



Bone marrow mesenchymal stem cells for tissue engineered pulmonary valves (TEPV)

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Abstract

Heart valve tissue engineering and regeneration represents a novel, and viable alternative treatment modality for valvular heart disease. Tissue-engineered pulmonary valves (TEPV) have been constructed from autologous cells grown on 3D biodegradable scaffolds or decellularized xeno- or homografts and surgically implanted in animal models. From a translational stand point, constructed valves must maintain regenerative competency in recipients, with minimum risk for stenosis over the long term. Stem cells are an attractive cell source for this proposed solution; candidate cells are expected to maintain robust differentiation and transdifferentiation capacity. Transplanted cells must persist, engraft, and couple electro-mechanically with endogenous tissue. Here, we present a discussion on the clinical applicability of bone marrow mesenchymal stem cells in TEPV.

Keywords: Tissue engineered heart valves, stem cells, mesenchymal stem cells, cardiomyoplasty

Introduction

Valvular heart disease is a significant cause of morbidity and mortality; annually, there are over 100,000 valve replacements in the US [1]. Heart valve tissue engineering has captured the popular imagination of investigators and patients alike. Expectation and hope is however countered by realistic and legitimate concerns [1-9]. The fundamental objective is extension of life expectancy. Traditional surgical replacement takes the form of mechanical or biological valves [3]. Thromboembolic complications, the need for anticoagulation therapy, sub-optimal hemodynamic performance and prosthetic endocarditis [1-11] are a drawback to mechanical valves. Durability, deterioration, and the absence of growth which necessitates re-replacement is an issue for non-living biological valves [1-9]. Postoperative calcification which leads to valve degeneration is also a major issue for bioprosthetic heart valves. Glutaraldehyde fixation spurs cell devitalisation and facilitates the reaction of calcium-containing extracellular fluid with membrane-associated phosphorus to produce calcium phosphate mineral deposits [12-13]. Young recipient age, along with other factors such as mechanical stress is thought to enhance the calcification process [12-13]. As a result, there is a need for living autologous equivalents. This is especially true for congenital heart malformations in pediatric patients [14]. Tissue-engineered heart valves (TEHV) have emerged in recent years as a viable and logical treatment modality to meet this demand [1-11,13-14]. Tissue engineered pulmonary valves (TEPV) have been tested as a replacement valve in defective pulmonary valve (PV) outflow track models [15-18]. The thin tricuspid structured

PV is a semilunar valve. It lies between the pulmonary trunk and the right ventricle, and functions to prevent regurgitation of blood back into the right ventricle from the low-pressure pulmonary circulation [19-21]. The aortic and atrioventricular valves supersede the PV in terms of importance [19-20].

Stem cells have been used in tissue engineering of all three cardiac valves. While the discussion here is focused on the PV, bone marrow mesenchymal stem cells (BM-MSCs) can certainly be used for tissue engineering of the other three heart valves with equal success. The clinical sequelae following impaired PV function is life threatening [19-21]. Right sided-volume overload, and dilatation, supraventricular or ventricular arrhythmias, hepatic congestion, peripheral edema and subsequent heart failure (HF) result from the excessive regurgitation seen with an impaired PV [19-21]. PV insufficiency, regurgitation, and dysfunction of implanted biologic valved conduits which are designed to establish continuity between the right ventricle and the pulmonary artery remain a chief reason for reoperations in pediatric congenital cardiac surgery [16,20-22]. TEPVs represent a viable alternative to the current percutaneous approach to PV replacement [22]. From a tissue engineering perspective, it is quite complex to simultaneously meet all functional requirements of the heart. The specific tissue components of the valve leaflet, sinus and root, and pulmonary artery conduit each play a vital role with unique tissue requirements. Beyond the anatomic requirements, there are neurological and vasoactive requirements which the proposed engineered valve must respond to and replicate [2,23]. We have to be realistic about what is possible. The specific *in vivo* physiological and

anatomical requirements present a formidable hurdle when scaling up *in vitro* models in terms of design and subsequent performance. Practical issues must be considered—ideal scaffold thickness, and leaflet shape based on native PV functionality [24-28]. In this review, we focus primarily on being able to develop a stem cell based living PV replacement from BM-MSCs.

