

Targeted Delivery of Factors and Cells for Improving Cardiac Tissue Regeneration and Heart Function Following Myocardial Infarction

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Abstract

Cardiovascular disease is one of the leading causes of death globally, accounting for almost one-third of total deaths in 2019. Myocardial infarction (MI), characterized by irreversible cardiac tissue death and scar tissue formation, is the main contributor to CVD mortality. Targeting post-MI fibrosis, considering its pathophysiology, is a promising therapeutic strategy. In this study, it was hypothesized that a new chitosan-based cryogel delivery system enabling sequential administration of cytokines, growth factors, and pre-differentiated stem cells would enhance post-MI restoration of cardiac function by decreasing the scar tissue area. Murine MI models were prepared by ligating the left anterior descending artery. Post-MI murine models were assessed for heart function under ultrasound at weeks 1 and 4. Mesenchymal stem cells were isolated from the compact bones of mice and pre-differentiated prior to transplantation. Combination therapy using cryogel loaded with interleukin-10, transforming growth factor- β , vascular endothelial growth factors, and fibroblast growth factor-2, followed by injection of pre-differentiated stem cells, was administered sequentially. This approach improved cardiac function and decreased fibrotic tissue area compared to the control group. These findings suggest that the chitosan-based cryogel delivery system is a promising approach for controlled therapeutic delivery and enhanced myocardial regeneration following MI.

Introduction

Cardiovascular disease (CVD) is one of the leading causes of death among the elderly, with an estimated global prevalence of 523 million by 2019 (Nedkoff et al., 2023). CVD accounts for 28,6% of total deaths, which was 19.4 million people in 2021 (Xia et al., 2025). Myocardial infarction (MI) is one of the main contributors to CVD mortality. According to Krittanawong et al. (2023), atherosclerosis from plaque rupture or erosion has increased in both elderly and those under 45 years. MI develops when acute coronary syndrome or prolonged supply-demand oxygen imbalance results myocardial ischemia (Smit et al., 2020). Every minute after the heart attack, cardiac tissue deteriorates, and thousands of cells die, making restoration of blood flow and oxygen supply essential. MI treatment includes pharmacological therapy (e.g., thrombolytics, antiplatelets, ACE inhibitors, statins) and surgical interventions like coronary angioplasty and bypass surgery. ("Heart attack - Diagnosis and treatment - Mayo Clinic", 2023). However, these approaches cannot restore full cardiac function because the adult heart regenerates poorly, replacing necrotized tissue with noncontractile fibrotic scars. (Gentek & Hoeffel, 2017). Therefore, it is very important to consider the ways of cardiac tissue regeneration to restore cardiac function and improve the quality of life of patients after MI.

Cell-based therapy is one of the prospective approaches in the treatment of MI. It is also important to modulate the inflammatory reaction caused by MI, which results in the development of fibrotic tissue and impaired heart function. Studies show that adult stem cells, such as mesenchymal stem cells (MSCs), cardiac progenitor cells, bone marrow cells (BMCs), and bone marrow-purified haematopoietic stem cells (HSCs) demonstrated limited effectiveness in animal models to improve cardiac regeneration after MI (Wang et al., 2023). On the other hand, Jiang and Lian (2020) assert that pluripotent stem cell-derived cardiomyocytes obtained *in vitro*

and transplanted *in vivo* demonstrated greater potential in cardiac function restoration. Numerous studies incorporated MSCs within hydrogels, as their enhanced paracrine secretion has a significant contribution to cardiac tissue repair (Gao et al., 2024). However, some studies show that encapsulation may affect stem cell proliferation and paracrine activity since the hydrogel environment can restrict cell-to-cell interactions, leading to decreased cytokine release (Qazi et al., 2017). This study aims to investigate the effect of sequential delivery of immunomodulators (IL-10, TGF- β), proangiogenic factors (VEGF-A, FGF-2), and stem cells on cardiac function and regeneration after MI.

