University of Texas Congenital Heart Regenerative and Restorative Therapies Laboratory

Cellular Signaling and Stem Cell Therapy in Congenital Heart Disease

Graeme-McDaniel Foundation (GMF) Research Grant Progress Report

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Background:

Endocardial fibroelastosis (EFE) is defined as collagen and elastin deposition within the endocardium. EFE primarily affects the immature left ventricle and left atrium but has also been described in the right ventricle[1-3] or involving only the left atrium.[4] On gross examination of the heart cavity, the endocardium looks opaque or pearly white as it loses its transparency.[5] Although linked to many diseases, distinct pathophysiologic mechanisms still need to be determined. The term "EFE" was coined in 1943, but there are other names including endocardial sclerosis, fetal endocarditis, idiopathic cardiac hypertrophy, cardiac enlargement of indeterminate cause, or congenital anomaly of the endocardium.[6-8] The layer of EFE causes diastolic stiffness and systolic impairment often resulting in congestive heart failure and sudden death in early infancy or childhood.[9-10] No definitive treatment is currently available.

Gap in Scientific Knowledge:

The exact pathophysiologic pathways driving the formation of EFE is unknown. There have been studies showing an endocardial-mesenchymal transition that occurs and appears to be mediated by the TGF-β/BMP inflammatory pathway (below). However, this fibrotic process continues to be poorly understood.

Specific Aim (Progress Report):

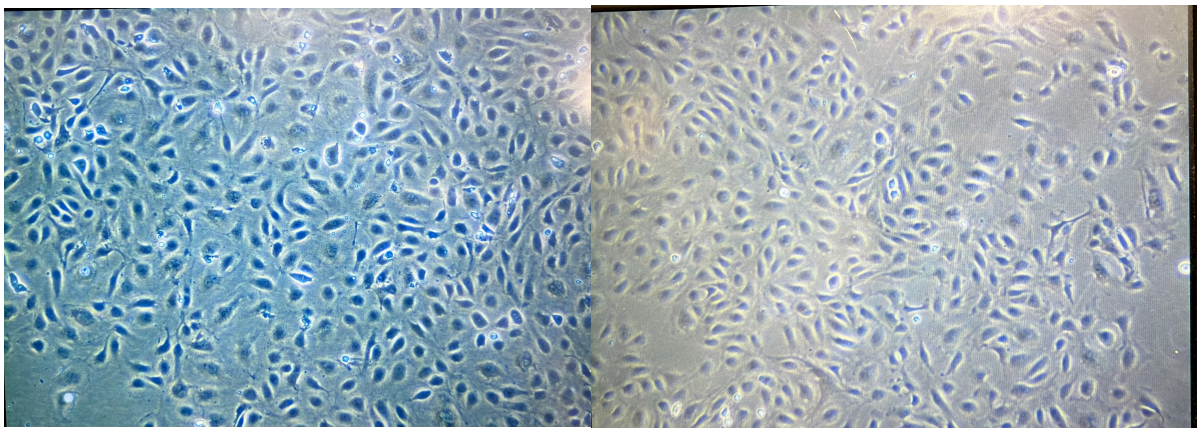
Specific Aim 1: To investigate in vitro inflammatory signaling in patient-derived EFE tissue.

We have successfully achieved the primary objectives of this Specific Aim. We have obtained human EFE and EFE-like tissue samples from living donors who have consented to donating EFE tissue to our lab. This is obtained in the operating room when EFE tissue is surgical removed from the inside of the heart chamber to improve diastolic function to allow the heart to expand and fill more successfully. These samples are processed in our lab to obtain RNA from them. We freeze the samples down, and then we mechanically disintegrate the samples and perform RNA purification. We obtain RNA from EFE tissue, surrounding healthy tissue, and a mixed sample of the two.

Specific Aim 2: To establish a novel *in vitro* model of EFE for stem cell therapy. We been successful in achieving the primary objectives of this Specific Aim. Since obtaining critical funding support, we have work to develop, and are currently refining, an *in vitro* model of EFE using human coronary artery endothelial cells (HCAECs). To date, human endocardial cells have not been able to be successfully cultured. However, HCAECs have been shown to originate largely from endocardial cells and have thus been used when studying cardiac fibrosis.[11] A study published in 2007 showed that HCAECs retain the biological potential to undergo endocardial-mesenchymal transition (EndMT), which is a major inflammatory process in the formation of endocardial fibroelastosis.[12] Due to these prior findings, there have been studies to evaluate the EFE inflammatory pathway by utilizing HCAECs as their *in vitro* model.[13,14] The first of these studies found the TGFβ/BMP pathway played a large role in the EFE process, most notably BMP7.[13] The latter study showed that mechanical stretching of HCAECs caused the EndMT pathway.[14]

Cell Culture

Utilizing direct GMF research funding, HCAECs were purchased from Lonza along with their endothelial growth media (EGM-2 MV). The media and contents were then thawed and prepared per manufacture instructions. Per 500 mL of media, the media was supplemented with 5% fetal bovine serum, 0.04% hydrocortisone, 0.4% human fibroblastic growth factor, 0.1% vascular endothelial growth factor, 0.1% recombinant analog of human insulin-like growth factor-I, 0.1% ascorbic acid, 0.1% human epidermal growth factor, and 0.1% gentamicin sulfate/amphotericin (GA-1000). Cells were maintained in this culture medium in a 37°C, 5% CO_2 incubator. The culture medium was changed every 48 to 72 hours. Cells were expanded up to 3 passages. Below are microscopic images obtained.



Future Experiments/Progress

Since 08/1/2022, we have procured four human EFE tissue samples that have been prepared for RNA sequencing to better understand what inflammatory mediators are present in these tissues. We will continue to obtain more samples. The HCAEC cells have been purchased and prepared, and plated on 4/9/23. Inflammatory cytokines will be added after they adhere to the wells. Three wells will be used – 1 control, 1 that will undergo agitated flow, and 1 that will undergo turbulent flow. TGFβ will be measured via ELISA after the experiments take place. We will also stain the cells with mesenchymal signal markers and evaluate them with immunohistochemistry.

Summary Statement

We anticipate the collection of significant experimental data related to the development of our in vitro EFE model over the next several weeks to months both from our *in vitro* model and live tissue samples. These data will not only serve a foundational role in establishing the first experimentally reproducible EFE in vitro model for further testing of EFE cellular signaling cascades, but will serve as an important scientific contribution to the field of congenital heart disease through the presentation of and publication of these data at a national research meeting and international medical journal.

In addition, we the experimental data we have generated will serve to provide an important platform for future experiments, including the development an *in vivo* translational model of EFE to demonstrate and test cellular therapeutic approaches to halting EFE development in pre-clinical scientific environment. In doing so, our laboratory will strive to identify and test positive and negative mediators of the EFE process to develop exogenous therapy to mitigate and/or arrest the formation of EFE. These ongoing efforts, will undoubtedly necessitate and be dependent upon additional funding in the future.

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