Review

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Review

Circular RNAs in Cardiovascular Disease: From Pathophysiological Regulators to Novel Biotechnological Tools for Diagnosis and Therapy

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Abstract

Background: Cardiovascular diseases (CVDs) remain the leading cause of global mortality, creating an urgent need for innovative diagnostic and therapeutic strategies. Circular RNAs (circRNAs), a class of covalently closed non-coding RNAs, have recently emerged as critical regulators in cellular pathophysiology. Their unique structural stability and tissue-specific expression patterns position them as highly promising molecules for biotechnological applications in cardiology. **Methods:** This review systematically synthesizes the current body of literature on circRNAs in the context of cardiovascular health and disease. We analyze their biogenesis, molecular mechanisms of action, and specific roles in key cardiac pathologies, including myocardial infarction, heart failure, and fibrosis. Furthermore, we evaluate the evidence supporting their use as clinical biomarkers and explore the cutting-edge technologies for their therapeutic delivery. **Results:** The evidence demonstrates that circRNAs function through diverse mechanisms, most notably as microRNA (miRNA) sponges and as scaffolds for RNA-binding proteins (RBPs), thereby modulating complex signaling networks that govern cardiomyocyte survival, apoptosis, inflammation, and regeneration. A multitude of specific circRNAs have been identified with dichotomous roles, either promoting or protecting against cardiac injury. Their exceptional stability in circulation supports their development as high-fidelity biomarkers, with meta-analyses confirming their diagnostic potential. Concurrently, advances in viral vectors, lipid nanoparticles (LNPs), and extracellular vesicles (EVs) are overcoming the critical hurdle of cardiac-specific delivery for circRNA-based therapeutics. **Conclusion:** CircRNAs represent a paradigm-shifting class of molecules in cardiovascular medicine. They are no longer considered molecular curiosities but are now recognized as fundamental regulators and powerful biotechnological tools. The convergence of circRNA biology with advanced nanodelivery platforms heralds a new era of precision medicine, offering the potential to diagnose CVD earlier and develop novel therapies aimed at cardiac repair and regeneration.

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Key Contribution: The breakthroughs or highlights of the manuscript. Authors can write one or two sentences to describe the most important part of the paper.

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1. Introduction

1.1. The Unmet Clinical Need in Cardiovascular Disease (CVD)

Cardiovascular diseases (CVDs) represent the foremost public health challenge of the 21st century, collectively standing as the leading cause of morbidity and mortality worldwide [1].

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Pathologies such as myocardial infarction (MI), heart failure (HF), and atherosclerosis impose a staggering burden on healthcare systems and diminish the quality of life for millions of individuals [2,3]. Despite significant advances in pharmacological interventions, surgical procedures, and device-based therapies over the past several decades, the prognosis for many patients, particularly those with advanced heart failure, remains poor [3]. The adult mammalian heart possesses a notoriously limited capacity for self-repair; following an ischemic insult like MI, the loss of terminally differentiated cardiomyocytes is largely irreversible, leading to the formation of a non-contractile scar, adverse ventricular remodeling, and a progressive decline in cardiac function [6]. This fundamental biological limitation underscores a critical unmet clinical need: the development of novel diagnostic tools for early disease detection and innovative therapeutic strategies that can move beyond symptom management to target the underlying molecular drivers of disease and promote true cardiac repair and regeneration [5]. This imperative has catalyzed a paradigm shift in cardiovascular research, moving focus toward the vast, largely unexplored territories of the non-coding genome [72,73].

1.2. The Expanding Universe of Non-Coding RNAs

For decades, the central dogma of molecular biology placed protein-coding genes at the heart of cellular function. However, the completion of the Human Genome Project revealed a surprising truth: protein-coding sequences account for less than 2% of the genome. The vast majority is transcribed into non-coding RNAs (ncRNAs), a diverse and complex class of molecules once dismissed as transcriptional "noise" or "junk" [9,72]. It is now unequivocally clear that this non-coding transcriptome represents a sophisticated and deeply integrated layer of genetic regulation. NcRNAs are broadly categorized by size and function and include well-studied players like microRNAs (miRNAs)—short ~22-nucleotide RNAs that mediate post-transcriptional gene silencing—and long non-coding RNAs (lncRNAs), which are transcripts longer than 200 nucleotides with diverse regulatory roles [6]. The discovery and characterization of these molecules have revolutionized our understanding of gene expression, revealing that ncRNAs are critical governors of nearly every cellular process, from development and differentiation to homeostasis and disease [10]. Within this expanding universe, a particularly intriguing class of ncRNAs has recently come into sharp focus: circular RNAs.

1.3. Emergence of Circular RNAs: From Splicing Anomaly to Key Regulator

The history of circular RNAs (circRNAs) is a compelling narrative of scientific rediscovery, fueled by technological innovation. These molecules were first observed in the 1970s as viroids in plants and were later detected in eukaryotic cells via electron microscopy [2,3], but were largely relegated to the status of molecular curiosities or rare by-products of aberrant pre-mRNA splicing events [1]. This perception persisted for decades until the advent of high-throughput RNA sequencing (RNA-seq) combined with the development of specialized bioinformatics algorithms in the early 2010s [4,5,7,13]. This technological leap enabled a global and unbiased view of the transcriptome, revealing that circRNAs are not rare artifacts but a widespread, abundant, and evolutionarily conserved class of RNA molecules present across diverse species, from fungi to humans [1,34].

Structurally, circRNAs are distinct from their linear counterparts. They are formed through a non-canonical splicing process known as "back-splicing," [8] where a downstream 5' splice donor site is covalently linked to an upstream 3' splice acceptor site [1]. This event generates a continuous, covalently closed loop structure that lacks the 5' cap and 3' polyadenylated (poly(A)) tail characteristic of linear mRNAs [12]. This unique circular topology is not merely a structural quirk; it confers remarkable biochemical properties. The absence of free ends renders circRNAs highly resistant to degradation by exonucleases, such as RNase R, which are responsible for degrading most linear RNAs [9]. Consequently, circRNAs exhibit exceptional stability, with half-lives that can be significantly longer—in some cases more than 48 hours compared to less than 10 hours—than their

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cognate linear mRNAs produced from the same gene locus [15]. This inherent stability is a foundational property that underpins their immense potential as both robust clinical biomarkers and durable therapeutic agents [38,39].