Review

In vitro modeling and fabrication

The drive towards clinical application of a stem cell based heart valve technology requires a thorough understanding and control over the modeling and fabrication aspect. The primary modes of heart valve fabrication have been constructs made via polymerization of biosynthetic material, decellularized xeno and allografts, and biodegradable scaffolds [1,6-8,28-29]. *In vitro* design and fabrication of TEPVs should be done from a translational perspective. The optimum replacement valve should be autologous—both the scaffold material and the seeded cells. The traditional design is for the scaffold to degrade over a period of time in a bioreactor system as the seeded cells develop into viable valve tissue [29]. The TEHV bioreactor provides mechanical, physiological and biochemical conditioning akin to the hemodynamic milieu of the heart [30-31]. The *in vitro* bioreactor system is designed to control all relevant cardiac variables—pO₂ and pCO₂ concentration, pH, temperature, metabolic requirements, and simulation of *in vivo* physiological hemodynamics [30-31]. Mechanical and physiological stimuli with regard to cytokine and growth factor secretion are integral components of heart valve development. A mechanically active milieu is critical for the appropriate gene expression profile and tissue maturation [29-32]. The advent of pulsatile dynamic bioreactors suits the need for mechanical conditioning within the context of heart valve tissue engineering [29-34]. The typical dynamic bioreactor system provides mechanical stimuli in the form of shear stress and strain as well cardiac parameters (stroke volume and cycle number) [30-32]. Eliciting physiologically suitable tissue formation and maturation of the valve leaflets requires continuous perfusion. It also requires dynamic strain over the duration of *in vitro* culture [29-30]. Fabrication and design of the trileaflet heart valve should factor in the effects of both flow over, and strain within the leaflets [29-31,34,36]. Both factors are known to govern tissue formation and development [29,34,36].

Collagen and elastin are key load-bearing proteins critical for mechanical and tensile strength of the valve leaflet [29-31,34-38]. Proteoglycans and hyaluronan in particular, form the greater portion of ground substance in the valve extracellular matrix (ECM) [29,34-38]. From a structural standpoint, glycosaminoglycans play an important role along with elastin with regard to stabilization of the valve cusps [39-40]. Hyaluronic acid, chondroitin and dermatan sulfates contribute to the mechanical behaviour of the extracellular matrix of native

valve cusps [39-40]. Their inherently large concentration of negative charges and subsequent hydrophilicity enables absorption of water [39-40]. They are therefore able to hydrate the spongiosa layer of the valve leaflet and decrease the stress associated with cuspal flexure [39-40]. Endocardial cushion formation in the cardiac outflow (OFT) tract initiates morphogenesis of the pulmonary and aortic valves [35-38]. Endocardial-mesenchymal transition leads to invasion of the trileaflet ECM by transformed atrioventricular (AV) canal cushion cells [35-38]. The AV cushion mesenchyme, under the influence of a variety of cytokine signals forms the basis for restructuring and elongation of the primitive valve structure into mature leaflets [35-38]. The exact mechanistic detail of leaflet elongation (refinement of leaflet shape, thinning, and tapering of the edges) [36] is not clear. Presumably, it involves proliferative and apoptotic events along with interactions between myocardial, endocardial and mesenchymal cells [35-38]. Fabrication must also take into consideration the differences in the shearing effects of flow. For example, the ventricular aspect of the valve leaflet is composed primarily of flattened endothelial cells due to the shearing effect of ventricular ejection [36]. In contrast, the arterial aspect consists primarily of cuboidal endothelial cells [36]. The prominent elastic lamellae of the ventricular aspect are also consistent with its continued exposure to the dynamic tension of systole [36]. On the other hand, the stagnant tension of diastole necessitates a primarily collagenous plate on the arterial side of the valve leaflet [36]. TEPVs are expected to persist, remodel and attain a degree of homeostasis in recipient hearts post transplantation. Design should therefore factor in anatomical and physiological determinants of valve growth. For example, a fundamental physical constraint on leaflet growth and elongation will be the diameter of the valve orifice [36].

