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EDITORIAL COMMENT

Exploring Cardiac Fibrosis

A Novel Ex Vivo Model Using Whole Mouse Hearts

Rudolf A. de Boer, MD, PhD,[a] Reza Ardehali, MD, PhD[b]

When the heart is injured, its intricate network of cells and blood vessels undergo substantial remodeling, resulting in changes to both its physical structure and its function. Pathological stress from injury or sustained pressure leads to the proliferation and activation of cardiac fibroblasts, ultimately leading to interstitial and/or perivascular fibrosis and adverse remodeling. Pathological stressors vary widely and can include mechanical stress and biochemical signals, which in concert trigger cardiac fibrosis. Various stressors activate multiple signaling pathways, yet many converge via the transforming growth factor beta pathway.[1] This pathway is considered as an integral molecular component that initiates and coordinates myocardial fibrosis. Historically, researchers have employed lineage tracing experiments alongside in vitro assays to explore the cellular contributors to cardiac fibrosis and the molecular pathways governing this phenomenon.[2]

Therefore, fibrosis has been considered a primary culprit and a target for treatment in adverse cardiac remodeling and heart failure. However, its origin, complexity, and progression remain only partially understood.[3] Despite the therapeutic advancement in heart failure treatment, including successful pharmacological and device therapies, none target myocardial fibrosis specifically. There are several knowledge gaps, particularly concerning time-dependent changes, where various cells and glycoproteins participate in the fibrotic process at different stages, posing challenges in fibrosis research. In addition, the complex cell-cell interactions preclude simple modeling, such as cultured fibroblasts. Genetic differences may also explain the various degree of fibrotic response.[4] Finally, different etiologies underlying the remodeling process and different comorbidities may also explain, at least in part, the complexity of fibrosis. Several animal models, especially mouse and rat models, have been used in the study of myocardial fibrosis. However, the intrinsic limitations of such models are well-known: they are expensive, time consuming, and most studies are performed in young animals, without background therapies and comorbidities, limiting their translational capacity. There is a clear need for more high-throughput in vivo systems to accelerate the study of myocardial fibrosis.

The study by Kruithof et al[5] in this issue of *JACC: Basic to Translational Science* offers a comprehensive exploration of cardiac remodeling in ex vivo cultured hearts. They adapted the Miniature Tissue Culture System to examine changes in cellular composition, extracellular matrix remodeling, and signaling pathways associated with cardiac fibrosis. The ex vivo heart culture was used to illustrate the following points. 1) Myofibroblasts originate from resident proliferating fibroblasts rather than other cell types. 2) Mechanical stressors within this ex vivo heart system provoke a fibrotic response and are related directly to the degree of fibrosis. 3) Activation of transforming growth factor beta signaling induces myocardial fibrosis, and pharmacological inhibition of this pathway can mitigate fibrosis in this model. Kruithof et al[5] provide extensive experimental proof, with controlling for several potential confounding factors. Although these discoveries have been shown previously through conventional in vivo and in vitro models, their innovative ex vivo system presents a valuable instrument for dissecting the intricate interaction of cellular responses

From the [a]Cardiovascular Institute, Thorax Center, Department of Cardiology, Erasmus Medical Center, Rotterdam, the Netherlands; and the [b]Department of Medicine-Cardiology, Baylor College of Medicine, Texas Heart Institute, Houston, Texas, USA.

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TABLE 1 Scoring Table of Different Experimental Fibrosis Models

	Costs	Time Dependency	Cell-Cell Interactions	Possibility to Evaluate Drugs	Overall Translational Capacity
Cultured fibroblasts	+	+++	-	++	+
Ex vivo model (Kruithof)	++	++++	++	+++	+++
In vivo models (animals)	++++	++++	++++	+++	++++
Human biopsies	+++	++	++++	+++	NA

Various aspects (Costs, Possibility to Study Time Dependency, Possibility to Study Cell-Cell Interactions, Possibility to Evaluate Effects of Drugs, and Overall Translational Capacity) of 4 experimental systems to assess cardiac fibrosis: Cultured (Cardiac) Fibroblasts, the Ex Vivo Model by Kruithof et al,[5] Animal Models, and Human Cardiac Biopsies. (-): not possible; (+) minor; (++) moderate; (+++) substantial; (++++) very strong.