1.4. Scope and Aims of the Review

The rapid acceleration of circRNA research has unveiled their critical involvement in the pathophysiology of numerous diseases, with the cardiovascular system being a particularly active area of investigation.[6] The heart expresses thousands of distinct circRNA species, many of which exhibit tissue-specific and developmentally regulated expression patterns, hinting at their specialized roles in cardiac biology.[21] The purpose of this review is to provide a comprehensive and expert-level synthesis of the current state of knowledge regarding circRNAs in cardiovascular disease, with a specific focus on their potential as biotechnological tools.

This paper will: (1) detail the biogenesis and multifaceted functional mechanisms of circRNAs active in the cardiac milieu; (2) critically analyze the growing body of evidence defining their often-dichotomous roles in key cardiac pathologies like myocardial infarction and heart failure; (3) evaluate their burgeoning application as a new generation of high-fidelity clinical biomarkers; and (4) provide an in-depth assessment of the biotechnological frontiers in designing, engineering, and delivering circRNA-based therapeutics. By bridging the gap between fundamental circRNA biology and its translation into clinical cardiology, this review aims to provide a valuable resource for researchers and clinicians seeking to understand and harness the power of these remarkable molecules.

2. The Functional Repertoire of circRNAs in the Cardiac Milieu

The functional impact of circRNAs is intrinsically linked to their unique biogenesis and molecular architecture. While initially thought to be non-functional, it is now clear that they participate in a wide array of regulatory activities. Their mechanisms of action are diverse, ranging from acting as molecular sponges for other non-coding RNAs to serving as scaffolds for protein complexes and, in some cases, even being translated into functional peptides. Understanding this functional repertoire is essential for deciphering their roles in cardiac pathophysiology and for designing effective therapeutic interventions.

2.1. Biogenesis: The Back-Splicing Mechanism

The formation of a circRNA is a departure from the canonical, linear splicing pathway that generates most messenger RNAs. In canonical splicing, the spliceosome recognizes and removes intronic sequences, ligating the exons in a linear, head-to-tail fashion to produce a mature mRNA with a 5' cap and a 3' poly(A) tail [12,36,40]. In contrast, circRNAs are generated through a non-canonical process termed "back-splicing," where the spliceosome joins a splice donor site (typically at the 3' end of a downstream exon) to a splice acceptor site (at the 5' end of an upstream exon) [15]. This head-to-tail linkage creates the characteristic covalently closed loop [38,39].

Several models have been proposed to explain how the splicing machinery is directed toward this alternative pathway. The two most prominent models are "lariat-driven circularization" (or exon skipping) and "intron-pairing-driven circularization" [15]. In the lariat-driven model, canonical splicing machinery skips one or more exons, generating an exon-containing lariat intermediate that is then processed to remove the introns, leaving a circularized exon or exons [26]. In the intron-pairing model, complementary sequences within the introns flanking the exons to be circularized (such as inverted Alu repeats) hybridize, bringing the back-splice sites into close proximity and facilitating their ligation by the spliceosome [18]. The biogenesis of circRNAs can also be influenced by RNA-binding proteins (RBPs) that bind to specific motifs in the flanking introns and promote circularization [15]. Depending on their genomic origin, circRNAs are classified into three main types: exonic circRNAs (ecRNAs), which are the most common and are composed entirely of one or more exons; circular intronic RNAs (ciRNAs), which are derived from introns; and exon-intron circRNAs (EIciRNAs), which contain both exonic and intronic sequences [26].

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2.2. The "miRNA Sponge": A Dominant Mechanism of Action

Perhaps the most extensively studied and well-characterized function of circRNAs is their role as microRNA (miRNA) sponges, a concept central to the competing endogenous RNA (ceRNA) hypothesis.[1] MiRNAs are small ncRNAs that regulate gene expression post-transcriptionally by binding to complementary sequences, known as miRNA response elements (MREs), typically located in the 3' untranslated region (UTR) of target mRNAs, leading to mRNA degradation or translational repression.[15] Many circRNAs, particularly those located in the cytoplasm, are rich in MREs and can competitively bind to specific miRNAs, effectively sequestering them and preventing them from interacting with their cognate mRNA targets.[2] By acting as a "sponge," the circRNA titrates the available pool of a given miRNA, thereby de-repressing the expression of that miRNA's target genes.[15] This mechanism is a major driver of circRNA function in the cardiovascular system. Several key examples illustrate its importance.

2.2.1. ciRS-7

ciRS-7 (also known as Cdr1as), is one of the most famous circRNAs and serves as a powerful "super-sponge" for miR-7. It contains over 70 conserved binding sites for miR-7 [27,35]. In the context of myocardial infarction, miR-7a has a protective role by repressing pro-apoptotic genes. The upregulation of ciRS-7 in the ischemic heart sponges miR-7a, leading to the de-repression of its targets, including poly(ADP-ribose) polymerase (PARP) and the transcription factor SP1. This action ultimately promotes cardiomyocyte apoptosis and exacerbates infarct size, highlighting ciRS-7 as a pathogenic factor [29].

2.2.2. Heart-Related circRNA (HRCR)

Identified as a key regulator in cardiac hypertrophy, HRCR acts as a sponge for miR-223. MiR-223 is known to target the apoptosis-related gene ARC (apoptosis repressor with CARD domain). By sequestering miR-223, HRCR prevents the degradation of ARC mRNA, thereby inhibiting cardiac hypertrophy and protecting against heart failure [16].

2.2.3. circNfix

This circRNA plays a detrimental role in the post-MI heart, in part by sponging miR-214. This sequestration of miR-214 leads to the upregulation of its target, glycogen synthase kinase 3β (GSK3β), a kinase known to promote apoptosis and inhibit pro-survival β-catenin signaling. This action contributes to the pro-apoptotic effects of circNfix in cardiomyocytes [10].

