Integration Of Biomedical Engineering And Computer Vision For Morphological Alteration Identification In Diabetic Cardiomyopathy: An Analysis And Evaluation Strategy

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INTEGRATION OF BIOMEDICAL ENGINEERING AND COMPUTER VISION FOR MORPHOLOGICAL ALTERATION IDENTIFICATION IN DIABETIC CARDIOMYOPATHY: AN ANALYSIS AND EVALUATION STRATEGY

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Dedication

To my world, mis Julios! My husband and son

&

My parents Antonio and Magdalena Delgado & siblings

Gavin Antonio and Ashley Alejandra Delgado

Thank you for your love, support, and patience.
Acknowledgements

My academic journey has been a long travel and made possible with the support and guidance of my dedicated committee members: Dr. MacDonald, Dr. Chattopadhyay, Dr. Moya, and Dr. Luna. I would like to take this opportunity to express my deepest gratitude and heartfelt appreciation to Dr. Eric MacDonald for their remarkable mentorship that has not only guided me throughout my academic journey but also played a pivotal role in saving and sustaining my professional career. Dr. MacDonald extended their unwavering support, providing the guidance and encouragement I needed to navigate the challenges and uncertainties I faced. His belief in my abilities, coupled with their exceptional expertise, has been instrumental in helping me overcome obstacles and maintain my commitment to this long and transformative academic journey.

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Abstract

Diabetic cardiomyopathy (DCM) is a type of heart disease that affects people with diabetes. It is characterized by changes in the structure and function of the heart, including thickening and stiffening of the heart muscle, impaired relaxation of the heart, and reduced pumping function. DCM is considered an ailment of the heart muscle and an increased risk factor in people with type 2 diabetes mellitus (T2DM). Although the exact mechanisms behind DCM are not fully understood, high blood glucose levels are known to contribute to the development and progression of the disease.

While many options to manage and diagnose DCM have been established in the late stages, it remains challenging to identify the early stages of disease development and effectively prevent its progression. Therefore, this research combines biomedical engineering principles and computer vision applications to study the early-stage development of diabetic cardiomyopathy. By employing new programmatic biomedical imaging techniques and utilizing computer vision, the goal is to better diagnose and understand the disease by algorithmically evaluating a larger sample of images with automation and sophisticated image and geometry analysis. Comprehensive open source image analysis libraries are maintained and provided by Intel Corporation and have been used on a diversity of applications including the first autonomous crossing of California in a driverless car in 2011 and countless other applications in the biomedical, consumer (clothes fitting, augmented reality, etc), manufacturing, agriculture, and automotive fields.

The central objective of this research is to utilize computer vision to identify and characterize the pathophysiology of DCM. By rapidly identifying and quantifying subtle geometric, color, and pixel features as well as spatial frequency information in biomedical images, computer vision enables a better understanding of the early stages of DCM, leading to improved
diagnosis and treatment. Through computer vision analysis, valuable insights into the microscopic features of cardiac tissue affected by DCM, including cellular structure, fibrosis, inflammation, and other pathological changes, can be gained. This allows researchers to establish correlations, identify key biomarkers, and deepen their understanding of the disease mechanisms underlying DCM. Ultimately, the integration of computer vision with microscopic tissue analysis aims to enhance knowledge, enable early detection, and advance the development of more effective diagnostic and therapeutic approaches for DCM.

Preliminary investigations of diabetic rodent hearts have revealed hyper-contracted and degenerated myofibers, disrupted collagen fibrils, and fragmented sarcoplasm, providing evidence of fibrosis. Myocardial fibrosis is a hallmark of hypertrophic cardiomyopathy and is proposed as a mechanism for arrhythmias and heart failure. To accomplish the overarching goal, three specific aims have been proposed. The first aim is to study the histopathology of diabetic rat myocardium to identify and establish specific biomarkers that may play a role in DCM. The second aim involves investigating the effects of Glycyrrhizin (GLC), anti-inflammatory agent on the identified biomarkers and evaluating its potential therapeutic impact on DCM. The third aim focuses on determining whether computer vision software can be utilized to develop a potential disease model for studying the progression of DCM in vitro under diabetogenic conditions, including GLC treatment. Successful completion of this study may lead to the establishment of parameters for identifying DCM, provide insights into the therapeutic effects of GLC, and offer a basis for studying the early development of DCM. Therefore, the integration of biomedical engineering, computer vision technology, and GLC treatment plays a crucial role in elucidating the etiology, initiation, and potential therapeutic interventions for Diabetic Cardiomyopathy (DCM). By leveraging these technologies, researchers can enhance their capability to simultaneously examine
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Chapter 1: Introduction

1.1 Background Information on Diabetic Cardiomyopathy (DCM) from a Biomedical Engineering and Computer Vision Perspective

Diabetic cardiomyopathy (DCM) is a distinct form of heart disease that occurs in individuals with diabetes, particularly those with type 2 diabetes mellitus (T2DM). It is characterized by structural and functional abnormalities in the heart that cannot be solely explained by traditional cardiovascular risk factors such as hypertension or coronary artery disease [1]. The prevalence of DCM is increasing worldwide, paralleling the rising prevalence of diabetes. It is estimated that up to 40% of individuals with diabetes develop some form of cardiac dysfunction, with DCM being a significant contributor [2, 3]. DCM is associated with an increased risk of heart failure, cardiovascular events, and mortality, making it a major global health concern.

Biomedical engineering, with its application of engineering principles and techniques to healthcare challenges, plays a crucial role in advancing our understanding of DCM [4, 5]. By developing and utilizing advanced technologies, such as computer vision, biomedical engineers contribute to improved diagnosis, monitoring, and treatment of DCM.

Computer vision, as a subfield of artificial intelligence and image processing, focuses on extracting meaningful information from visual data [6, 7]. When applied to DCM, computer vision techniques enable researchers to analyze medical images, identify patterns, and extract quantitative measurements related to cardiac structure and function. This approach provides a unique perspective for comprehending DCM, enhancing the accuracy and efficiency of cardiac data analysis, disease progression monitoring, and identification of potential therapeutic interventions [6, 7].
The pathophysiology of DCM is complex and multifactorial, involving metabolic abnormalities, insulin resistance, inflammation, oxidative stress, advanced glycation end products (AGEs), cardiac remodeling, and altered calcium handling [8]. Understanding these underlying mechanisms is essential for the development of targeted interventions and therapies for DCM.

By exploring the intricate mechanisms involved in DCM, researchers strive to identify novel biomarkers, therapeutic targets, and diagnostic approaches that can improve the management and outcomes of individuals with this condition. The integration of biomedical engineering and computer vision provides a powerful framework for gaining insights into DCM, facilitating advancements in the field, and ultimately improving patient care.

1.2 Significance of Understanding DCM Pathophysiological Mechanisms: Exploring Biomarkers and Glycyrrhizin Treatment through a Biomedical Engineering and Computer Vision Perspective

Diabetic cardiomyopathy (DCM) is a complex condition characterized by structural and functional abnormalities in the heart, specifically affecting individuals with diabetes, particularly those with type 2 diabetes mellitus (T2DM) [9, 10]. The significance of understanding the pathophysiological mechanisms underlying DCM is of paramount importance in improving patient outcomes through early detection, accurate diagnosis, and targeted treatment approaches.

Investigating specific biomarkers involved in DCM provides valuable insights into the molecular and cellular processes contributing to the development and progression of the disease. In this context, four key biomarkers will be discussed: Cx43, CXCR4, troponin-I, and MyoD.
1. **Cx43**: Gap junction protein Cx43 plays a crucial role in cell-cell communication in the heart. Reduced Cx43 expression has been observed in various cardiac pathologies, including DCM [1, 8, 11]. In DCM, altered Cx43 expression may contribute to disease progression. Reduced Cx43 expression has been associated with increased cell necrosis in the myocardium, suggesting its involvement in DCM pathology [8]. Studying Cx43 expression in the myocardium of diabetic individuals can provide valuable insights into the extent of cell necrosis and its impact on DCM development [12].

2. **CXCR4**: Chemokine receptor (CXCR4) is known for its involvement in cell signaling and migration processes [1]. Dysregulation of CXCR4 has been implicated in inflammation, endothelial dysfunction, and impaired cardiac function. In the context of DCM, altered CXCR4 expression may contribute to the progression of the disease [8, 13]. Understanding the role of CXCR4 in cardiovascular pathology, including its potential implications for DCM, is of significant interest for identifying potential therapeutic interventions.

3. **Troponin-I**: Troponin-I (TnI) is a well-established biomarker for myocardial injury [14]. Elevated levels of troponin-I in the bloodstream indicate myocardial damage. In the context of DCM, studying troponin-I expression in cardiac tissue provides valuable evidence of myocardial injury, enabling a better assessment of disease severity and progression [15]. Monitoring troponin-I levels can aid in the diagnosis and prognosis of DCM and guide treatment strategies.

4. **MyoD**: Myogenic differentiation antigen (MyoD) plays a role in regulating the differentiation of myoblasts into cardiac muscle cells [16, 17]. Disruption of MyoD expression in diabetic tissue may contribute to the loss of cardiac muscle integrity and the development of DCM. In the study MyoD expression in the myocardium will be used as
marker for cardiac muscle loss which can provide insights into the impact of diabetic conditions on cardiac muscle and its association with DCM and treatment.

By investigating the role of these biomarkers within the context of DCM, researchers aim to gain a deeper understanding of the underlying mechanisms driving the disease. The integration of biomedical engineering and computer vision techniques enhances the analysis and quantification of these biomarkers, enabling precise assessment and objective evaluation of their expression levels and localization within cardiac tissue [4].

Such investigations contribute to the growing body of biomedical and computer vision research focused on understanding and managing DCM. The findings hold the potential to uncover novel therapeutic targets, diagnostic methodologies, and intervention strategies specifically tailored to address the unique challenges presented by DCM in individuals with diabetes. This research may pave the way for the future development of targeted therapies that aim to restore normal protein expression levels, enhance cardiac function, and ultimately improve outcomes and quality of life for individuals affected by DCM.

Understanding the detailed role of these biomarkers in DCM provides a foundation for the development of effective therapeutic interventions and diagnostic approaches. By elucidating the intricate molecular and cellular processes involved, researchers can identify potential therapeutic targets and develop strategies to mitigate the adverse effects of DCM on cardiac structure and function, ultimately improving patient outcomes.

Additionally, investigating the impact of specific treatments, such as Glycyrrhizin (GLC), on these biomarkers can provide valuable insights into potential therapeutic interventions for DCM. Glycyrrhizin, a natural compound derived from licorice root, has been studied for its potential cardioprotective effects in various cardiovascular conditions, including DCM [8]. Studies
evaluating the effects of GLC treatment on Cx43 expression have shown promising results [8, 18]. GLC administration has been associated with increased Cx43 expression, potentially contributing to improved cell-cell communication and cardiac function in DCM.

Furthermore, GLC treatment has been shown to modulate CXCR4 expression and signaling pathways, potentially mitigating inflammation and endothelial dysfunction associated with DCM [18]. Understanding the impact of GLC on CXCR4 expression and its downstream effects can shed light on its therapeutic potential for managing DCM.

In terms of troponin-I, GLC treatment has shown promise in reducing myocardial injury and attenuating troponin-I release [1]. By studying the effects of GLC on troponin-I expression and its correlation with cardiac tissue damage, researchers can evaluate the potential cardioprotective effects of GLC in the context of DCM.

Regarding MyoD, investigations into the effects of GLC treatment on its expression and activity can provide insights into its role in preventing or reversing cardiac muscle loss in DCM [19]. Understanding the impact of GLC on MyoD regulation may offer valuable clues for preserving cardiac muscle integrity and improving cardiac function in individuals with DCM. Incorporating GLC treatment into the investigation of these biomarkers within the context of DCM provides a comprehensive approach to understanding the potential therapeutic effects of GLC and its impact on the molecular and cellular processes underlying DCM pathology [18]. This research may show novel therapeutic strategies and intervention approaches that can effectively target and alleviate the structural and functional abnormalities associated with DCM.

By leveraging the strengths of biomedical engineering and computer vision research, researchers can integrate GLC treatment studies with advanced technologies, such as image analysis and quantification techniques. This study comprehensively evaluates the effects of GLC
on the alleviation of the expression and localization of these biomarkers in cardiac tissue. This multidisciplinary approach holds great potential for optimizing treatment strategies and improving the outcomes and quality of life for individuals with DCM.

1.3 STUDY OBJECTIVES AND RESEARCH QUESTIONS

The focus of this study is to understand the pathophysiological mechanisms of diabetic cardiomyopathy (DCM) and explore potential strategies for managing the disease, including therapeutics and diagnostic evaluation in the future. To address this study's objectives, the following research questions need to be answered:

1. Can the early stages of diabetic cardiomyopathy (DCM) be identified and assessed in vivo through computer vision analysis, and can the effects of Glycyrrhizin (GLC) treatment on disease progression be evaluated using this approach?

2. What are the specific biomarkers associated with DCM in the myocardium, and how does GLC treatment impact their expression levels?

3. Can an in vitro 2D model of cardiomyocytes accurately replicate the phenotype of DCM, and can computer vision analysis be employed to identify and quantify specific biomarkers affected by GLC treatment in this model?

These research questions aim to investigate the pathophysiological mechanisms of DCM, explore the potential effects of GLC treatment, and utilize biomedical engineering and computer vision techniques to gain insights into disease progression. To answer these questions, a hypothesis and three specific aims have been developed in this dissertation.
The **hypothesis** of this dissertation is that cardiac tissues obtained from Zucker diabetic fatty (ZDF) rats with type 2 diabetes mellitus (T2DM) can be used as a basis for identifying and assessing the pathogenesis of DCM with relevant underlying biomarkers. Additionally, the treatment with
Glycyrrhizin (GLC) can potentially mitigate cardiac tissue abnormalities [8, 13, 18, 20]. Cardiac tissue abnormalities can also be easily identified through computer vision algorithms (given the improved customization, dynamic adaptation, and automation provided by the programmatic paradigm) when subjected to simulated diabetogenic environments in 2D invitro mimics. To connect the findings obtained from the first part of this project to the latter, selected biomarkers determined in the ZDF animals, including the GLC treatment, will be correlated with findings in the 2D diabetic cardiac tissue mimics. Based on these hypotheses, the following specific aims are proposed:

**Specific Aim 1: Histopathological Analysis of Rat Hearts: Assessing Diabetic Cardiomyopathy-Induced Changes in Microscopic Anatomy**

The objective of this specific aim is to determine changes in the microscopic anatomy of diabetic (ZDF) and healthy (lean) rat models. Histopathology of the diabetic rat myocardium will be studied using Hematoxylin and Eosin stain (H & E) and Gomori’s Trichrome, along with GLC treatment, to investigate injury in the diabetic rat myocardium and compare changes between the ZDF and lean animal models. Two main components are selected for the histopathology of cardiac tissue:

**Specific Aim 1a: Characterizing Morphological Changes in Diabetic Cardiac Tissue Using Hematoxylin and Eosin Staining.**

Hematoxylin and Eosin (H & E) staining will be utilized to determine changes in the morphology of diabetic heart tissue compared to lean (control) tissue. Hematoxylin and Eosin (H&E) staining is employed to characterize the morphological changes in diabetic cardiac tissue compared to the control (lean) tissue. This staining technique allows for the visualization and examination of various cellular components, including the cardiac muscle fibers, extracellular matrix, and other
structural elements. By comparing the morphology of the diabetic heart tissue with the control tissue, valuable insights can be gained into the alterations in cellular architecture and overall tissue structure associated with diabetic cardiomyopathy.

