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Advancing the Assessment of Cardiac Injury and Function

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Editorial

While heart transplantation is the most effective therapeutic modality for end-stage heart failure, less than 50% of potential donors become actual heart donors. The main cause of donor heart underutilization is the maximum 4 h ischemic time allowed during mechanical arrest with cold storage, which limits the geographic distribution of hearts due to transportation time limitations. Importantly, prolonged ischemic time is an important risk factor for primary graft dysfunction (PGD), when the donor heart output is insufficient for organ perfusion. PGD is a significant challenge that occurs in 10%–20% of heart transplants. While the 1 month heart transplant mortality rate is approximately 8%, PGD accounts for 39% of these deaths.

The recent clinical use of commercially available normothermic *ex situ* perfusion devices has extended the "out of body" time of donor hearts while reducing the cold ischemia time. This technology has yielded significant benefits in terms of allowing donors to be matched with recipients over long geographical distances. *Ex situ* perfusion has also facilitated the use of "marginal" donor hearts with features such as ventricular hypertrophy, longer ischemic times, and older donor age. In a recent clinical trial of an *ex vivo* cardiac perfusion system, the rates of severe left ventricle (LV) or right ventricle (RV) PGD remained high at 10.7% for deceased brain death (DBD) donor hearts despite normothermic cardiac perfusion during organ transport to the recipient hospital[1]. Indeed, the rates of severe PGD requiring mechanical circulatory support are even higher for deceased cardiac death (DCD) donor hearts. Furthermore, continuous hypothermic perfusion of the donor heart with cold preservation solution has been utilized for transport. These novel approaches center around perfusion under several conditions: perfusion with autologous blood or preservation solution, perfusion at normothermic (37 °C) or cold (4–10 °C) temperatures, and perfusion *ex situ* using a pump apparatus or *in vivo* using normothermic regional perfusion (NRP). The precise clinical advantages of certain approaches over others are not clearly defined, but research models will allow the systematic study of each combination of approaches.

The use of murine cardiac assessment models has the advantage of the availability of a wide range of transgenic mice models for the detailed study of the molecular mechanisms of PGD and other conditions involving impaired cardiac function (e.g., myocardial infarction). Li et al.[2] describe an elegant technique for transplanting murine donor

hearts using a "cuff" technique in the cervical region of the recipient mice. This has significant advantages in terms of the technical ease of performance, the accessibility for graft evaluation, and the ability to carry out intravital two-photon imaging (e.g., for the tracking of leukocytes). Noly et al.[3] also report the use of mice in *ex situ* studies of cardiac function, with the similar advantage of access to a broad array of transgenic models. *Ex situ* perfusion with Krebs buffer allows the study of the preservation biology of the native cardiac cells without recipient cellular responses. This will allow researchers to further examine the preservation biology of the native heart without infiltrating leukocytes adding complexity to the reperfusion event. Furthermore, Noly et al.[3] illustrate a method for assessing the cardiac function of both the left and right heart using a small balloon in this *ex situ* model. Given the frequency of isolated right heart failure in clinical heart transplantation, this is a very relevant technique for assessing univentricular versus biventricular graft failure.

DCD heart donation is gaining momentum as an important source of donor hearts for transplantation. The rat DCD heart transplant model presented by Quader et al.[4] provides an important medium-sized animal platform for studying DCD donor hearts and allows for the testing of a variety of agents that may mitigate cardiac injury during DCD donation. The rodent DCD donation protocol mirrors that of clinical organ procurement procedures. Rodent models of cardiac assessment are critical for studying molecular mechanisms and screening for potentially efficacious therapeutics, and they have the advantage of scale in terms of allowing for the use of relatively large experimental groups. However, whether the insights obtained from rodents are relevant for human patients is a valid concern. Thus, large-animal (including human hearts used for research) platforms serve to validate the knowledge gained from smaller-animal models. Khalil et al.[5] describe a porcine model of NRP with pig hearts using extracorporeal membrane oxygenation. Their group also highlights important differences from human heart responses regarding cardiac arrhythmogenicity. This system provides a means to test various pharmacological and nonpharmacological strategies to rehabilitate DCD hearts for transplantation in this setting. Dang Van et al.[6] further add to the sophistication of the hemodynamic assessment of large-animal hearts by incorporating pressure-volume loops using conductance catheters and surface echocardiography. NRP has the distinct advantage of allowing the assessment of hearts in "loaded" working conditions compared with the "nonworking" Langendorff setups using conventional *ex situ* perfusion.

Aside from the study of whole hearts, contractile function can be evaluated at the single-cell level, as illustrated by Lavey et al.[7]. Sarcomere shortening and calcium fluxes can be measured with great accuracy in single myocytes. Molecular editing at the cellular level can be achieved efficiently using various gene transfer techniques (e.g., viral transfection), and the effects can be evaluated at the cellular level. This is an important screening tool for novel therapeutics and for understanding the underlying mechanisms of cardiac dysfunction. Bermea et al.[8] provide a reliable method for studying immune components and their role in mediating cardiac injury. The ability to capture all the immune cells of interest in the heart is a prerequisite for developing an accurate understanding of the immune responses to cardiac injury. The described techniques can be combined with the aforementioned *ex situ* and NRP cardiac platforms for the study of immunity in cardiac preservation, injury, and repair.

This methods collection collates some of the state-of-the-art techniques for the assessment of cardiac injury and function.

While many of the models are designed for the study of PGD following cardiac preservation, this collection remains relevant for the study of cardiac function in other settings (e.g., global or regional warm ischemia, genetic cardiomyopathies). We are optimistic that these research tools will improve the understanding of PGD and facilitate the discovery of novel therapeutics to preserve cardiac function following injury.

Disclosures

The authors have nothing to disclose.

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