Materials and methods

Murine Models of Myocardial Infarction

Overall, 25-30 mice are required for one experiment. Mouse acute myocardial infarction is induced by ligation of the left anterior descending (LAD) coronary artery. It includes several steps. First, mice are anesthetized in the induction chamber with 4% isoflurane and 100 % oxygen for 2 minutes. Following induction, mice are endotracheally intubated, and the intubation tube is connected to the MINIVENT mouse ventilator using the following settings: tidal volume – 240 μ L/stroke and ventilation rate – 140 strokes/minute with 2% isoflurane and 100% oxygen. Mice are positioned and disinfected before the first incision is made. Next, the thoracotomy through the 3rd intercostal space is performed, and ribs retracted using the chest retractors to keep it open. Pericardium is removed, and LAD is found under the microscope (ZEISS Stemi 508, Carl Zeiss AG, Germany). Ischemia is induced by ligating the vessel using a 7-0 suture 2-3mm below the auricle of the left atrium. If the ligation is successful, the left ventricle turns pale. The rib cage is then closed using the 5-0 surgical sutures, and the animal is observed until spontaneous breathing returns. Extubated mice are placed into the clean cage and supplied with food and water. Transthoracic echocardiography was performed each week using the VEVO 3100 Preclinical Imaging System under anesthesia. Injections into the myocardial tissue were administered at three different time points using ultrasound guidance. First is administered right after the LAD ligation into three sites of the infarcted zone borders, 10 μ L each. The second (VEGF and FGF, 30 μ L) and third injections (differentiated cells, 30 μ L) were delivered using a 27-G needle with an ultrasound-guided closed-chest approach. The syringe was fixated and held with a micromanipulator (VisualSonics, Canada) and the needle and RMV scanhead probe were aligned with the left ventricular long axis.

Post-operative Assessment of the Cardiac Function of Murine Models of MI

Cardiac function of murine myocardial infarction models was evaluated by transthoracic echocardiography at 1 and 4 weeks after surgery. Echocardiography was done under general anesthesia using the VEVO 2100 Preclinical Imaging System (VisualSonics, Canada). First, mice were placed in an induction chamber and exposed to 4% isoflurane for two minutes to induce general anesthesia. Once sedated, they were transferred onto the imaging system. Ultrasound gel was applied to the skin of mice to obtain optimal cardiac imaging quality. The imaging

platform with the mice was adjusted by tilting forward and rotating counterclockwise in the frontal plane to position the heart parallel to the imaging surface. Cardiac function parameters specifically end-systolic volume, end-diastolic volume, and ejection fraction, were obtained using both M-mode and B-mode imaging at the papillary muscle level. The contractile function of the LV was assessed using VEVO Lab software. After the procedure was done, mice were removed from the imaging platform, the gel was wiped off, and mice were returned to the cage. Each mouse was monitored until full recovery from anesthesia was observed.

Synthesis of Cryogel

Briefly, cryogels were prepared using the 2% w/v Chitosan (Sigma, United States) solubilized in 1% v/v acetic acid (Millipore, United States) and mixing it using a magnetic stirrer at 500rpm on a hot plate for 2 hours. Then, 5% precooled w/v polyvinyl alcohol (Fisher Scientific, UK) and 0.1% heparin w/v were placed on a 600-rpm magnetic stirrer at room temperature. 400 μ l of 25% glutaraldehyde was pipetted immediately after the dissolved chitosan was added to the prepared solution of PVA-heparin. The final mixture was aliquoted into two 50 mL Eppendorf tubes and placed into a -12°C refrigerated circulator (Julabo F34 HE, Germany) for 24 hours. After incubation, cryogels are washed with distilled water 3 times for 10 minutes each. Glutaraldehyde, which was not reacted during the preparation of the cryogel, was neutralized using 50 mM NaBH₄ (Sigma-Aldrich, USA) at room temperature on a shaker for 3 hours. After the procedure, the product was washed with distilled water again for 2 times. Removing excess water, cryogels were frozen at -20°C for 12 hours and then lyophilized for 48 hours in a freeze dryer (Lyotrap™, VWR International, USA).

Isolation of mesenchymal stem cells from mouse compact bones

A 2-3-week-old male C57BL/6 strain mouse was euthanized via cervical dislocation. To reduce the risk of contamination, the mouse was sterilized post-mortem by washing in 70% ethanol for 3 minutes. The mouse was placed in a stable position on the 90mm culture plate for further dissection. Long bones from limbs were carefully removed, and humeri, tibiae and femurs were cleaned from surrounding skin and muscles. After the isolation of bones, they were washed with DMEM (Gibco, USA) solution with 2% antibiotics, specifically with penicillin and streptomycin (Thermo Fisher, USA). To minimize the risks of cross-contamination with hematopoietic cells, the cavity of the mouse long bones was washed with DMEM solution supplemented with 2% FBS(Sigma-Aldrich, USA) + 1mM EDTA (Thermo Fisher, USA) 3 times. An insulin syringe with a 30G needle was used for the procedure. After, the muscles and tendons were gently removed from long bones with the help of sterile medical gauze. The prepared bones were cut into chips with sterile scissors and rinsed with DMEM supplemented with 2% FBS + 1mM EDTA solution 3 times. The cleaned bone chips were placed into a 50 ml flask with a Collagenase solution (DMEM, 10% FBS, 1% antibiotics, 2% collagenase type 2) and incubated in a 37 °C shaker for an hour at 160rpm. Following the incubation, bone chips were centrifuged and washed with DMEM solution containing 20% FBS and 1% antibiotics. The complete DMEM resuspended bone fragments were placed into a fresh T25 flask for further incubation at 37 °C in a 5% CO₂ incubator. On day 3, the culture medium was replaced and left for further incubation, allowing the MSCs to fully migrate

out of the bones. On day 7, cell culture was transferred into a new T25 flask, changing the media to DMEM supplemented with 10% FBS and 1% antibiotics to ensure uniform and homogenous growth of MSCs. On day 14, cells underwent immunofluorescence analysis and were used for further experiments.