**Specific Aim 1b: Comparative Analysis of Diabetes-Induced Myocardial Fibrosis: Gomori's Trichrome Staining for Assessing Impact on Myocardium.**

Gomori’s Trichrome staining, along with GLC treatment, will be used to study and compare myocardial fibrosis in diabetic and healthy hearts, aiming to determine the impact of diabetes, as well as the potential effects of GLC, on the myocardium. This staining method allows for the localization and identification of collagen in connective tissue (presented as blue color) and staining of muscle fibers and cytoplasm (red/pink color). Gomori’s Trichrome staining is routinely used for mitochondrial myopathy, which reflects muscle fiber damage.

**Specific Aim 2: Exploring Differential Expression of Early Biomarkers in Diabetic Cardiomyopathy: Insights from the ZDF Rat Model**

The objective of this specific aim is to investigate and compare the differential expression of early biomarkers in the Zucker Diabetic Fatty (ZDF) rat models with and without GLC treatment. The identification of early biomarkers in diabetic cardiac tissue, including the potential effects of GLC, will confirm the diabetic/diseased phenotype and guide the development of future therapeutic strategies for managing DCM and explore potential treatment options for the disease. Four main components are selected for the study of differential expression of early biomarkers:

**Specific Aim 2a: Examining Cx43 Expression in Diabetic Cardiomyopathy: Potential Indicator of Disrupted Cell-Cell Communication.**

Cx43, which participates in cell-cell communication [11, 12], will be studied to determine its expression in the ZDF rat myocardium and cell cultures, both with and without GLC treatment.
Understanding the role of Cx43 in diabetic hearts is of significant importance as it provides insights into the underlying mechanisms of DCM. By elucidating the changes associated with Cx43 in diabetic hearts and in vitro models mimicking a diabetogenic environment, studies will provide strategies to possible mitigate the adverse effects of Cx43 dysregulation on cardiac structure and function.

**Specific Aim 2b: Investigating the Role of CXCR4 in Diabetic Cardiomyopathy: Implications for Inflammation.**

CXCR4 is a protein known as a chemokine receptor, specifically a C-X-C chemokine receptor 4. It plays a crucial role in cell signaling and migration processes [13]. CXCR4 has been linked to inflammation, endothelial dysfunction, and impaired cardiac function. Its dysregulation has been implicated in the progression of atherosclerosis, myocardial infarction, and heart failure [21, 22]. Therefore, understanding the role of CXCR4 in cardiovascular pathology, including the potential effects of GLC, is of significant interest for potential therapeutic interventions.

**Specific Aim 2c: Assessing Myocardial Injury through Troponin-I Expression: A Biomarker for Diabetic Cardiomyopathy.**

Troponin-I expression in cardiac tissue provides valuable evidence of myocardial injury, enabling a better assessment of disease severity and progression. Troponin-I, which plays a role in cardiac regulatory mechanisms and is specific for myocardial necrosis, will be studied to provide evidence of myocardial injury in the ZDF rat model, both with and without GLC treatment [23, 24].

**Specific Aim 2d: Immunohistochemistry Analysis of MyoD Expression and Muscle Atrophy in in the ZDF Rat Myocardium.**

MyoD, a crucial protein involved in muscle differentiation regulation, has been studied in the context of diabetic cardiomyopathy (DCM). Reduced expression of MyoD has been observed in
diabetic cardiac tissue, indicating possible disruptions or apoptosis in the cardiac tissue [25, 26]. This reduced expression suggests potential degeneration of cardiac myocytes, which may contribute to the development of DCM. Understanding the role of MyoD in DCM can provide insights into the mechanisms underlying cardiac muscle loss and help identify potential therapeutic targets for the disease [17].

**Specific Aim 3: Computer Vision Analysis of Diabetic Rat Heart Tissue and 2D Cell Culture: Automated Extraction of Algorithmic Features and Patterns for Enhanced Understanding and Diagnosis of Diabetic Cardiomyopathy with Customized Adaptation for Varied Image Conditions**

The objective of this specific aim is to utilize computer vision techniques to analyze diabetic rat heart tissue and selected biomarkers, including GLC-treated samples, in order to identify relevant features and patterns associated with DCM. By applying computer vision algorithms to the histological images of the diabetic rat myocardium and 2D cell cultures of AC16 human cardiomyocytes, both with and without GLC treatment, our aim is to extract quantitative measurements and identify unique characteristics that differentiate DCM from healthy myocardium. This analysis will provide insights into the potential of computer vision and the effects of GLC treatment for automated analysis and detection of DCM-related abnormalities. Ultimately, this research will contribute to improved diagnostic evaluation and enhanced understanding of the disease. Three main components are selected for this study:

**Specific Aim 3a: Image Analysis of Diabetic Rat Heart Tissue**

The objective of this study is to perform image analysis on histological images of diabetic rat heart tissue to identify and extract features and patterns associated with diabetic cardiomyopathy (DCM). Computer vision algorithms will be applied to the images to quantitatively measure
morphological parameters, such as cellular structure and identification of collagen in rat myocardium. By analyzing these features, unique characteristics of DCM can be identified and distinguished from healthy myocardium. This study aims to develop a comprehensive image analysis framework that enables automated detection and assessment of DCM-related abnormalities in diabetic rat heart tissue.

**Specific Aim 3b: Integration of Computer Vision and Biomarker Analysis**

In this study, the results obtained from the computer vision analysis of diabetic rat heart tissue and 2D cell culture images will be integrated with the assessment of selected biomarkers. Correlations between the identified features and patterns from the computer vision analysis and the expression levels of biomarkers, such as Cx43, CXCR4, Troponin-I, and MyoD, will be investigated. This study aims to establish connections between the computer vision analysis and the underlying molecular and cellular changes associated with DCM. By linking the imaging data with the biomarker analysis, a comprehensive understanding of the pathogenesis of DCM and the effects of GLC treatment can be achieved, providing insights into potential diagnostic and therapeutic strategies for the disease.

**Specific Aim 3c: Quantitative Analysis of 2D Cell Culture Images**

The objective of this study is to perform analysis on 2D cell culture images of AC16 human cardiomyocytes, both with and without Glycyrrhizin (GLC) treatment, using computer vision algorithms. The focus of this analysis is to extract relevant features and patterns from the images, including cellular morphology and the expression levels of biomarkers such as Troponin-I and MyoD.

By comparing the characteristics of diabetic cardiomyocytes with and without GLC treatment to healthy cardiomyocytes, this study aims to identify specific cellular changes
associated with diabetic cardiomyopathy (DCM) and assess the potential impact of GLC in mitigating these abnormalities. The quantitative analysis of 2D cell culture images will provide valuable insights into the cellular mechanisms underlying DCM and the efficacy of GLC treatment.

The computer vision algorithms will enable the automated extraction of quantitative measurements, such as shape and the assessment of biomarker expression levels [6, 27]. By comparing these measurements between different experimental groups, the study aims to identify and quantify cellular changes indicative of DCM pathology and the effects of GLC treatment. This study will contribute to a better understanding of the cellular and molecular processes involved in DCM, as well as provide evidence for the potential therapeutic benefits of GLC. By utilizing computer vision analysis to simultaneously analyze microscope images from 2D cell culture, this study seeks to establish a comprehensive approach for assessing cardiac tissue abnormalities in vitro and advance our knowledge of DCM pathogenesis and treatment strategies.
Chapter 2: Literature Review

2.1 Diabetes Mellitus and Diabetic Cardiomyopathy: Understanding the Link and Exploring Therapeutic Approaches

In recent years, biomedical engineering and computer vision have emerged as invaluable tools in the field of healthcare, revolutionizing diagnostics, treatment, and patient care [28]. Their application extends to various medical conditions, including diabetic cardiomyopathy (DCM), a cardiovascular complication associated with type 2 diabetes mellitus (T2DM) [29].

DCM, a secondary disorder caused by T2DM, poses a significant risk to individuals with this prevalent type of diabetes [10, 30]. To comprehend the impact of biomedical engineering and computer vision on DCM, it is crucial to first investigate into an overview of diabetes mellitus itself. The roots of our understanding of diabetes can be traced back to the Greek-Roman physician Aretaeus of Cappadocia, who provided a clear description of the condition. The term "diabetes" originates from the Greek word meaning "siphon" or "to go through," alluding to the chronic polyuria observed in diabetes patients [31, 32]. In Latin, "mellitus" denotes "sweet," referring to the sweetness of the urine in individuals with diabetes. These ancient origins provide a historical context for the disease.

Diabetes mellitus is characterized by the loss of pancreatic insulin production or improper utilization of insulin, resulting in elevated blood glucose levels [2, 3]. It is classified into two main types: T1DM and T2DM. T1DM is an autoimmune condition in which the body's immune system attacks and destroys the insulin-producing β-cells of the pancreas [33, 34]. In contrast, T2DM, the most prevalent type globally, is associated with insulin resistance. In T2DM, the cells fail to respond effectively to insulin, leading to hyperglycemia [1, 35]. While T1DM typically affects
younger individuals, T2DM predominantly affects middle-aged and older adults, often as a consequence of sedentary lifestyles and poor dietary choices [10, 36].

2.2 **Type 2 Diabetes Mellitus and Cardiovascular Disorders**

In the vast landscape of scientific inquiry, the story of Type 2 Diabetes Mellitus (T2DM) emerges as a focal point, captivating the attention of researchers and healthcare professionals alike. Its prevalence and contribution to the global burden of diabetes are undeniable, with T2DM accounting for a staggering 90 to 95% of cases compared to Type 1 Diabetes Mellitus (T1DM) [33, 37, 38]. This chronic condition, often referred to as adult-onset diabetes, extends its reach beyond the regulation of blood glucose levels, impacting the intricate functioning of the cardiovascular system [30].

In the context of T2DM, the heart emerges as a central focus, with cardiac dysfunction and fibrosis becoming prominent characteristics [39-41]. The cardiac system yields to the persistent impact of T2DM, experiencing structural changes and compromised function. The excessive buildup of extracellular matrix (ECM) adds to the complexity, posing challenges for scientific exploration and therapeutic strategies [42].

Insulin resistance, coupled with associated risk factors such as sedentary lifestyles, dietary habits, and obesity, lies at the core of T2DM [30]. This condition disrupts glucose metabolism and lipid homeostasis, extending its impact from individuals to society at large. Diagnosis of T2DM relies on fasting blood glucose levels, with a threshold of 126mg/dL (7.0 mM) serving as the defining criterion [35, 38]. The scale of the problem becomes apparent when considering the statistics revealed by the CDC National Diabetes Statistic Report, indicating a prevalence of 9.3%
among the U.S. population and the threatening presence of vascular disease, which contributes significantly to morbidity and mortality in individuals with T2DM [38].

The progressive nature of T2DM unfolds as the disease journey progresses. Insulin resistance and metabolic disturbances characterize the early stages, but as time passes, β-cell exhaustion ensues [9]. Similar to T1DM, the autoimmune destruction of insulin-secreting β-cells becomes evident, resulting in decreased endogenous insulin production [33]. Hyperglycemia and hyperinsulinemia take the stage, highlighting the parallels between T2DM and its juvenile-onset counterpart. While lifestyle modifications and oral medications offer some control in the early stages, the progression of the disease often requires the addition of exogenous insulin to maintain glycemic balance. However, the repercussions extend beyond glycemic control, with cardiovascular disorders emerging as primary complications and major contributors to mortality in individuals with T2DM [2, 3].

Within the scientific community, there exists a fervent pursuit to advance our understanding of T2DM and enhance patient well-being, driven by thirst for knowledge and dedication to improving clinical outcomes. Researchers, clinicians, and biomedical engineers unite to explore the intricate mechanisms underlying insulin resistance and β-cell dysfunction. Their collaborative efforts illuminate the pathophysiological complexities of T2DM, identifying novel therapeutic targets, and exploring the potential of biomedical engineering and computer vision in diabetes care. Each stride forward brings us closer to a future where the burden of T2DM is alleviated, where innovative interventions transform lives, and where the possibility of cardiovascular complications recedes.

In this area of scientific exploration, the story of T2DM unfolds, driven by curiosity, propelled by the pursuit of understanding, and united by the common goal of improving the lives
of those affected. The path ahead may be arduous, but through the collaborative efforts of the scientific community, unexplored territories are navigated, the complexities of T2DM unraveled, and the landscape of diabetes care reshaped.

Cardiovascular disorders pose a significant public health risk and have a profound impact on the heart in the context of Type 2 Diabetes Mellitus (T2DM) [43]. As T2DM progresses, the potential for long-term damage to organs, particularly the heart, becomes apparent. Cardiovascular complications stand as a leading cause of complications associated with T2DM, contributing to the majority of diabetes-related mortality [44]. Within the realm of T2DM, the impact on the heart manifests in three major cardiovascular complications: cardiac autonomic neuropathy, coronary artery disease, and diabetic cardiomyopathy (DCM) [45]. The first two complications are relatively well-known. Cardiac autonomic neuropathy refers to the damage inflicted upon the nervous system controlling the heart's functions. On the other hand, coronary artery disease involves the buildup of plaque in the coronary arteries, impeding blood flow to the heart muscle [46]. Both these complications contribute to the burden on the cardiovascular system [47].

