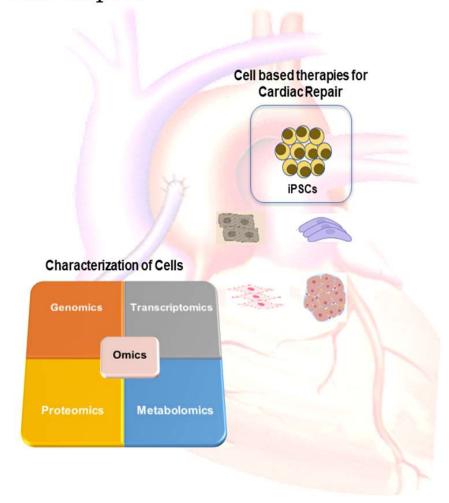
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Regenerative Therapies for Cardiac Repair



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From the Publisher's Desk

Welcome to Biotechnology Kiosk!

This issue includes a letter on the latest advances in Chromothripsis and genomic instability in cancer and editor picks on the positive effects of drugs on reducing obesity and affordable production of antibodies.

We hope our readers will enjoy reading these news and views on the current cutting-edge topics that include latest research breakthroughs in different areas of medicine and biotechnology. We look forward to receiving your feedback. We do hope that you will enjoy reading this issue of Biotechnology Kiosk. Please do write to us with your comments. Your suggestions are always appreciated.

Dr. Megha Agrawal & Dr. Shyamasri Biswas.

Editors-in-Chief, Biotechnology Kiosk





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Applications of Omics Technologies in Regenerative Therapies for

Cardiac Repair

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Abstract

Lately, cardio regenerative therapeutic approaches in clinic have received strong boost from promising research advances in stem cells. Researchers have shown applications of the first generation of clinical trials using cell-based therapies in the heart that have been performed with bone marrow and adipose tissue derived mesenchymal stem cells shortcomings in the first generation cells. Second generation cell therapies are considered superior and being considered towards the use of cardiac-committed cell populations. These include cardiac progenitor cells and pluripotent stem cell derived cardiomyocytes. However, translating the research laboratory results along with pre-clinical data into effective clinical treatments is still thought to be challenging. This is due to the existing lack of knowledge on the regenerative mechanisms of action of these therapeutic products in addition to the stringent regulatory and safety concerns. This has prompted researchers to consider advanced analytical methods for characterization of such complex products and a deep understanding of their therapeutic effects at the cell and molecular level. This characterization is considered very critical to overcome the challenges and make these cells compatible with the advanced therapies. To this end, omics technologies including proteomics and glyco(proteo)mics that are based on state-of-the-art massspectrometry have shown tremendous promise to generate novel data on cell biology along with the required assessment of cell based-products that are applied in cardiac regeneration strategies. In this perspective, we have described proteomics and glyco(proteo)mics and discuss the impact of omics technologies on cardiac progenitor cells and pluripotent stem cell derived cardiomyocytes biology cardiac regenerative therapies.

Keywords: Cardiac repair, proteomics, glycoproteomics, stem cells, regenerative therapies.

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Introduction

It is estimated that life expectancy worldwide will continue to increase in the upcoming decades, which may lead to a higher prevalence of age-related diseases such as cancer, neurodegenerative disorders and cardiovascular diseases. Among all these diseases, Acute Myocardial Infarction (AMI), which belongs to one of the severe cardiovascular medical conditions is considered lethal and does not give much time to recover. AMI or heart attack is known to cause millions of deaths worldwide each year. AMI causes serious damage to the patient's health as it results in irreversible myocardial tissue damage with loss of cardiomyocytes (CMs) that are the main cell type in the heart. Human body responds to this ischemic attack and the affected area becomes fibrotic with accumulation of extracellular matrix proteins to compensate the damage. Studies conducted on the fibrotic tissue have revealed that such tissue is stiffer with different electromechanical comparison healthy properties in to myocardium. Consequently, this leads to impaired cardiac output. To overcome this medical problem, several therapeutic strategies have been suggested that include medications, such as beta-blockers and angiotensin converting enzyme inhibitors. While these therapies have proven to be successful in reducing mortality, they do not allow for the complete restoration of normal heart muscle function. This includes preventing progression to chronic heart failure that gives rise to with high mortality rates. The only available treatment in such scenario is heart transplantation or the use of mechanical circulatory assist devices [1, 2].