2.3. Interaction with RNA-Binding Proteins (RBPs): Scaffolds and Decoys

Beyond their interaction with miRNAs, circRNAs exert significant regulatory influence by binding to RNA-binding proteins (RBPs) [2]. RBPs are crucial regulators of RNA processing, transport, stability, and translation. CircRNAs can modulate RBP function in several ways. They can act as molecular "decoys" or "sponges" for RBPs, sequestering them away from their canonical linear RNA targets and thereby altering post-transcriptional gene regulation [27]. Alternatively, circRNAs can function as dynamic "scaffolds," bringing multiple proteins into close proximity to facilitate the assembly of enzymatic complexes or to shuttle proteins to specific subcellular locations [18]. This mode of action is also highly relevant in the cardiac context.

2.3.1. circFndc3b

This cardioprotective circRNA, which is downregulated after MI, exerts its pro-angiogenic effects by interacting with the RBP Fused in Sarcoma (FUS). The circFndc3b-FUS complex appears to regulate the expression of vascular endothelial growth factor (VEGF), a critical signaling molecule for angiogenesis. Overexpression of circFndc3b promotes angiogenesis and improves cardiac function post-MI, a function mediated through this RBP interaction [16].

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2.3.2. circFoxo3

This circRNA is implicated in cellular senescence, a process relevant to cardiac aging and heart failure. It has been shown to interact with several RBPs, including the anti-senescence protein ID-1 and the transcription factor E2F1. By binding to and sequestering these proteins in the cytoplasm, circFoxo3 prevents them from carrying out their nuclear functions, thereby promoting a senescent phenotype in cardiomyocytes [17].

2.3.3. circAmotl1

Highly expressed in cardiac tissue, circAmotl1 promotes cardiomyocyte survival by acting as a scaffold. It binds to both PDK1 and AKT1, two key kinases in the pro-survival PI3K/AKT pathway. This interaction is thought to facilitate the phosphorylation and activation of AKT, promoting its translocation to the nucleus where it can activate downstream survival genes [17].

The functional plasticity of circRNAs is a testament to their complex regulatory capacity. A single circRNA can often engage in multiple types of interactions simultaneously. For instance, circNfix not only sponges miR-214 but also interacts with the RBP Y-box-binding protein 1 (Ybx1), promoting its ubiquitination and degradation [16]. This demonstrates that circRNAs can orchestrate complex biological outcomes by concurrently modulating both miRNA and RBP networks. This functional multiplicity underscores the need for a systems-level understanding when studying these molecules and carries significant implications for their therapeutic targeting, as manipulating one interaction may have unforeseen consequences on others.

2.4. Emerging and Less-Characterized Functions

While miRNA and RBP sponging are the most documented functions, the regulatory repertoire of circRNAs continues to expand. Two emerging areas of research are particularly noteworthy. First, some circRNAs, especially EIciRNAs that are retained in the nucleus, can regulate the transcription of their own parent genes [15]. These nuclear circRNAs can interact with the U1 small nuclear ribonucleoprotein (snRNP) and the RNA polymerase II (Pol II) complex at the gene's promoter, enhancing the expression of the linear mRNA transcript from which they were derived [2]. This creates a positive feedback loop where the circRNA can amplify its own parent gene's expression [47].

Second, a fascinating and more recent discovery is that a subset of circRNAs can be translated into proteins or peptides [1]. Although circRNAs lack a 5' cap, which is required for canonical cap-dependent translation, some contain an internal ribosome entry site (IRES) or other sequence motifs that can recruit the ribosomal machinery and initiate cap-independent translation [13]. While this function appears to be less common than their non-coding roles, the peptides produced from circRNA translation have been shown to be functional in some contexts. This discovery blurs the traditional lines between coding and non-coding RNAs and opens up an entirely new dimension of circRNA biology that is just beginning to be explored in the cardiovascular system.

3. The Dichotomous Roles of circRNAs in Cardiac Pathophysiology

The discovery of a rich and diverse circRNA landscape in the heart has paved the way for investigating their roles in the molecular pathogenesis of cardiovascular disease. Extensive research has revealed that circRNAs are not merely passive bystanders but are active and potent modulators of the cellular processes that underpin cardiac injury and remodeling [20,21,23,24,52]. Following an event like myocardial infarction, the expression profile of cardiac circRNAs is dramatically altered, with hundreds of species being up- or downregulated [22]. These changes are not epiphenomenal; they are integral to the heart's response to stress. A striking theme that has emerged from this body of work is the dichotomous nature of circRNA function. Many circRNAs act as pathogenic factors, exacerbating cell death, inflammation, and fibrosis, while others serve as endogenous protective molecules, promoting survival, angiogenesis, and regeneration. The balance between these opposing circRNA networks likely dictates the ultimate fate of the heart, determining whether it succumbs to

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pathological remodeling or successfully adapts and repairs. This section will systematically review the evidence for the specific roles of key circRNAs in the critical processes of myocardial infarction, cardiac fibrosis, and regeneration.

3.1. Myocardial Infarction (MI) and Ischemia-Reperfusion (I/R) Injury

Myocardial infarction, resulting from the occlusion of a coronary artery, triggers a cascade of detrimental events, including massive cardiomyocyte death (via apoptosis and necrosis), an intense inflammatory response, and subsequent ischemia-reperfusion (I/R) injury upon restoration of blood flow [16]. CircRNAs have been identified as crucial regulators at every step of this injurious cascade, acting as molecular switches that can either amplify or dampen the pathological response [16].

3.1.1. Pro-Apoptotic and Pro-Injury circRNAs

A significant number of circRNAs are upregulated following MI and function to exacerbate cardiac injury, primarily by promoting cardiomyocyte apoptosis [53]. These pathogenic circRNAs represent attractive targets for therapeutic silencing. As one of the most well-studied pro-apoptotic circRNAs, **circNfix** is upregulated in the post-MI heart. Its pathogenic effects are mediated through a sophisticated dual mechanism. It sponges miR-214, leading to increased expression of the pro-apoptotic kinase GSK3β. Simultaneously, it facilitates the degradation of the RBP Ybx1, which suppresses the expression of cell cycle promoters Cyclin A2 and Cyclin B1. The net result is a powerful block on cardiomyocyte proliferation and a strong push towards apoptosis [10].

Cdr1as (ciRS-7) is a potent miR-7a sponge is also upregulated during MI. By sequestering miR-7a, Cdr1as de-represses the expression of PARP and SP1, two key proteins involved in apoptotic signaling pathways. Overexpression of Cdr1as in vivo has been shown to increase cardiac infarct size, confirming its detrimental role [17].

