**Cell counting analysis**

To evaluate the cellularity of the native ovine pulmonary roots (non-implanted), the implanted cryopreserved ovine pulmonary roots, and recellularisation of the explanted acellular porcine pulmonary roots, one section at each of the three levels from each explanted and non-implanted sample was stained with DAPI, or antibodies for cell markers (CD80, CD163, MAC, CD271, CTGF, CD3, CD19, CD34 and Ki67).

For counting of total cells (DAPI stained images) and cells labelled with antibodies to CD80, CD163 and MAC, fields of view (FoV) were selected using a pre-determined template from the three different levels of each pulmonary root assigned for analysis. Cell counting was performed in nine areas of the pulmonary wall (three areas of each of the adventitial, medial and intimal layers, each at three levels) and two areas of the leaflet at 100 x magnification using “Image J” software. The total number of cells in the DAPI stained sections were counted and all the cells expressing a given marker stained with antibodies were counted within each FoV. The area of a field of view was 0.576 mm2. The mean number of cells per FoV stained with DAPI (total cells) and antibodies to CD80, CD163 and MAC in the adventitia, media, intimal regions of the pulmonary artery wall and leaflets were calculated as indicated below. The mean number of cells per FoV were then multiplied by 1.74 to give the mean number of cells per mm2.

* Adventitia mean of cell count for FoV`s A level 1, 2 and 3; D level 1, 2 and 3 and G level 1,2 and 3
* Media mean of cell count for FoV`s B level 1, 2 and 3; E level 1, 2 and 3, H level 1, 2 and 3
* Intima mean of cell count for FoV`s C level 1, 2 and 3; F level 1, 2 and 3 I level 1, 2 and 3
* Leaflet mean of cell count for FoV`s K level 1, 2 and 3 and J level 1, 2 and 3.



**Scanned image of a section of native ovine pulmonary root showing the fields of view (100 x magnification) used for counting cell nuclei (DAPI) and positively labelled cells following immunohistochemical staining for cell markers. (**A) Distal wall adventitia, (B) Distal wall media, (C) Distal wall intima, (D) Mid-wall adventitia (E) Mid-wall media, (F) Mid-wall intima (G) Junction adventitia, (H) Junction media, (I) Junction intima, (J) proximal leaflet, (K) Distal leaflet.

For counting of cells labelled with antibodies to CD271, CD34, CTGF, CD3, CD19 and Ki67, fields of view (FoV) were selected using the same pre-determined template from the middle levels of each pulmonary root assigned for analysis. Cell counting was performed in nine areas of the pulmonary wall (three areas of each of the adventitial, medial and intimal layers) and two areas of the leaflet at 100 x magnification using “Image J” software. The total number of cells expressing a given marker stained with antibodies were counted within each FoV. The area of a field of view was 0.576 mm2. The mean number of cells per FoV stained with antibodies to CD271, CD34, CTGF, CD3, CD19 and Ki67 in the adventitia, media, intimal regions of the pulmonary artery wall and leaflets were calculated as indicated below. The mean number of cells per FoV were then multiplied by 1.74 to give the mean number of cells per mm2.

* Adventitia mean of cell count for FoV`s A level 2; D level 2 and G level 2.
* Media mean of cell count for FoV`s B level 2; E level 2 and H level 2.
* Intima mean of cell count for FoV`s C level 2; F level 2 and I level 2.
* Leaflet mean of cell count for FoV`s K level 2 and J level 2.