Results

LAD model of Myocardial infarction

The study used the murine models of myocardial infarction to evaluate the therapeutic efficiency of different treatment options on heart function. The model was created according to a previously established protocol (Jimi et al., 2020) to maintain consistency and reproducibility across experimental groups (Figure 1). Following induction of anesthesia, the mice model was intubated, and tidal volume and respiratory rate were properly provided using the mouse ventilator. To expose the heart for MI induction, left thoracotomy was done. The left descending artery of the murine model was tied up to induce the myocardial infarction. Success of the procedure was confirmed by the pale-colored appearance of the ischemic region. After the closure of the chest wall, animals were closely observed to ensure a good recovery after the procedure and to detect any immediate post-operative complications.

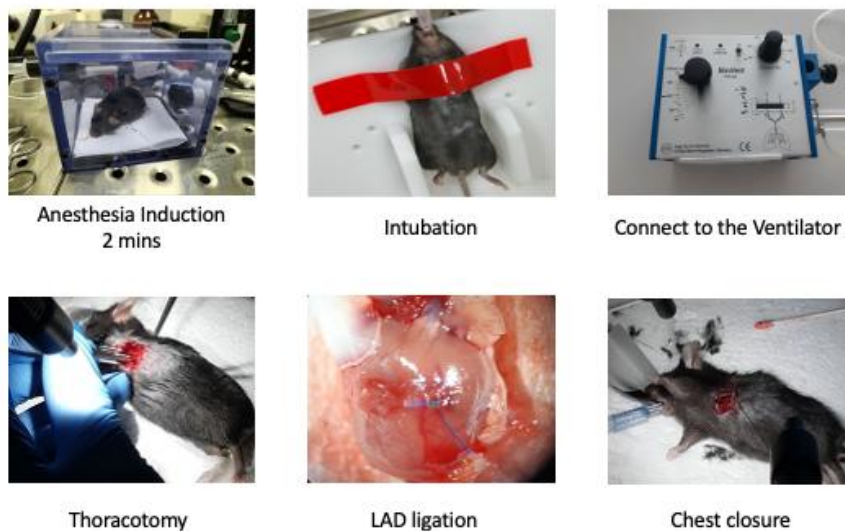


Figure 1: A brief overview of the LAD ligation method and the materials used in the procedure

Mesenchymal Stem Cells isolation and culture

The MSC culture was isolated using the previous protocol that was published in Nature Protocols (Zhu et al., 2010). Figure 2 depicts the MSCs isolation procedure step-by-step: euthanized mouse, bone extraction, cleaning process, washing the bones, culturing them in a T25 flask and microscopic examination. During the first week, the cells were cultured in the DMEM solution

with 1 % antibiotics and 20% FBS to provide optimal conditions for vigorous cell growth and survival. Isolated cells were observed under the light microscope for 2 weeks, and some morphological changes were depicted. On the first day, cells were round as they were just leaving the bones and adapting to the in vitro flasks. Light microscopy was done on Day 3 showed that some of the isolated cells started to elongate, reflecting their MSC characteristics and plasticity (Figure 2). By day 7 and 14, cells showed pronounced fibroblast-like features, becoming more cells that are spindle-shaped and with enhanced cell-to-cell contact.