Upregulated under hypoxic conditions, **circNCX1** contributes to cardiomyocyte apoptosis by binding to and inactivating miR-133a-3p. The loss of miR-133a-3p function leads to increased expression of its target, CDIP1, which aggravates ischemic myocardial injury [16].

A growing list of other circRNAs has been implicated in promoting cardiac cell death. **circACAP2** mediates apoptosis by sponging miR-29 [16]. **circMFACR** (Mitochondrial-fission-and-apoptosis-related circRNA) promotes MI by sponging miR-652-3p, which upregulates the mitochondrial fission regulator MTP18 [16]. **circPVT1** acts as a sponge for both miR-125b and miR-200a, attenuating their protective effects [16]. **circJARID2** promotes hypoxia-induced injury by sponging miR-9-5p to upregulate BNIP3 [16]. **circROBO2** enhances TRADD expression by sponging miR-1184, exacerbating apoptosis and cardiac dysfunction [16].

3.1.2. Cardioprotective and Pro-Survival circRNAs

In contrast, another set of circRNAs acts as endogenous guardians of the heart, with expression levels often decreasing after injury. Restoring the levels of these protective circRNAs is a promising therapeutic strategy.

The expression of **circFndc3b** is dramatically downregulated in both human ischemic cardiomyopathy and in mouse models of MI. This circRNA is profoundly cardioprotective. It enhances endothelial cell function and protects cardiomyocytes from death by interacting with the RBP FUS to positively regulate the expression of the pro-angiogenic factor VEGF. Overexpression of circFndc3b via AAV9 vectors has been shown to reduce apoptosis, promote angiogenesis, and significantly improve left ventricular function post-MI [16].

ACR (Autophagy-Related circRNA) is a circRNA significantly reduced during I/R injury. Its protective function lies in its ability to suppress excessive and detrimental autophagy. ACR directly binds to the DNA methyltransferase Dnmt3B, blocking it from methylating and silencing the promoter of the *Pink1* gene. The resulting sustained Pink1 expression helps to modulate autophagy and reduce autophagic cell death, thereby protecting the heart from I/R injury [16].

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Downregulated in MI models, **circSNRK** exerts a protective effect by reducing cardiomyocyte apoptosis and promoting proliferation. It functions as a sponge for miR-103-3p, leading to increased levels of its parent gene protein, SNF1-related kinase (SNRK), which in turn modulates the activity of the pro-apoptotic kinase GSK3β [16].

Several other circRNAs have been identified as having protective roles. **circCNEACR** (Cardiac-necroptosis-associated circRNA) inhibits Ripk3-dependent programmed necrosis by binding to HDAC7 in the cytoplasm [16]. **circCDYL**, which is downregulated in MI, promotes myocardial regeneration by sponging miR-4793-5p [10]. **circMACF1** functions as a sponge for miR-500b-5p to upregulate EMP1 and mitigate apoptosis [16].

3.2. Cardiac Fibrosis and Pathological Remodeling

Following the acute phase of MI, the heart enters a chronic phase of adverse remodeling, characterized by cardiac hypertrophy and the excessive deposition of extracellular matrix proteins by activated fibroblasts, leading to fibrosis [6]. This stiffening of the ventricle impairs both systolic and diastolic function, serving as the primary substrate for the development of heart failure. CircRNAs are deeply involved in regulating the fibrotic process, with specific species either promoting or inhibiting fibroblast activation and collagen synthesis [54].

3.2.1. Pro-Fibrotic circRNAs

The expression of **circPAN3** is upregulated in the hearts of MI rats and in cardiac fibroblasts stimulated with the pro-fibrotic cytokine TGF-β1. It promotes fibrosis by sponging miR-221, which leads to increased expression of the autophagy-related genes Foxo3 and ATG7. This activation of autophagy in fibroblasts enhances their pro-fibrotic activity [16]. **circHIPK3** is a multifaceted circRNA is upregulated in the infarcted heart and contributes to fibrosis. It can aggravate myocardial injury by sponging miR-124-3p. Furthermore, exosomal circHIPK3 derived from cardiomyocytes can influence endothelial cells via the miR-29a/IGF-1 axis, contributing to the maladaptive remodeling environment [10].

3.2.1. Anti-Fibrotic circRNAs

In contrast to the pro-fibrotic circRNAs, circNFIB expression is reduced in post-MI heart tissue. It acts as a brake on the fibrotic process. Overexpression of circNFIB can resolve the pro-fibrotic effects induced by TGF-β signaling pathways. It achieves this by acting as a competitive inhibitor of miR-433, preventing this pro-fibrotic miRNA from repressing its anti-fibrotic target genes [10].

3.3. Cardiac Regeneration: Proliferation and Differentiation

The ultimate goal of cardiac repair is to replace lost cardiomyocytes, a process that requires the stimulation of proliferation in remaining cardiomyocytes or the differentiation of cardiac progenitor cells. While the adult heart has very limited regenerative potential, the neonatal heart can regenerate, and this process is governed by specific molecular programs that are now being elucidated. CircRNAs have emerged as key players in these regenerative pathways, offering exciting therapeutic targets to "reawaken" the heart's dormant regenerative capacity [7,19,74,75,77,78].

circHipk3 is a circRNA is highly abundant in the fetal and neonatal mouse myocardium, a period of active cardiomyocyte proliferation, but its expression declines sharply in the adult heart. Si et al. demonstrated that re-introducing circHipk3 into the adult mouse heart post-MI via an adenoviral vector had remarkable regenerative effects. It induced cardiomyocyte proliferation, promoted angiogenesis, and decreased fibrosis. Its mechanism is dual: it promotes angiogenesis by sponging miR-133a, while its pro-proliferative effect on cardiomyocytes is achieved by increasing the stability of the Notch1 intracellular domain, a key component of a major developmental signaling pathway [10].

