

P44

Ultrastructural Localisation And Quantification Of Xeno-antigen Galactose α 1,3 Galactose In Decellularised Porcine Valves

Padmini Sarathchandra¹, Patricia Taylor¹, Adrian Chester¹, Steven Goldstein², Al Heacox², Magdi Yacoub¹

¹Heart Science Centre/ Imperial College, Harefield/Middlesex, United Kingdom, ²CryolifeInc, Kennesaw, Georgia, United States

Objectives:

Decellularisation is a novel way of preparing porcine valve bioprostheses, utilizing reduction of cellular antigen rather than crosslinking by glutaraldehyde. If processing is successful, the xeno-antigen galactose α 1,3 galactose (α -gal) should not be present in these valves. This study evaluates the presence of α -gal in porcine decellularised valve leaflets and sinus wall tissue. Immunoelectron microscopy was performed to determine whether α -gal was localised within the cell or associated with extracellular matrix.

Method:

Non-decellularised/cryopreserved (n=2) and decellularised/cryopreserved/irradiated porcine pulmonary valves (n=2) were studied. Decellularisation was carried out by hypotonic lysis, DNaseI/RNaseA digestion and isotonic washout. Gamma irradiated valves were subjected to 25-40 kGy. The belly region of the valve leaflet and mid-sinus wall were processed into Lowicryl HM20 resin. Immunogold labelling was performed using an antibody to α -gal epitopes (M86) and anti-mouse IgM gold. The mean number of cell and matrix associated gold particles was determined.

Results:

Intact cells and cell remnants were evident in the decellularised samples suggesting the method of decellularisation was suboptimal. The number of cell-associated gold particles was significantly higher than the low number of matrix-associated particles ($p < 0.001$) in cusp tissue from both non-decellularised/cryopreserved and decellularised/irradiated/cryopreserved valves. α -Gal was predominantly localised within the cells in sinus adventitia and was not present in medial cells.

Conclusions:

This is the first report on ultrastructural quantification of α -gal in decellularised porcine valves prepared for clinical use. The persistence of α -gal despite a marked reduction in intact cells may be a contributing factor to immune system reactivity to such valves following implantation in humans.