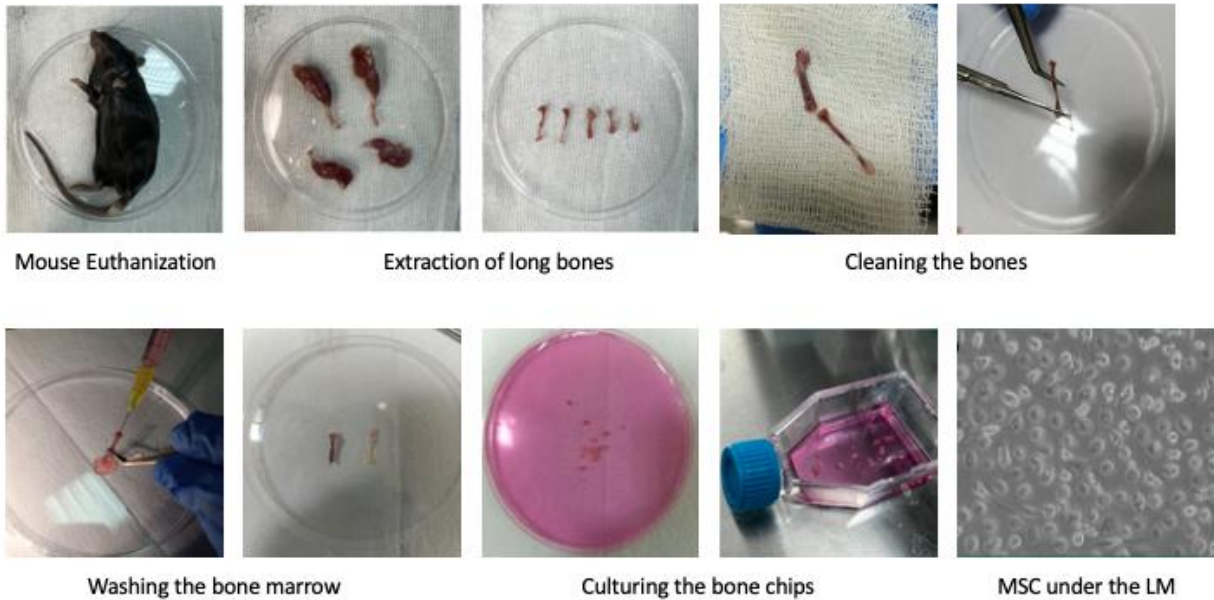


Figure 2: Comprehensive procedure for mesenchymal stem cell isolation

Preparation and characterization of cryogel

A chitosan-cryogel based system was created to enable the delivery of biomolecules like immunomodulatory factors (IL-10, TGF- β) and pro-angiogenic factors (VEGF-A, FGF-2) to the infarcted myocardium. In this study, cryogels were powdered and sterilized by my teammates before using them for further experiments (Raziyeva *et al.*, 2024). Figure 3 shows surface electron microscopy (SEM) of the syringe-injectable cryogel microparticles, which are 50-100 μm in size. The synthesized microparticles were examined under the SEM (ZEISS Crossbeam 540, Carl Zeiss AG, Germany) with different magnifications to see the structural features. The synthesized cryogel microparticles exhibited a rough surface texture, which is very important for the cytokine and growth factor binding efficiently. Its irregular surface pattern and uniform distribution of pores allow efficient potential biomolecule release and the scaffold's mechanical stability. Moreover, image analysis verified that particles' size range is consistent to achieve predictable and controlled cytokine release profile.

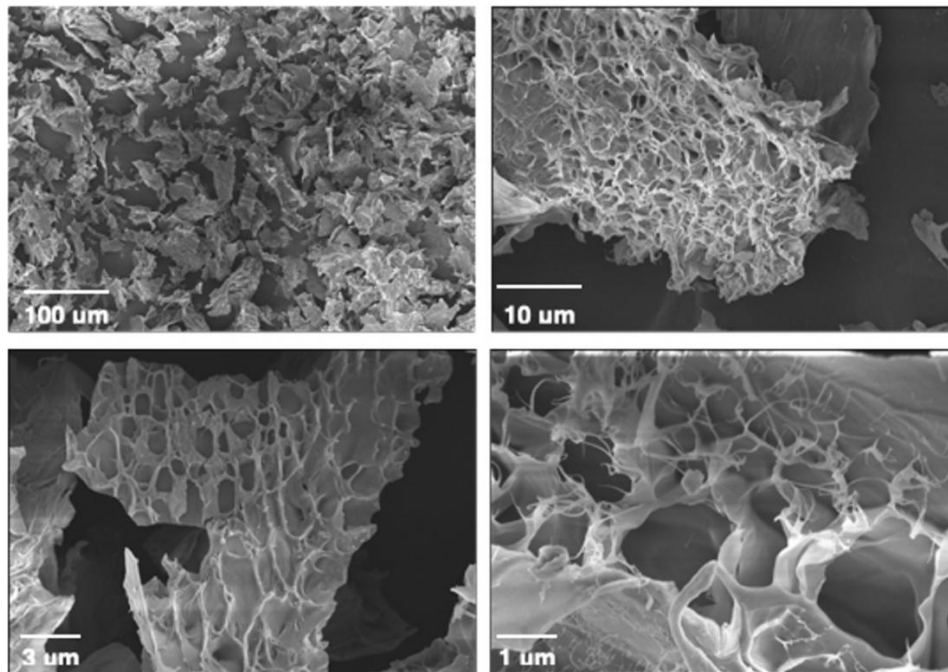


Figure 3. Cryogel microparticles under the SEM imaging at 4 different resolutions: 100 μm , 10 μm , 3 μm , 1 μm . (Obtained by me in collaboration with Raziyeva *et al.*, 2024)