While the upregulation of **circNfix** is detrimental post-MI, the converse is also true. The targeted knockdown of circNfix in a murine MI model was shown to unleash a regenerative response,

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characterized by increased cardiomyocyte proliferation and improved cardiac function [10]. This highlights how inhibiting a "stop" signal can be as effective as activating a "go" signal. Similar to circHipk3, **circCDYL** appears to be a pro-proliferative factor. Its overexpression in adult mouse cardiomyocytes in vitro promoted their proliferation, while its overexpression in vivo after MI led to an increase in ejection fraction [10].

The regenerative potential of the heart can also be approached through cell-based therapies using stem cells like mesenchymal stem cells (MSCs) [46]. CircRNAs are crucial for maintaining the "stemness" and differentiation capacity of these cells. For example, **circFOXP1** and **CDR1as** are both highly expressed in MSCs and are essential for their proliferation and pluripotency. Silencing these circRNAs impairs the regenerative potential of MSCs [10]. This indicates that modulating circRNA expression could be a strategy to enhance the efficacy of cardiac stem cell therapies.

The context-dependent and often opposing roles of circRNAs in cardiac pathophysiology are striking. A circRNA that is pro-apoptotic in a cardiomyocyte may be pro-fibrotic in a fibroblast. The balance of expression of hundreds of these molecules creates a complex regulatory network that fine-tunes the heart's response to injury. This complexity presents both a challenge and an opportunity. The challenge lies in the need for highly specific therapeutic targeting to avoid unintended off-target effects. The opportunity lies in the potential to precisely modulate these networks to shift the balance away from pathological remodeling and toward adaptive repair and regeneration.

3.4. Summary of Key circRNAs in Cardiac Pathophysiology

Table 1. Key Circular RNAs Implicated in Myocardial Infarction and Heart Failure.

circRNA Name	Change in CVD	Primary Mechanism	Key Molecular Target(s)	Downstream Pathway/Effect	Overall Pathophysiological Role
Pro-Pathogenic circRNAs					
circNfix [10]	Upregulated in MI	miRNA sponge; RBP interaction	Sponges miR-214; Promotes Ybx1 degradation	Upregulates GSK3β; Suppresses Cyclin A2/B1	Promotes cardiomyocyte apoptosis, inhibits proliferation
Cdr1as (ciRS-7) [16]	Upregulated in MI	miRNA sponge	Sponges miR-7a	De-represses PARP and SP1	Promotes cardiomyocyte apoptosis, increases infarct size
circACAP2 [16]	Upregulated in MI	miRNA sponge	Sponges miR-29	Modulates apoptosis pathways	Promotes hypoxia-induced cardiomyocyte apoptosis
circMFACR [16]	Upregulated in H/R	miRNA sponge	Sponges miR-652-3p	Upregulates MTP18	Promotes mitochondrial fission and apoptosis
circPAN3 [16]	Upregulated in MI	miRNA sponge	Sponges miR-221	Enhances Foxo3 and ATG7 expression	Promotes autophagy-mediated cardiac fibrosis

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circHIPK3 [10]	Upregulated in MI	miRNA sponge	Sponges miR-124-3p, miR-29a	Aggravates I/R injury; promotes fibrosis	Pro-fibrotic and pro-injury
Cardioprotective circRNAs					
circFndc3b [16]	Downregulated in MI	RBP interaction	Interacts with FUS	Regulates VEGF expression	Protects cardiomyocytes, promotes angiogenesis
ACR [16]	Downregulated in I/R	RBP interaction	Binds to Dnmt3B	Blocks methylation of *Pink1* promoter	Suppresses excessive autophagy and cell death
circSNRK [16]	Downregulated in MI	miRNA sponge	Sponges miR-103-3p	Increases SNRK levels, modulates GSK3β	Reduces apoptosis, promotes proliferation
circNFIB [10]	Downregulated in MI	miRNA sponge	Sponges miR-433	Inhibits TGF-β/Smad3 pathway	Anti-fibrotic, resolves collagen deposition
circCDYL [10]	Downregulated in MI	miRNA sponge	Sponges miR-4793-5p	Increases amyloid precursor protein	Promotes cardiomyocyte proliferation and regeneration
circHipk3 (neonatal) [10]	High in neonates	miRNA sponge; Protein stabilization	Sponges miR-133a; Stabilizes Notch1	Promotes endothelial proliferation; Induces cardiomyocyte proliferation	Pro-regenerative in the neonatal context

4. Biotechnological Application I: circRNAs as High-Fidelity Biomarkers

The quest for ideal biomarkers is a cornerstone of modern medicine. An ideal biomarker should be sensitive, specific, easily accessible through non-invasive means, and provide accurate diagnostic, prognostic, or predictive information. Traditional cardiac biomarkers, such as cardiac troponins, have revolutionized the diagnosis of acute myocardial infarction, but they have limitations, including a delayed rise after injury and potential elevation in non-cardiac conditions [8,29,48]. The unique biochemical properties of circRNAs position them as a potentially superior class of biomarkers for a wide spectrum of cardiovascular diseases [3,30,32].

4.1. The Rationale: Why circRNAs Are Superior Biomarkers

Several intrinsic features make circRNAs exceptionally well-suited for biomarker applications. The primary advantage of circRNAs is their remarkable stability. The covalently closed loop structure, devoid of free 5' and 3' ends, renders them highly resistant to degradation by RNase enzymes that are abundant in body fluids like blood plasma and serum [9]. This resistance means that circRNAs persist in circulation for much longer than their linear RNA counterparts, providing a more stable and reliable signal for detection.

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The heart is a rich source of circRNAs, with thousands of distinct species expressed, many of which show tissue- and disease-specific expression patterns [1]. For example, a circRNA that is specifically upregulated in cardiomyocytes during ischemia offers a much more precise diagnostic window into cardiac injury than a generic marker of cell death [14]. This specificity can potentially distinguish between different types of CVD or different stages of disease progression.

CircRNAs are not confined to their cells of origin. They are actively packaged into extracellular vesicles (EVs), including exosomes, and released into the circulation [9]. Encapsulation within these lipid bilayer vesicles provides an additional layer of protection from degradation and offers a means for non-invasive "liquid biopsies." By analyzing the circRNA content of plasma, serum, or even saliva, it is possible to obtain a molecular snapshot of the pathological processes occurring within the heart, without the need for invasive tissue biopsies.