Interestingly, Maron et al., [36] report the absence of capillaries or blood vessels and nerve fibres in developing embryonic and fetal valve leaflets which suggests that the leaflets and cusps can be nourished via diffusion. But adult human heart valves are known to have interlinking muscle and nerve fibres, and blood vessels [41-47]. The larger proportion of leaflet vasculature and innervation is situated close to the base and annular region of the valve leaflet [42,44-47]. Valve nerve fibres are a mix of thin non-myelinated and thick myelinated fibres [46-47]. The key question here is how to overcome the seemingly complex and specialized nature of the heart valve in our attempt to accurately recapitulate its structure and function. Decellularized PV scaffolds have emerged as a promising alternative to overcome the inherent microstructural complexity of the trileaflet valve [13,24,49-51]. Fundamentally, the technique of decellularization removes the cellular and antigenic components, leaving an intact ECM scaffold that can be repopulated with autologous cells [49-51]. The advantage to the decellularized PV scaffold is that it closely mimics the native valve in structure and function. It also enables regeneration, growth and potential endothelialisation by endogenous

cells [49-51]. Decellularized xenografts can however be problematic [52-55]. The main issues with transplanted decellularized TEPVs derived from xenogenous tissue are fibrosis, distal obstruction, inflammation, thrombogenicity, and immunogenicity, resulting in aneurysm, stenosis and regurgitation [52-54]. The vast majority of problems associated with transplanted decellularized PVs appear to be associated with the clinical introduction aspect. Issues associated with surgical implantation in terms of sizing, placement, anatomic and physiological dynamics and the valve's ability to merge harmoniously with the endogenous micro-milieu from a biochemical and hemodynamic stand point [52-55]. Not with standing, decellularized pulmonary valves such as the CryoValve SG pulmonary human valve have been previously cleared and are currently being studied prospectively to determine mortality, reoperation, structural deterioration, thromboembolism, conduit failure, calcification among other adverse events [56-58]. Replacement TEPVs developed from biodegradable scaffolds seeded with autologous cells have proven successful in large animal and human models [29,31, 59-61]. From a fabrication stand point, the scaffold design is critical; the 3D structure guides cellular migration, attachment and growth [62-64]. Ideal synthetic biodegradable scaffolds are typically porous and are designed to enable diffusion of both nutrients and waste products [29,59,65]. The ideal scaffold should be optimally biodegradable—the timing of absorption into endogenous tissue should match tissue formation. Scaffolds have been developed from polyglycolic acid (PGA), polylactic acid (PLA), combined PGA-PLA and polyhydroxyoctanoate (PHO) [29,59-65]. The advantage with these materials is thermoplasticity, biodegradability and biocompatibility [29,60-65]. Irrespective of the chosen *in vitro* modelling and fabrication system, the overarching question is the degree of *in vivo* persistence, homeostasis and remodelling. Specifically, we ought to have a clear picture of the time course of TEPV cell remodelling into the 3D biodegradable scaffold material following transplantation. Also of consequence are the specific morphological and molecular level changes associated with remodelling TEPV in recipients.

Bone marrow mesenchymal stem cells

Barring the determination of an ideal cell type, the concept of isolating a patient's own stem and progenitor cells, expanding them *in vitro* and creating a viable living valve replacement is feasible [66-67]. The ideal cell type need not be pluripotent; but must maintain multi-differentiation capacity with minimal risk for immunogenicity. The fundamental question with regard to determining the appropriate stem cell population is its ability to recapitulate the individual cell types of the pulmonary valve leaflet. Della Rocca et al., [68] showed that the largest density of cells in the PV leaflet is located in the anterior, posterior and septal cusps. The three layers of the valve leaflet (fibrosa, spongiosa and ventricularis) each express varying amounts of vimentin and non-muscle myosin which

