Table 4 Summary of recent approaches to LAHVA development

Cell source (VICs)	Cell source (VECs)	Scaffold material	Signals	Model / observation period	Functionality	Morphology / structure	Mechanical strength	ECM production / DNA content	Scaffold degradation time	Limitations	Reference
Ovine femoral artery smooth muscle cells / fibroblasts	Ovine femoral artery endothelial cells	Woven PGLA / non-woven PGA leaflet construct (3.2mm thickness)	-	*In vivo* allogeneic and autogeneic ovine pulmonary models / up to 21 days	Autogeneic leaflets shown to function appropriately using Doppler echocardiography	Shrinkage and deterioration of allogeneic leaflets Poorly developed matrix	Maximal tensile strength pre-implantation of 3.6MPa compared to 3.1MPa for native valve	30% of native valve collagen after 7 days No values reported for DNA content	Not reported	Serious infectious complications in allogeneic models Inflammatory reaction Moderate regurgitation	Shinoka *et al*., 1995
-	-	Valve matrix decellularised using hypo-/hypertonic KCl solutions, Triton X-100, SDS, DNase and RNase	-	*In vivo* allogeneic canine pulmonary model / explantation at 1 month	Leaflet motion demonstrated at 1 month	Partial endothelialisation Ingrowth of cells at leaflet base	Not reported	Not reported	-	Requires long-term follow-up studies	Wilson *et al*., 1995
Ovine femoral artery smooth muscle cells / fibroblasts	Ovine femoral artery endothelial cells	Woven PGLA / non-woven PGA leaflet construct (3.2mm thickness)	-	*In vivo* autogeneic ovine pulmonary model / up to 11 weeks	Competent in pulmonary circulation	Resemblance to native valve architecture	Maximal tensile strength of 2.68MPa after 11 weeks Increase in strength over time	Evidence for elastin and collagen production Increase in collagen content over time	Scaffold persisted for up to 6 weeks	Thickness and stiffness of leaflets Long-term growth and durability unknown	Shinoka *et al*., 1996
Mixed population of dermal fibroblasts and endothelial cells		Woven PGLA / non-woven PGA leaflet construct (3.2mm thickness)	-	*In vivo* autogeneic ovine pulmonary model / explantation at 8-10 weeks	Evidence for contracted immobile leaflets	Less organised structure than leaflets of arterial cell origin	Maximal tensile strength of 1.27MPa	Evidence for elastin and collagen production Collagen values approached 56% of native valve collagen	Scaffold persisted for up to 8 weeks	Thick and contracted leaflets Disorganised matrix Mild regurgitation	Shinoka *et al*., 1997
-	Human saphenous vein endothelial cells	Valve matrix decellularised using Triton X-100, DNase and RNase	-	*In vitro* xenogeneic porcine model / up to 3 days in culture	Not implanted	Grossly loosened matrix structure Confluent monolayer of viable endothelial cells	-	-	-	Presence of immume-stimulating cell remnants could not be excluded	Bader *et al*., 1998
Ovine carotid artery myofibroblasts	Ovine carotid artery endothelial cells	Trileaflet valve scaffold composed of non-woven PGA mesh coated with P4HB	*In vitro* pulse duplicator system – 14 days pre-conditioning	*In vivo* autogeneic ovine pulmonary model / up to 20 weeks	Synchronous opening and closing of leaflets Leaflets competent during valve closure	Tissue maximally organised after 14 days pre-conditioning Uniform laminated structure after explantation	Suture retention strength >50g after 14 days pre-conditioning Tensile strength 130% that of native tissue at 20 weeks post-implantation	180% of native valve collagen values after 8 weeks 150% of native valve DNA content, 140% of native valve GAG content after 20 weeks Elastin detectable by 6 weeks	Complete degradation of PGA by 4 weeks and of P4HB by 8 weeks	Moderate regurgitation reported	Hoerstrup *et al*., 2000
Ovine carotid artery myofibroblasts	Ovine carotid artery endothelial cells	Valve matrix decellularised using 0.05% trypsin and 0.02% EDTA under continuous shaking	-	*In vivo* allogeneic ovine pulmonary model / up to 12 weeks	Mild to moderate regurgitation in unseeded control valves Severe regurgitation in 1 of 6 animals in seeded group	Moderate thickening of valves in seeded group Complete endothelial lining at 4 and 12 weeks Repopulation of valve matrix	Not reported	Active matrix synthesis indicated by procollagen I-staining	-	Calcification of conduit tissue Inflammatory reaction Long-term fate of grafts remains unclear	Steinhoff *et al*., 2000