Discussion

According to the World Health Organization, cardiovascular disease accounts for 1/3 of all deaths around the world, with an estimated 20 million deaths in 2022 (Marcos-Garcés *et al.*, 2025). Among all cardiovascular diseases, acute myocardial infarction is associated with the highest mortality level (World Health Organization, 2025). Despite advances in clinical therapeutic approaches in reperfusion of tissue by coronary artery bypass grafting and percutaneous coronary intervention, cardiac tissue remodeling leads to the progression of heart failure. Therefore, the pathophysiological mechanisms of MI, such as ischemia–reperfusion–induced oxidative stress, inflammatory and immune cell activation, cardiomyocyte necrosis and apoptosis, fibroblast activation with extracellular matrix remodeling, microvascular injury, and limited cardiomyocyte regenerative capacity are the key targets of novel therapeutic approaches (Frantz *et al.*, 2022).

First, a murine model of myocardial infarction was produced using the previously established protocol of Jimi and colleagues (2020). Mice are widely used in cardiovascular research because they are small, cost-effective and easily modifiable animals that can help in studying disease mechanisms and evaluating therapeutic approaches (Lindsey *et al.*, 2021). According to the literature, MI models of mice can be created by several methods: permanent ligation of Left Anterior Descending (LAD) Coronary Artery; Ischemia-Reperfusion model, in which the LAD is temporarily ligated and then released; and chemical or cryoinjury models (Lindsey *et*

al., 2021). This research used the permanent ligation method, which mimics a complete heart attack which leads to ischemia, cardiomyocyte death and fibrosis of myocardium - key target mechanisms of this study. Moreover, the method used in my research is considered less invasive because it involves endotracheal intubation instead of tracheotomy and requires only a single incision at the site of thoracotomy, without making additional incisions for effective blood removal from the surgical site (Johnny & Dutta, 2022). This approach has been associated with decreased mortality and a smaller number of post-surgical complications, such as pneumothorax, hemorrhage and ventricular rupture, in MI models (Gao et al., 2017). In addition, use of isoflurane alone for induction reduces the drug-induced complications and increases survival rates after surgery (Johnny & Dutta, 2022; Reichert et al., 2017). Some studies have used ketamine and xylazine in combination with isoflurane to prolong duration of anesthesia, whereas use of isoflurane alone allows rapid recovery from anesthesia once administration is stopped (Buitrago et al., 2008).

The established mice MI model is used to investigate post-infarction remodeling and to evaluate the molecular and cellular regenerative strategies. Over the past few decades, many studies have focused on the molecular mechanisms of myocardial infarction and tissue regeneration after myocardial infarction. Cardiac tissue regeneration is a very complex process that involves many molecular signaling mechanisms, cells, and genes. Studies showed that noncardiomyocyte cells, such as skeletal myoblasts, bone marrow-derived cells, and mesenchymal stem cells can be used in cell-based therapy of cardiac tissue regeneration. Skeletal muscle cells transplanted in animal models were successfully differentiated into myogenic lineage and improved cardiac function in both ischemic cardiomyopathies (Durrani et al., 2010). Moreover, bone-marrow-derived mononuclear cells showed some beneficial effects increasing the ejection fraction after MI. However, randomized clinical trials showed no significant improvement in left ventricle function after bone-marrow derived mononuclear cell transplantation therapy (Sürder et al., 2013; Traverse et al., 2012).

Another cellular approach for cardiac tissue regeneration focused on cardiac stem cells. Both preclinical and clinical trials carried out using the cardiac stem cells defined by cell surface marker mast/stem cell growth factor receptor KIT showed beneficial effects increasing the left ventricular ejection fraction and decreasing the infarct size (Ellison et al., 2013). The use of human induced pluripotent stem cell-derived cardiomyocytes significantly improved cardiac outcomes, including ejection fraction, and increased the thickness of the infarct and muscle content (Chow et al., 2017).

Mesenchymal stem cells were used in this study because they are among the most extensively studied stem cell types and they possess multipotent, immunomodulatory, and pro-angiogenic properties (Gupta et al., 2021). Although some studies suggest that pre-differentiation of stem cells into cardiomyocyte-like cells before transplantation may reduce therapeutic efficiency, research by Razzaq and colleagues (2025) demonstrated that transplantation of pre-differentiated hUC-MSCs into the myocardial infarction site improved

both systolic and diastolic cardiac function and decreased the fibrotic tissue area (Gupta et al., 2021). These findings further support the view that stem cell transplantation is one of the highly promising approaches in the treatment of the post-MI fibrosis, tissue regeneration. However, there are some constraints like poor engraftment, low efficiency in cell differentiation to cardiac cells (He et al., 2024). Therefore, optimization of post-MI microenvironment of myocardial tissue is essential for effective cell-based therapeutic approaches.