suggest the presence of mesenchymal cell, smooth muscle (SM) and non-muscle cell phenotypes [68-69]. Overall, the PV leaflet consists primarily of SM cells, myofibroblasts and fibroblasts [68]. Myofibroblasts and SM cells take up the bulk of the fibrosa which is consistent with the pressure differences [29,68-71]. Fibroblasts localize predominantly to the ventricularis [68] and are chiefly responsible for the maintenance of the ECM [29,68-70]. In thinking about optimal seeding and layering methods, it should be noted that cell alignment and patterning in the valve leaflet is perpendicular to the direction of flow [29]. Seeded cells must quickly adhere, spread out, proliferate and subsequently develop into tissue [73]. From a structural standpoint, it is important to note that valve leaflet myofibroblasts express varying amounts of skeletal and cardiac muscle proteins (myosin heavy chain, SM myosin heavy chain, desmin, calponin, and α -smooth muscle actinin) [68,71]. Based on this cell phenotype, the traditional approach has been to isolate endothelial cells and myofibroblast-like cells from vascular tissue [66-67]. This cell population is however limited with regard to atherosclerotic and diabetic risk and the need to sacrifice intact healthy human vascular tissue [66,72]. There is therefore a need to determine an alternative and optimum cell source. A variety of different stem cell types have been tested—umbilical cord, chorionic villi, amniotic fluid, and bone marrow stem cells [66-67]. We make a case here for bone marrow derived mesenchymal stem cells (BM-MSCs).

Mesenchymal stem cells (MSCs) are a multipotent fibroblast-like non-hematopoietic stem cell isolated from a number of different tissue sources. BM-MSCs have been extensively studied and are currently being tested in several on-going clinical trials [74-76]. Positive expression of non-hematopoietic surface makers such as (CD105, CD90, CD106, CD73, CD44, CD29, etc) and null expression of hematopoietic cell surface markers (CD45, CD34, CD11b, CD14, etc) is a hallmark of MSCs [77-78]. Irrespective of tissue source, MSCs maintain extensive differentiation, proliferative and clonogenic capacity *in vitro* [78-79]. They differentiate into adipocytes, chondrocytes, cardiomyogenic cells (Figure 1), neurogenic cells, endothelial cells and have demonstrated angiogenic capacity [78-81]. Data shown in (Figure 1) demonstrates the ability of BM-MSCs to form mesodermal tissue—specifically mesenchymal, cartilaginous, ECM components, SM and non-smooth muscle which makes them a viable cell source for heart valve engineering. In terms of valve engineering, BM-MSCs are well fitted for this purpose. Isolation, purification and expansion to obtain clinically applicable numbers can be done in a matter of days (Figure 2) [78-80]. BM-MSCs are a heterogeneous population, very responsive to external stimuli, and easy to direct in terms of lineage specification [77-83]. Their ability to secrete an array of cytokines [80-81], and ECM proteins (collagen I, IV, fibronectin and laminin) [82] which drive the formation of an underlying framework for tissue shape and formation makes them a viable option