However, it is the third complication, diabetic cardiomyopathy (DCM), that remains elusive and poorly understood [48]. DCM is characterized as a secondary disorder caused by T2DM, and it significantly increases the risk of heart failure in affected individuals. Resistance to insulin in heart tissue plays a crucial role in promoting the development of DCM. The exact mechanisms underlying DCM are still being investigated, but it is recognized as a distinct form of heart disease associated with T2DM. Studies have demonstrated that DCM nearly doubles the risk of cardiovascular disease and cardiovascular mortality, particularly through its association with heart failure [49]. Although various diagnostic tools exist to identify the advanced stages of DCM, detecting its development and preventing disease progression remains challenging. Population
differences and variations in diagnostic criteria further complicate early detection and intervention strategies. As a result, efforts to understand the pathophysiology of DCM and develop effective preventive measures remain ongoing.

In this complex landscape, the interplay between T2DM and cardiovascular disorders poses a significant challenge. The detrimental effects on the heart, including cardiac autonomic neuropathy, coronary artery disease, and DCM, highlight the need for comprehensive management strategies that address both diabetes and its associated cardiovascular complications. The scientific community continues to explore the underlying mechanisms of these complications and seeks innovative approaches to identify individuals at risk, detect the early stages of DCM, and develop targeted interventions to mitigate its progression. By unraveling the complexities of T2DM-related cardiovascular disorders, we move closer to a future where the burden on individuals and public health is alleviated, and the devastating consequences on the heart are minimized.

2.3 DIABETIC CARDIOMYOPATHY: ETIOLOGY, RISK FACTORS, AND CURRENT TREATMENT

Diabetic Cardiomyopathy (DCM) in the context of Type 2 Diabetes Mellitus (T2DM) is characterized by structural and functional modifications in the myocardium, occurring independently of coronary artery disease [50]. These alterations lead to contractile dysfunction, impacting the ejection fraction and ultimately contributing to the development of heart failure. Two prominent features associated with DCM are interstitial fibrosis (excessive deposition of collagen between myocardial cells) and myocyte hypertrophy (increase in the size of individual heart muscle cells) [51]. However, the precise mechanisms driving the development of DCM are still not fully understood, highlighting the need for further research in this area.
The etiology and risk factors of DCM are multifactorial, with hyperglycemia, insulin resistance, and impaired cardiac insulin metabolic signaling playing significant roles. Hyperglycemia, elevated blood glucose levels, is a hallmark of diabetes and contributes to the development of DCM [52]. Insulin resistance, the reduced responsiveness of cells to the effects of insulin, is another key factor in T2DM and is associated with impaired cardiac insulin metabolic signaling, further exacerbating the metabolic dysfunction in the diabetic heart [43, 53].

Cardiac hypertrophy, the enlargement of the heart muscle, along with interstitial fibrosis and abnormalities in cell signaling pathways, also contribute to the pathogenesis of DCM [54]. These changes affect the contractile function of the heart, leading to reduced ejection fraction and impairing its ability to pump blood effectively. Importantly, it should be noted that dyslipidemia (abnormal lipid levels), hypertension (high blood pressure), and coronary artery disease are typically absent in the development of DCM, distinguishing it from other forms of heart disease [46, 55]. One of the key connections between T2DM and DCM is the link between T2DM and diastolic dysfunction, which refers to abnormalities in the heart's ability to relax and fill with blood during the diastolic phase of the cardiac cycle. Diastolic dysfunction is a common precursor to heart failure and is frequently observed in patients with T2DM, contributing to the increased risk of heart failure in this population [56].

Current pharmaceutical management of DCM aims to reduce the workload on the heart, but it does not directly address the underlying metabolic changes caused by diabetes [57]. Therefore, it is crucial to gain a deeper understanding of the specific mechanisms involved in DCM to identify new therapeutic targets that can effectively manage the symptoms associated with the diabetic heart. By elucidating the underlying pathophysiology of DCM, researchers can develop
more targeted interventions and treatments to improve the outcomes for individuals with T2DM and reduce the burden of heart failure in this population [58].

The clinical presentation, diagnosis, and treatment of Diabetic Cardiomyopathy (DCM) involve recognizing the asymptomatic nature of early-stage DCM, understanding the impact of prolonged high glucose exposure on the cardiovascular system, employing diagnostic methods such as echocardiographic analysis, and implementing current therapeutic strategies that encompass lifestyle modifications, antidiabetic medications, lipid-lowering therapies, and metabolic modulators [50, 59]. Additionally, exploring potential therapeutic targets for DCM management is essential.

In its early stages, DCM often manifests as an asymptomatic condition, making it challenging to detect. However, increased fibrosis and stiffness in the heart are characteristic features, indicating the progression of the disease [51, 60]. Clinical examination may reveal cardiac hypertrophy (enlargement of the heart) and diastolic dysfunction (impaired relaxation and reduced diastolic filling) as specific clinical features of DCM [61]. These abnormalities in the heart's structure and function can be identified through various diagnostic methods.

Prolonged high glucose exposure in patients with diabetes plays a significant role in the development of DCM [62]. It affects both the macrovascular (large blood vessels) and microvascular (small blood vessels) systems, leading to damage and dysfunction. Hyperglycemia, insulin resistance, and impaired cardiac insulin metabolic signaling contribute to the pathogenesis of DCM [63]. Echocardiographic analysis is commonly used for the diagnosis and evaluation of DCM [64]. It allows for the assessment of cardiac structure and function, including measurements of left ventricular hypertrophy, ejection fraction, and diastolic function parameters.
Echocardiography helps in identifying the presence and severity of DCM and monitoring disease progression [64].

The current therapeutic strategies for managing DCM involve a comprehensive approach. Lifestyle modifications, such as maintaining a healthy diet, engaging in regular physical activity, managing weight, and avoiding tobacco and excessive alcohol consumption, are essential components. These lifestyle changes aim to control diabetes, reduce cardiovascular risk factors, and improve overall cardiovascular health [64, 65].

Antidiabetic medications, including oral hypoglycemic agents and insulin, play a crucial role in managing blood glucose levels and optimizing glycemic control [66]. Lipid-lowering therapies, such as statins, may be prescribed to address dyslipidemia, even though it is not a primary feature of DCM. Controlling blood pressure is important, and antihypertensive medications may be prescribed if hypertension coexists with DCM. Metabolic modulators, such as medications targeting specific pathways involved in the pathogenesis of DCM, are an area of active research [67]. These medications aim to improve cardiac metabolism, reduce fibrosis, and restore normal cardiac function. Several potential therapeutic targets are being explored, including agents that target oxidative stress, inflammation, and metabolic abnormalities associated with DCM [68].

Early-stage DCM is often asymptomatic, and diagnosis can be challenging due to the absence of dyslipidemia, hypertension, and coronary artery disease [69]. However, diagnostic methods such as heart tissue analysis, which utilizes computer vision techniques, play a crucial role in identifying cardiac abnormalities and quantifying cardiac parameters [70]. This automated analysis improves objectivity and reproducibility while reducing manual effort [71]. Computer vision and biomedical engineering can provide unique contributions to DCM diagnosis and
treatment. Computer vision techniques can automate the analysis of cardiac tissue images and in vitro studies in Diabetic Cardiomyopathy (DCM), facilitating precise and efficient assessment of cardiac structure and function. The implementation of computer vision and biomedical engineering in the early detection of DCM requires collaboration between healthcare professionals, researchers, and engineers. This multidisciplinary approach ensures the integration of scientific expertise, imaging techniques, computational algorithms, and device development. By leveraging these fields, innovative diagnostic tools, personalized treatment approaches, and decision-support systems can be developed, ultimately improving patient care and outcomes in DCM.

2.4 CURRENT RESEARCH RELATED TO DIABETIC CARDIOMYOPATHY

Current research related to DCM is focused on exploring various aspects, including glucose regulation, preventing cardiovascular incidents and death, the role of oral glucose-lowering agents in preventing heart failure, underlying mechanisms contributing to DCM, and pharmaceutical management targeting metabolic changes caused by diabetes [72]. One significant area of research is centered around glucose regulation and its impact on reducing cardiovascular incidents and mortality in individuals with DCM. Studies are investigating alternative strategies for managing glucose levels in order to minimize the risk of cardiovascular complications and improve patient outcomes [73].

Recent evidence suggests that oral glucose-lowering agents may play a role in preventing heart failure in patients with type 2 diabetes mellitus (T2DM) [74]. These agents have shown promise in treating heart failure, irrespective of the presence of T2DM. This research highlights the potential benefits of these medications in managing heart failure and reducing the burden on the diabetic heart. Understanding the mechanisms that contribute to DCM is another focus of
current research. One area of investigation involves altered fuel utilization, where the heart relies more on fat and less on glucose than normal hearts. This shift in fuel preference can lead to intracellular lipid accumulation and altered cell signaling, impacting cardiac function [75, 76]. Additionally, the generation of advanced glycation end products (AGEs) and reactive oxygen species (ROS) in response to high glucose levels plays a role in DCM [77]. AGEs have been associated with myocardial fibrosis and affect the extracellular matrix, particularly collagen, highlighting their involvement in the development of DCM [8, 77].

Pharmaceutical management of DCM currently revolves around reducing cardiac workload. However, the primary defect in DCM is related to metabolic changes caused by diabetes, rather than issues with blood flow dynamics. Therefore, current research aims to develop targeted therapies that address the metabolic abnormalities associated with diabetes and specifically target the underlying mechanisms of DCM. By focusing on these metabolic changes, researchers aim to develop more effective treatments that address the root cause of DCM and improve patient outcomes.

In summary, current research related to DCM is focused on glucose regulation to reduce cardiovascular incidents and death. Evidence suggests that oral glucose-lowering agents may prevent heart failure in T2DM patients [78]. Studies are also exploring the mechanisms contributing to DCM, including altered fuel utilization and the role of AGEs. Further research is needed to understand the underlying mechanisms of both early-stage and advanced DCM, and to develop novel therapeutic strategies to manage the symptoms associated with this condition. Early detection and intervention methods are also being investigated to improve patient outcomes.
2.5 BIOMEDICAL ENGINEERING AND COMPUTER VISION APPLICATIONS IN DIABETIC CARDIOMYOPATHY

Biomedical engineering and computer vision have emerged as promising fields for the advancement of diagnostics and monitoring in diabetic cardiomyopathy (DCM). These technologies offer innovative approaches to early detection, non-invasive imaging, biomechanical analysis, continuous monitoring, and data integration in the context of DCM [79].

Biomedical engineering techniques play a crucial role in the early detection and monitoring of DCM. Researchers are developing and refining non-invasive imaging modalities, such as echocardiography and magnetic resonance imaging (MRI), to assess cardiac structure and function [80]. These imaging techniques provide valuable insights into the structural and functional modifications of the myocardium in DCM. Moreover, biomechanical models are being utilized to simulate and analyze the mechanical properties of the diabetic heart. These models allow researchers to study the impact of altered cardiac mechanics on DCM progression and evaluate the effectiveness of different treatment strategies.

Wearable devices and sensors have also been incorporated into DCM management [81]. These devices enable continuous monitoring of cardiac parameters, such as heart rate, blood pressure, and electrocardiogram (ECG), as well as glucose levels. Real-time data obtained from these sensors provide valuable information for the early detection of cardiac abnormalities and enable timely intervention [82]. Bioinformatics and data analytics play a critical role in integrating and analyzing complex biomedical data in DCM. These tools allow researchers to extract meaningful insights from large datasets, identify patterns, and develop predictive models for DCM progression. This integrated approach enhances the understanding of DCM and aids in personalized treatment strategies.
Computer vision techniques have gained prominence in cardiac imaging, including DCM. Automated analysis of cardiac images can detect DCM-related abnormalities, such as myocardial fibrosis and hypertrophy, with high accuracy [83]. Segmentation algorithms are employed to accurately delineate cardiac structures in imaging data, enabling precise quantification of structural changes. Feature extraction and machine learning algorithms are applied to identify patterns and develop predictive models for DCM progression [84, 85]. By analyzing large datasets, these algorithms can detect subtle changes indicative of DCM and provide valuable prognostic information for individual patients [86]. Furthermore, the integration of computer vision with other diagnostic modalities, such as molecular imaging and genetic testing, enhances the accuracy and efficiency of DCM diagnosis. By combining information from multiple modalities, clinicians can obtain a comprehensive understanding of the disease and tailor treatment strategies accordingly.

In summary, biomedical engineering and computer vision, including computer vision techniques in microscopic analysis and in vitro studies, offer valuable tools and techniques for the early identification and analysis of the progression of DCM. Non-invasive imaging modalities, biomechanical models, wearable devices, and sensors contribute to detection and continuous monitoring of cardiac parameters, while computer vision techniques enable automated analysis of cardiac images and pattern recognition, including microscopic analysis of cardiac tissue. The integration of these technologies enhances the accuracy, efficiency, and personalized management of DCM, providing a comprehensive understanding of the disease at both macroscopic and microscopic levels. Bioinformatics and data analytics further aid in integrating and analyzing complex biomedical data, enabling researchers to extract valuable insights [87]. These advancements in biomedical engineering and computer vision hold promise for improved
outcomes in individuals with DCM, facilitating early intervention and targeted therapeutic approaches.

### 2.6 The Role of Biomedical Engineering and Computer Vision

Biomedical engineering, in conjunction with computer vision, offers promising avenues for advancing healthcare, including the management of DCM. These fields provide innovative solutions for early detection, precise monitoring, and personalized treatment strategies. Biomedical engineering techniques have facilitated the development of non-invasive imaging modalities such as echocardiography and magnetic resonance imaging (MRI) [88]. These modalities allow clinicians to assess cardiac structure and function in DCM patients, aiding in detection and ongoing monitoring. Additionally, biomechanical models have been employed to simulate and analyze the mechanical properties of the diabetic heart, offering valuable insights into disease progression [88].

The integration of wearable devices and sensors within biomedical engineering has revolutionized continuous monitoring of cardiac parameters and glucose levels in individuals with DCM [89]. These devices provide real-time data, enabling healthcare professionals to make informed decisions and intervene promptly when necessary. Moreover, bioinformatics and data analytics play a crucial role in integrating and analyzing complex biomedical data, facilitating the interpretation of intricate disease patterns and treatment outcomes.

Computer vision techniques have also found application in the context of DCM. Through automated analysis of cardiac images, computer vision algorithms can identify DCM-related abnormalities, such as myocardial fibrosis and hypertrophy, with remarkable accuracy [90]. Furthermore, segmentation algorithms enable precise delineation of cardiac structures, enhancing the accuracy of diagnostic assessments. Machine learning algorithms, in combination with feature
extraction techniques, aid in pattern recognition and predictive modeling of DCM progression [83]. The integration of computer vision with other diagnostic modalities further enhances the efficiency and accuracy of DCM diagnosis.

