VIEWPOINT

Heart-on-a-Chip

Leveraging Technology for Sickle Cell Disease

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BURDEN OF SICKLE CELL DISEASE

Sickle cell disease (SCD) is caused by a single point mutation in the hemoglobin β subunit, resulting in red blood cells (RBCs) polymerizing into stiff crescent-shaped RBCs. It is characterized by intermittent vaso-occlusive events and chronic hemolytic anemia. The former results in tissue ischemia causing damage to numerous organ systems including the heart, brain, spleen, bones, liver, and kidneys.

In the United States, roughly 1 in every 360 African Americans will be born with SCD, and global incidence is estimated to be 300,000 to 400,000 babies born each year with SCD.[1] Between 2015 and 2017, chronic cardiovascular disease was the most common cause of death at 27%.[2] Despite this, the mechanisms involving chronic cardiac stress are poorly understood, and the early diagnosis and treatment of SCD-related heart failure (HF) are similarly lacking.

IMPRECISE PHENOTYPING AND TREATMENT OF SICKLE CELL CARDIOMYOPATHY

In SCD patients, cardiac complications are treated as they arise similar to how they are treated in non-SCD patients. Cardiac ischemic events are treated with nitroglycerin, aspirin, beta-blockers, and angiotensin-converting enzyme inhibitors.

Congestion in acute cardiac failure is managed with loop diuretics and supportive care. However, no modality is specifically directed at preventing the complications from happening or treating SCD-specific cardiac complications.

Cardiovascular complications are thought to be due to the sickled shape and adhesion properties of sickled RBCs. Because of their deformity, sickled RBCs are often destroyed prematurely when they reach the spleen, resulting in hemolytic anemia. The heart compensates for the decrease in oxygen delivery by increasing the cardiac stroke volume, leading to left ventricular dilation. Continuous ventricular wall stress eventually leads to eccentric hypertrophy.

Prolonged periods of dysfunction in the heart from anemia and multiple vaso-occlusive crises cause chronic inflammation pathways due to increased reactive oxidative stress, vasoconstriction, and can lead to both micro-infarcts in the heart and diffuse myocardial fibrosis. SCD patients have been observed to have higher extracellular volume fractions (a measure of amount of fibrosis) than their healthy counterparts, according to Niss et al.[3] Increased fibrosis causes increased stiffness in the heart, correlating with poor prognosis.

Multiple factors may predispose SCD patients to maladaptive cardiac remodeling and HF. The chronic volume overload, which compensates for the reduced blood oxygen-carrying capacity, may lead to progressive myocardial damage and subsequent exercise intolerance and HF. Roughly 50% of HF in those with SCD have preserved ejection fractions in the left ventricle, causing delays in diagnosis and limited treatment, as echocardiograms and HF treatment are designed for reduced ejection fraction HF. Instead, SCD-associated HF may be suspected upon onset of nonspecific symptoms of exercise intolerance, fatigue, and dyspnea among SCD patients.

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OPPORTUNITIES TO LEVERAGE TECHNOLOGY FOR MANAGEMENT

Researchers have identified the basic process of SCD—vaso-occlusions, hemolytic anemia and increased reactive oxidative stress, chronic inflammation—however, the precise mechanisms that contribute to heart disease within SCD remain unclear. The development of physiologically relevant SCD models can enhance studies aimed at understanding the disease mechanism and developing new drugs. In the past, either the monolayer cell monoculture studies or animal models were used but not without any drawbacks. The former lacks the complexity of multicellular structure and interaction found in cardiac tissues while the latter is genetically different from humans.

Genetically altered mouse models have been instrumental in our understanding of SCD progression and treatment, but currently the Food and Drug Administration is pushing away from animal use and more toward alternative research platforms.[4,5] Organ-on-a-chip (OoC), and in this case heart-on-a-chip (HoC), technology implementing microfluidics is the next big step for research.

The HoC model entails fabricating functional cardiac microtissues within a small chamber "chip." The microtissues are often composed of human- or rodent-derived cardiomyocytes, extracellular matrix-like hydrogels, and other cardiovascular cells. HoCs using human cell lines are naturally more biologically relevant relative to rodent-based models due to species differences in terms of biologic demands, receptor presentation, and heart rate which may present limitations. With SCD, different physiological parameters can be evaluated and adjusted, such as but not limited to: altering the amount of free heme available, creating oxygen gradients, and changing the amount of inflammatory or vasoconstrictive molecules within the system. This functionality permits ease of probing cell-specific effects. Furthermore, HoCs offer control of the ischemic microenvironment and allow the concurrent study of Ca^{2+} dynamics and pharmacological intervention effects, which is helpful in modeling Ca^{2+} overload and arrhythmia.

