilazane) for 10 min, air dried in an exhaust hood at room temperature [1], gold coated, studied and photographed in SEM Tesla BS 301. In order to define integrity of endothelium and quality of surface area under SEM, a special score system (from 1 to 6) was implemented: 1. morphologically intact endothelium, 2. confluent endothelium with membrane irregularity, 3. disruption of intercellular contacts, 4. separation of endothelial cells, 5. complete loss of endothelium with the basal lamina exposed, 6. damage of subendothelial layers. The leaflets of the aortic valve (specimens harvested from cadaveric “beating heart” donor, initially stored in saline at ambient temperature for 20 min) were investigated as control samples. However, the endothelial pattern was irregular, with frequent central excavations on cell membranes in the perinuclear area (Figure 1a, Score 2). On aortic and pulmonary valves from non heart-beating donors (harvested at autopsy, 12 h after death) the ventricular surface showed initial separation of endothelial cells from the basal membrane (Figure 1b,1c, Score 3–4). On the other specimens of this group (autopsy, 48 h after death), more severe changes were found (Score 4 and 5) – from shrinkage and stripping of endothelial cells to complete denudation of the endothelium (Score 5). On surface of specimens stored in saline a wide spectrum of changes was visible, from patchy losses of endothelium (Figure 1d, Score 4) to completely stripped endotheliocytes. In the group of antibiotic-sterilized allografts all specimen surfaces were completely devoid of endothelium (Figure 1e, Score 5). Samples of thawed valves showed complete stripping of the endothelial cells from both ventricular and vascular surfaces of aortic and pulmonary valve leaflets (Score 5), patches of damaged subendothelial layers (Figure 1f, Score 6) were occasionally detected. Our bank uses the protocol [2] which provides AHV of reasonable quality from the surgical point of view. Retrospective and morphological studies gave evidence that the integrity of the endothelial covering is one of the decisive factors determining the further fate of the transplanted cardiac valve [3]. In spite of its immunogenity, the intact endothelium of AHV is able to slow degeneration and calcification of implanted grafts [4], and decreases the thrombogenicity. We were able to detect early changes in the endothelium right after harvesting, after few hours of warm ischemia. Denudation of the endothelial covering appeared during processing and
preservation with and without freezing. This work has shown that initial washing of allografts in saline destroyed the endothelium during harvesting. Processing and cryopreservation caused destruction of endothelium and basal membrane. This means that we are implanting AHV without any endothelium. In summary, our simplified method of scanning electron microscopy, using fast drying with HMDS may be helpful for morphological control of processing, cryopreservation and liquid nitrogen storage of AHV. Further combination of morphological investigations with other methods will help to increase quality of AHV transplants.

References

Figure 1. Bar 80 μm in A; bars = 20 μm in B,E; bar = 40 μm in C; bars = 16 μm in D, F. Arrowheads: lower edge of endothelial covering in D, borders of subendothelial damage in F.