Scisense PV Technical Note

Cardiac Hemodynamic Assessment using PV Loops in Stem-Cell Research and Tissue Engineering

The heart has unique biomechanical properties. Each ventricular chamber (LV and RV) has a slightly different muscular/extracellular matrix (ECM) composition. The cellular composition of the heart also varies between species based on the amount of cardiomyocytes vs. supporting cells and also based on the amount and composition of the ECM (13). Studies show that cardiomyocytes behave poorly on man-made ECM surfaces (14). For that reason it is important to mimic the native cardiac mechanical environment.

The function of the supporting ECM is to stop or slow down the remodeling and scar formation process by preventing the dilation of the heart muscle chambers. Meanwhile, delivered cells replace the dead cardiomyocytes/supporting cells and integrate with the neighboring cardiac tissue. The supporting ECM should have niches to sustain the survival of the delivered cells to facilitate regeneration process.

The definitive objective of post-MI therapy is to attenuate the remodeling process and regenerate the new cardiomyocyte/support cell based muscle. This can be achieved by a cell delivery system consisting of a supporting matrix and suitable cells. Currently, most strategies fail to address all the important factors for successful regeneration including: the loss of cardiomyocytes, attacks of inflammatory cells on unprotected vulnerable tissue, cell isolation and expansion, immunogenicity of grafted cells or matrix, cell survival, biomechanical/electrical coupling properties of the tissue constructs, cytotoxicity levels and degradation properties.

The active mechanical function of cardiac tissue is mostly delivered during systole. If the myocardium is replaced by a noncompliant scar tissue systolic contraction is decreased. At the same time, not only contraction but also relaxation (diastole) is affected by the inability to accommodate all of the blood volume inside the cavity as the heart chamber stiffens. Using invasive PV hemodynamic assessment within stages of regenerative therapy, especially the load-independent parameters and contractility, increases the ability to properly measure and compare pathophysiological cardiac function.

As delivered cells have very low retaining capacity, researchers are making a variety of biomaterial scaffolds. Biomaterials often exhibits an intrinsic stiffness that may compromise diastolic function. Biodegradation of the scaffold materials often remains incomplete, adding to the potential problems with diastolic function.

Diastolic dysfunction (DD) is characterized by myocardium that has decreased ability to generate force and is unable to accept an adequate volume of blood during diastole at normal diastolic pressure. This results in an inability to maintain stroke volume (SV). Degradation of scaffold causes:

- Poor relaxation (impaired lusitropy)
- Decreased compliance

DD occurs when these scaffold degradation processes are prolonged, slowed and/or incomplete. DD generally depends on the onset, rate and extent of decline of pressure in ventricles and the relationship between pressure and volume, stress, or strain during diastole.

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Hemodynamics in Cardiac Tissue Engineering Cont.

PV CHARACTERISTICS OF DD (LV LOAD-DEPENDENT MEASUREMENTS):

- LV EDP (end diastolic pressure) is increased with diastolic dysfunction as compared to healthy control. As the LV EDP rises left atrial and pulmonary venous pressures rise leading to pulmonary congestion and edema.
- Depending on the relative changes in SV and EDV, small decrease in EF and CO can be also observed. Because SV is decreased, decrease in ventricular SW can be also noticed on PV loop examination.
- Minimal/maximal rate of LV pressure change (dP/dt_{min}, dP/dt_{max}) is decreased.