HoC models have shown success in assessing drug responses and recapitulating features of the heart and Zhang et al fabricated a microfluidic HoC model with human-induced pluripotent stem cells on a hydrogel scaffold with the added ability to record electrical signals of cardiac muscles. The results showed appropriate response to verapamil and isoprenaline, which indicated its reliability for drug testing.[6] Co-cultures with cardiac muscle cells, endothelial cells, and fibroblasts have also been demonstrated by Hussain et al[7] by creating a three-dimensional cardiac co-culture using bioactive chitosan, which resulted in polarized cardiomyocyte morphology and exhibited synchronized contractions in large tissue-like cellular networks.
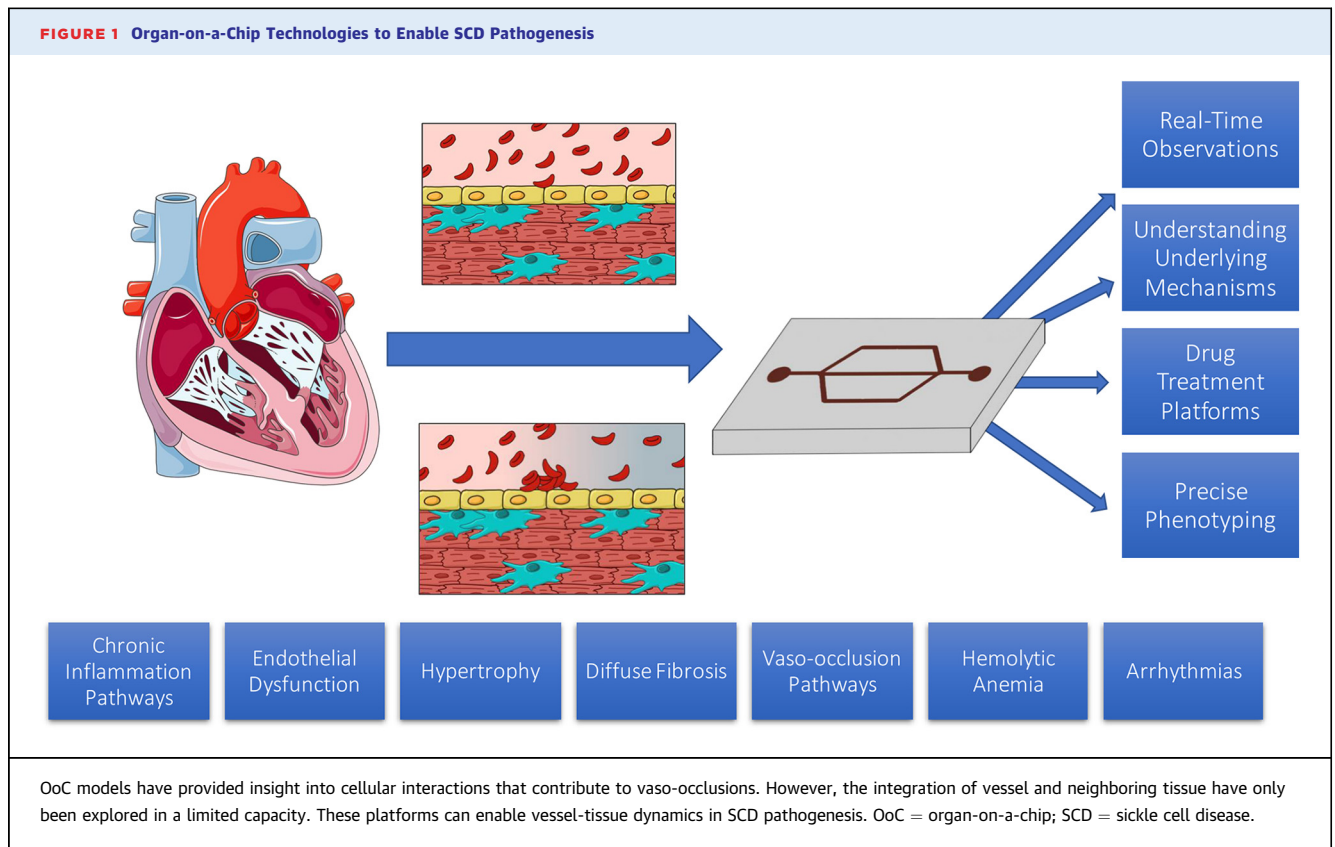
Microfluidic models have been used to study vaso-occlusions mechanisms within SCD. Dong et al[8] developed a microvasculature model with an oxygen gradient to determine when vaso-occlusions are most likely to occur. Loiseau et al[9] showed that vaso-occlusion preferentially occur at or near corners using their microfluidic models composed of arrays of corners and branching pathways. Kucukal et al[10] developed an RBC adhesion assay by treating microchannels of human endothelial cells with increasing concentrations of heme to monitor resulting vaso-constrictive molecules caused by increased oxidative stress. Mathur et al[11] implemented patient-derived endothelial cells into a microfluidic platform to quantify differences in interactions of sickled RBCs and found greater endothelial activation among severe SCD patients compared to patients with milder symptoms.

Understanding cellular interactions within the SCD vasculature can lead to insights into the underlying mechanisms, signal cascades of chronic stress, and ischemic-like events that lead to the often serious heart complications in SCD. OoC models can support phenotypic and cellular interaction investigations during disease progression as well as provide pharmacological and genetic assessments.

CONCLUSIONS

Complications such as vaso-occlusive crisis, hemolytic anemia, ventricular dysfunction, chronic inflammation, and diffuse fibrosis constitute significant disease manifestations caused by SCD, all of which result in detriment in quality of life for a patient. Despite the severity of cardiovascular complications, specific mechanisms of cardiac remodeling as a response to this unique vaso-occlusive stress and resulting phenotypes remain poorly understood. Emerging technologies and models may be leveraged to understand these mechanisms. More specifically, OoC models utilizing engineered human tissues can be utilized to

FIGURE 1 Organ-on-a-Chip Technologies to Enable SCD Pathogenesis

OoC models have provided insight into cellular interactions that contribute to vaso-occlusions. However, the integration of vessel and neighboring tissue have only been explored in a limited capacity. These platforms can enable vessel-tissue dynamics in SCD pathogenesis. OoC = organ-on-a-chip; SCD = sickle cell disease.

quantify genetic, phenotypic, and functional changes in the heart (**Figure 1**). With a better understanding of underlying mechanisms, designing therapies that not only treat complications but also prevent them from arising may be achieved.

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