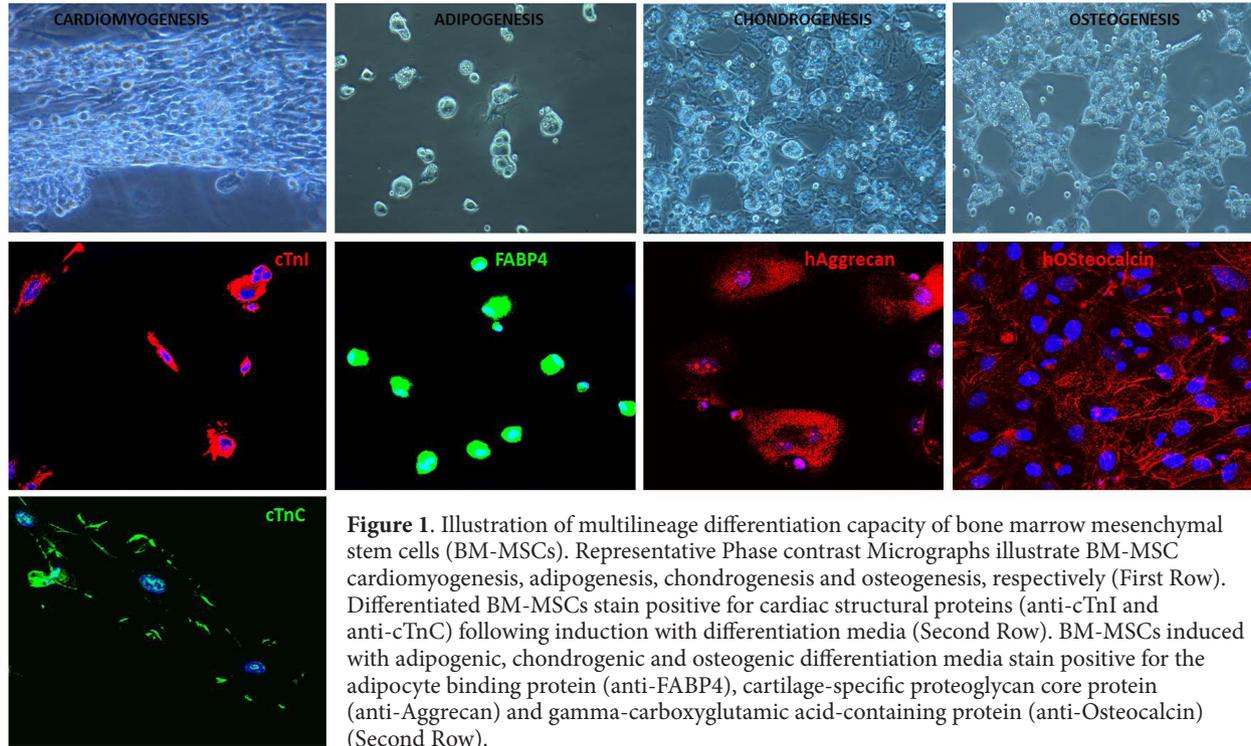


Figure 1. Illustration of multilineage differentiation capacity of bone marrow mesenchymal stem cells (BM-MSCs). Representative Phase contrast Micrographs illustrate BM-MSC cardiomyogenesis, adipogenesis, chondrogenesis and osteogenesis, respectively (First Row). Differentiated BM-MSCs stain positive for cardiac structural proteins (anti-cTnI and anti-cTnC) following induction with differentiation media (Second Row). BM-MSCs induced with adipogenic, chondrogenic and osteogenic differentiation media stain positive for the adipocyte binding protein (anti-FABP4), cartilage-specific proteoglycan core protein (anti-Aggrecan) and gamma-carboxyglutamic acid-containing protein (anti-Osteocalcin) (Second Row).

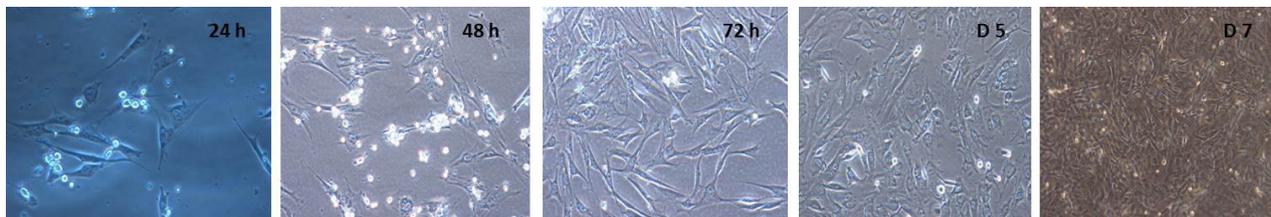


Figure 2. Representative Micrographs show bone marrow mesenchymal stem cells (BM-MSCs) at 24 hours (24h), 48 hours (48h), 72 hours (72h), 5 days (D 5) and 7 days (D 7) post primary isolation. BM-MSCs expand to over 90% confluency one week post isolation. BM-MSCs were isolated from Sprague Dawley rats (4 months).

[84-86]. BM-MSCs have a demonstrated ability to differentiate into each of the mesodermal lineages (mesenchymal, SM and non-muscle) [67-71] that constitute the valve leaflet [77-81].