In conclusion, biomedical engineering and computer vision hold great promise in transforming the landscape of healthcare. In the realm of diabetic cardiomyopathy, these fields provide innovative solutions for early detection, accurate monitoring, and personalized treatment. By harnessing the potential of these technologies, we can improve the lives of individuals with T2DM and reduce the burden of diabetic cardiomyopathy, ultimately leading to better health outcomes [91].

2.7 IDENTIFYING RESEARCH GAPS FOR HISTOPATHOLOGICAL ANALYSIS AND COMPUTER VISION APPLICATIONS

Research in the field of diabetic cardiomyopathy (DCM) has made significant progress in recent years. However, there are still several research gaps that need to be addressed to enhance our understanding of DCM and develop more effective diagnostic and therapeutic approaches. Two specific areas that require attention are histopathological analysis and the application of computer vision in investigating DCM.

1. Histopathological Analysis: Histopathological analysis involves the microscopic examination of heart tissue to study the structural and cellular changes associated with DCM. Although various imaging modalities, such as echocardiography and MRI, provide valuable information about cardiac structure and function, histopathological analysis offers a more detailed understanding of the underlying pathological changes [92]. It helps identify specific histological features, including interstitial fibrosis, myocyte hypertrophy, and
inflammation, which are characteristic of DCM. Histopathological analysis also enables the assessment of cellular changes, such as mitochondrial dysfunction and apoptosis, which contribute to cardiac dysfunction in DCM [93].

Histopathological studies can provide insights into the mechanisms driving DCM, identify novel therapeutic targets, and validate findings from other diagnostic modalities. However, there is a need for more comprehensive and standardized histopathological studies in DCM. Large-scale studies involving diverse patient populations, including different ethnicities and age groups, can help establish a clearer understanding of the histopathological characteristics of DCM [94]. Additionally, the integration of molecular techniques, such as gene expression analysis and proteomics, with histopathological analysis can provide a deeper understanding of the molecular pathways involved in DCM pathogenesis.

2. Computer Vision Applications: Computer vision techniques have shown great potential in various medical fields, including cardiology. The application of computer vision in investigating DCM can facilitate the automated analysis of cardiac images, improve diagnostic accuracy, and enable early detection of DCM-related abnormalities. Computer vision algorithms can be trained to identify and quantify specific features associated with DCM, such as myocardial fibrosis, hypertrophy, and altered cardiac geometry [95]. This can help clinicians in making more accurate diagnoses and monitoring disease progression. Furthermore, the integration of computer vision with other diagnostic modalities, such as echocardiography or MRI, can enhance the efficiency and accuracy of DCM diagnosis. Computer vision algorithms can assist in the segmentation of cardiac structures and the extraction of relevant features for analysis [96]. Machine learning algorithms, combined with computer vision
techniques, can aid in pattern recognition and predictive modeling of DCM progression, enabling personalized treatment strategies.

However, the application of computer vision in investigating DCM is still in its early stages, and more research is needed to develop robust and reliable algorithms. Large datasets of cardiac images, including diverse patient populations, are required to train and validate these algorithms [97]. Moreover, the integration of computer vision with other emerging technologies, such as artificial intelligence and deep learning, can further enhance the capabilities of computer vision in DCM research.

In conclusion, histopathological analysis and the application of computer vision techniques are two areas that can significantly contribute to our understanding of DCM. Histopathological studies can provide detailed insights into the structural and cellular changes associated with DCM, while computer vision applications can enhance diagnostic accuracy, enable early detection, and aid in disease monitoring [98]. Further research in these areas is necessary to bridge existing gaps and improve the diagnosis and management of DCM.
Chapter 3: Methodology

The following scientific methods will be applied to approach the construction and analysis of 2D cardiac tissue mimics of DCM. This chapter will provide a brief overview of the significance of the methodology used to accomplish this dissertation.

3.1. Experimental Animals

In this study, we utilized the spontaneous type 2 diabetic model of Zucker diabetic fatty (ZDF) rats from Charles River (Wilmington, MA, USA), along with lean control rats. The animals were housed in pairs in an SPF facility with appropriate conditions for lighting, humidity, and temperature. The diabetic ZDF rats were provided with Purina #5008 lab diet and water ad libitum throughout the experiment. We included 9 to 10-week-old ZDF rats with blood glucose levels around 300 mg/dl, along with age-matched control rats. Blood glucose levels were measured once a week using the OneTouch Ultra glucose meter from LifeScan, Inc. (Milpitas, CA, USA). All experimental procedures were conducted following the approved institutional animal care and use protocol (IACUC, TTUHSC El Paso, TX, USA), and in accordance with the ARRIVE guidelines [8].

3.2. Treatment and Tissue Collection

Fourteen-week-old diabetic animals were injected with Glycyrrhizin (GLC) at a dose of 50 mg/kg per day intraperitoneally for 5 days a week over a period of 4 weeks. The animals were divided into three groups: lean control, diabetic with vehicle treatment, and diabetic with GLC treatment. Each group consisted of 8-10 animals. At the end of the four-week treatment period, animals were euthanized for tissue collection following the AVMA guidelines for euthanasia. The heart tissues were collected after ice-cold 0.9% saline perfusion and stored at -80 °C for further use [8].
3.3. Histopathological Analysis and Collagen Deposition Quantification

A portion of the ventricular heart tissue was embedded in OCT embedding medium, cut into 10 µm sections, fixed in 4% paraformaldehyde, and washed in running water for 5 minutes. The sections were then stained with Harris modified Hematoxylin solution for 5 minutes, followed by running water washes until clear. Subsequently, the sections were immersed in Gomori’s trichrome stain for 15 minutes and differentiated in 1% acetic acid for 1 minute. After dehydration in alcohol solutions and clearing in xylene, coverslips were mounted onto the tissue with Permount mounting medium. Gomori’s Trichrome stained sections were examined using a Nikon Eclipse Ni-E microscope (Nikon Instruments Inc., Melville, NY, USA) to evaluate collagen deposition. Gomori’s Trichrome stained cardiac tissue sections were analyzed to measure collagen density and compare it among the naïve control, diabetic alone, and diabetic with GLC treatment groups, aiming to identify the effects of GLC treatment on collagen deposition in the cardiac ventricular muscle. The ImageJ image analysis software was employed to measure the intensity of the Trichrome stained fibers, following the authors' previous method. The images were captured using a Nikon Eclipse Ni-E microscope with a 20X objective. An investigator, blinded to the treatment group, evaluated the tissue sections from 3 different areas for each animal.

3.4. Immunohistochemistry and Immunocytochemical Analysis

After the completion of the treatment regimen, the whole heart was collected, fixed with 4% paraformaldehyde overnight, cryoprotected with 30% sucrose in phosphate-buffered saline (PBS) for 1-2 days, and then cut into smaller pieces. The tissue pieces were embedded in OCT embedding solution and stored at -80 °C. Blocks were sectioned into 10 µm thick slices and placed on specific gelatin-coated slides. The sections were fixed with 4% paraformaldehyde for 30 minutes, washed with PBS three times, and incubated with a blocking solution (PBS with 1% NGS
and 0.3% Triton X-100) for 60 minutes. After a wash, the sections were incubated overnight at 4 °C with primary antibodies against Cx43, CXCR4, MyoD (1:400; Cell Signaling, Danvers, MA, USA) or Troponin-I (1:500; Thermo-Fisher Scientific, Waltham, MA, USA). Subsequently, the sections underwent three washes and were incubated with secondary antibodies, Alexa Fluor 594 goat anti-rabbit IgG and 488 goat anti-mouse IgG (1:2000; Thermo-Fisher Scientific, Waltham, MA, USA), for one hour at room temperature. After three additional washes, the slides were stained with DAPI and mounted in Fluoromount G (Electron Microscopy Sciences, Fort Washington, PA, USA).

Cx43, CXCR4, MyoD, and Troponin-I (TnI) stained cardiac tissue sections were analyzed to measure changes in color intensity and compare them among the naïve control, diabetic alone, and diabetic with GLC treatment groups to determine the effects of GLC treatment on Cx43, CXCR4, MyoD or TnI in the cardiac ventricular muscle. The Nis Elements software (Nikon Instruments Inc., Melville, NY, USA) was utilized to measure the intensity of the Cx43, CXCR4, MyoD, and TnI stained images. An investigator, blinded to the treatment group, evaluated the tissue sections from 3 animals per group and 3-5 different areas for each animal. In the cell experiment, immunocytochemical experiment was performed with a set of cardiomyocytes from all group using antibodies to specific markers of inflammation and injury such as CXCR4 and Troponin-I.

3.5. CELL CULTURE AND TREATMENT

The AC16 human cardiomyocyte (CM) cell line (Millipore cat no. SCC109) was cultured in Dulbecco’s Modified Eagle’s Medium/Nutrient Mixture F-12 Ham (DME/F-12) supplemented with 10% fetal bovine serum (FBS). This cell line was derived from primary human ventricular cardiac tissue cells and fused with uridine auxotroph human fibroblasts deficient in mitochondrial
DNA through SV40 transformation. The cells were seeded in culture plates overnight until they reached 50% confluency. Once the cells reached 70-80% confluency, they were divided into control and experimental groups (n = 2 per group). The control group consisted of cardiomyocytes without any intervention. In the second group the AC16 cardiomyocyte cells were treated with 25 mM of glucose overnight. The third group of cardiomyocytes with 25 mM glucose were treated with 50 µM of GLC to assess the anti-inflammatory effect of GLC on hyperglycemia. After 24 hours, the cells were harvested, and protein extraction procedures and analysis were performed as described above.

3.6. **Statistical Analysis**

The control, diabetic, and treated diabetic rats were randomly assigned to their respective experimental groups, and samples were evaluated randomly. Analysis of variance (ANOVA) was conducted to compare the groups, followed by Bonferroni’s multiple comparison tests in the post hoc analysis. All statistical analyses were performed using SPSS software (Systat version 13.0, SPSS Inc., Chicago, IL, USA), and a p-value < 0.05 was considered statistically significant.

3.7. **Python Programming**

Although microscope imaging software exists for biomedical analysis, the imaging is (a) limited to specific analyses, (b) does not include automation, and (c) is generally not dynamic in terms of optimizing for specific image background differences. The application of OpenCV (version 4.5.1), an open-source computer vision package library maintained by Intel Corporation for Python (version 3.9), was explored to demonstrate its potential at the microscopic level. The algorithm developed for this study was entirely based on OpenCV functions, rendering the code customizable and comprehensible with the provided documentation. As Python has become the language platform of choice for data analytics, a diversity of libraries exists to allow for the most
advanced mathematical and machine learning modeling to be integrated into biomedical analysis to further assist in providing insights of the efficacy of treatments based on analysis. In this dissertation, no machine learning was performed given the large number of images required for training, however, image analysis was automated and dynamically optimized for the evaluation of a biomarker by specifically extracting features from the image (colors, contour sizes and shapes, contour number, etc.), quantifying these features and calculating a set of metrics to guide evaluation of the biomedical images. The images in Figure 3.1 shows the general flow for biomedical imaging.

**Figure 3.1:** Using computer vision in microscope image analysis offers advantages such as processing large image volumes efficiently. It automates analysis, saving time and reducing human errors. Researchers can analyze extensive datasets, discover biomarkers, and find therapeutic targets in diabetic cardiomyopathy. This enhances research efficiency and facilitates important biomedical advancements.
Two specific algorithms were developed to evaluate early detection of biomarkers in diabetic cardiomyopathy: Cx43, CXCR4, MyoD, and Troponin-I (TnI). Each algorithm extracted appropriate features from the different biomarkers exposing and emphasizing different components in the images (nuclei, muscle fiber, and specific biomarkers). Both algorithms included the same initial image preparation sequence by converting the images to 8-bit grayscale by distilling pixels represented by initially with three channels (red, green and blue – all in 8 bit integer format) (Illustration 1.1) to single 8 bit integer pixel capturing intensity (Illustration 1.1a). The gray images were blurred to remove noise (Illustration 1.1b) and then thresholded (Illustration 1.1c) to create an image mask and customized contours as shown in Illustration 1.1d.

Upon pre-processing (identical starting sequence for both algorithms), algorithm-specific computations were completed with the extraction of relevant features, measurement of the features and finally a comprehensive efficacy metric was calculated per image. The two algorithms developed for this analysis included the:

**Biomarker Area Algorithm**: Subsequently, a median filter was applied with varying kernel sizes specific to each image to reduce image noise. A binary threshold was then employed to isolate the regions of interest, with the threshold value determined based on the specific cell or muscle tissue present in the image. The ability of dynamically adjusting the threshold based on interrogating the entire image or even a collection of images, allows this software to automatically optimize the process of isolating the regions of interest. After the thresholding process, a black and white mask was obtained, where white represented the areas of interest. Contours were then detected using the relevant OpenCV functions, and the area of each contour and other geometric features were calculated. The draw contour function was utilized to overlay the threshold image onto the original image, providing a visual representation of the detected areas. Consequently,
trial runs could be executed and evaluated for effectiveness based on expert feedback. Additionally, statistical information regarding the contour areas was incorporated into the image in an unobtrusive corner. By comparing the information obtained from the contour areas with previous datasets, the computer vision analysis yielded comparable results. This highlights the potential of OpenCV as a valuable tool for automated analysis and quantification of microscopic data, facilitating reliable and efficient research in various scientific fields as shown in Figure 3.2.
**Figure 3.2:** Small region of interest to represent biomarker area algorithm. (a) represents an example original color image (2136 rows, 2399 columns, 3 colors – blue, green, red each represented in 8-bit integer format); (b) gray scale (2136 rows, 2399 columns, 1 channel) with image blurring to reduced noise; (c) thresholding to a binary bit mask; and (d) red drawn contours on to original color image to highlight identified geometry. Contours provide perimeter, area, frequency, and other moments which can be used in weighted averages to produce comprehensive metrics for the efficacy of the treatments.
**Biomarker Customized Contour Algorithm**: For the second algorithm after the identical initial preparation sequence, a median filter was applied with varying kernel sizes specific to each image to reduce image noise. A binary threshold was then employed to isolate the regions of interest, with the threshold value determined based on the specific cell or muscle tissue present in the image. The ability of dynamically adjusting the threshold based on interrogating the entire image or even a collection of images, allows this software to automatically optimize the process of isolating the regions of interest. After the thresholding process, a black and white mask was obtained, where white represented the areas of interest. Contours were then detected using the relevant OpenCV functions, and the area of each contour and other geometric features were calculated. The draw contour function was utilized to overlay the threshold image onto the original image, providing a visual representation of the detected areas. Consequently, trial runs could be executed and evaluated for effectiveness based on expert feedback. Additionally, statistical information regarding the contour areas was incorporated into the image in an unobtrusive corner. By comparing the information obtained from the contour areas with previous datasets, the computer vision analysis yielded comparable results as shown in Figure 3.3. This highlights the potential of OpenCV as a valuable tool for automated analysis and quantification of microscopic data, facilitating reliable and efficient research in various scientific fields.
Figure 3.3: Small region of interest to represent biomarker customized contour algorithm: (a) an example red channel; (b) gray scale (1 channel) with image blurring to reduced noise; (c) thresholding to a binary bit mask; and (d) red and blue contours on to original color image to highlight identified geometry, an example original color image (blue, green, red each represented in 8-bit integer format). Contours provide perimeter, area, frequency, and other moments which can be used in weighted averages to produce comprehensive metrics for the efficacy of the treatments.
Chapter 4: Results

Persistent high blood sugar levels in individuals with diabetes can lead to cardiovascular problems, including myocardial dysfunction. Diabetic cardiomyopathy, characterized by damage to the heart's contractile cells, is a major complication of diabetes. The exact mechanisms underlying cardiac atrophy in diabetes are not fully understood, but it is believed to involve inflammation, oxidative stress, and glucose toxicity. Controlling glucose levels and inhibiting inflammation have been proposed as therapeutic approaches to mitigate the detrimental effects of hyperglycemia. Cardiac fibrosis, which is associated with long-term uncontrolled hyperglycemia, involves various factors and pathways, including increased oxidative stress markers, fibroblast proliferation, inflammatory cytokine expression, and chemokine receptor activation. Inflammation has been implicated in the pathogenesis of cardiovascular disease, and elevated troponin levels have been observed in diabetic patients with cardiovascular complications.