4.2. The Rationale: Why circRNAs Are Superior Biomarkers

A growing body of research has moved from simply identifying circRNAs in the heart to demonstrating their potential as clinical biomarkers [4]. Several studies have used high-throughput screening to identify panels of circRNAs in the peripheral blood of acute myocardial infarction (AMI) patients that can distinguish them from healthy controls with high accuracy [51]. One study identified a five-circRNA panel (circTMEM165, circUBAC2, circZNF609, circANKRD12, and circSLC8A1) that demonstrated high sensitivity and specificity for diagnosing AMI [16]. Another important biomarker is the **myocardial infarction-related circular RNA (MICRA)**. Vausort et al. found that lower circulating levels of MICRA in patients following an MI were significantly associated with a higher risk of developing left ventricular dysfunction, suggesting it has prognostic as well as diagnostic value [28]. Other circRNAs implicated in apoptosis, such as circNFIX and MFACR, are also being investigated as sensitive biomarkers for MI [22].

The utility of circRNA biomarkers extends to chronic conditions. For example, **HRCR**, the circRNA that protects against hypertrophy by sponging miR-223, could potentially serve as a biomarker to monitor the progression of heart failure [16]. In the context of atherosclerosis, **circ-ANRIL**, which is associated with the disease process, and **circRNA-284**, which may indicate carotid plaque rupture, are promising candidates for risk stratification and diagnosis.

The maturation of the circRNA biomarker field is evidenced by the emergence of higher-level evidence synthesis. A meta-analysis published in 2022 by Wang et al. aggregated data from 27 eligible articles, encompassing 47 individual studies and over 6,800 participants, to evaluate the overall diagnostic accuracy of circRNAs for CVD [39]. The pooled results were highly encouraging, demonstrating a summary sensitivity of 0.81 (95% CI: 0.78-0.83) and a specificity of 0.74 (95% CI: 0.68-0.78). The positive likelihood ratio was 3.1, indicating that a positive circRNA test is moderately good at ruling in disease. This meta-analysis provides robust, evidence-based confirmation that circRNAs, as a class, hold significant diagnostic potential. The analysis also revealed that serum-based measurements may provide higher accuracy than whole blood and identified factors like detection method and sample size as sources of heterogeneity, providing valuable guidance for the design of future studies.

4.3. Challenges and Path to Clinical Implementation

Despite the immense promise, the translation of circRNA biomarkers from research laboratories to routine clinical practice faces several significant hurdles that must be addressed [26]. A major challenge is the lack of standardized, universally accepted protocols. Different studies use varying methods for sample collection, circRNA isolation, and detection (e.g., qRT-PCR vs. RNA-seq). This variability makes it difficult to compare results across studies and establish consistent diagnostic thresholds. The development of standardized operating procedures and reference materials is critical for clinical adoption.

While many studies have identified promising candidate biomarkers in small cohorts, these findings must be validated in large-scale, multi-center, prospective clinical trials. These trials are

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essential to definitively establish the diagnostic and prognostic accuracy of specific circRNAs or circRNA panels in diverse patient populations and to compare their performance against existing gold-standard biomarkers.

The accurate identification and quantification of circRNAs from complex RNA-seq data remains a bioinformatic challenge [14]. The back-splice junction is the unique signature of a circRNA, but algorithms must be robust enough to distinguish true back-splice events from sequencing artifacts or trans-splicing events. Continued improvement in computational tools is necessary to ensure the reliability of sequencing-based biomarker discovery.

The journey of circRNAs from discovery to validated clinical tools is well underway. The field is clearly transitioning from an initial phase of discovery-based research to a more rigorous phase of clinical validation. The next wave of research must focus on tackling these challenges of standardization and large-scale validation to translate the remarkable potential of circRNA biomarkers into tangible benefits for patients with cardiovascular disease.

5. Biotechnological Application II: Therapeutic Frontiers in circRNA Engineering and Delivery

Beyond their utility as passive biomarkers, circRNAs represent a new and exciting class of therapeutic molecules. Their ability to modulate fundamental disease pathways makes them powerful tools for intervention. The dichotomous nature of their function provides a clear therapeutic rationale: pathogenic circRNAs that drive disease can be silenced, while protective circRNAs that promote repair can be administered as therapeutic agents. This has opened up a new frontier in RNA-based medicine for cardiovascular disease [3,55–57,82]. However, realizing this therapeutic potential hinges entirely on solving the single greatest challenge in RNA therapeutics: safe and efficient delivery of the RNA cargo to the target tissue—in this case, the heart [19,58,59].

5.1. Therapeutic Modalities: Silencing vs. Overexpression

Two primary strategies are being pursued for circRNA-based therapy [44]. For circRNAs that are upregulated in disease and contribute to pathology (e.g., circNfix or Cdr1as in MI), the goal is to reduce their expression. This can be achieved using nucleic acid-based drugs like small interfering RNAs (siRNAs) or antisense oligonucleotides (ASOs). A key advantage here is specificity. Because these silencing agents can be designed to target the unique back-splice junction sequence found only in the circRNA, they can knock down the circular form without affecting the linear mRNA transcribed from the same gene locus. This minimizes off-target effects on the parent gene's protein product [44].

For circRNAs that are protective but downregulated in disease (e.g., circFndc3b or ACR), the strategy is one of replacement or overexpression. This involves synthesizing the beneficial circRNA in vitro and delivering it to the heart as a drug. An alternative and more sophisticated approach is to design and deliver synthetic "circRNA sponges." These are engineered circRNAs designed with multiple binding sites for a specific pathogenic miRNA (e.g., a sponge for miR-221 to treat fibrosis). This allows for highly specific inhibition of a single miRNA, which may offer a better safety profile than using small molecule inhibitors that can have off-target effects [40].

5.2. The Critical Hurdle: In Vivo Delivery to the Heart

Naked RNA molecules are rapidly degraded in the bloodstream and cannot efficiently cross cell membranes. Therefore, the development of effective delivery vehicles, or vectors, is paramount. The ideal cardiac delivery system must protect the RNA cargo from degradation, evade the immune system, and specifically deliver its payload to the cells of the heart (cardiomyocytes, fibroblasts, or endothelial cells, depending on the target). Research is actively exploring three major classes of delivery platforms: viral vectors, lipid nanoparticles, and extracellular vesicles [5].

