Injectable Collagen-Chitosan Hydrogel loaded with Human Induced Pluripotent Stem-Cells for Myocardial Regeneration: Phase I/II Clinical Trial Design

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Problem statement: Myocardial infarction

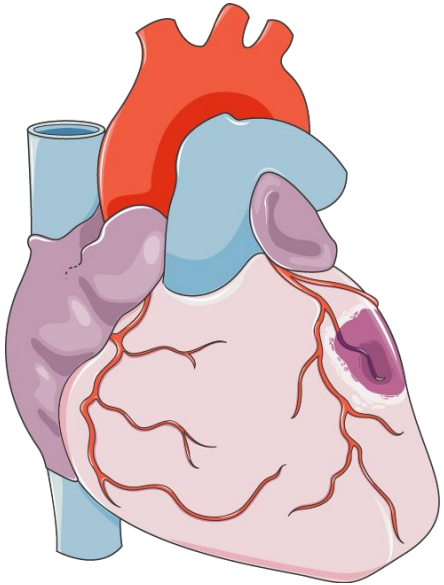
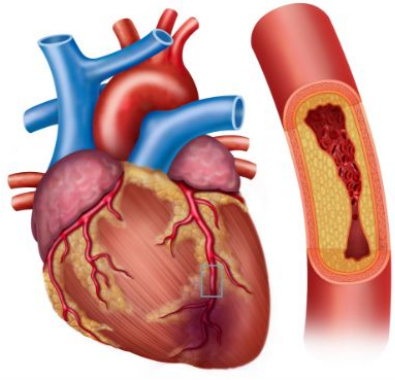


Figure 1. Myocardial infarction.
Source: [1]

"Heart attack"

Caused by decreased or complete cessation of blood flow to a portion of the myocardium.

Myocardial infarction is usually due to thrombotic occlusion of a coronary vessel caused by rupture of a vulnerable plaque.



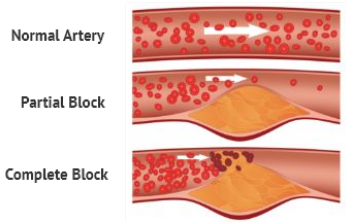


Figure 2. Heart attack.
Source: [2]

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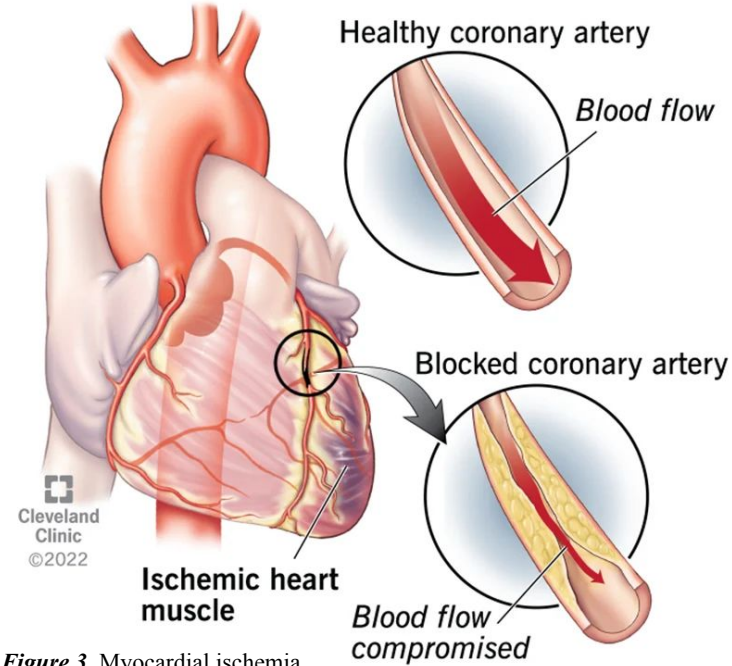


Figure 3. Myocardial ischemia.
Source: [3]

Ischemia induces profound metabolic and ionic perturbations in the affected myocardium and causes rapid depression of systolic function.