According to Sultankulov *et al.* (2019), the use of modern delivery systems with anti-inflammatory cytokines and pro-angiogenic growth factors facilitates the optimization of the microenvironment for further stem cell incorporation. There are several advanced delivery systems, including the cryogel, which is biodegradable, non-cytotoxic, and immunotolerant. Chitosan cryogel is the best-fit delivery system for this research as it has two important properties for regenerative medicine. First, it acts as an injectable, shape-memory macroporous scaffold that can hold and protect cells inside its structure. Secondly, it is a local reservoir that can control the sequential release of immunomodulators (Jimi *et al.*, 2020). This kind of control aligns with the biological restoration of cardiac function and facilitates this process. Moreover, unlike conventional nanoporous hydrogels that restrict the cellular migration and cell growth, the pore size of cryogels can be easily manipulated so that the macroporous architecture allows passage of cells and vascular ingrowth (Xuan *et al.*, 2024; Jimi *et al.*, 2020). In addition, according to Ayaz, Demir, & Bölgen (2021), chitosan-based cryogel showed immunosuppressive effect due to the chitosan composition, a natural polysaccharide derived from chitin, by decreasing the macrophage inflammatory activity after an ischemic event. The properties discussed above make chitosan-based cryogel an appropriate delivery system for regenerative therapy.

Moreover, complexity of the cardiac tissue regeneration after MI must be addressed by targeting multiple biological pathways simultaneously. Therefore, designing a combinatorial therapeutic approach, rather than relying on monotherapy, is necessary to effectively use the available treatment options (Poustchi et al., 2021; Tenreiro et al., 2021). The data presented in the final paper of our research, conducted by my colleagues (Raziyeva *et al.*, 2024) showed that the pre-differentiated stem cells, in combination with anti-inflammatory and pro-angiogenic factors delivered by the chitosan cryogel, markedly improved the myocardial tissue repair and cardiac function. This research assumed that the effectiveness of therapeutic intervention can be strengthened by using the immunomodulatory and pro-angiogenic factors followed by the injection of pre-differentiated cardiomyocytes, endothelial cells, and vascular smooth muscle cells via the chitosan cryogel delivery system.

Heart function of post-MI and post-treatment mice was evaluated after 1 week and 4 weeks, comparing the groups with treatment options of cells alone (pre-differentiated cardiomyocytes, endothelial cells, and vascular smooth muscle cells), cryogel loaded with growth

factors (cryogel+growth factors), and sequential delivery of cryogel loaded with growth factors followed by injection of pre-differentiated stem cells (cryogel+growth factors+cells). Echocardiographic findings showed that heart function parameters of the group with the treatment option of sequential delivery of cryogel+growth factors+cells improved significantly. At week 1, there were no significant differences between the groups with various treatment options and the control group without treatment. However, by week 4, all treatment groups were demonstrated significant improvement compared to the control group. Although there was no statistically significant difference between treatment groups, the cryogel+growth factors+cells group showed a higher level of significance ($p < 0.001$) compared to other groups ($p < 0.05$ and $p < 0.01$). Figure 4B represents the M*Mode echocardiography for each group which demonstrates the cardiac wall motion indicating the functional capacity of heart muscles after treatment.