5.2.1. Viral Vectors (AAVs, Lentivirus)

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Viral vectors leverage the natural ability of viruses to efficiently deliver genetic material into cells. **Adeno-Associated Viruses (AAVs)** are the leading viral vector for in vivo gene therapy. They are attractive due to their low immunogenicity and their ability to transduce both dividing and non-dividing cells, like adult cardiomyocytes [33]. Certain serotypes, particularly AAV9, exhibit a natural tropism for the heart, making them well-suited for cardiac delivery [46]. Successful proof-of-concept has already been demonstrated in preclinical models, where AAV9-mediated cardiac overexpression of circFNDC3B and circITCH was shown to be protective in mouse models of MI and cardiotoxicity, respectively [33]. Lentiviruses are notable for their ability to integrate their genetic payload into the host cell's genome, which allows for stable, long-term expression of the therapeutic circRNA [33]. This could be advantageous for treating chronic conditions like heart failure.

Despite their efficiency, viral vectors face significant challenges. Their cargo capacity is limited, which can be a problem for delivering large circRNAs or complex expression cassettes. More importantly, they carry safety risks, including the potential to trigger an immune response, which can limit their efficacy and prevent re-dosing. Lentiviral vectors also carry a risk of insertional mutagenesis, where the random integration into the genome could potentially disrupt a tumor suppressor gene and lead to cancer [33].

5.2.2. Lipid Nanoparticles (LNPs)

LNPs are non-viral vectors composed of a mixture of lipids that self-assemble into a nanoparticle, encapsulating the RNA cargo within a protective core [63–65]. The clinical viability and scalability of LNP technology were spectacularly proven by the rapid development and deployment of the mRNA-based COVID-19 vaccines [46]. LNPs are attractive because they are non-viral, have lower intrinsic immunogenicity than viruses, and can carry large RNA payloads [48]. Their lipid composition can be tuned to optimize stability, and their surface can be modified to improve targeting to specific tissues [46]. Excitingly, recent research has shown that fibroblast-targeting LNPs can be used to deliver a combination of circTbx5 and a siYbx1, successfully inducing direct cardiac reprogramming in a mouse model of MI and improving cardiac function [7].

While promising, LNPs are not without hurdles. They can exhibit some level of cytotoxicity, and without specific targeting moieties, they tend to accumulate non-specifically in the liver and spleen. A key step in their mechanism is endosomal escape—the ability to exit the endosome and release their cargo into the cytoplasm—which remains a challenge to optimize [46].

5.2.3. Extracellular Vesicles (EVs)/Exosomes

EVs are nature's own nanocarriers. These small, membrane-bound vesicles are secreted by cells to mediate intercellular communication, transporting proteins, lipids, and nucleic acids (including circRNAs) to recipient cells [46,66–71].

As a biological delivery system, EVs are inherently biocompatible and have very low immunogenicity, making them less likely to be rejected by the immune system [43]. Their natural role in cell-to-cell signaling suggests they have evolved efficient mechanisms for cellular uptake and cargo delivery [46]. Preclinical studies have already shown their therapeutic potential. For instance, EV-mediated delivery of the regenerative circRNA circWhsc1 has been shown to promote cardiomyocyte proliferation and improve cardiac repair in mouse MI models [51]. Furthermore, EVs can be engineered; for example, their surface can be decorated with targeting ligands (like the rabies virus glycoprotein for neural targeting) to enhance delivery to specific tissues [47].

The primary obstacles for EV-based therapies are manufacturing and cargo loading. It is difficult to isolate a pure, homogenous population of EVs and to produce them at the large scale required for clinical use. Moreover, efficiently and consistently loading a specific therapeutic circRNA into EVs remains a significant technical challenge [43].

The choice of delivery system is not a one-size-fits-all decision. It involves a complex series of trade-offs between efficiency, safety, expression duration, and manufacturability. For treating an acute condition like MI, a transient but potent effect delivered by an LNP might be ideal. For a chronic

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disease like heart failure, the long-term expression afforded by an AAV vector might be more appropriate. The future of circRNA therapy will likely involve a sophisticated toolbox of these different delivery platforms, each tailored to a specific clinical application.

5.3. Comparative Analysis of Delivery Systems

To provide a clear overview of the relative merits and drawbacks of each major delivery platform, Table 2 compares them across several key translational parameters. This comparison highlights the critical trade-offs that researchers and drug developers must consider when selecting a delivery strategy for a circRNA-based therapeutic.

Table 2. Comparison of Delivery Systems for circRNA-Based Therapeutics.

Delivery Platform	Mechanism	Delivery Efficiency	Immunogenicity	Cargo Capacity	Expression Duration	Scalability/Manufacturing
Viral Vectors (AAV) [33]	Viral transduction, episomal DNA formation	High	Moderate-High	Limited (~4.7 kb)	Long-term/Stable	Complex, established protocols
Lipid Nanoparticles (LNP) [7]	Endocytosis, membrane fusion, endosomal escape	Moderate-High	Low-Moderate	High	Transient	Highly Scalable
Extracellular Vesicles (EV) [46]	Endocytosis, membrane fusion (natural)	Variable	Low	Moderate	Transient	Challenging, issues with purity/yield

6. Discussion

The field of circRNA biology has undergone a remarkable transformation, evolving from the study of obscure splicing by-products to a frontier of cardiovascular medicine. The accumulated evidence, synthesized in this review, firmly establishes circRNAs as a new and critical paradigm of gene regulation within the heart. Their unique stability, diverse functions, and dynamic expression in response to pathological stress position them at the nexus of disease pathogenesis and therapeutic opportunity. This discussion aims to synthesize the key themes that have emerged, address the most significant challenges, and outline the future trajectory of the field.