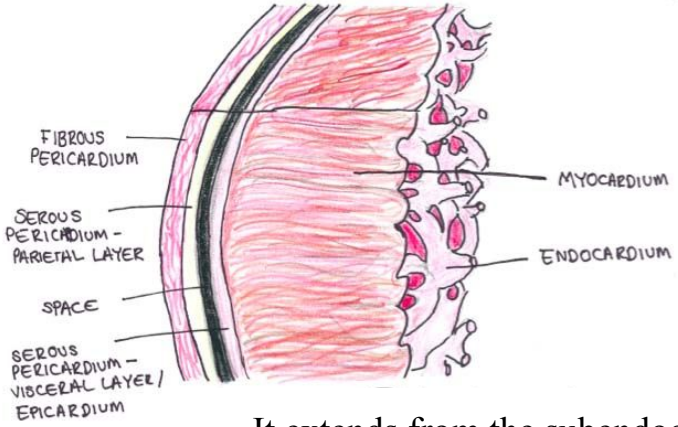


Figure 4. Heart walls.
Source: [4]

It extends from the subendocardium to the subepicardium

Some statistics: Myocardial infarction

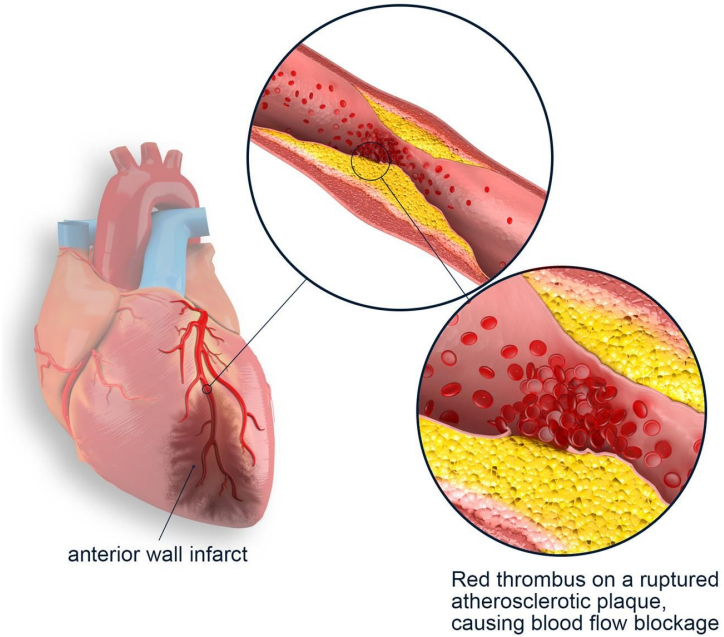


Figure 5. Thrombotic occlusion.
Source: [5]

The leading cause of death worldwide, causing around

17.3 million deaths annually

(representing **30%** of all registered deaths),
making it one of the major public health problems

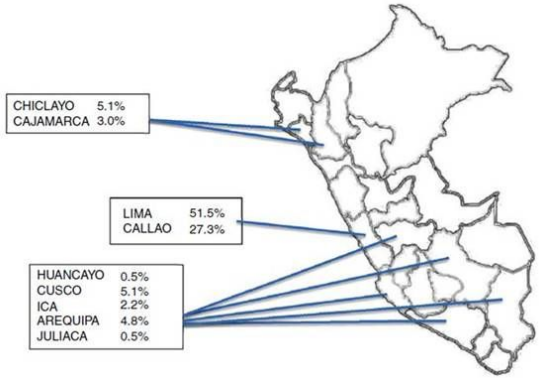


Figure 6. Percentage of patients in the registry according to geographic location.
Source: [6]