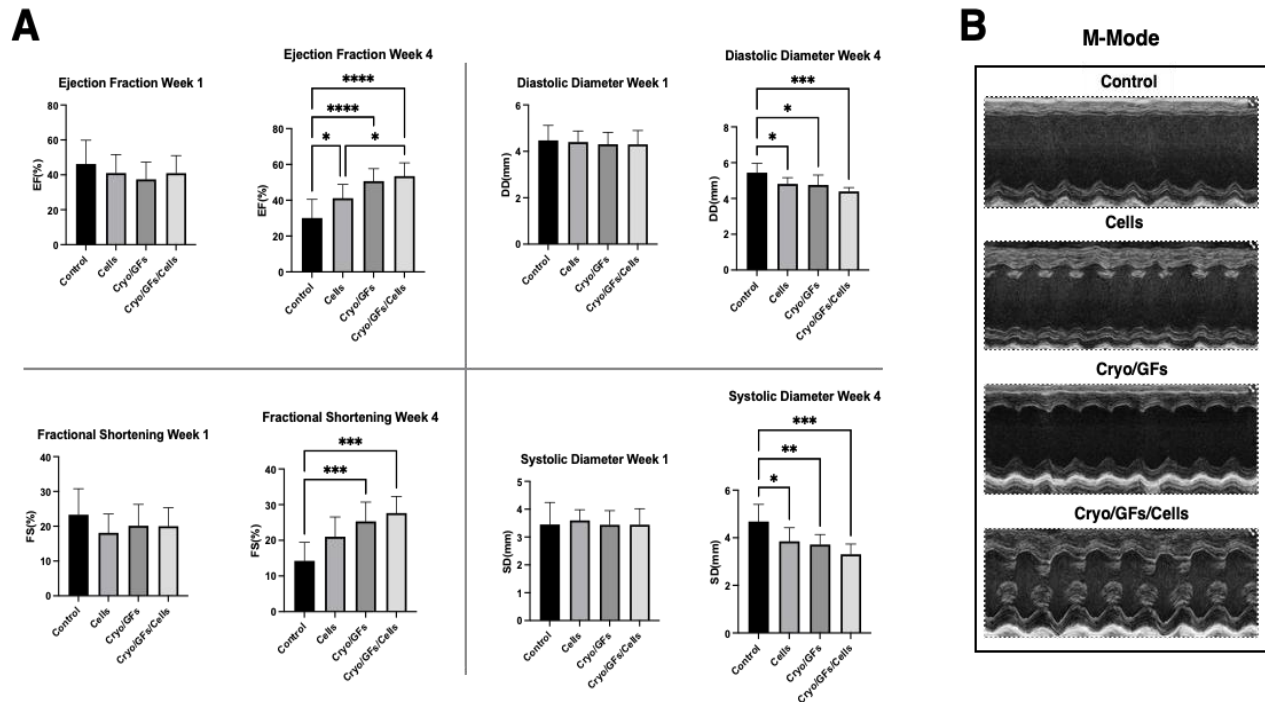


Figure 4. Post-operative Assessment of the Cardiac Function of Murine Models after the cardiac repair interventions. Data is presented as mean+SD where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Raziyeva *et al.*, 2024).

In addition, in this study, the level of myocardial tissue fibrosis was analyzed and compared between different groups by staining the myocardial tissue with Masson's trichrome. The average percent area of the scar tissue was almost 2 times less for cryogel+growth factors+cells than the control group ($31.6 \pm 9.5\%$ vs $60.8 \pm 3.7\%$) (Fig. 5B). Other therapeutic options also demonstrated significantly reduced scar tissue area after intervention compared to the control group. Even if

there was no substantial difference between the results of these three treatment options, the cryogel+growth factors+cells group suggested the highest statistical significance with the p-value < 0.0001 .

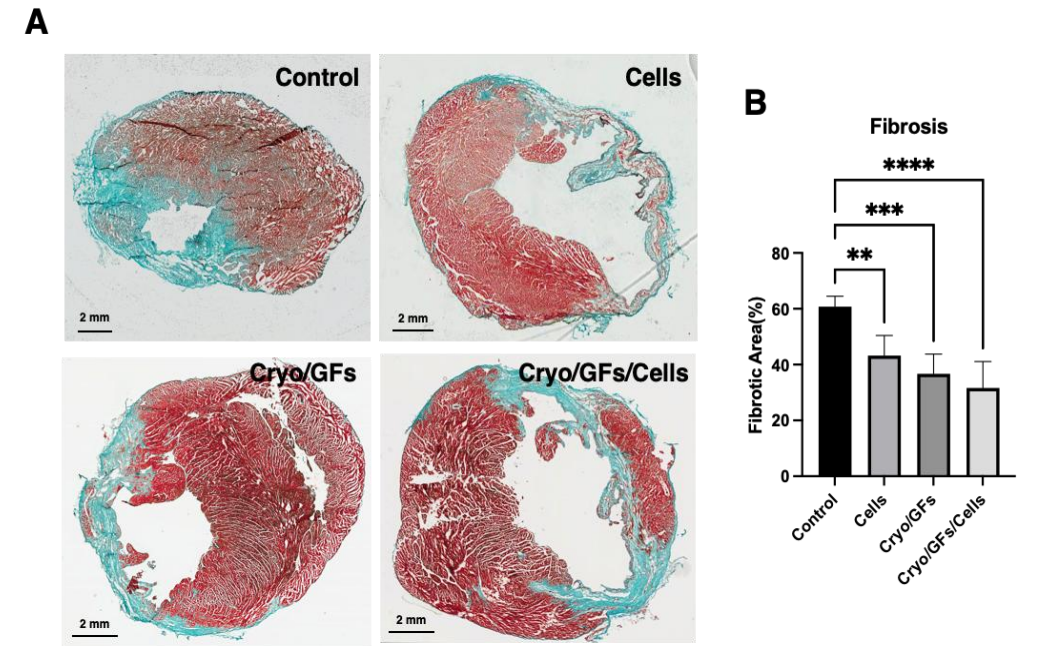


Figure 5. Fibrosis level of myocardial tissue (A) Masson's Trichrome staining which indicates the fibrotic tissue as blue and healthy myocardium as red (B) Statistical analysis (** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). (Raziyeva *et al.*, 2024)

According to the study, combined cell therapy using the sequential delivery of factors and cells enhanced cell survival, improved the microenvironment, and supported the angiogenesis of the tissue. The macroporous structure of the chitosan based cryogel supported cell retention and perfusion, while the release of factors inhibited the inflammatory process and promoted vascular formation, and decreased fibrotic area.