BM-MSCs maintain a selective advantage in terms of genetic stability [86-87] and immunosuppression [88-89]. In determining an ideal stem cell source, we have to be mindful of the tumorigenic potential of stem cells [86-87]. The risk of spontaneous transformation by BM-MSCs to cause oncologic issues in the clinical setting has so far been negligible. The data for human BM-MSC tumorigenesis is murky and questionable at best. Uncontaminated, low passage BM-MSCs isolated from an appropriate source, expressing the appropriate panel of surface markers do not pose an oncologic risk [86-87]. The need for living tissue autologous equivalents in pediatric

valve replacement necessitates a cell with extensive long term expansion capacity and stable growth kinetics [6,14]. Fibrosis, inflammation, and immunogenicity with potential for aneurysm and stenosis are a major hurdle for valve engineering [8]. Vascular tissue derived endothelial and myofibroblast-like cells which have been traditionally employed pose an atherosclerotic and diabetic risk [72]. By virtue of their unique immune privilege and immunosuppressive ability [88-89], BM-MSCs might be the key to overcoming these barriers. They suppress the proliferation of T-lymphocytes, and express negligible and high levels of immunogenic MHC-Ia, and immunosuppressive MHC-Ib, respectively [88-89]. BM-MSC based engineered functional living heart valves have been developed and tested in large animal models [84-86]. These

preliminary studies show that, BM-MSCs pose no inflammatory risk. BM-MSCs are particularly responsive to mechanical and biophysical stimuli [83,90-91] which are crucial for *in vitro* structural valve design and patterning. Stress and geometrical variables (flow, flexure, and stretch), scaffold materials, and the mechanically active milieu of the bioreactor system each play distinct but interdependent roles that drive the growth process [84-86]. In scaling up from an *in vitro* system, BM-MSC engineered valves will have to respond to hemodynamic conditions in a way that ensures efficient opening and closure. This, along with differences in collagen levels, ECM patterns, and trileaflet stiffness are greatly influenced by mechanotransduction [83,90-91]. Central to this process are fundamental questions about BM-MSC differentiation into myofibroblasts and endothelial cells, and cell distribution within the leaflet structure. Also of importance is the ability of BM-MSCs to survive, proliferate, instigate angiogenesis and ultimately differentiate into functional valve tissue capable of withstanding *in vivo* mechanical and hemodynamic stress. The key to this is their collagen and elastin forming ability [4-7,82] which will determine long term survivability. Questions about cell seeding density, vessel type, and *in vitro* morphogenesis are easily addressed during the *in vitro* culture phase using pre-validated methods based on other adult stem cells.

A major challenge to the use of TEPVs in the clinical setting has been the need to answer questions pertaining to valve persistence, homeostasis and remodelling post implantation. There are still unanswered questions in terms of the trans-differentiation of BM-MSCs following transplantation. Elucidating the specific mechanistic details of BM-MSC biology on TEPVs following implantation is an even more important proposition. Addressing these issues requires a detailed mechanistic look at cells once the valve has been transplanted. There is a need to determine the exact location of BM-MSCs over time to ascertain whether they remain on the scaffold. Also of importance is the long-term quantification of seeded BM-MSCs to determine differentiation, survival and their interaction with the microenvironment. These are fundamental but critical questions; the advantage to BM-MSCs is that enough is known to enable detailed mechanistic studies of this sort. As with cellular cardiomyoplasty where

great strides have been made with regard to modes and routes of cell application, the engineering aspect of valve development with stem cells can be efficiently controlled. But a potentially limiting factor is longitudinally studying the BM-MSCs once the valve has been implanted in a living recipient. One option is to transfect cells with a reporter gene before valve implantation [92] and subsequently track the cells with positron emission tomography camera [93]. This can also be accomplished via visualization and cell tracking by MRI [94-95]. It is important to assess and quantify matrix elements such as collagen, elastin and glycosaminoglycans following transplantation. For example a comparison of native normal pulmonary valves to implanted equivalents should reveal degree of SM cell differentiation, macrophages, progenitor cells and monocyte formation. Basic mechanistic studies such as these are necessary; a determination of endothelialisation with CD31 and eNOS (nitric oxide), von Willbrand factor, and determination of levels of immune cell invasion by staining for neutrophils, monocytes and T cells with NIMP-1, Mac-1 and CD3 respectively [98].