Treatments: Myocardial infarction

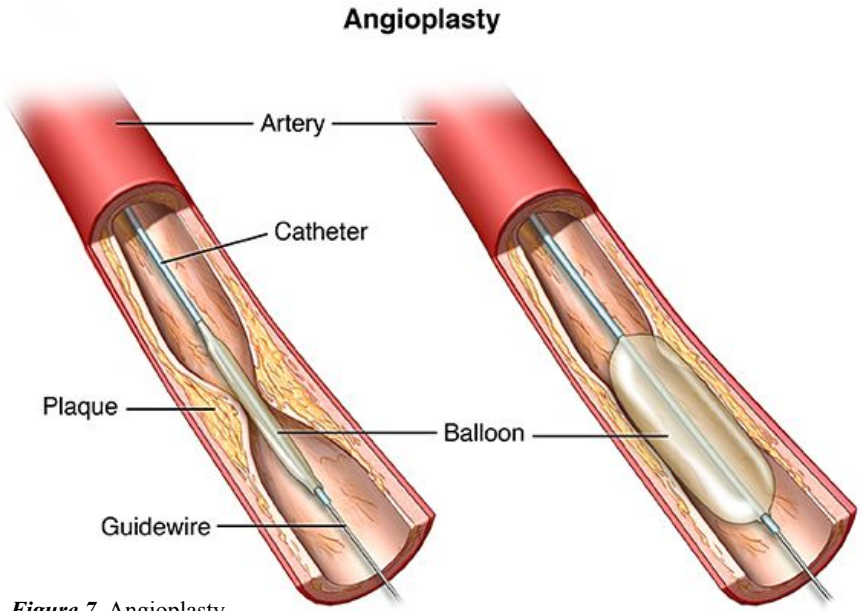


Figure 7. Angioplasty.
Source: [7]

- The painkillers such as morphine or meperidine can also be administered to relieve pain.
- A nitroglycerin and antihypertensive drugs such as Beta-blockers, ACE inhibitors or Calcium channel blockers may be administered to lower the blood pressure and to reduce the heart's oxygen demand.
- Anticoagulants such as heparin, aspirin or warfarin may also be administered to reduce the risk of blood clots.
- Dopamine or dobutamine is administered to increase the blood flow to the heart and strengthen the heartbeat.