The recent concept of Advanced Therapy Medicinal Products (ATMPs) for cardio regenerative medicine has emerged due to the increasing incidents of heart failure and the scarcity of donor organs for heart transplantation. ATMPs are considered promising for heart disease treatments that include cell transplantation-based strategies with cardiac progenitor cells (CPCs) or pluripotent stem cell derived cardiomyocytes (PSC-CMs). These treatment methodologies have been used in a number of pre-clinical and clinical trials. Further, it has been shown that extracellular vesicles through biomaterial-based delivery systems can also preserve cardiac tissue after myocardial infarction [1]. Especially, studies have suggested that exosomal microRNAs can play a key role in cardiac regeneration. Despite these advances, there are still challenges involving preclinical studies and clinical trials that do not show consistent physiological improvements and benefits over standard pharmacological treatment procedures. In other words, the goal is to translate the potential of stem cells and other ATMPs pre-clinical data into effective treatments that are used in the clinical set. To achieve this goal, there is a need for analytical methods that permit a deep characterization of such complex ATMPs along with determining their potency and the assessment of their mechanisms of action in humans [3, 4].

Recent advances in omics technologies have fulfilled the demand of accurate and reliable analytical techniques and software. These technologies have allowed comprehensive and non-targeted

investigation of these cell populations, which enable accomplishing a more complete molecular characterization and reveal key mechanisms of action. The most well-known omics technologies are proteomics and glyco(proteo)mics based tools. The workflows of these omics' tools are based on state-of-the-art mass-spectrometry that have paved way to some of the major breakthroughs in cell biology and a detailed assessment of cell based-products applied in cardiac regeneration strategies. These omics approaches are focused on the profiling of protein and glycan signatures for identification and characterization of cell populations. This can potentially lead to the discovery of pluripotency and differentiation biomarkers along with providing new insight into paracrine mechanisms and signaling cascades that are involved in cardiac repair. It is believed that the gained knowledge from the omics-based characterization and analysis would pave the way to a more rational therapy design in ATMPs [1].

In this short perspective, we have discussed recent trends and applications of omics technologies in cardiac repair.

Cell-Based Therapies for Cardiac Repair

Researchers employed multiple cell types in several pre-clinical studies and clinical trials (Figure 1). These approaches were mostly based on cells including skeletal myoblasts, mesenchymal stem cells, bone marrow mononuclear cells, and bloodcirculating progenitors [1]. While, several studies showed most of these firstgeneration cell-based therapies to be safe, there were some data that showed poor clinical effects. Researchers attributed the

failures of these therapies to the cell heterogeneity, lack of cell engraftment, and also inability of cells to differentiate and replace cardiac tissue. To overcome these challenges, current research has focused on second-generation cell-based therapies that include CPCs and PSC-CMs, which give more purified, homogenous and cardiaccommitted cell populations [5-9]. To this end, several in-vitro and preclinical studies have been conducted that have shown activation of different populations of CPCs upon AMI. It has been shown that these secondgeneration cells proliferate and migrate to the site of injury. Subsequently, these cells release important paracrine factors and microvesicles that are involved in angiogenesis, vasculogenesis, immunomodulation and CM cytoprotection [10-12].

Researchers have also demonstrated injection of human CPCs (hCPCs) in animal models of AMI that have resulted in an overall reduction of scar tissue size and improvements in heart function. In these studies, preclinical meta-analysis has been done that has shown an estimated improvement of 10.7% of heart left ejection fraction [1]. These promising preclinical results have initiated growth in research and developments in rapid translation of laboratory research into the clinic. This has been achieved with different populations of hCPCs in clinical trials that consist of both in autologous and allogeneic settings. To this end, pluripotent stem cells (PSCs) that include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have shown tremendous promise in proliferative potential. This also include the capacity to

differentiate into all cells from the three primary germ layers involving mesoderm, ectoderm and endoderm and CMs. Studies have clearly indicated ability of PSCs to theoretically provide an unlimited number of CMs that has made PSC-CMs. This is potentially very exciting as a novel clinical option for the replacement of the endogenous CMs that are lost in AMI and chronic heart failure patients (Figure 1) [1, 13-16]. However, analytical characterization is the major issue/requirement that we will discuss next.