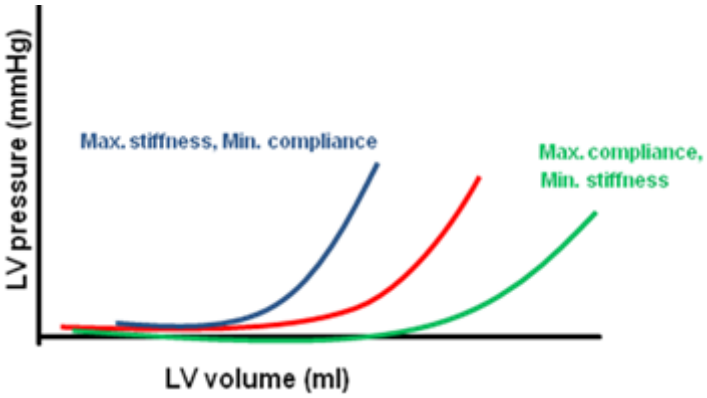

Fig. 1: Diastolic dysfunction (DD) impacts load independent properties of the left ventricle (EDPVR) during cardiac diastole characterized by the compliance and stiffness.

PV CHARACTERISTICS OF DD (LV LOAD-INDEPENDENT MEASUREMENTS):

- EDPVR (end diastolic pressure volume relationship) represents relation between EDP-EDV points described by LV PV relationship (Fig 1). EDPVR is characterized by initial large increases in volume at low pressures. As volume increases further past the initial stage, pressure raises rapidly while volume increases slow as it is restrained by native ECM (e.g. collagen, proteoglycans, and glycoproteins). EDPVR curve fits are discussed in more depth in the publication by Burkhoff from 2005 (15). The EDPVR fits a non-linear curve that represents the diastolic stiffness (inverse of diastolic compliance) having the exponential fit EDP=A*exp (k*EDV), where k represents chamber stiffness or diastolic stiffness constant. K represents EDPVR slope, the change of ventricular pressure relative to a change in volume of the ventricular chamber (dP/dV).
- When a tissue engineered construct is attached to the LV, compliance of the chamber often decreases. This overall chamber stiffening leads to a decrease of myocardial relaxation properties called lusitropy. Lusitropy is characterized by unwinding of individual sheets of myocardium proceeding into partial or complete relaxation. For more about lusitropy please see “Understanding Lusitropy (RPV-8-tn).” The unique lusitropic properties of myocardium change during heart development and aging; further challenging tissue construct site selection and final implantation.
- Isovolumic relaxation time (IVR) is the time from aortic valve closure to mitral valve opening. During DD, IVR might be prolonged. IVR or Tau (isovolumic pressure decay) is caused by uncoupling helically woven layers of myocardial fibers (including extracellular matrix) assembled in linked sheets. Myocardial fibre arrangement generates unique (heart specific) relaxation pattern accounting for observed IVR pressure gradient during LV emptying and filling. Therefore, IVR (Tau) will increase post-cardiac patch implantation as compared to non-injured heart. For more information about IVR please see Tech Note: “Understanding Lusitropy (RPV-8-tn).”
- Ventricle elastance (Ees) describes the transmission of mechanical energy from the ventricle into the arterial system. Effective arterial elastance (Ea) can be derived from the ratio of ESP to SV (Ea = ESP/ SV). Healthy arterial system works with maximum coupling efficiency, where Ees/ Ea = 0.3 to 1.3 (16). However, values outside of this range have to be thoroughly examined before deemed pathological. This unitless ratio of coupling (Ees/ Ea) increases during diastolic insufficiency since both the systolic and diastolic ventricular efficiencies decline while there is an increase in afterload.

Hemodynamics in Cardiac Tissue Engineering Cont.

Difficulties and Considerations for Development of Functional Myocardium

CELL SOURCE & TYPE

CATEGORY	TYPE	LIMITATION
Source of cells	Autologous	Difficult to harvest in numbers
	Allogenic	Immunology roadblocks
	Xenogenic	Rejections
	Syngenic	Cloning, limited translational value
Type of cells	Harvested primary cells	Difficult to expand/organ specific
	Secondary from cryopreserved cell banks	Immunology roadblocks
	Adult stem cells	Source and type to use
	Embryonic stem cells	Purification, potential malignancy

AMOUNT & METHOD OF CELLS TO DELIVER

Some investigators use delivery of isolated stem cells (1-6), others use in vitro-designed tissue equivalents (7-10).