Potential and drawbacks: Myocardial infarction

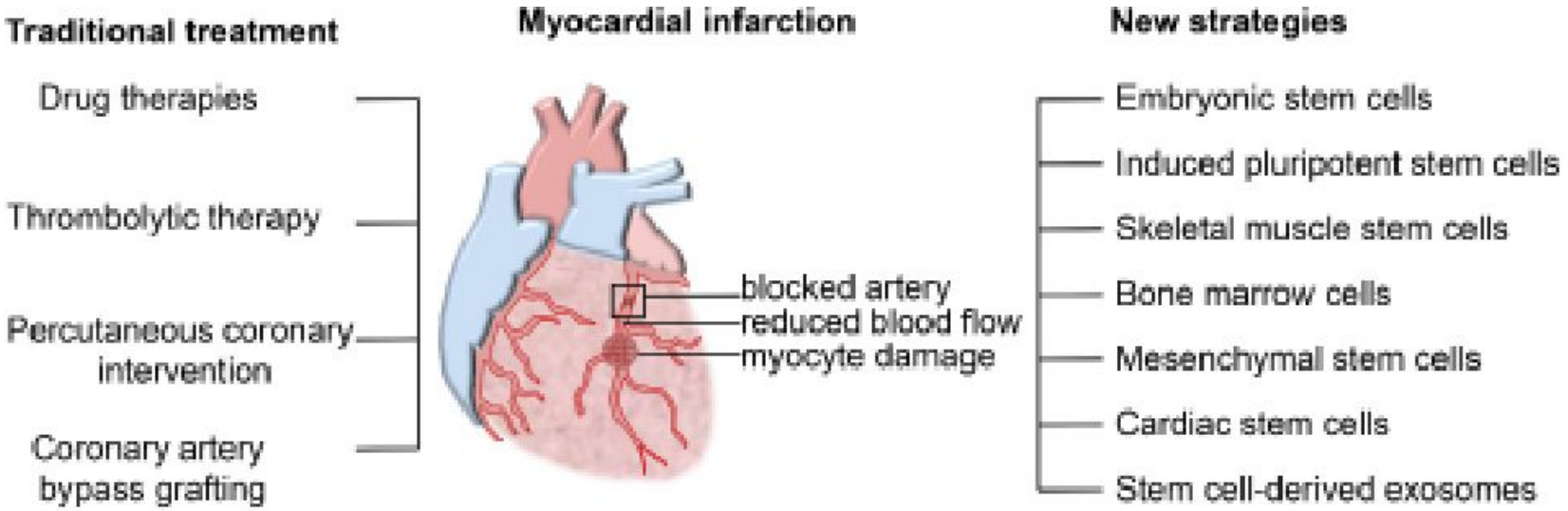
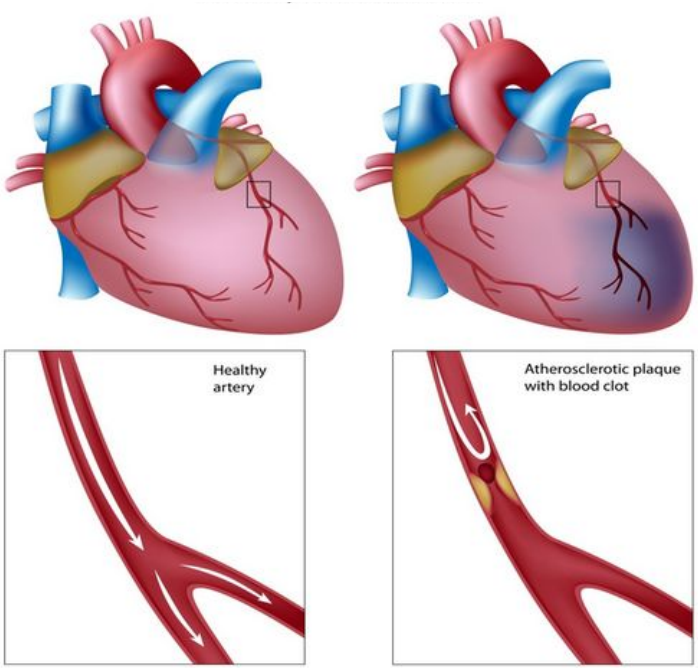


Figure 8. New strategies for myocardial infarction treatment.
Source: [8]

Potential and drawbacks: Myocardial infarction



- The cell microenvironment does not release determined factors or control cellular adhesion or mechanical function.
- Matrix does not include the decrease of inflammatory response and prolongation of the absorption delay by additional chemical and/or physical cross-linking treatments.
- Electrostimulation and shear stress are not incorporated to precondition matrices.

Figure 9. Anatomy of a heart attack.
Source: [9]

Objectives

- **General objective:**

  Design a phase I/II clinical trial to test the effectiveness of a tissue engineering based product for the treatment of acute myocardial infarction (MI).

- **Specific objectives:**

- Define a suitable biomaterial for scaffolding construction and other additional components to enhance the regeneration process.
- Determine the inclusion and exclusion criteria to select the participants for the clinical trial.
- Determine the primary and secondary outcomes that indicate the safety and effectiveness of the proposed therapy.
- Determine the techniques and assays for patient monitoring and outcomes measure.

Scaffolds may enable myocardial regeneration by mimicking the cardiac extracellular matrix