Available stem cell sources for TEPV

(Table 1) A variety of stem cells derived from different sources have been used in TEPVs. The ideal stem cell need not be pluripotent but must maintain extensive differentiation and proliferative capacity which has been demonstrated in BM-MSCs (Figure 1). The prototypical cell source must also be well characterized; it has to be easily accessible and easy to culture to obtain clinically relevant numbers. BM-MSCs are particularly advantageous in this regard (Figure 2) [98-100]. From the standpoint of cardiac tissue specificity, cardiovascular progenitor cells taken from vascular structures have been extensively used for valve engineering [5-8,10,66-67,94]. The disadvantage with this cell source is that the population is mixed and extensive cell sorting and purification has to be carried out [10]. Furthermore, tissue source for vascular derived endothelial cells is scarce—more often than not, whole vessels must be sacrificed to obtain a large enough cell population [10]. The immunogenicity of this cell source means there is an increased risk of immune-rejection and possible thromboembolic events following transplantation

Table 1. Available Stem cell sources for pulmonary valve tissue engineering.

Stem Cell Source	Isolated Cell	Cell Phenotype	Reference
Umbilical cord blood	Mesenchymal Stem Cells	CD105+ CD54+ CD59+ CD29+ CD31- CD34- CD45- OCT4+	[66-67,105-108,112]
	Endothelial Progenitor Cells	CD133+ CD31+ CD34+ OCT4- Nanog-	[66-67,106,109-113]
Chorionic villi	Endothelial-Mesenchymal Progenitor Cells	CD105+ CD106+ CD146+ STRO1+ CD117- CD34-	[66-67,104,109-113]
Amniotic fluid	Embryogenic-Mesenchymal Cells	CD105+ CD29+ CD34- CD117- OCT4 SSEA+ Nanog+	[101-103]
Peripheral blood	Mesenchymal Stem Cells	CD133+ CD105+ CD44+ CD45- CD34- CD14- OCT4+	[1,66-67,105,108,112]
	Endothelial Progenitor Cells	CD133+ CD31+ CD34+ OCT4- Nanog-	[107,109-113]
Bone Marrow	Mesenchymal Stem Cells	CD105+ CD90+ CD44+ CD29+ CD45- CD34- CD31- OCT4+ SOX2+ Nanog+	[77-80,82-83,85]

[5-8,54,96-97]. In the presence of atherosclerotic and vascular disease, this cell source cannot be used, since vessels would have undergone atherosclerotic damage. A number of different stem cells derived from amniotic fluid have also been employed for heart valve engineering. Majority are of an endothelial-like progenitor lineage [5-8,10,66-67,101-103]. Typical amniotic fluid stem cells express CD29, CD166, CD44 and SSE4 along with a number of stemness genes such as NANOG, OCT-4 and SOX-2 [67,101]. Amniotic fluid stem cells also maintain extensive differentiation capacity [67,102-103]. This cell source appears to be the most ideal for pediatric heart valve engineering but unfortunately they are not well characterized and isolation requires invasive surgical intervention [67,102-103]. The issue of phenotypic characterization is critical because within amniotic fluid, mesenchymal stem cells, cells of endothelial-like progenitor lineage and mature endothelial cells can be isolated [67,102-103]. Cryo-preserved amniotic fluid stem cells have been tested in TEPV as a possible life-long cell source [66-67]. While amniotic fluid stem cells hold great potential for TEPVs, there are still unanswered questions about their growth kinetics, the effects of long term passaging and carcinogenicity [66-67]. Mesenchymal stem cells have also been isolated from foetal chorionic villi for heart valve engineering. The isolation process typically involves prenatal chorionic villus sampling and serial passaging to obtain a large enough cell population [5-8,66-67,104]. This cell population is also not well characterized and has been shown to contain foetal capillary endothelial cells [10,66-67]. Umbilical cord blood stem cells with fibroblast like features, expressing CD105, CD73, CD29 and CD13 and null expression of CD45 have also been isolated and used in heart valve engineering [10,66-67,105-108]. This cell source has properties akin to BM-MSCs and has thus been extensively employed in autologous valve engineering. The key barrier to the use of these cells in the clinical setting has been the issue of purification and *in vitro* culture expansion of the adherent cell population as opposed to the mononuclear population [66-67,105-109]. Endothelial progenitor cells have also been isolated from umbilical cord blood [66-67,105-113]. This particular cell source has shown tremendous promise for surface endothelialisation and covering of the valve leaflet during *in vitro* fabrication [10,66-67]. Despite their ease of isolation and expansion, it is still unclear if umbilical cord blood endothelial progenitor cells transdifferentiate into a bona fide mesenchymal-myofibroblast-like phenotype which is critical for heart valve tissue engineering [66-67,105-113]. The main drawback to stem cells derived from amniotic fluid, chorionic villi and umbilical cord is that accessibility is potentially invasive and dependent on pregnancy. Isolated stem cells must be cryo-preserved for long term use which is also predicated on the assumption that they will remain viable over the long term. Induced pluripotent stem cells (iPS) [114] are also a possible alternative but their inherent genome instability and risk of tumorigenesis makes BM-MSCs