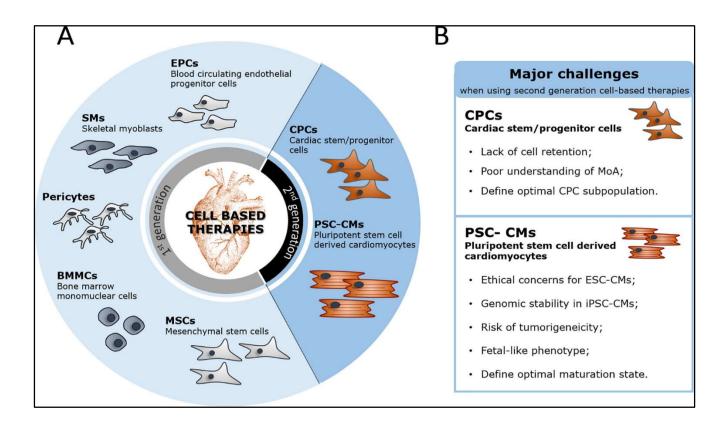


Figure 1: Schematic depiction of cell transplantation therapies that can be implemented for cardiac repair. (A) First generation of cell therapies is shown for myocardium repair (SMs, EPCs, BMMCs, Pericytes and MSCs). Second generation of cardiac committed cell populations including CPCs and PSC-CMs is also shown. (B) A list of major challenges when using CPC and PSC-CMs for clinical applications [**Source:** Biotechnology Advances (2021)].

Omics Technologies for Characterization of Cells

Several studies have been conducted using different omics methodologies (Figure 2) to the characterization of CPC and PSC-CMs [1]. These studies include gene expression profile employing transcriptomics, epigenomic modifications by epigenomics, analysis of nutrients and other small molecules that arise from the cell's energy metabolism by metabolomics, proteins using proteomics and protein post translational modifications by employing glycomics. Researchers have also employed complex multi-omics approaches for analyzing different datasets and their interactions. These different types of omics platforms are known to generate big amounts of data. These datasets are analyzed by using different omics methodologies that include bioinformatic processing tool and deconvolute and perform functional annotation of experimental data [17-21].

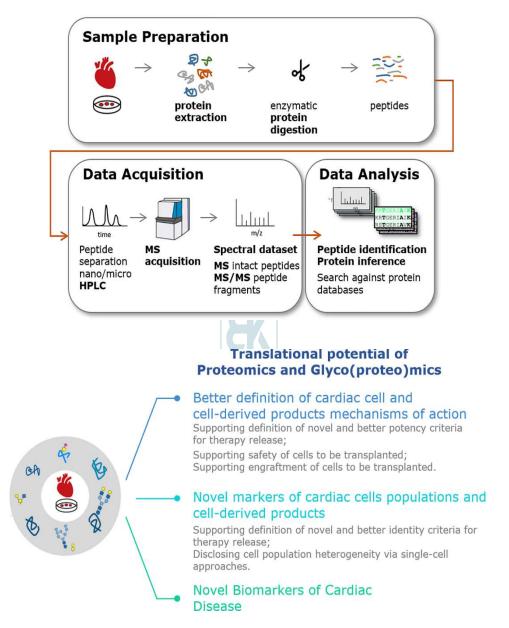


Figure 2. (Top) Non-targeted proteomic approaches are shown that include biological samples derived from tissues or cell cultures. High-performance liquid chromatography (HPLC) is employed to generate peptide mixture. Peptides are ionized and separated by mass spectrometry (MS). (Bottom) An overview is presented that shows translational potential for proteomics and glyco(proteomics) tools in cardiac cells and cardiac cell-derived products [**Source:** Biotechnology Advances (2021)].

Researchers have shown Single Proteomics by MS (SCoPE-MS) for quantification of thousands of proteins in single cell samples. This has enabled to measure proteome fingerprints of single cells and linking them to different other phenotypes. On the hand. glycoproteomics is another form of proteomics, which is focused on identifying glycosylated proteins and mapping their sites of glycosylation. This helps to gain a better of the understanding function of glycoproteins in biological or disease states. One of the applications of glycoproteomics is in genome-editing technologies that has been shown to reduce glycosylation facilitate glycoproteomic complexity to analysis [1, 22].

It is believed that progress of cell transplantation and cell-free clinical therapies for heart regeneration will be substantially moved forward the by applications of proteomics and glyco(proteo)mics methodologies. These omics technologies are supposed to assist in translation of these therapeutic approaches to practical applications, for example in novel biomarkers of heart disease (Figure 2) [1]. In addition, studies based on proteomics and glyco(proteo)mic are considered to play key roles in developing strategies to better characterize and interrogate PSC-CMs and CPCs populations. This is expected to reveal important insights that can help to elucidate their membrane molecular landscape, molecular processes regulating cardiac differentiation. This also include integration into the target tissue, and regenerative molecular mechanisms of those cells and

corresponding cell-derived products [1, 23-25].