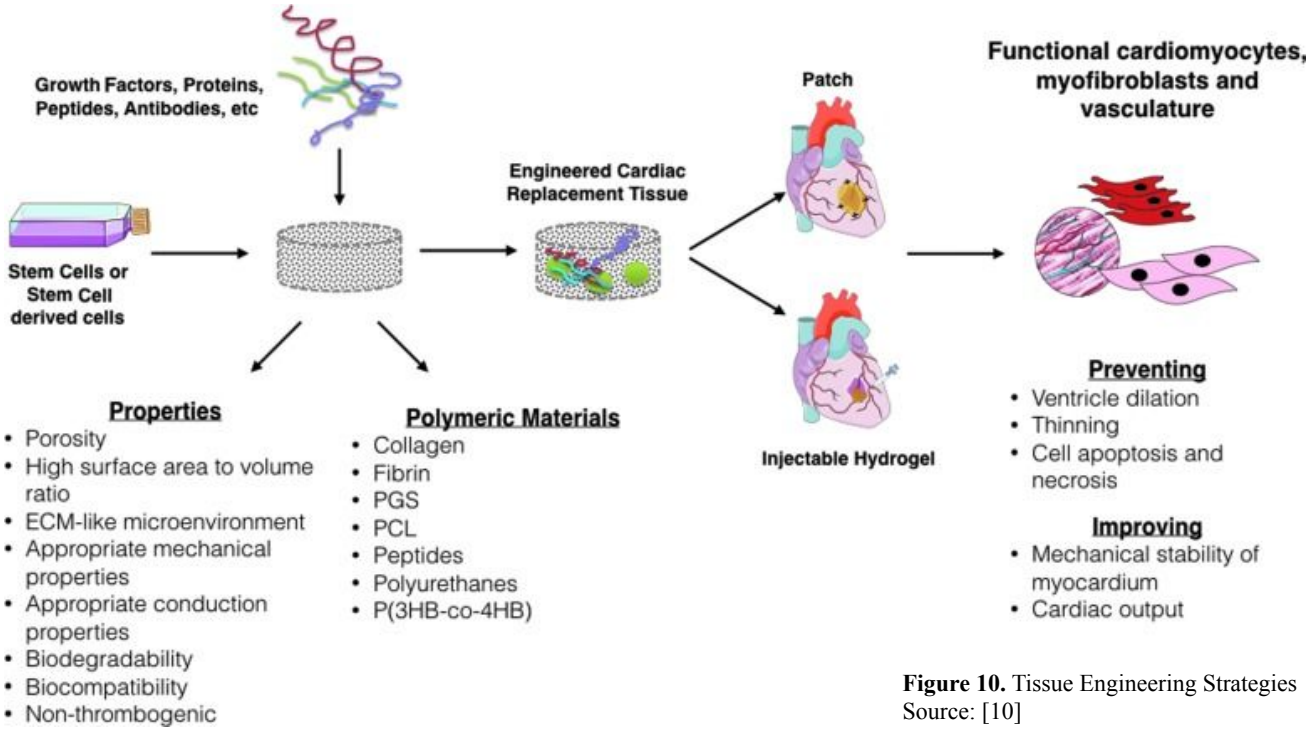
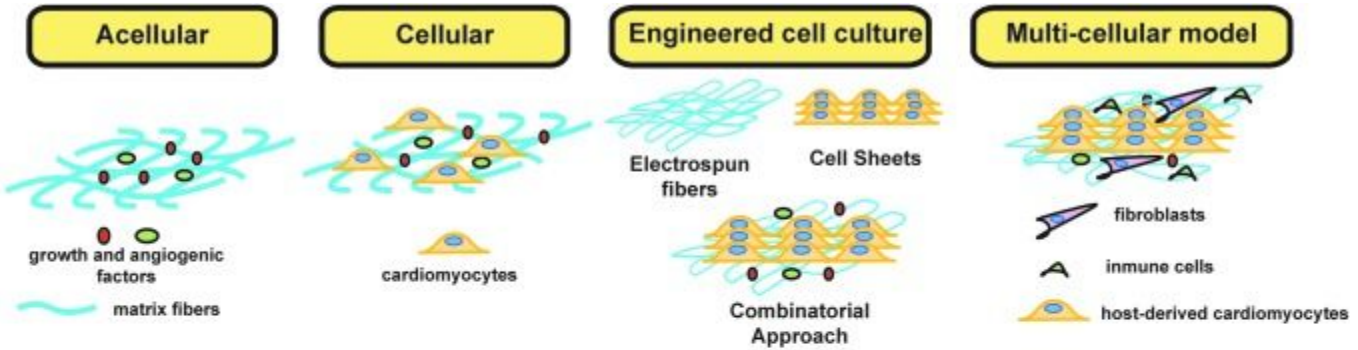