NA = not applicable.

and molecular signaling pathways underlying cardiac remodeling.

Indeed, the establishment of a novel ex vivo model and its comprehensive characterization of fibrotic remodeling represents a significant advancement in the field of cardiac fibrosis research. This study offers an innovative tool to investigate the molecular and mechanical factors involved in cardiac fibrosis. It establishes a novel ex vivo fibrosis model for intact mouse hearts, providing a platform to study cardiac fibrosis in a controlled environment outside the body. This model enables researchers to induce fibrosis in initially healthy hearts, replicating pathological conditions observed in vivo, and examine progression of fibrosis while exploring potential therapeutic interventions. This work could facilitate the development of novel treatment approaches for cardiac fibrosis, addressing an unmet clinical need.[6] Although, this study uses mouse hearts that may not mimic human hearts perfectly, some aspects of their findings, particularly regarding collagen expression patterns and myofibroblast activation, are consistent with observations in human cardiac fibrosis, enhancing the translational relevance of the ex vivo model.[7]

Although the ex vivo fibrosis model described by Kruithof et al[5] provides valuable insights, it may not replicate the complexity of in vivo cardiac fibrosis fully. The absence of physiological factors such as neurohormonal regulation, systemic inflammation, and dynamic changes in blood flow could limit the generalizability of the findings. Clearly, systemic effects of comorbid conditions associated with fibrosis (diabetes, renal disease, aging, obesity) cannot be recreated in this model. Additionally, the culture duration, although sufficient for observing initial fibrotic changes, does not capture the long-term effects or chronic fibrotic processes seen in vivo. Prolonged culture periods or repeated interventions may provide a more comprehensive understanding of fibrotic progression and potential therapeutic interventions.

The authors showed that the extent of cardiac fibrosis depends on the level of mechanical stress on the ex vivo cultured heart. However, the model may oversimplify the mechanical environment compared with in vivo conditions. Factors such as cardiac contractility, hemodynamic forces, and tissue stiffness may interact in complex ways to influence fibrotic remodeling. This system could offer the platform to identify and validate biomarkers of cardiac fibrosis and correlating with clinical settings.

This study may also open several areas for future research and development. First, the refinement of the ex vivo heart culture system with improvement in its physiological relevance and reproducibility is necessary. This work could involve optimizing culture conditions, such as perfusion parameters and media composition (ie, the inclusion of the immune system and inflammatory response), to better mimic the in vivo cardiac microenvironment. Additionally, investigating the long-term effects of fibrotic remodeling in the ex vivo heart culture could provide valuable insights into the progression and chronicity of cardiac fibrosis. This, in turn, could offer a valuable tool for preclinical screening of potential drug candidates and targets. One can envision development of high-throughput screening platforms to assess the efficacy of various compounds in inhibiting fibrosis progression in a controlled environment. Finally, integrating advanced imaging techniques, such as live-cell imaging and multiphoton microscopy, with the ex vivo model could provide real-time visualization of fibrotic processes at the cellular and subcellular levels. This would enable dynamic monitoring of fibrosis progression and response to therapeutic interventions.

We believe that this innovative system provides an excellent platform to advance myocardial fibrosis research. In effect, future studies of myocardial

fibrosis may use such ex vivo systems with validation of key findings obtained from in vivo models (Table 1). Given the global importance and presence of the fibrotic reaction in response to organ damage, targeting organ fibrosis has the potential to become an effective therapeutic modality, for myocardial fibrosis, but also pulmonary fibrosis, liver fibrosis, and kidney fibrosis.[8]

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ADDRESS FOR CORRESPONDENCE: Dr Rudolf de Boer, Erasmus University Medical Center, Department of Cardiology, Dr. Molewaterplein 40, PO Box 2040, Rotterdam, Zuid Holland 3015 GD, the Netherlands. E-mail: r.a.deboer@erasmusmc.nl.

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