Conclusion and Outlook

It is believed that applications of omics analytics technologies will help advance analytical assays to identify stem cell population subtypes. This can also help understand the regenerative mechanisms of action of stem cells and their impact on the cardiac tissue. This understanding is critical because it can enable researchers to better define the quality requirements for an accelerated clinical translation of stem-cell based products in cardiac repair. With further developments, proteomic and glyco(proteo)mic profiling strategies are supposed to become more sensitive. and high throughput accurate methodologies. This is expected to pave the way to advanced characterization of stem cells and stem cell derived products in homeostasis and disease settings. To this researchers demonstrated end. have potential of untargeted high-throughput proteomic and glyco(proteo)mic characterization of cardiac populations and cardiac cell derived products. Studies have been conducted to reveal key molecular pathways and proteomic/glycan signatures that leverage strategies for differentiation, purification and application of CPCs, PSC-CMs and/or cell derived products in clinical applications. Future studies are anticipated to focus on a better definition of critical quality attributes of these cells and cell-based products. This is believed to lead to future regulatory frameworks that could accelerate the clinical applications of stem cell based cardiac repair

and therapies based on omics methodologies.

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Biotechnology Advances around the World Editor's Picks

Every issue of Biotechnology Kiosk presents select latest research news picked by the editorsin-chief on significant research breakthroughs in different areas of biotechnology around the world. The aim is to promote further R&D in all of these cutting-edge areas of biotechnology. The editors have compiled and included the following innovations and breakthroughs to highlight the latest biotechnology advances.



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Cataract

New therapy for the treatment of eye cataract

Cataract is known to form clouding of the eye lens that gradually develops over time and affects the quality of vision. An accumulation of protein in the lens causes cataract, which reduces the transmission of light to the retina. The current treatment for a significantly developed cataract is only by a surgical procedure that is employed to remove the cloudv lens and insert an artificial replacement. In a recent study by worldleading eye experts have shown breakthrough that could potentially change the way cataracts are treated. The method has the potential for drug therapy to replace surgery.

A team of international scientists has recently published research (Aquaporins Have Regional Functions in Development of Refractive Index in the Zebrafish Eye Lens. Investigative Opthalmology & Visual Science, 2021; 62 (3): 23 DOI: <u>10.1167/iovs.62.3.23</u>) that shows the sophisticated optics of the lens develops much earlier in gestation than previously been thought. Researchers also demonstrated how a particular protein (aquaporin) responsible for water passage in the lens that can disrupt the optical development, leading to cataract formation.

This study was conducted using the most precise measurements on optics of the lens at SPring-8, the world's largest and most powerful synchrotron, in Japan. The synchrotron is a particle accelerator that produces powerful X-rays by accelerating electrons to the speed of light. Subsequently, the X-rays allow measurements to be taken with the highest accuracy on optical properties of the eye.

This study indicated the role of the aquaporin proteins. It also showed the crucial importance for the lens to work correctly and the eye to see clearly.

Food & Nutrition

Adverse effects of western diet on gut health

It is known that inflammatory bowel disease is a common problem globally. A recent study by US researchers has indicated that eating a Western diet could impair the immune system in the gut in ways that could potentially increase risk of infection and inflammatory bowel disease. Their research demonstrated that long-term consumption of a Western-style diet high in fat and sugar could potentially impair the function of immune cells in the gut that could promote inflammatory bowel disease or even increase the risk of intestinal infections.

This study was done in mice and people that showed that a diet high in sugar and fat causes damage to Paneth cells, immune cells in the gut. These cells are known to keep inflammation in check. For example, people with Crohn's disease, which is a kind of inflammatory bowel disease characterized by abdominal pain, diarrhea, anemia and fatigue has been shown with non-functional Paneth.

Researchers showed that nonfunctional Paneth cells can lead to the gut immune system excessively prone to inflammation. This can put people at risk of inflammatory bowel disease and undermining effective control of diseasecausing microbes. The findings were published in Cell Host & Microbe (Western diet induces Paneth cell defects through microbiome alterations and farnesoid X receptor and type I interferon activation. Cell Host & Microbe, 2021; DOI: <u>10.1016/j.chom.2021.04.004</u>). This study opens up new approaches to regulating gut immunity by restoring normal Paneth cell function.

In their study, researchers analyzed a database that contained demographic and clinical data on 400 people. This included an assessment of each person's Paneth cells. The researchers then found that high body mass index (BMI) was associated with Paneth cells that looked abnormal and unhealthy under a microscope. They found out that the higher a person's BMI, the worse his or her Paneth cells looked. Researchers then studied two strains of mice that were genetically predisposed to obesity. Such mice chronically overeat because they carry mutations that prevent them from feeling full even when fed a regular diet. Researchers found that the obese mice had Paneth cells that looked normal. In the next series of experiments, researchers fed normal mice a diet in which 40% of the calories came from fat or sugar, similar to the typical Western diet and after two months on this chow, the mice had become obese and their Paneth cells looked decidedly abnormal. This suggests the adverse effects of western diet on the gut health.

Compiled and Edited by Dr. Megha Agrawal and Dr. Shyamasri Biswas.

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