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Review article

Carbon nanomaterial-enhanced scaffolds for the creation of cardiac tissue constructs: A new frontier in cardiac tissue engineering

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ABSTRACT

Carbon nanomaterials (CNMs) have outstanding mechanical and electrical properties, making them ideal candidates for improving conventional cardiac tissue scaffolds. The improved cardiac tissue constructs have the potential to advance drug discovery and eventually allow for myocardial tissue regeneration, improving the treatment of heart diseases. This review first outlines the major research directions in the treatment of heart diseases, including surgical methods and pharmacological therapies, as well as gene and cell therapy. Afterwards, the review focuses on the use of CNMs in the construction of scaffolds for engineering cardiac tissue constructs, which could offer promising solutions to address the challenges in cell therapy. A series of studies in the past five years have shown that the incorporation of CNMs in tissue scaffolds enhances the survival, retention, organization, and physiological functions of cardiomyocytes.

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1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of deaths in the world, currently accounting for 17.3 million deaths globally (~30% of global deaths). This number is expected to rise to 23.6 million by 2030.[†] The global economic burden from CVDs is very significant, with over half of the cost coming from the developed nations (Table 1), and there is a strong incentive to invest in research to improve treatments of CVDs. A significant portion of this investment is specifically related to the CVDs that affect the myocardium, the muscle tissue of the heart that forms a thick middle layer between the outer epicardium layer and the inner endocardium layer.

Several key research directions have been pursued to improve the treatment of patients suffering from the myocardial damage. Surgical methods, implantation of the left ventricular assistance device (LVAD), pharmacological therapy, gene therapy, and cell therapy are the leading treatment methods [2]. Each method has

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had certain clinical success along with challenges and limitations. Surgical methods, such as transplantation, have been limited by the shortage in supply of donated organs [3]. LVAD implantation has had major problems linked to events such as thrombosis, infection, bleeding, neurologic events, and device malfunction [4]. Current pharmacological therapies for heart diseases require strict treatment schedules decreasing patient compliance and, therefore, limiting clinical efficiency [5–8].

Gene therapy represents a relatively recent advancement in the treatment of heart diseases [9]. In this type of treatment, a gene is incorporated into a viral vector, transferred into cells by the vector, and then incorporated into the cells' DNA, replacing the function of a faulty gene. The vectors currently in use are recombinant adenoassociated viruses. Unfortunately, circulating neutralizing antibodies bind to adenoassociated viruses, excluding about 50% of the population from this treatment [10]. Therefore, while gene therapy has the potential to gain clinical significance, it can only be used to treat a limited population.

In addition to their limitations, the abovementioned techniques focus on the treatment of the symptoms of myocardial damage rather than fixing the damage itself. An attractive alternative is cell therapy which focuses on the replacement or regeneration of the damaged tissue. The following section outlines recent progress in cell therapy and discusses the major challenges related to this type of treatment.

1.1. Cardiac cell therapy

Myocardial damage usually results from a loss of specialized cardiac muscle cells, known as cardiomyocytes, which can be caused by a variety of cardiovascular diseases and by aging. For example, acute myocardial infarction (MI) can destroy 25% of the left ventricular myocardium, roughly 0.5 to 1 billion cardiomyocytes, in a few hours. Unfortunately, the human heart has little inherent capacity for repair. Therefore, the goal of treating myocardial damage is to replenish functional and physiologically fused cardiomyocytes. Cardiac cell therapy has been recently under intensive investigation for this purpose, although it is not yet a standard clinical practice [11–15]. There is a strong drive to accelerate promising cell therapy techniques to clinical trials, due to the increasing economical burden of heart diseases and poor early diagnosis [11].

Cardiac cell therapy involves direct delivery of cells into a host heart, such as cardiac progenitor cells, bone marrow cells, and pluripotent stem cells, and programming their differentiation into cardiomyocytes [9]. Cell therapies for MI using human adult bone marrow-derived stem cells and skeletal myoblasts are currently undergoing clinical trials [16]. Researchers believe that other stem cell therapies should proceed to clinical trials as well, to allow for further understanding of cardiac regeneration and the development of new cell therapy techniques [11]. Early clinical studies indicate that cell therapy is a relatively safe treatment method. However, low cell survival rate and efficacy are major setbacks, keeping these technologies from being adopted into standard clinical practices [11,17,18].

In early studies, cells suspended in a saline solution were directly injected into the heart or administered systemically through intravenous injections. For example, in 2007, van Laake et al. reported the direct injection of differentiated human embryonic stem cells (HESCs) into mouse heart [5]. The transplanted cell population was mixed, with 20–25% of cells being cardiomyocytes (HESC-CMs). Four weeks post-injection, there was improved cardiac function; however, this improvement declined after 12 weeks. It was suggested that the improvement in function might have been limited by the small graft size and the low efficiency of differentiating HESCs into HESC-CMs. Notably, even though the total cell survival (of all types of cells) diminished over time with a final efficiency of approximately 2.3%, the cardiomyocyte population remained stable. Van Laake et al. suggested performing long-term *in vivo* studies to accurately characterize changes in cardiac function after transplantation, noting also that transplantation of a larger population of cardiomyocytes might lead to long-term improvement in cardiac function.

More recently, cell reprogramming has been developed as an alternative method for the cardiac cell therapy [19–24]. This approach is aimed at eliminating the challenges faced in cell transplantation, such as the immunogenic response and lack of host integration. In 2010, Ieda et al. reported that the combination of transcription factors Gata4, Mef2c, and Tbx5 could reprogram fibroblasts into beating cardiomyocyte-like cells *in vitro* [25]. Their *in vivo* results also suggested that fibroblasts could differentiate into cardiomyocyte cells within two weeks post transplantation. In 2013, Olsen et al. reported on cardiac reprogramming of fibroblasts to treat MI in mice [18]. Reprogramming fibroblasts into cardiomyocytes reduced the post-MI fibrosis and increased the number of functional cardiomyocytes. Although cardiac programming was shown to be superior to direct stem cell transplantation in terms of functional improvement, the conversion efficiency was very low. In 2016, Zhou et al. reported that the reduction of Bmi1, a polycomb complex gene, significantly increased the efficiency of reprogramming neonatal and adult rat fibroblasts into induced cardiomyocytes [26]. This study revealed that small hairpin RNA (shRNA) screening can give rise to epigenetic barriers in fibroblast reprogramming; however, the screening done in this study was not genome wide. This new approach to improve reprogramming efficiency *in vivo* is a promising research avenue. In summary, current cell therapy methods, either cell transplantation or cell reprogramming, lack the ability to regenerate a sufficient number of cardiomyocytes. Further research is required to improve the regeneration capability in order to make this approach clinically viable.

1.2. Cardiac tissue engineering for cell therapy

In contrast to cell therapy, which is based on the direct delivery of cells into a host heart, cardiac tissue engineering relies on the creation of cardiac tissues *in vitro*. These artificial tissues can be used as either biologically relevant models for *in vitro* studies or replacement of damaged tissues for regenerative therapy.

Table 1
Impact of cardiovascular diseases globally and on developed countries [1].[a,b,c,d,e,f]

	Prevalence	Death	Total Cost
Canada	1.6 million	66,000	$16B USD
United States	85.6 million	787,000	$320B USD
European Union	12 million (hospital discharges)	1.9 million	$219B USD
Worldwide	~67 million (new cases/year)	17.3 million	$906B USD

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