While this study focused on the anti-inflammatory, pro-angiogenic and regenerative capacity of myocardial tissue, oxidative stress is another critical mechanism that limits regenerative efficacy. There are many studies that show the impact of ischemia–reperfusion–induced oxidative stress on cardiac function restoration. Most of the contemporary MI therapeutic approaches try to improve the coronary blood flow and tissue perfusion by CABG and percutaneous coronary intervention right after the MI. These procedures sharply increase the oxygen level in ischemic tissue, which leads to the elevated formation of the reactive oxygen species (ROS), which leads to ischemia reperfusion injury (IRI) exerting cytotoxic effect on

cardiomyocytes (Rodius et al., 2020). Now there are numerous studies going on aiming to suppress the production of ROS and genes that are responsible for its formation. Liu et al. (2020) investigated the brahma-related gene 1 (BRG1) in peri-infarct zone. This BRG1 was upregulated in infarcted zones in association with NRF2 and HO1, a crucial antioxidant pathway which drive the expression of more than 250 antioxidant enzymes (Zhang et al., 2019). Intramyocardial injection of the BRG1 gene by an adenoviral vector leads to the overexpression of the gene translocation of Nuclear erythroid 2-related factor 2 (Nrf2), which activates the Heme oxygenase 1 (HO1), attenuating the ROS cytotoxicity and cell death. The study demonstrated that the overexpression of these genes enhanced the heart's contractility and its function and decreased the area of the infarcted zone. On the other hand, the Lenti-Brg1 shRNA injection, which leads to *Brg1* knockdown, demonstrated the opposite effect. Another research was done to investigate the impact of Zinc finger protein ZBTB20 on the cardiac tissue regeneration after MI. Results indicated that the upregulation of ZBTB20 in post-infarcted tissue elevated the superoxide dismutase (SOD) enzymatic activity, which is a key antioxidant that converts ROS to oxygen molecules (Li et al., 2020; Tanwir et al., 2021).

Furthermore, miRNAs are very crucial regulators of oxidative stress post-MI (Çakmak & Demir, 2020). For example, expression of miR-323-3p, Bax, Bcl-2, SOD1, and SOD2 genes and ROS were evaluated in cardiac cells of miR-323-3p-transfected mice and non-transfected mice. The research showed that miR-323-3p is downregulated in post-MI mice heart tissue, and upregulation of the gene reduced the Bax expression and facilitated the rise of Bcl-2, SOD1, and SOD2 expression, consequently declining the oxidative stress products (Shi et al., 2020). Moreover, there are many other studies done on the effect of miRNA and antioxidant gene regulators on ROS elimination and downregulation of production, including the miRNA-187, miR-340-5p and miRNA-124 (Zhu et al., 2021; Li et al., 2019; Ellery et al., 2018). Although the current study focused on immunomodulatory, angiogenic, and regenerative pathways, it can be improved using gene therapy strategies, including miRNA-based approaches, to eliminate ROS and increase the regenerative capacity of myocardium.

There are several limitations of the project that can be acknowledged. First, assessment of the cardiac function was limited to 4 weeks, long term outcomes and assessment should be done. Second, the arrhythmogenic effect of the integrated factors and cells remain unknown; it needs further evaluation. Third, research on the dose optimization is required and application of ROS elimination approaches should be explored. Finally, as the study was done using the murine models, further research is needed on the applicability of this model to the human population.

Conclusion

Myocardial infarction remains a leading cause of mortality and morbidity around the world due to the limited regenerative capacity of adult human cardiac tissue. Recent research in regenerative medicine and delivery systems show the role of combinational therapeutic

approaches in optimization of post-MI microenvironment. Consistent with these developments, this research demonstrated chitosan-based cryogel sequential delivery of anti-inflammatory cytokines, pro-angiogenic growth factors, and pre-differentiated stem cells notably improved the heart function and decreased the fibrotic tissue area following MI. These findings support the concept that successful post-MI regeneration requires not only restoration of blood flow and reperfusion of tissue but also modulation of inflammation, enhancement of angiogenesis, and cellular replacement for regeneration. These research findings establish a basis for further future research into cell delivery systems and regenerative strategies in post-MI cardiac tissue. Future studies should focus on optimization of doses, evaluation of long-term outcomes, and investigation of miRNA use along with anti-inflammatory and pro-angiogenic factors.

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