Ovine carotid artery myofibroblasts	Ovine carotid artery endothelial cells	Conduit: non-porous PHO film (240µm thick) between 2 layers of PGA felt (1mm thick) Leaflet: porous PHO film (120µm thick)	-	*In vivo* autogeneic ovine pulmonary model / up to 24 weeks	Valve competence demonstrated One non-functioning leaflet fused to conduit wall	Thickened laminated structure in conduit wall lined by endothelial cells Leaflets showed less tissue maturity than conduit wall	Not reported	Stainable collagen and PGs in leaflets reported No stainable elastin in leaflets	PHO material still evident in conduit and leaflets at 24 weeks	Long-term degradation period of PHO may have potential to augment host-tissue reactions	Stock *et al*., 2000
Ovine carotid artery myofibroblasts	Ovine jugular vein endothelial cells	Moulded porous PHO trileaflet valve scaffold	-	*In vivo* autogeneic ovine pulmonary model / up to 17 weeks	Synchronous opening and closing of leaflets	Smooth flow surfaces on leaflets and conduit walls Capillary ingrowth	Maximal tensile strength decreased from 967±99kPa (1 week) to 648±52kPa (17 weeks) Constructs became more elastic	116% of native valve collagen, 73% of native valve DNA content after 17 weeks	Only 30% degradation of scaffold after 17 weeks in vivo	Mild stenosis and regurgitation in all animals	Sodian *et al*., 2000
Human ascending aorta myofibroblasts	-	Moulded fibrin gel trileaflet valve scaffold	-	*In vitro* human model / up to 4 weeks in culture	Not implanted	Gross appearance comparable to native valve	Tissue could be sutured but strength was too low for direct implantation	Well-developed ECM with organized collagen bundles	Degradation rate can be controlled *in vitro* using aprotinin	Low initial stiffness	Jockenhoevel *et al*., 2001
Porcine thoracic aorta myofibroblasts	Porcine thoracic aorta endothelial cells	Type I collagen scaffold derived from bovine skin tissue	-	*In vitro* xenogeneic porcine model / up to 4 weeks in culture	Not implanted	Several layers of cells separated by extensive ECM	Not reported	Evidence for the production of PGs, and ECM proteins fibronectin and thrombo-spondin	Not reported	Confluent, intact endothelium not achieved	Rothenburger *et al*., 2001
Human bone marrow stromal cells (MSCs)		Trileaflet valve scaffold composed of non-woven PGA mesh coated with P4HB	*In vitro* pulse duplicator system – 14 days pre-conditioning	*In vitro* human model / up to 21 days in culture	Not implanted	Synchronous opening and closing of leaflets in bioreactor system Leaflets competent during valve closure	Conditioned leaflets displayed maximum stress 92±12% that of native leaflets, and Young's modulus 139±14% of native leaflets	Evidence for production of collagen types I and III Collagen content reached 25% and GAG content 37% that of native valve DNA content reached >300%	Not reported	Minimal characterization studies of MSCs before and after seeding of scaffolds Low ECM production	Hoerstrup *et al*., 2002
Mixed population of human umbilical cord artery, vein and Wharton's jelly cells		Scaffold patches composed of non-woven PGA mesh coated with P4HB	*In vitro* laminar flow system – 14 days pre-conditioning	*In vitro* human model / up to 21 days in culture	Not implanted	-	Layered tissue structure with irregular cellular ingrowth	Production of collagen types I and III GAG content reached 34% that of native pulmonary artery DNA content reached 361%	Not reported	'Mixed' cell population was used, with minimal characterization studies performed before and after cell seeding	Kadner *et al*., 2002

List of abbreviations: VICs: valvar interstitial cells; VECs: valvar endocardial cells; ECM: extracellular matrix; DNA: deoxyribonucleic acid; PGLA: polyglactin; PGA: polyglycolic acid; KCl: potassium chloride; SDS: sodium dodecyl sulphate: DNAse: deoxyribonuclease: RNAse: ribonuclease; P4HB: poly-4-hydroxybutyrate; GAG: glycosaminoglycan; EDTA: ethylene diamine tetraacetic acid; PHO: polyhydroxyoctanoate; PGs:proteoglycans