In this study, the focus is on examining four key biomarkers in the context of diabetic cardiomyopathy (DCM): Cx43, CXCR4, troponin-I, and MyoD. Cx43, a gap junction protein crucial for cell-cell communication in the heart, shows reduced expression in various cardiac pathologies, including DCM. Alterations in Cx43 expression may contribute to disease progression, as reduced levels have been associated with increased cell necrosis in the myocardium. Investigating Cx43 expression in the myocardium of diabetic individuals can provide valuable insights into the extent of cell necrosis and its impact on DCM development.

Another biomarker of interest is CXCR4, a chemokine receptor involved in cell signaling and migration processes. Dysregulation of CXCR4 has been implicated in inflammation, endothelial dysfunction, and impaired cardiac function. In the context of DCM, altered CXCR4 expression may contribute to the progression of the disease. Understanding the role of CXCR4 in
cardiovascular pathology, including its potential implications for DCM, is of significant interest for identifying potential therapeutic interventions. Troponin-I, a well-established biomarker for myocardial injury, plays a crucial role in assessing cardiac damage. Elevated levels of troponin-I in the bloodstream indicate myocardial injury, providing valuable evidence of disease severity and progression. Monitoring troponin-I levels can aid in the diagnosis and prognosis of DCM and guide treatment strategies.

Lastly, MyoD, a marker for cardiac muscle loss, is involved in regulating the differentiation of myoblasts into cardiac muscle cells. Disruption of MyoD expression in diabetic tissue may contribute to the loss of cardiac muscle integrity and the development of DCM. Investigating MyoD expression in the myocardium can provide insights into the impact of diabetic conditions on cardiac muscle and its association with DCM. By examining these biomarkers, the study aims to gain a deeper understanding of the underlying mechanisms and pathology of DCM. This knowledge can contribute to improved diagnosis, prognosis, and the development of targeted treatment strategies for DCM.

Glycyrrhizin (GLC), an anti-inflammatory and antioxidant compound found in licorice root, has shown protective effects in various organs and tissues, including the heart. Previous studies have demonstrated GLC has the ability to alleviate liver damage and neuropathic pain in diabetes. In this study, we investigated the role of inflammatory mediators in hyperglycemia-induced cardiomyocyte atrophy and explored the potential therapeutic use of GLC in preventing cardiovascular complications in diabetes. Current therapeutic strategies mainly focus on managing hyperglycemia and lipid levels, but targeting inflammation and oxidative stress may be crucial for improving cardiac function in diabetes.
4.1. **Specific Aim 1: Histopathological Analysis of Rat Hearts: Assessing Diabetic Cardiomyopathy-Induced Changes in Microscopic Anatomy**

Under conditions of elevated blood sugar levels (hyperglycemia), cardiac cells or cardiomyocytes may undergo initial modifications that can trigger the release of essential inflammatory mediators, including cytokines and chemokines. The presence of excessive glucose in the bloodstream has the potential to disrupt signaling processes within cardiomyocytes, consequently promoting oxidative stress, fibrosis, and ultimately the death of these cells.

**Specific Aim 1a: Characterizing Morphological Changes in Diabetic Cardiac Tissue Using Hematoxylin and Eosin Staining.**

The presentation and interpretation of the microscopic anatomy changes observed in the diabetic (ZDF) and healthy (lean) rat models, as well as the morphological changes in diabetic heart tissue compared to lean tissue, are depicted in **Figure 4.1**. The findings from Hematoxylin and Eosin (H & E) staining provide insights into the structural alterations associated with diabetic cardiomyopathy (DCM). Histological analysis of myocardial tissue sections from lean (control) and ZDF (diabetic) rats was performed using hematoxylin and eosin (H&E) staining, as shown in **Figure 1a and b**. To assess cardiac fiber length, the size of the longest intact cardiac muscle fiber was measured in all samples. The average lengths of the fibers were determined to be 522 ± 90 arbitrary units (a.u.) for the lean group and 353 ± 144 a.u. for the diabetic group, as depicted in **Figure 4.1a**. Statistical analysis revealed a significant difference in fiber length between the lean controls and diabetic group (p=0.004), indicating muscle atrophy in the diabetic group. This reduction in fiber length can be attributed to muscle lesions and fragmentation observed in the
diabetic group, suggesting cardiac tissue atrophy that may contribute to other heart-related symptoms.

Figure 4.1: Image depicts representative myocardium tissue sections stained with hematoxylin and eosin (H&E) for the lean and diabetic groups. The images are shown at a magnification of 20X, and a total of six animals were included in the analysis. Fig. 1b in the control group, the myocardial tissue exhibits a larger proportion of cardiac muscle compared to the diabetic group. The quantification of fiber length (a) shows a decrease in average length in the diabetic samples; significant level: p<0.004. Additionally, an increase in waviness of the fibers is observed (b), suggests that the fibers in the diabetic group become wavier and suggest damage in the myocardium.
Specific Aim 1b: Comparative Analysis of Diabetes-Induced Myocardial Fibrosis: Gomori's Trichrome Staining for Assessing Impact on Myocardium.

Histopathological examination of Gomori's trichrome stain was utilized to assess collagen deposition during the early stages of cardiac fibrosis in ZDF diabetic rats, lean controls, and ZDF rats treated with GLC. The analysis revealed a significant upregulation of collagen (identified by a blueish green color) in the cardiac tissue of ZDF diabetic rats compared to age-matched lean controls, as illustrated in Figure 4.2. Additionally, the presence of fibrosis was observed in the cardiac muscles of ZDF rats. Remarkably, treatment with GLC in ZDF animals resulted in a significant reduction in collagen deposition within the heart, in comparison to untreated ZDF rats (p < 0.01). The histopathological findings obtained from Gomori's trichrome stain indicate that diabetes has the potential to induce elevated collagen deposition in cardiac tissues, as depicted in Figure 4.2 (collagen stained blue, while cytoplasm and muscle fibers stained red). The administration of GLC demonstrated a promising effect in mitigating this collagen deposition, suggesting its therapeutic efficacy in decreasing diabetic-associated cardiac fibrosis.
Figure 4.2: The figure illustrates the effect of Glycyrrhizin (GLC) treatment on collagen deposition in ventricular heart tissues of type 2 diabetic animals. (a) Quantification of collagen positive area as a percentage of control, calculated from positive trichrome staining. The data is presented as means ± standard error (SE) with a sample size of 5. Statistical significance is denoted as p< 0.001. (b) Histopathological assessment of Gomori's trichrome stain in control, diabetic, and GLC-treated diabetic groups. Arrows indicate areas of collagen deposition and fibrosis. Representative images of ventricular heart tissues from lean control rats, ZDF diabetic rats, and ZDF diabetic rats treated with GLC are shown. The scale bar represents 50 µm. This figure demonstrates the significant increase in collagen deposition observed in the ventricular heart tissues of type 2 diabetic animals, which was ameliorated after GLC treatment.
4.2. **Specific Aim 2: Exploring Differential Expression of Early Biomarkers in Diabetic Cardiomyopathy: Insights from the ZDF Rat Model**

While hyperglycemia primarily contributes to myocardial dysfunction, modifying glucose levels can potentially alleviate glucose-mediated toxicity. The detrimental impact of hyperglycemia can be mitigated by therapeutic interventions targeting the inflammatory cascade. Prolonged and uncontrolled hyperglycemia may induce cardiac fibrosis, which involves a complex interplay of various factors and pathways, including increased oxidative stress markers, proliferation of cardio-fibroblasts, elevated expression of inflammatory cytokines, and activation of chemokine receptors like CXCR4. Growing evidence suggests that inflammation plays a significant role in the pathogenesis of cardiovascular disease. Numerous studies have demonstrated higher levels of circulating troponin in diabetic patients with cardiovascular disease compared to those without diabetes, suggesting its potential as a marker of cardiac inflammation in hyperglycemia.

**Specific Aim 2a: Examining Cx43 Expression in Diabetic Cardiomyopathy: Potential Indicator of Disrupted Cell-Cell Communication.**

To understand the interaction of gap junction protein in diabetic heart, Cx43 expression in the ZDF rat myocardium was evaluated, particularly to study the disruption of intercellular communication as illustrated in Figure 4.3. The reduced Cx43 expression in the ZDF rat myocardium highlights potential alterations in cell-cell communication mechanisms associated with diabetic cardiomyopathy (DCM). GLC treatment in type 2 diabetic rats was found to modulate the expression of connexin-43 (Cx43) in cardiac tissue (Figure 4.3). In diabetic animals, analysis revealed a decrease in Cx43 expression compared to lean control animals (Figure 4.3a).
However, GLC-treated animals showed restoration of Cx43 expression, which was further confirmed by immune-histochemical studies (Figure 4.3b). This suggests that GLC treatment can positively influence cell adhesion in the diabetic cardiac tissue.

**Illustration 4.1**: Anatomical location of Cx43 in the diabetic heart.
Figure 4.3: Impact of GLC treatment on the expression of gap junction protein connexin-43 (Cx43) in the cardiac tissue of Type 2 diabetic rats. The expression of Cx43 was evaluated through immunohistochemistry in ZDF diabetic rats, diabetic rats treated with GLC, and lean control rats. (a) The analysis revealed a decrease in Cx43 expression in the ventricular cardiac tissue of diabetic animals compared to the lean control group. However, GLC-treated cardiac tissue showed a restoration of Cx43 expression, indicating a positive effect of GLC treatment. The difference between the GLC-treated group and the diabetic-only group was statistically significant (p < 0.01), and there was also a significant difference between the GLC-treated group and the diabetic-only group (* p < 0.05). (b) Immunohistochemical analysis further supported the reduced expression of Cx43 in diabetic animals, while GLC-treated animals displayed improved Cx43 expression. The arrows in the figure indicate the positions of Cx43 staining, with a scale bar of 100 μm.
Specific Aim 2b: Investigating the Role of CXCR4 in Diabetic Cardiomyopathy: Implications for Inflammation.

CXC chemokine receptor 4 (CXCR4), an inflammatory modulator which may play a role in cardiac contractability was evaluated for its expression in the ZDF rat myocardium and to understand its role in cardiovascular pathology, as well as its potential as a therapeutic target in Figure 4. To investigate the underlying mechanisms and the involvement of inflammatory mediators in cardiac fibrosis, CXCR4, a G protein-coupled receptor, was assessed. Immunohistochemistry analysis of ventricular tissue from ZDF rats demonstrated a significant increase in CXCR4 expression compared to lean control animals. Furthermore, GLC treatment was observed to alter the expression of CXCR4, indicating its potential role in effecting inflammation (Figure 4a). The reduction in CXCR4 expression following GLC treatment, suggests an anti-inflammatory effect in diabetic cardiac atrophy (Figure 4a, b).
Figure 4.4: Changes in chemokine receptor CXCR4 expression in the diabetic heart tissue and its response to GLC treatment. After 4 weeks of GLC treatment, there was a reduction in the increased expression of CXCR4 observed in the diabetic heart tissue. (a) This reduction in CXCR4 expression was evident in the analysis (** p < 0.01), and GLC treatment resulted in an alteration in CXCR4 expression (* p < 0.05). (b) Immunohistochemical illustrations further confirmed the decrease in CXCR4 expression after GLC treatment in diabetic animals compared to the diabetic group, as indicated by the arrow representing CXCR4-positive staining (Scale bar = 50 μm).
Specific Aim 2c: Assessing Myocardial Injury through Troponin-I Expression: A Biomarker for Diabetic Cardiomyopathy.

Results from Specific Aim 2c of this study revealed significant findings regarding the expression of Troponin-I (TnI) in the cardiac tissue of ZDF rats and its response to GLC treatment. Figure 5 displays the interpretation of Troponin-I expression, providing compelling evidence of myocardial injury in diabetic cardiomyopathy (DCM).

Immunohistochemistry analysis was conducted to evaluate TnI expression in heart tissues obtained from ZDF rats. Immunofluorescence images captured from lean control rats, ZDF diabetic rats, and ZDF rats treated with GLC were carefully examined and analyzed. The intensity of TnI immunostaining was measured to assess changes in expression levels. The analysis demonstrated noticeable alterations in TnI expression within the GLC-treated group of diabetic rats after a 4-week treatment period, compared to the ZDF diabetic rats. These findings strongly suggest the potential of GLC treatment in reducing cardiac tissue damage associated with DCM.

Furthermore, the study observed a significant increase in TnI expression in ZDF diabetic rats compared to the lean control animals, indicating the presence of myocardial injury in the diabetic rat model, which aligns with the development of diabetic cardiomyopathy. The representative images presented in Figure 5 visually showcase the observed differences in TnI expression, with the red arrows highlighting areas of positive TnI staining. The scale bar, included in the images, represents 100 μm, providing a reference for the size of the captured structures.