Figure 10. Tissue Engineering Strategies
Source: [10]

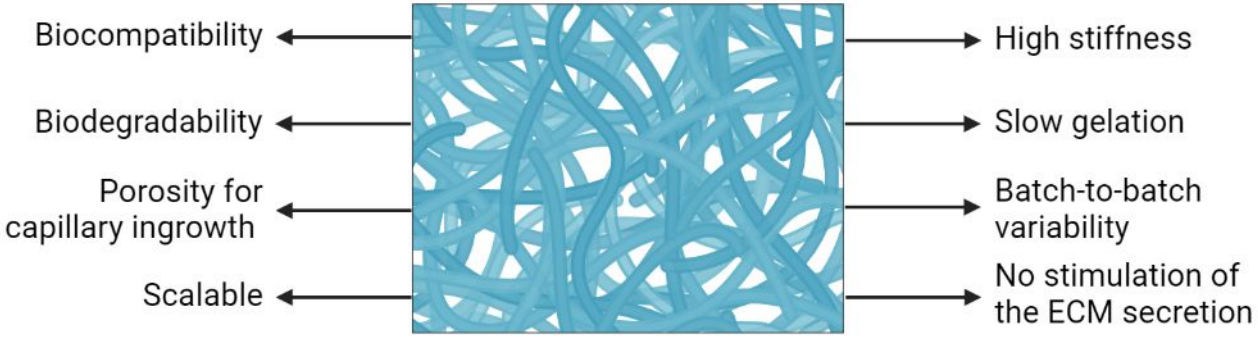
There are different approaches for myocardial regeneration



	Acellular	Cellular	Engineered cell culture	Multi-cellular model
Main Advantages:	Reduced Ventricular wall remodeling Mechanical support Limited vascularization Off-the-shelf availability Limited immune reaction Low cost and extended shelf-life	Reduced Ventricular wall remodeling Mechanical support Improved vascularity	Reduced Ventricular wall remodeling Improved mechanical stability Partial host-tissue integration	Sustained cardiac function in vitro Limited inmune reaction Reduced Ventricular wall remodeling/stability? Improved vascularity? Complete host-tissue integration?
Challenges:	Lack of host cardiac tissue regeneration Limited host cell integration Variable mechanical stability	Limited shelf availability Potential inmune reaction High cost and decreased shelf-life Time-limited patch contractility	Limited shelf availability Potential immune reaction High cost and decreased shelf-life Time-limited patch contractility Lack of cardiac tissue regeneration Limited vascularity	High cost and decreased shelf-life

M. Domenech, et al. Tissue engineering. Part B, Reviews, 22 (2016)

Collagen Scaffolds present optimal physical and chemical properties for cardiac tissue regeneration