a superior choice from a clinical standpoint.

The key issue with each of the outlined cell sources in comparison to BM-MSCs is accessibility for primary isolation and *in vitro* culture, and availability in clinically relevant numbers. Accessing tissue for cell isolation requires potentially invasive clinical procedures which pose a risk to the developing foetus and mother. If the goal is to develop an autologous valve for pediatric patients, then isolation can only be carried out at birth or during pregnancy in the case of cells derived from amniotic fluid, chorionic villi and umbilical cord blood [66-67]. Phenotyping is also an issue—exactly what markers define the majority of these cell populations is still unclear and there is no definitive differentiation protocol to test multilineage differentiation capacity in most cases. The advantage that BM-MSCs have over these other stem cell sources is that they have been extensively and rigorously tested through the pre-clinical and now the clinical stages. Overall, our knowledge of these stem cells at the mechanistic level is still patchy, but we know enough about BM-MSCs that their use in the clinical setting for heart valve engineering makes sense. In general, stem cells are a very sensitive cell population and the risk of uncontrolled growth along with genetic alterations should make us wary of using untested cells. There is a need to increase our knowledge of how these cells behave specifically when applied to biodegradable or decellularized scaffolds in terms of inflammation, toxicity and interaction with the specific components of the scaffold [5-8,10,66-67]. As discussed, BM-MSCs are not without issues—oncogenesis, and questions surrounding transdifferentiation post transplantation are the key issues from a clinical standpoint. But in terms of our understanding of their basic cell biology and mechanism of action, more is known about BM-MSCs which makes their use in this technology practical. BM-MSCs are currently being tested in several on-going clinical trials for their ability to restore function and regenerate tissue post myocardial infarction. The drive towards their clinical application in heart valve tissue engineering is therefore a realistic goal that could occur in the next five to ten years.

Conclusion

Clearly, congenital heart valve disease in pediatric patients is an issue [1-15]. For example left and right heart failure resulting from pulmonary and aortic stenosis and ventricular dysfunction post pulmonary valve regurgitation in conditions such as tetralogy of Fallot [16-22]. Transitioning from *in vitro* development and fabrication of replacement TEPVs to their clinical application will require a realistic and thoughtful approach. One that takes into consideration the potential *in vivo* risks—in the case of MSCs, calcification, ectopic tissue formation, and aging, transdifferentiation into the appropriate cell type, survival and engraftment are potential issues. There is still a major question mark on the issue of BM-MSC transdifferentiation into viable and functional valve tissue [96]. An alternative to *in vitro* fabrication in the bioreactor

model as demonstrated by Vincentelli and colleagues [85] is direct injection of BM-MSCs into decellularized scaffolds prior to implantation. The idea here is that pre-seeding just before implantation will enable valve tissue formation under the appropriate physiological conditions [85]. Additionally genetic engineering and growth factor based systems can be used to enhance BM-MSC formation of valve tissue [85]. Overall, fabrication of PV leaflets using BM-MSCs as the prime cell source is feasible; the clinical application of such valves as autologous replacements both in pediatric and adult patients is a practical therapeutic option.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	FZA	VL
Research concept and design	✓	✓
Collection and/or assembly of data	✓	--
Data analysis and interpretation	✓	--
Writing the article	✓	--
Critical revision of the article	✓	✓
Final approval of article	✓	✓
Statistical analysis	✓	--

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