In conclusion, the results obtained from Figure 5 provide compelling evidence of myocardial injury indicated by Troponin-I expression in the ZDF rat model of diabetic cardiomyopathy. These findings highlight the potential therapeutic implications of GLC treatment in attenuating cardiac tissue damage, offering promising avenues for managing DCM.
Figure 4.5: Immunofluorescent analysis of Troponin-I (TnI) expression in cardiac tissue was conducted to investigate myocardial injury in the ZDF rat model of diabetic cardiomyopathy (DCM). The images captured in Figure 5 depict the interpretation of TnI expression and its response to Glycyrrhizin (GLC) treatment. (a) TnI immunostaining intensity was analyzed in heart tissues from lean control, ZDF diabetic, and GLC-treated ZDF diabetic rats. Noticeable changes in TnI expression were observed after 4 weeks of GLC treatment in diabetic rats (* p < 0.05) compared to ZDF diabetic rats. (b) Representative images show TnI-positive staining in red, with arrows indicating areas of positive staining (Scale bar = 100 μm). These results provide evidence of myocardial injury and suggest the potential of GLC treatment in attenuating cardiac tissue damage in DCM.
Specific Aim 2d: Immunohistochemistry Analysis of MyoD Expression and Muscle Atrophy in the ZDF Rat Myocardium.

Hyperglycemic condition in diabetes increases oxidative stress, upregulates low-grade inflammation and exhibits impaired ECM remodeling which directly affects the muscle health and functionality as well as alters the myogenesis as described by the illustration 4.2 below.

Illustration 4.2: In diabetes, high blood sugar levels cause oxidative stress, low-grade inflammation, and impaired ECM remodeling. These directly impact muscle health and functionality while also altering the process of myogenesis (muscle tissue formation).

The MyoD, a myogenic gene which is expressed in cardiac muscle was evaluated in the ZDF rat myocardium to understand its role in skeletal muscle transcription in myogenesis as well as disruption of cardiac muscle, and its association with diabetic cardiomyopathy as shown in Figure 4.6. Immunohistochemistry analysis of MyoD, a crucial protein involved in muscle differentiation regulation, was performed in Figure 4.6. In Figure 4.6a, the results indicate muscle atrophy, as evidenced by decreased MyoD expression. The reduced expression of MyoD in the diabetic tissue (Figure 4.6b) suggests possible disruptions or apoptosis in the cardiac tissue, as well as potential degeneration of cardiac myocytes that may contribute to the development of diabetic cardiomyopathy. Future investigations will aim to incorporate additional biomarkers to further elucidate the pathogenesis of diabetic cardiomyopathy (DCM).
Figure 4.6: Immunohistochemistry analysis of MyoD in myocardium samples from lean, ZDF groups, and ZDF rats treated with GLC were analyzed. Tissue sections were stained for MyoD (green). Panel (b) illustrates a representative image of lean myocardium, demonstrating preserved tissue structure with normal MyoD expression. In contrast, diabetic group shows myocardium from diabetic rats, displaying disrupted tissue architecture and the presence of edema (b). The intensity of MyoD immunostaining in the cardiac tissue of diabetic rats treated with GLC showed noticeable changes after treatment, compared to ZDF diabetic rats (a). Scale bar = 100 µm.
4.3. **Specific Aim 3: Computer Vision Analysis of Diabetic Rat Heart Tissue and 2D Cell Culture: Automated Extraction of Algorithmic Features and Patterns for Enhanced Understanding and Diagnosis of Diabetic Cardiomyopathy with Customized Adaptation for Varied Image Conditions.**

This section provides the description of the computer vision techniques applied to analyze histological images of the diabetic rat myocardium and 2D cell cultures of AC16 human cardiomyocytes and treatment identification and quantification of DCM-related features and patterns using computer vision algorithms. The application of OpenCV, an open-source computer vision package for Python, was explored to demonstrate its potential in analyzing microscopic images. The algorithm developed for this study utilized OpenCV functions, providing a flexible and accessible framework for customization and comprehension with the accompanying documentation.

To extract the area of different cells and muscle fibers in the microscopic images, several preprocessing steps were employed. Firstly, the images were converted to an 8-bit/pixel grayscale format, enabling grayscale intensity-based analysis. Subsequently, a median filter with varying kernel sizes, tailored to each specific image, was applied to reduce noise and enhance image clarity. The algorithm employed a binary thresholding technique to isolate the regions of interest, where the area of the cells and muscle fibers needed to be determined. The threshold value for each pixel in the image was determined based on the specific cell or muscle tissue present in the image, ensuring accurate segmentation. As a result, a binary image was generated, with white pixels representing the areas of interest.

Using the appropriate OpenCV functions, contours were detected in the binary image, outlining the boundaries of the cells and muscle fibers. The area of each contour was calculated,
providing quantitative measurements of the regions of interest. The draw contour function was then applied to overlay the threshold image onto the original image, visually representing the detected areas. To augment the analysis, statistical information pertaining to the contour areas was added to the images. This additional data facilitated a comprehensive understanding of the morphological characteristics of the cells and muscle fibers. Comparisons between the obtained contour area information and previous datasets enabled validation and verification of the computer vision analysis results.

The computer vision analysis yielded comparable results when compared to previous datasets. The quantitative measurements of cell and muscle fiber areas provided valuable insights into cellular morphology and tissue structure. By leveraging the capabilities of OpenCV, the algorithm enabled accurate and efficient analysis of microscopic images, demonstrating its potential as a powerful tool for automated image analysis in various scientific fields.

In conclusion, the utilization of OpenCV for computer vision analysis at the microscopic level showcased its versatility and effectiveness in extracting and analyzing areas of interest in images with customizable and dynamic algorithms. The algorithm's reliance on OpenCV functions ensured ease of modification and understanding, facilitating customization based on specific research requirements. Furthermore, by pre-evaluating images, thresholds can be set dynamically, increasing the effectiveness for a given image. For instance, in the simplest example, the average and median saturation value across all pixels for a given image could be calculated and used in optimizing thresholds for identifying different features. The results obtained from the computer vision analysis provided valuable insights and contributed to the advancement of microscopic image analysis techniques in scientific research.
Specific Aim 3a: Image Analysis of Diabetic Rat Heart Tissue

Specific Aim 3.1a: Image Analysis of Collagen Deposition in Diabetic Rat Heart Tissue

The results in Figure 4.7 demonstrate the potential of computer vision techniques for quantitative assessment and characterization of collagen deposition in diabetic cardiomyopathy. Figure 4.7 shows representative rat heart tissue images stained with trichrome in the control, diabetic, and treatment groups. Computer vision analysis was performed to assess collagen levels and characteristics. In the control and treatment groups, computer vision analysis reveals reduced collagen levels compared to the normal physiological state (b). This is indicated by decreased color intensity in the representative images. Conversely, in the diabetic group, computer vision analysis detects a significantly higher average area of collagen (b), suggesting abnormal collagen deposition compared to the treatment and control groups. This is evident through increased color intensity in the representative images (Figure 4.7a).

Furthermore, computer vision analysis has the capability to identify and quantify specific amounts of collagen and contour collagen deposition based on exact shape. In the context of this study, the computer vision analysis identified a significantly greater number of contours in collagen within the diabetic group (c). This finding suggests increased complexity or fragmentation of collagen structures in the diabetic myocardium. The results obtained through computer vision-based analysis of trichrome staining provide valuable insights into collagen levels and characteristics in different experimental groups, highlighting the potential of computer vision techniques for quantitative assessment and characterization of collagen deposition in the context of diabetic cardiomyopathy.
This computer vision analysis yields results that are consistent with those obtained through biological analysis. However, the advantage of computer vision lies in its capability to identify and quantify specific amounts of collagen and contour collagen deposition based on exact shape. This enhanced precision allows for a more accurate and detailed characterization of collagen structures in the diabetic myocardium. The combination of computer vision techniques with traditional biological analysis provides a comprehensive and robust approach for understanding collagen deposition in the context of diabetic cardiomyopathy.
Figure 4.7: Computer Vision-Based Trichrome Staining Analysis for Collagen Detection. Representative rat heart tissue images stained with trichrome in the control, diabetic, and treatment groups (a). Computer vision analysis reveals reduced area of collagen (blue) in the control and treatment groups compared to the normal physiological state (a). Increased area of blue color intensity in the diabetic group indicates abnormal collagen deposition (b). Computer vision analysis identifies a greater number of collagen contours in the diabetic group (c), suggesting altered collagen clusters.
Specific Aim 3b: Integration of Computer Vision and Biomarker Analysis

Specific Aim 3.1b: Examining Cx43 Expression in Diabetic Cardiomyopathy: Potential Indicator of Disrupted Cell-Cell Communication.

The results from Figure 4.8 demonstrate the successful application of computer vision techniques for the extraction and analysis of Connexin 43 (Cx43) protein expression in both the red channel and the RGB (Red, Green, Blue) channel. The red channel extraction, as shown in Figure 4.8a, provides representative images illustrating the computer vision extraction of Cx43 protein expression. This extraction allows for the visualization and analysis of Cx43 protein based on its intensity or specific color representation in the red channel. On the other hand, Figure 4.8b demonstrates the computer vision extraction of Cx43 protein within the RGB channel. In this extraction, Cx43 protein is detected and distinguished within the full-color RGB image, enabling its localization and expression analysis using computer vision methods.

The findings from Figure 4.8 highlight the effectiveness of computer vision techniques in accurately extracting and analyzing Cx43 protein expression in both the red channel and the RGB channel. This approach provides valuable insights into the distribution and localization patterns of Cx43 protein within the studied samples. Furthermore, the analysis of Cx43 expression revealed interesting results. The number of contours of Cx43 expression was found to be lower in the diabetic groups compared to the control (c). This indicates that the treatment with GLC in the diabetic group helps to recover the overall expression level of Cx43 compared to the control group. In addition, the mean area occupied by Cx43 protein was found to be lower in the diabetic group compared to the control group (d). This quantitative assessment suggests a potential decrease in
the spatial distribution and density of Cx43 protein expression in the diabetic myocardium, which may have implications for intercellular communication and cardiac function.

The analysis reveals that, on average, Cx43 expression covers approximately 38% of the identified red color protein regions in the control and treatment groups (Figure 4.8c), confirming the presence of Cx43 protein in these regions as supported by the biological analysis (Figure 4.3). Furthermore, the computer vision-based analysis of Cx43 expression in the diabetic group shows that, on average, Cx43 expression covers approximately 24% of the identified Cx43 protein regions (Figure 4.8c). This finding aligns with the results obtained through biological analysis, indicating a relatively lower extent of Cx43 protein presence within these regions, as depicted in Figure 4.3 and Figure 4.8. The consistent findings between the computer vision-based approach and the biological analysis validate the reliability and accuracy of the computer vision technique for assessing Cx43 expression.

These results enhance our understanding of Cx43 expression patterns and provide valuable information regarding its association with the studied conditions. The computer vision analysis contributes to the comprehensive characterization of Cx43 expression in rat cardiac tissue, supporting the significance of this protein in the context of the experimental groups and their implications for cardiac function and pathology. Overall, the results demonstrate the efficacy of computer vision techniques in accurately extracting and analyzing Cx43 protein expression, providing insights into its distribution and potential alterations in diabetic cardiomyopathy.
Figure 4.8: Computer Vision Extraction of Cx43 Protein Expression in the Red Channel and RGB Channel. (a) Red channel extraction demonstrates the visualization and analysis of Cx43 protein expression (white arrows) based on its intensity and specific color (red) representation within the Red channel. (b) RGB channel extraction reveals the detection and distinction of Cx43 protein within the full-color RGB image, enabling its localization and expression analysis using computer vision methods. Panel (c)
displays the number of contours of Cx43 found in the rat myocardium, showing a lower number of contours in the diabetic group compared to the control group. Panel (d) illustrates the median area (µm) of Cx43 expression identified as red color protein regions, which was found to be higher in both the control and treatment groups.

In addition, results from the application of computer vision techniques for the extraction and quantification of nuclei in rat cardiac tissue yielded valuable insights into their characteristics and their association with inflammation in the context of diabetic cardiomyopathy. The results in Figure 4.9a demonstrates the computer vision-based analysis, enabling the identification and analysis of nuclei within the tissue samples. This approach proved to be effective in accurately extracting and quantifying the nuclei. Interestingly, the diabetic group exhibited a higher area expression intensity compared to both the control and diabetic + GLC groups as shown in Figure 4.9b. This finding suggests potential changes in nucleus size and distribution in the diabetic myocardium.

Furthermore, Figure 4.9c illustrates the mean area of identified blue color regions covered by nucleus expression. It was observed that the control and diabetic + GLC groups had a lower percentage of nucleus identification within these regions. In contrast, the diabetic group showed a higher percentage of nucleus identification within these regions. This indicates an immune response and inflammation-mediated increase in nucleus presence in the diabetic myocardium.

In summary, the results from Figure 4.9 highlight the capability of computer vision techniques to accurately extract and quantify nuclei in rat cardiac tissue, considering their specific geometry within fiber bundles. These findings provide valuable insights into potential changes in nucleus characteristics and their association with inflammation in the context of diabetic cardiomyopathy.
Figure 4.9: Computer Vision Analysis of Nuclei in Rat Cardiac Tissue. (a) Computer vision-based extraction by contour (green) and identification of nuclei (blue) within the tissue expressing Cx43 (b) Number of nuclei in each image of rat myocardium. (c) Mean area of blue regions covered by number of nuclei. This suggests the changes in immune response and increase in inflammation cause changes of numbers of immune cells as represented in the increase in nucleus presence in the diabetic myocardium.

Immunofluorescent images of heart tissues from lean control, diabetic, and diabetic rats treated with GLC were analyzed using computer vision techniques in Figure 4.10. Representative immunofluorescent images of cardiac tissue from ZDF diabetic rats demonstrating a substantial increase in TnI expression compared to lean control rats. The computer vision analysis confirms the elevated intensity of TnI immunostaining in the cardiac tissue of ZDF diabetic rats. In Figure 4.10 GLC treatment group representative immunofluorescent images of cardiac tissue from GLC-treated ZDF diabetic rats. Computer vision analysis reveals noticeable changes in TnI expression of GLC treatment, compared to ZDF diabetic rats. The intensity of TnI immunostaining shows a significant decrease, as indicated by the reduced red signal in the representative images. The computer vision analysis supports the observed changes in TnI expression in response to GLC treatment and validates the substantial increase in TnI expression in ZDF diabetic rats compared to lean control rats, as depicted in Figure 4.5.
Figure 4.10: Findings from computer vision analysis that support the changes observed in Troponin-I (TnI) expression in ZDF diabetic rat cardiac tissue and its response to GLC treatment. Panel (a) presents the results of computer vision analysis, which validate the observed alterations in TnI expression in the cardiac tissue of ZDF rats and demonstrate the influence of GLC treatment. Panel (b) displays the average contour number of troponin, indicating the density of troponin expression. Panel (c) shows the mean area of Troponin, indicating the extent of troponin expression. Notably, the results indicate higher TnI expression in the diabetic group compared to the control and treatment groups, confirming the impact of diabetes on troponin levels.
**Specific Aim 3.3b: Immunohistochemistry Analysis of MyoD Expression and Muscle Atrophy in the ZDF Rat Myocardium.**

The application of computer vision analysis in Figure 4.11 supports the immunohistochemistry analysis of MyoD expression in diabetic rat cardiac tissue. In the control group, representative images of lean myocardium display preserved tissue structure with normal MyoD expression. The computer vision analysis accurately detects and quantifies the color areas corresponding to MyoD expression, providing an objective percentage measure of its presence in the myocardium (Figure 4.11b).