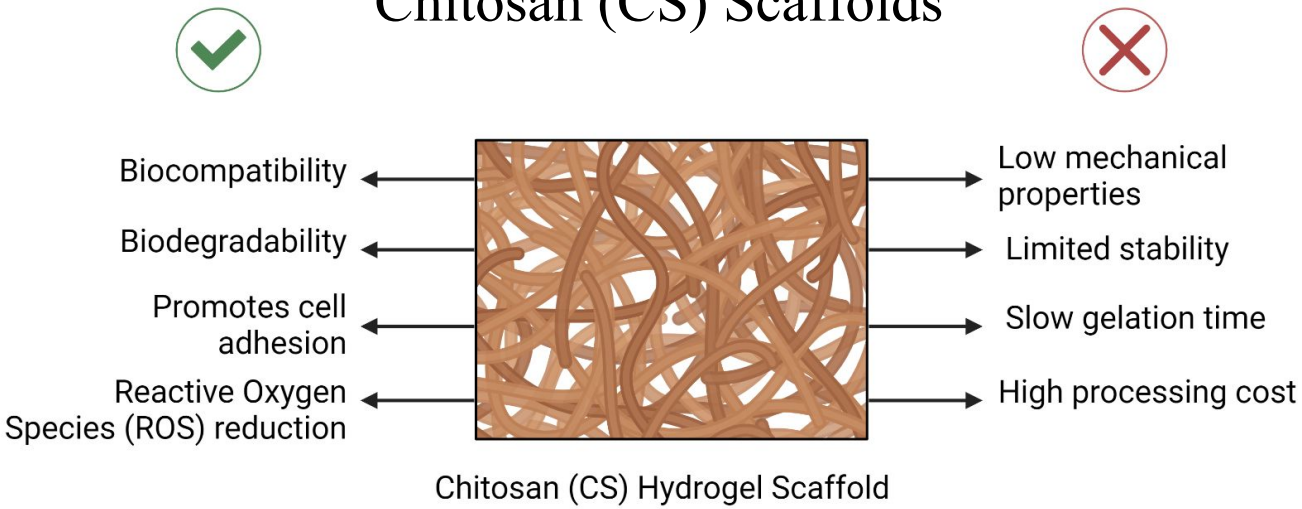


Collagen type I is the most abundant structural protein in mammalian myocardium

There is currently no single biomaterial that fully recapitulates the architecture or properties of adult myocardium [11]

Figure 11. Advantages and disadvantages of Collagen Hydrogel Scaffold
Source: Created in BioRender.com

Chitosan (CS) Scaffolds



CS has a high degree of hydrophilicity, which can enhance cell binding and proliferation.

CS hydrogels can be enhanced in the MI microenvironment to improve engraftment, survival, and stem cell homing by removing ROS and increasing cytokines [12].

Figure 12. Advantages and disadvantages of Chitosan Hydrogel Scaffold
Source: Created in BioRender.com

CROSSLINKING AGENT

Tripolyphosphate (TPP) [13]

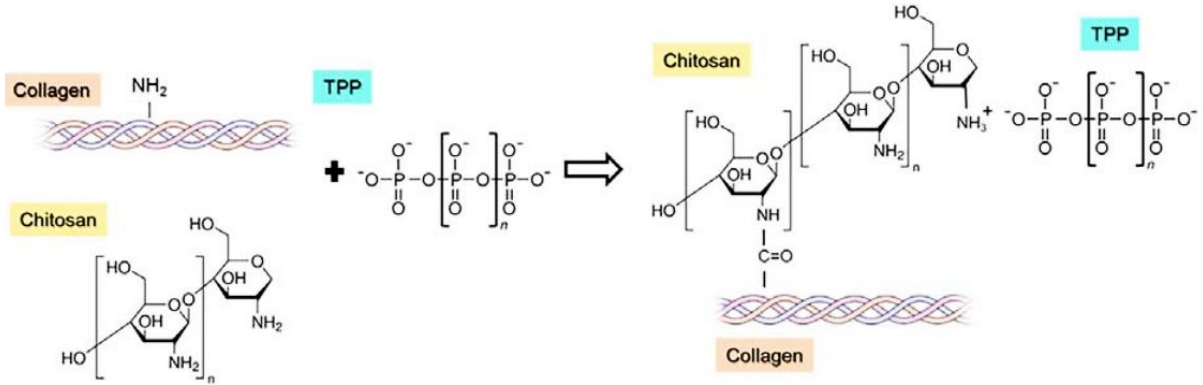


Figure 13. Schematic illustration of crosslinking reaction. Source: [13]

- Adequate porosity (>65%) and swelling ratio
- Better biocompatibility - Higher cell viability
- Highest expression of cardiac-specific marker protein and the best contractile performance
- Suitable for the growth and structural maturation of cardiomyocytes
- Elastic modulus (81.0 ± 8.1 kPa) in the physiological range of native myocardium
- Lower crosslinking degree and a higher degradation rate

DELIVERY AGENT

Human Induced Pluripotent Stem-Cell Derived Cardiomyocytes (hiPSC-CMs) [14]