Cells delivered without a scaffold (intravascular, intracoronary, intramyocardial, transendocardial, epicardial) are prone to large losses. Within minutes 85-90% of cells injected intravascularly are lost, almost all cells are trapped in lungs (11) with less than 1% found in heart (12). While, larger animal model cell-retention rates are usually higher, optimal delivery method is still elusive.

Isolated stem cell (derived-cardiomyocytes, skeletal myoblasts, fibroblasts, mesenchymal, adipose stem cells etc.) delivered directly to the infarct site have low cell survival and poor cell engraftment due to a lack of functional vasculature at the implant site, inflammation, and constant tissue remodeling (7, 17).

TIMING OF CELL DELIVERY

Timing of cell delivery is impacted by animal model/physiology and therapeutic target (limiting scar extension, limiting inflammation, improving angiogenesis, vascularization). The best timing of implanted cells delivery is still under discussion as all above-mentioned factors that are in play (1,5, 6, 8).

METHOD OF GROWING CELLS

Despite being able to grow functional cardiomyocytes in culture, the re-establishment of a contracting cardiac tissue (patch), including cardiac fibroblast and endothelial cells, is still elusive. Cardiac myocytes cultured in the standard 2D culture with the presence of growth-promoting medium lean towards dedifferentiation and are often overgrown by non-myocytes. This has been largely overcome by using 3D culture environment. Additionally, the important influence of active or passive forces on cardiac myocyte growth, morphology, orientation, gene expression etc. has been demonstrated (7, 17). Substrates with a stiffness very close to that of the native adult rat myocardium were found to be favorable for heart cell morphology and function seen by cellular elongation, high contractile force and striations development (14).

Hemodynamics in Cardiac Tissue Engineering Cont.

TYPE OF SCAFFOLD

Most cardiac tissue engineering groups use scaffold proteins (e.g. collagen, gelatin, laminin, matrigel, hyaluronic acid (hyaluronan), alginate, and chitosan) or synthetic polymers (e.g. polylactic acid and polyglycolic acid) for tissue reconstitution from isolated cells.

Even the more common scaffolds, such as fibrin gel and matrigel, are far from ideal for cardiac tissue engineering. As the gelation rate of fibrin is slow and it lacks sufficient mechanical strength there is a loss of delivered cells and low cell retention during injection. Its breakdown during heart contraction and relaxation is another downside along its high innate fibrinolysis rate. In case of matrigel, a biosafety concern exists, as it is derived from tumors (17).

Stabilization of the infarct area is the key concept for scaffold cellular delivery along with cell retention in the area. Temperature sensitive hydrogels mixed with variety of pro-angiogenic factors are a promising scaffold option (8).

SEEDING & GROWING CELLS ON SCAFFOLD

Tissue engineered cardiac patches (implanted tissue graft seeded with cardiomyocytes) have much less compact myocyte bundles as compared to native myocardium with less ability to generate necessary contractile force, often with non matured M bands. Core ischemia of the implanted graft seeded with cells often occurs. Cardiomyocytes incorporated on or in gelatin meshes form a thick cell layer only on the outside without a homogeneous cell distribution. Theoretical nutrient diffusion limit is 100-200 µm, but the limit is lower for more specialized cells such as cardiomyocytes (7). Substrate selection is also an important determinant of cell phenotypic development (14).

ELECTRICAL COUPLING

Despite observing implanted cell endurance and differentiation, mechanical and electrical cell-cell contacts between graft-and-host, required for synchronous contractions, are only rarely observed. Scar tissue appears to account for this problem by inhibiting contact between grafted cells and host tissue.

Inability to reproduce propagation of action potential (AP) is another concern. Action potential (current) propagates from SA (sinoatrial) pace-making node by intracellular channels (gap junctions). Most cells that are injected into the damaged SA node are not retained and might become pro-arrhytmic (18).

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Hemodynamics in Cardiac Tissue Engineering Cont.

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