6.1. Synthesis: The circRNA-CVD Axis as a New Regulatory Paradigm

The data presented in Sections 2 and 3 paint a clear picture: the circRNA network constitutes a previously unappreciated layer of regulatory control that is integral to cardiac homeostasis and the response to injury [76,79–81]. This network is characterized by its complexity and its dichotomous nature. For nearly every pathogenic process—be it apoptosis, fibrosis, or inflammation—there exist circRNAs that promote it and others that inhibit it. For example, in the immediate aftermath of an MI, the upregulation of pro-apoptotic species like circNfix and Cdr1as pushes cardiomyocytes toward death, while the remaining levels of protective species like circFndc3b and ACR fight to keep them alive. The net outcome for the heart is likely determined not by any single molecule, but by the dynamic balance of this entire regulatory ecosystem. This understanding shifts the therapeutic goal from simply targeting a single gene to modulating a complex network, aiming to tip the balance in favor of the protective, pro-regenerative circRNAs.

6.2. The Biomarker-to-Therapy Pipeline: A Virtuous Cycle

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A powerful theme that emerges from this review is the synergistic relationship between the diagnostic and therapeutic applications of circRNAs. The search for biomarkers (Section 4) is not an isolated endeavor; it is the primary engine for therapeutic target discovery (Section 5). When a study identifies a circRNA, such as MICRA, whose levels in a patient's blood correlate with poor ventricular function post-MI [28], it does more than just provide a new diagnostic test. It simultaneously identifies MICRA as a potential pathogenic factor and, therefore, a prime candidate for therapeutic silencing. Conversely, identifying a protective circRNA like circFndc3b that is downregulated in diseased tissue immediately suggests a replacement therapy strategy. This creates a powerful and efficient "biomarker-to-therapy" pipeline, where clinical observation directly informs preclinical drug development in a virtuous cycle. This integrated approach can significantly accelerate the translation of basic biological discoveries into novel medicines.

6.3. Addressing the "Off-Target" Conundrum and Network Complexity

The very properties that make circRNAs potent regulators also make them potentially challenging therapeutic targets [37]. Their pleiotropic nature—the ability of a single circRNA to sponge multiple different miRNAs and bind to multiple different RBPs—means that they sit at the hub of complex interaction networks [53]. As discussed for circNfix, it simultaneously modulates a miRNA pathway (miR-214/GSK3β) and an RBP pathway (Ybx1 degradation) to achieve its pro-apoptotic effect [16]. While this makes it a powerful master regulator, it also raises a critical safety concern. A therapeutic designed to inhibit the circNfix-miR-214 interaction might have unintended and potentially detrimental off-target effects on the Ybx1 pathway or on other, as-yet-unidentified interaction partners.

This "off-target" conundrum is a significant hurdle for clinical translation [53]. It underscores a profound need for the field to move beyond single-interaction studies. Before a circRNA can be safely targeted in humans, its complete interaction network—its "interactome"—must be comprehensively mapped using advanced systems biology tools, including multi-omic analyses (transcriptomics, proteomics, metabolomics) and high-throughput interaction screening. Only by understanding the full spectrum of a circRNA's connections can we predict and mitigate the risks of therapeutic intervention.

6.4. Current Gaps and Unanswered Questions

Despite rapid progress, many fundamental questions in circRNA biology remain unanswered, representing key areas for future research. Thousands of circRNAs are expressed in the heart, but the function of the vast majority remains unknown. A systematic, large-scale effort is needed to functionally annotate the cardiac "circRNA-ome" to identify new regulators and therapeutic targets.

Questions regarding subcellular trafficking and secretion include: How are specific circRNAs sorted for their destination? What are the molecular signals that dictate whether a circRNA remains in the nucleus, is exported to the cytoplasm, or is packaged into an exosome for secretion? Unraveling these trafficking mechanisms is crucial, especially for understanding their roles in intercellular communication and for harnessing EVs as delivery vehicles.

While current delivery platforms are promising, the next generation of therapeutics will require "smart" delivery systems. Can we design LNPs or EVs that not only target the heart but also respond to the local disease environment, releasing their therapeutic cargo only in ischemic or fibrotic tissue? Developing such stimuli-responsive systems is a major challenge at the intersection of biotechnology and materials science. While many circRNAs show promise in rodent models, their efficacy and safety in larger animal models and ultimately in humans remain to be proven. Bridging this translational gap is the final and most critical step.

7. Conclusions and Future Perspectives

In the span of a single decade, circular RNAs have journeyed from the periphery of molecular biology to the forefront of cardiovascular research. They are no longer viewed as simple splicing

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artifacts but are now understood to be bona fide regulators of cardiac pathophysiology with immense and multifaceted biotechnological potential. This review has synthesized the compelling body of evidence that establishes circRNAs as a new class of molecules for both diagnosing and treating cardiovascular disease.

Their exceptional stability in circulation and their tissue-specific expression profiles make them near-ideal candidates for the development of a new generation of high-fidelity clinical biomarkers that could enable earlier and more precise disease detection. Concurrently, their ability to function as potent modulators of gene expression networks makes them an entirely novel class of RNA therapeutics.

The path to clinical translation is challenging, but clear. The primary bottleneck for circRNA-based therapy remains the development of delivery technologies that are safe, efficient, and capable of targeting the heart with high specificity. However, the rapid progress in the fields of viral vector engineering, lipid nanoparticle formulation, and exosome-based delivery provides a strong foundation for optimism.

Looking forward, the convergence of circRNA biology with these advanced nanodelivery platforms, alongside powerful tools like gene editing, holds the promise of creating a new generation of precision medicines for cardiovascular disease. We can envision a future where a simple blood test for a panel of circRNAs can predict an individual's risk of heart failure, and where a precisely delivered therapeutic circRNA can be administered to halt pathological remodeling, silence fibrotic pathways, and even stimulate the regeneration of lost heart muscle. By continuing to unravel the complexities of this fascinating class of molecules, we may one day be able to fundamentally change the prognosis for patients with cardiovascular disease, moving from a paradigm of disease management to one of true cardiac repair.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MDPI	Multidisciplinary Digital Publishing Institute
DOAJ	Directory of open access journals
TLA	Three letter acronym
LD	Linear dichroism

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Appendix A

Appendix A.1

The appendix is an optional section that can contain details and data supplemental to the main text—for example, explanations of experimental details that would disrupt the flow of the main text but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.

Table A1. This is a table caption.

Title 1	Title 2	Title 3
entry 1	data	data
entry 2	data	data

Appendix B

All appendix sections must be cited in the main text. In the appendices, Figures, Tables, etc. should be labeled starting with "A"—e.g., Figure A1, Figure A2, etc.

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