The diabetic group images of myocardium from ZDF diabetic rats show disrupted tissue architecture (Figure 4.11a). The computer vision analysis identifies and quantifies the color areas associated with MyoD expression, reflecting the altered expression pattern in the diabetic myocardium (Figure 4.11c). In the GLC treatment group, images of myocardium from ZDF diabetic rats treated with GLC demonstrate noticeable changes in the color areas corresponding to MyoD expression compared to ZDF diabetic rats. This quantitative analysis provides insights into the effects of GLC treatment on MyoD expression levels.

The computer vision analysis, incorporating a unique technique of color area detection, provides valuable support and enhances the observations made through immunohistochemistry analysis of MyoD expression in myocardium samples. This technique not only accurately detects and quantifies the color areas corresponding to MyoD expression but also enables the quantification of the exact geometry of fragmentation and tissue alignment. By analyzing the fragmentation and alignment of the myocardial tissue, the computer vision analysis offers insights into the structural changes associated with MyoD expression in the diabetic rat cardiac tissue. These findings, in conjunction with the biological analysis presented in specific aim 2, provide a
comprehensive understanding of MyoD expression and its potential implications in the context of diabetic cardiomyopathy. Overall, the integration of computer vision analysis enhances our ability to quantify the exact geometry of fragmentation and tissue alignment, contributing to a more comprehensive assessment of MyoD expression in the diabetic myocardium.
Figure 4.11: Computer Vision Analysis Supports Immunohistochemistry Analysis of MyoD Expression in Diabetic Rat Cardiac Tissue. Panel (b) displays the number of contour (blue color shown in panel a) associated with MyoD expression. Panel (c) presents the mean area of MyoD expression (green), providing quantitative measurements of the extent of MyoD expression in the diabetic rat cardiac tissue. This analysis incorporates color area detection, facilitating the evaluation of the distribution patterns and spatial characteristics of MyoD expression within the diabetic rat cardiac tissue.
**Specific Aim 3c: Quantitative Analysis of 2D Cell Culture Images**

**Specific Aim 3.1c: Assessing Myocardial Injury through Troponin-I Expression: A Biomarker for 2D Cell Culture of Diabetic Cardiomyopathy Analyzed by Computer Vision Techniques.**

The application of computer vision techniques in Figure 4.12 facilitated the accurate detection and quantification of Troponin-I (TnI) expression in 2D human cardiomyocytes cell culture, specifically in the high glucose cardiomyocytes cell culture. Results from Figure 4.12a demonstrate the utilization of computer vision techniques allowed for the detection and analysis of the red color intensity, representing TnI expression, in the cell culture samples. Additionally, the green color expression contour was identified and visualized, providing insights into the spatial distribution of TnI expression. Interestingly, the TnI expression was higher in the high glucose cardiomyocytes cell culture compared to the control (normal glucose) and high glucose with GLC treatment groups.

The mean area of TnI expression, represented by the red color, was measured, enabling the quantification of TnI expression extent in the cell culture samples (Figure 4.12b). The results indicated a higher mean area of TnI expression in the high glucose cardiomyocytes cell culture. The identified expression contour in green color was visualized and analyzed, allowing for the assessment of the spatial distribution of TnI expression within the cell culture samples. The spatial distribution analysis revealed a greater extent of TnI expression within the high glucose cardiomyocytes cell culture compared to the control and high glucose with GLC treatment groups (Figure 4.12c).

The computer vision analysis in Figure 4.12 provided valuable insights into the extent and spatial distribution of TnI expression in 2D human cardiomyocytes cell culture, particularly in the
context of high glucose conditions. These findings were consistent with the results obtained from the biological analysis, reinforcing the similarities between the computer vision-based quantification and traditional analysis methods.
Figure 4.12: Computer Vision Analysis of Troponin-I Expression in 2D Human Cardiomyocytes Cell Culture. (a) Utilization of computer vision techniques to detect and analyze the red color intensity, representing Troponin-I (TnI) expression (red), in the cell culture samples. The expression contour is identified and visualized in green color, providing insights into the spatial distribution of TnI expression. (b) Visualization and analysis of the identified expression contour in green color, allowing for the assessment of the spatial distribution of TnI expression within the cell culture samples. (c) Measurement of the mean area of TnI expression, represented by the red color, enabling the quantification of TnI expression extent in the cell culture samples.
**Specific Aim 3.2c**: Investigating the Role of CXCR4 in Diabetic Cardiomyopathy: Implications for Inflammation in 2D cell culture.

The application of computer vision analysis successfully detected high expression of CXCR4 in 2D human cardiomyocytes cell culture under high glucose conditions, consistent with the findings obtained in Specific Aim 1 (Figure 4.13). Results from Figure 4.13a demonstrated the effective utilization of computer vision techniques to analyze the red color intensity, representing CXCR4 expression, in the cell culture samples. The high glucose group exhibited significantly higher levels of CXCR4 expression (Figure 4.13b), supporting the findings from the biological studies conducted in Specific Aim 1. The identified expression contour in green color (Figure 4.13c) allowed for the visualization and analysis of the spatial distribution of CXCR4 expression within the cell culture samples.

Importantly, the results from Figure 4.13 suggest that high glucose conditions lead to higher expression levels of CXCR4 in the 2D human cardiomyocytes cell culture. The increased mean area of CXCR4 expression and the presence of larger contours in the high glucose group compared to other experimental groups indicate a potential relationship between glucose levels and CXCR4 expression in the cell culture environment. Furthermore, the response to GLC treatment can also be observed through changes in the CXCR4 expression pattern, as evidenced by alterations in the red color intensity and contour of CXCR4 in the GLC-treated group.

Overall, the computer vision analysis provides valuable insights into the expression dynamics of CXCR4 in 2D human cardiomyocytes cell culture, shedding light on the impact of glucose levels and the response to GLC treatment. These findings, in conjunction with the biological analysis presented in Specific Aim 1, highlight the role of computer vision techniques in accurately quantifying and analyzing CXCR4 expression patterns. The integration of computer
vision analysis enhances our understanding of the relationship between glucose levels, CXCR4 expression, and the potential implications for cardiac cell culture.
Figure 4.13: Illustrates the utilization of computer vision analysis to detect red color intensity, representing CXCR4 expression, in 2D human cardiomyocytes cell culture and its response to GLC treatment (a). Immunohistochemical images were subjected to computer vision techniques to quantify and visualize the expression of CXCR4 in the various experimental groups. The analysis included the contour represented by blue color (b) and the measuring of the mean area of CXCR4 expression represented by red color (c). Representative immunohistochemical images of the cell culture demonstrate the presence of red color regions corresponding to CXCR4 expression, along with the average contour of CXCR4.
Chapter 5: Discussion

The findings of this study contribute to our understanding of hyperglycemia-induced cardiomyocyte atrophy and provide insights into the potential therapeutic role of glycyrrhizin (GLC) in preventing cardiovascular complications in diabetes. The results demonstrate that GLC treatment effectively attenuates hyperglycemia-induced cardiomyocyte atrophy, as evidenced by the significant reduction in cardiomyocyte size and preservation of cellular integrity.

These findings are consistent with previous research highlighting the anti-inflammatory and antioxidant properties of GLC. Hyperglycemia-induced inflammation and oxidative stress are known to play a crucial role in the development of diabetic cardiomyopathy, contributing to cardiac remodeling, apoptosis, and fibrosis [99]. GLC, as a natural compound, has been shown to possess potent anti-inflammatory and antioxidant effects, which may underlie its protective effects against hyperglycemia-induced cardiomyocyte atrophy observed in this study [8, 18].

The study also provides evidence that GLC treatment inhibits the activation of transcription factor that regulates the expression of pro-inflammatory genes and is known to be activated in diabetes [8]. GLC may modulate the expression of inflammatory mediators, leading to the attenuation of cardiac inflammation and subsequent cardiomyocyte atrophy [100]. Importantly, the study highlights the potential of GLC as a therapeutic intervention for diabetic cardiomyopathy. Given the limited treatment options currently available for preventing and managing cardiovascular complications in diabetes, the identification of GLC as a promising agent offers new possibilities for therapeutic strategies. GLC’s natural origin, well-established safety profile, and demonstrated efficacy in mitigating hyperglycemia-induced cardiomyocyte atrophy make it an attractive candidate for further investigation. However, several aspects should be considered when interpreting the results of this study. Firstly, the study was conducted in a
preclinical setting using an animal model, and thus, the findings need to be validated in human studies. Translating the results from animal models to human patients is essential to determine the clinical relevance and applicability of GLC in the context of diabetic cardiomyopathy.

Secondly, the study primarily focused on the cellular level changes and did not assess the functional consequences of GLC treatment on cardiac performance. Further studies should investigate the effects of GLC on cardiac function parameters such as ejection fraction, contractility, and diastolic function to provide a comprehensive assessment of its therapeutic potential. Additionally, the optimal dosage and treatment duration of GLC need to be determined. The study utilized a specific dosage and treatment regimen, but exploring dose-response relationships and assessing the long-term effects of GLC treatment would provide valuable insights into its therapeutic efficacy and safety.

Furthermore, the mechanisms underlying the protective effects of GLC require further investigation. Elucidating the precise molecular mechanisms of GLC's action would deepen our understanding of its therapeutic potential and may even uncover novel therapeutic targets for diabetic cardiomyopathy. The findings of this study support the notion that GLC has the potential to prevent hyperglycemia-induced cardiomyocyte atrophy in diabetes. The observed attenuation of cardiomyocyte size reduction, preservation of cellular integrity, and further exploration of GLC as a therapeutic intervention for diabetic cardiomyopathy. However, additional research, including clinical trials, is needed to validate these findings, optimize treatment protocols, and unravel the precise mechanisms of action underlying GLC's therapeutic effects. In addition to the integration of computer vision and biomedical engineering in cardiac imaging, another novel technique that holds promise for studying diabetic cardiomyopathy is the application of computer vision in microscope image analysis. Microscopic imaging provides valuable insights into the cellular and
molecular changes that occur in the heart during diabetic cardiomyopathy. By combining computer vision algorithms with microscopic imaging, researchers can extract detailed information about cellular structures, protein expression, and tissue morphology, further enhancing our understanding of the disease.

Microscope image analysis using computer vision techniques offers several advantages in the study of diabetic cardiomyopathy at the cellular level. It enables the automated segmentation and quantification of various cellular components, such as cardiomyocytes, mitochondria, nuclei, and intracellular structures [101]. By accurately identifying and quantifying these components, researchers can gain insights into alterations in cellular organization, size, shape, and density that may occur in diabetic cardiomyopathy. Moreover, the use of computer vision allows for the automatic adjustment of saturation and thresholding, and finally, in the algorithm framework of a Python with many additional open source libraries and packaging, future work in this vein could be extended to deep machine learning and artificial intelligence algorithms to further assist in the identification of non-obvious trends in the data to help provide insights into the efficacies of different treatment options.

Moreover, computer vision algorithms can assist in the analysis of protein expression patterns within cardiac cells. By automatically detecting and quantifying the presence and distribution of specific proteins, researchers can uncover important molecular changes associated with diabetic cardiomyopathy. This information can contribute to identifying key signaling pathways, protein interactions, and cellular processes involved in the development and progression of the disease. The integration of microscope image analysis with computer vision techniques also enables the exploration of spatial relationships within cardiac tissue. By analyzing the spatial arrangement of cells, extracellular matrix components, and blood vessels, researchers can gain
insights into tissue remodeling, fibrosis, and angiogenesis that occur in diabetic cardiomyopathy. Computer vision algorithms can aid in the identification and quantification of these structural changes, providing valuable information about the progression of the disease and potential therapeutic targets.

One of the advantages of using computer vision in microscope image analysis is the ability to process large volumes of image data efficiently. Microscopic imaging generates vast amounts of high-resolution images, and manually analyzing these images can be time-consuming and prone to human error. Computer vision algorithms can automate the image analysis process, significantly reducing the time required for data analysis and increasing the throughput of experiments. This allows researchers to analyze large datasets and perform high-throughput screening, facilitating the discovery of novel biomarkers and therapeutic targets in diabetic cardiomyopathy.

However, challenges exist in applying computer vision to microscope image analysis. Microscopic images often suffer from various types of noise, artifacts, and variability in image quality, which can affect the performance of computer vision algorithms [102]. Pre-processing steps, such as image denoising and normalization, may be required to enhance the quality of the images and improve the accuracy of the analysis. Additionally, the development of robust and adaptable computer vision models that can handle the variability in image acquisition protocols and experimental conditions is essential for reliable and reproducible results. This dynamic nature of programmatically analyzing biomedical images allows for adjusting for background differences in the imaging due to collecting the data at different laboratories, for instance.

In summary, the integration of computer vision with microscope image analysis provides a novel and powerful approach for studying diabetic cardiomyopathy at the cellular and molecular levels. By automating the analysis of microscopic images, computer vision algorithms enable the
quantification of cellular structures, protein expression patterns, and tissue remodeling in a high-throughput manner. This technique has the potential to reveal critical insights into the underlying mechanisms of diabetic cardiomyopathy and contribute to the development of targeted therapies. Overcoming challenges related to image quality, variability, and model robustness will be crucial for the successful application of computer vision in microscope image analysis and advancing our understanding of diabetic cardiomyopathy.

The discussion chapter provides an in-depth analysis of the results obtained from the three specific aims of this study. Specific Aim 1 focused on histopathological analysis, revealing structural changes in diabetic cardiomyopathy. Specific Aim 2 explored the differential expression of biomarkers, highlighting their potential roles in diabetic cardiomyopathy. Specific Aim 3 employed computer vision analysis to extract features and patterns for improved diagnosis. These findings collectively enhance our understanding of diabetic cardiomyopathy:

5.1 Specific Aim 1: Histopathological Analysis of Rat Hearts: Assessing Diabetic Cardiomyopathy-Induced Changes in Microscopic Anatomy

The histopathological analysis of rat hearts aimed to assess the microscopic anatomy changes associated with diabetic cardiomyopathy (DCM) compared to healthy hearts. The findings from the histological analysis using Hematoxylin and Eosin (H&E) staining revealed significant structural alterations in the diabetic hearts. The average length of cardiac muscle fibers was significantly reduced in the diabetic group compared to the healthy control group, indicating muscle atrophy in diabetic cardiomyopathy. This reduction in fiber length can be attributed to the observed muscle lesions and fragmentation, suggesting cardiac tissue atrophy.
The presence of collagen deposition and fibrosis in the cardiac tissue was assessed using Gomori's trichrome stain. The analysis revealed a significant upregulation of collagen in the diabetic rat hearts compared to the healthy controls, indicating increased collagen deposition during the early stages of cardiac fibrosis in diabetic cardiomyopathy. Remarkably, treatment with GLC demonstrated a significant reduction in collagen deposition, indicating its potential therapeutic efficacy in decreasing diabetic-associated cardiac fibrosis.

Overall, these findings highlight the structural alterations in diabetic cardiomyopathy, including muscle atrophy, collagen deposition, and fibrosis. The histopathological analysis provides valuable insights into the pathological changes occurring in the diabetic heart and suggests potential therapeutic interventions for managing DCM.

5.2 Specific Aim 2: Exploring Differential Expression of Early Biomarkers in Diabetic Cardiomyopathy: Insights from the ZDF Rat Model

The aim of this specific aim was to explore differential expression of early biomarkers in diabetic cardiomyopathy using the ZDF rat model. Hyperglycemia is known to contribute to myocardial dysfunction, and the study aimed to investigate potential therapeutic interventions targeting the inflammatory cascade to alleviate glucose-mediated toxicity.

The expression of various biomarkers, including connexin-43 (Cx43), chemokine receptor CXCR4, and Troponin-I (TnI), was assessed to understand their roles in diabetic cardiomyopathy and evaluate the effects of GLC treatment. The reduced expression of Cx43 in the ZDF rat myocardium suggests potential disruptions in intercellular communication mechanisms associated with diabetic cardiomyopathy. GLC treatment restored the expression of Cx43, indicating a positive influence on cell adhesion in diabetic cardiac tissue.
The upregulation of CXCR4 expression in the diabetic rat myocardium indicates its potential involvement in the inflammatory processes of diabetic cardiomyopathy [103]. GLC treatment resulted in a reduction in CXCR4 expression, suggesting an anti-inflammatory effect and its therapeutic potential in attenuating diabetic cardiac atrophy.

The increased expression of Troponin-I in the ZDF rat myocardium, along with the observed myocardial injury, highlights the presence of cardiac tissue damage in diabetic cardiomyopathy. GLC treatment showed promising results in reducing cardiac tissue damage, offering potential therapeutic implications for managing diabetic cardiomyopathy.

Furthermore, the expression of MyoD, a crucial protein involved in muscle differentiation regulation, was analyzed. The reduced expression of MyoD in the diabetic tissue suggests possible disruptions or apoptosis in the cardiac tissue, potentially contributing to the development of diabetic cardiomyopathy. These findings demonstrate the involvement of various biomarkers in the pathogenesis of diabetic cardiomyopathy and highlight the potential therapeutic effects of GLC treatment in attenuating cardiac tissue damage and inflammation.

5.3 Specific Aim 3: Computer Vision Analysis of Diabetic Rat Heart Tissue and 2D Cell Culture: Automated Extraction of Algorithmic Features and Patterns for Enhanced Understanding and Diagnosis of Diabetic Cardiomyopathy with Customized Adaptation for Varied Image Conditions

Computer vision analysis techniques were employed to analyze histological images of diabetic rat myocardium and 2D cell cultures of AC16 human cardiomyocytes. The application of OpenCV, an open-source computer vision package, allowed for the extraction and quantification of DCM-related features and patterns. The computer vision algorithm utilized various
preprocessing steps, including grayscale conversion, median filtering for noise reduction, and binary thresholding to isolate regions of interest. Contour detection was employed to outline the boundaries of cells and muscle fibers, enabling quantitative measurements of their areas. The algorithm provided accurate and efficient analysis of microscopic images, demonstrating its potential as a powerful tool for automated image analysis in scientific research.

The computer vision analysis results yielded valuable insights into cellular morphology and tissue structure, providing information that aligns with previous datasets. The utilization of OpenCV facilitated customization and dynamic modification of the algorithm, making it adaptable to specific research requirements. The algorithm's reliability and the ability to set thresholds dynamically increased its effectiveness in identifying different features. In this dissertation, we investigated the microscopic anatomy and cellular structure changes associated with hyperglycemia-induced cardiomyocyte atrophy and the potential therapeutic use of glycyrrhizin (GLC) in preventing cardiovascular complications in diabetes.

Histopathological analysis using hematoxylin and eosin (H&E) staining revealed significant morphological changes in the myocardial tissue of diabetic (ZDF) rats compared to lean (control) rats. The cardiac muscle fibers in the diabetic group showed a reduction in length, indicating muscle atrophy. Moreover, muscle lesions and fragmentation were observed in the diabetic group, suggesting cardiac tissue damage and atrophy.

Gomori's trichrome staining was used to assess myocardial fibrosis in the diabetic and healthy hearts. The analysis showed a significant increase in collagen deposition in the cardiac tissue of diabetic rats compared to lean controls. This indicates the presence of fibrosis in the diabetic group. However, treatment with GLC in diabetic rats resulted in a significant reduction in
collagen deposition, suggesting its potential therapeutic efficacy in attenuating diabetic-associated cardiac fibrosis.

Furthermore, we examined the expression of key biomarkers in the diabetic myocardium. Immunohistochemical studies revealed that diabetic rats exhibited decreased expression of connexin-43 (Cx43), a gap junction protein involved in cell adhesion [104]. GLC treatment restored the expression of Cx43, indicating its positive effect on cell adhesion in diabetic cardiac tissue.

We also investigated the expression of CXC chemokine receptor 4 (CXCR4), a receptor involved in inflammation, and found a significant increase in its expression in the ventricular tissue of diabetic rats. However, GLC treatment resulted in a reduction in CXCR4 expression, indicating its potential anti-inflammatory effect in diabetic cardiac atrophy.

Additionally, the expression of troponin-I (TnI), a marker of myocardial injury, and MyoD, a transcription factor involved in muscle cell differentiation, were examined in this study. Immunofluorescent analysis was conducted to assess the levels and distribution of these proteins in the cardiac tissue of diabetic rats. The results revealed noticeable changes in TnI expression in diabetic rats treated with GLC compared to untreated diabetic rats, indicating a potential protective effect of GLC against myocardial injury. Furthermore, the expression and localization of MyoD were assessed, providing insights into the regulation of muscle cell differentiation and potential implications for cardiac health in the context of diabetes.

These findings suggest that hyperglycemia-induced cardiomyocyte atrophy involves changes in cellular structure, collagen deposition, and altered expression of key biomarkers. GLC treatment showed promise in mitigating fibrosis, restoring cell adhesion, reducing inflammation, and protecting against myocardial injury in diabetic rats. These results highlight the potential
therapeutic role of GLC in preventing cardiovascular complications associated with diabetes and emphasize the importance of targeting inflammation and oxidative stress for improving cardiac function in diabetes.

Overall, the computer vision analysis demonstrated the versatility and effectiveness of OpenCV in extracting and analyzing areas of interest in microscopic images. It showcased the potential of automated image analysis techniques for improved understanding and diagnosis of diabetic cardiomyopathy.

**CONCLUSION AND FUTURE DIRECTIONS**

In conclusion, the discussion chapter summarized the key findings from Specific Aims 1, 2, and 3. The histopathological analysis revealed significant structural alterations in the diabetic heart, including muscle atrophy, collagen deposition, and fibrosis. The differential expression of biomarkers provided insights into the pathogenesis of diabetic cardiomyopathy and the potential therapeutic effects of GLC treatment. The computer vision analysis showcased the effectiveness of OpenCV in extracting features and patterns from microscopic images, contributing to the advancement of image analysis techniques in scientific research.

Based on the findings of this study, there are several potential future directions that could be explored to further our understanding of hyperglycemia-induced cardiomyocyte atrophy and the therapeutic potential of glycyrrhizin (GLC) in preventing cardiovascular complications in diabetes:

1. Mechanistic studies: Investigating the underlying molecular mechanisms involved in hyperglycemia-induced cardiomyocyte atrophy and the protective effects of GLC. This
could involve exploring signaling pathways, gene expression profiles, and cellular processes associated with cardiac remodeling in diabetes.

2. Dose optimization and treatment duration: Assessing the optimal dosage of GLC and determining the ideal treatment duration to achieve maximum therapeutic benefits. This could involve conducting dose-response studies and evaluating the long-term effects of GLC treatment on cardiac structure and function.

3. Clinical trials: Translating the findings from preclinical studies to clinical trials involving human subjects with diabetes. Investigating the safety, efficacy, and tolerability of GLC in preventing cardiovascular complications in diabetic patients could provide valuable insights into its therapeutic potential in a clinical setting.

4. Combination therapies: Exploring the potential synergistic effects of GLC with other pharmacological agents commonly used in diabetes management. Combinations of GLC with existing medications such as antidiabetic drugs or cardiovascular drugs may offer enhanced protection against cardiac complications and improve overall patient outcomes.

5. Investigating alternative: Exploring alternative delivery methods or formulations of GLC that could improve its bioavailability, stability, and tissue targeting. Novel approaches such as nanoparticle-based drug delivery systems or sustained-release formulations may enhance the therapeutic efficacy of GLC in the context of diabetic cardiomyopathy.

6. Comparative studies: Comparing the effects of GLC with other natural compounds or pharmaceutical agents that have shown potential in preventing cardiac complications in diabetes. Comparative studies could help identify the most effective interventions for managing diabetic cardiomyopathy.
By pursuing these future directions, we can advance our knowledge of hyperglycemia-induced cardiomyocyte atrophy, refine the therapeutic potential of GLC, and develop more effective strategies for preventing and treating cardiovascular complications in individuals with diabetes. In addition to the aforementioned conclusions and future directions, the integration of computer vision techniques has provided valuable insights into the microscopic changes associated with hyperglycemia-induced cardiomyocyte atrophy and the effects of GLC treatment. Computer vision analysis has enabled a quantitative and objective assessment of cellular structure, collagen deposition, and biomarker expression, enhancing the overall understanding of diabetic cardiomyopathy and the potential therapeutic efficacy of GLC. Further exploration and integration of computer vision techniques can contribute to the advancement of research in this field. Some potential future directions involving computer vision in the study of hyperglycemia-induced cardiomyocyte atrophy and GLC treatment include:

1. Refinement of image analysis algorithms: Continual refinement of image analysis algorithms can improve the accuracy and efficiency of quantification methods. Developing advanced algorithms that can automatically segment cardiac tissue, identify cellular structures, and measure biomarker expression levels will streamline the analysis process and minimize potential bias.

2. Multimodal image analysis: Integration of multiple imaging modalities, such as brightfield microscopy, fluorescence microscopy, and confocal microscopy, can provide a more comprehensive assessment of cellular changes. Combining information from different imaging techniques through computer vision analysis can offer a holistic view of diabetic cardiomyopathy and the effects of GLC treatment.
3. 3D analysis: Extending the analysis to three-dimensional (3D) image data can capture the spatial distribution of cellular structures and biomarker expression patterns. Utilizing computer vision algorithms for 3D reconstruction and analysis can provide a more detailed understanding of cardiac tissue remodeling in diabetes.

4. Machine learning-based analysis: Leveraging machine learning algorithms, such as deep learning, can enhance the ability to extract complex features from microscope images. Training models on large datasets of annotated images can enable automated detection and quantification of specific cellular structures, fibrosis, and biomarker expression, reducing the manual effort required for analysis.

5. High-throughput analysis: Scaling up the analysis to handle large datasets can accelerate research progress. Developing high-throughput image analysis pipelines that can process a large number of microscope images efficiently will facilitate comprehensive studies involving multiple samples, time points, and treatment conditions.

6. Real-time imaging and analysis: Exploring real-time imaging techniques, such as live-cell imaging, can capture dynamic cellular processes in real-time. Integrating computer vision algorithms for real-time image analysis can enable the monitoring of cellular responses to stimuli or treatments, providing valuable insights into the temporal dynamics of diabetic cardiomyopathy and GLC effects.

Overall, the integration of computer vision techniques has proven invaluable in the study of hyperglycemia-induced cardiomyocyte atrophy and the evaluation of GLC as a potential therapeutic agent. Continual advancements in computer vision methodologies, algorithms, and imaging technologies will contribute to further advancements in our understanding of diabetic
cardiomyopathy and facilitate the development of effective interventions for preventing and treating cardiovascular complications in individuals with diabetes.
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Vita

Monica Delgado is a driven and accomplished Latina woman, breaking barriers as a first-generation student in her family, she has proven that determination, resilience, and talent know no bounds. Her graduate journey, marked by two master's degrees—one in Biological Sciences and another in Biomedical Engineering—from The University of Texas at El Paso, reflects her relentless pursuit of knowledge and commitment to excellence. This culminated in her graduation magna cum laude and her recognition as a top ten student during her undergraduate studies. Monica Delgado has continually defied limitations and pushed the boundaries of what is possible. Her accomplishments in obtaining internships at the Harvard Catalyst Program at Harvard Medical School and the University of California, San Francisco (UCSF), speak volumes about her capabilities and the high regard in which her work. Moreover, Monica Delgado has received multiple scholarships and fellowships such as the Gates Millennium Scholarship and National Science Foundation Fellowship in Graduate Teaching in K-12 Education (Gk-12), and her passion for research and innovation has led to several publications. Her desire to give back to her community and inspire others, particularly those from underrepresented backgrounds, is a reflection of her character and strength of purpose. Her journey, filled with determination and resilience, serves as a powerful reminder that nothing can hold back a motivated and talented individual. Monica Delgado journey is a testament to the strength and power of perseverance. Her accomplishments stand as an example of inspiration for others, motivating them to embrace their own abilities and strengths fearlessly. As she continues her academic and professional journey, Monica Delgado will undoubtedly leave an indelible mark, shaping the future of inclusivity for underrepresented backgrounds.