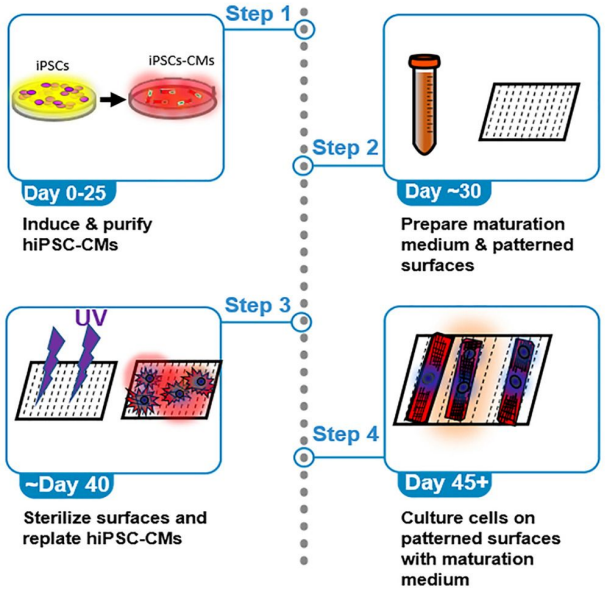


Figure 14. Protocol to produce mature human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) Source: [15]

- Effective integration with host myocardium
- Significant cardiac fibrosis reduction
- Improvement in cardiac function
- Cardiomyocyte marker expression
- No cardiac teratoma formation
- Cogent autocrine and paracrine mechanism for treating MI

Most myocardial regeneration studies have been implemented in small animals, including mice and rats.

Figure 15. Preclinical trial in rat. Adapted from Wu et al. 2022
Source: Created in BioRender.com

↑ cardiomyocyte fiber restoration

Novel fabrication of bioengineered injectable chitosan hydrogel loaded with conductive nanoparticles to improve therapeutic potential of mesenchymal stem cells in functional recovery after ischemic myocardial infarction [16].

There is a severe lack of valuable investigation in large animal models that can more closely mimic the cardiac pathophysiology of human patients [8].

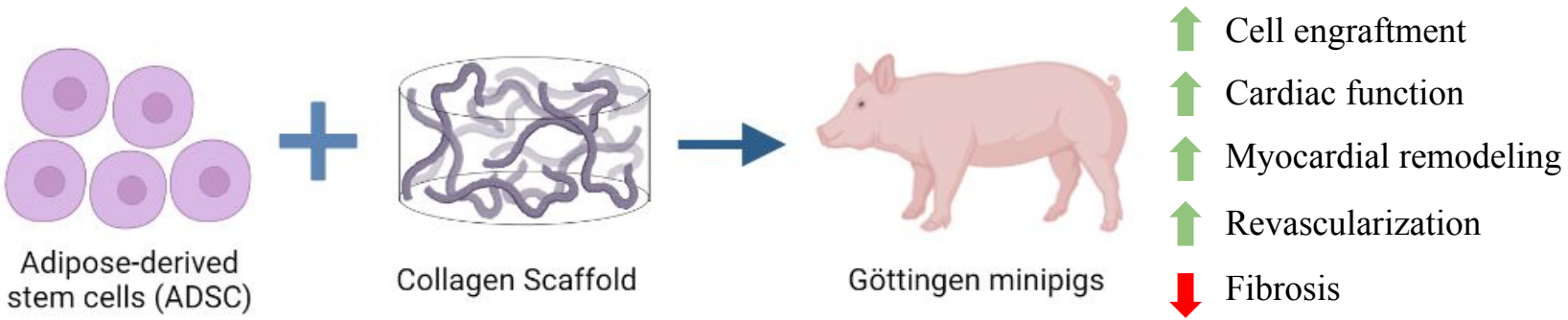


Figure 16. Preclinical trial in minipigs. Adapted from Araña et al. 2014
Source: Created in BioRender.com

Epicardial delivery of collagen patches with adipose-derived stem cells in rat and minipig models of chronic myocardial infarction [17].

BIOMATERIAL PROPOSAL: Collagen I/ Chitosan + hiPSC-CMs Cardiac Scaffold

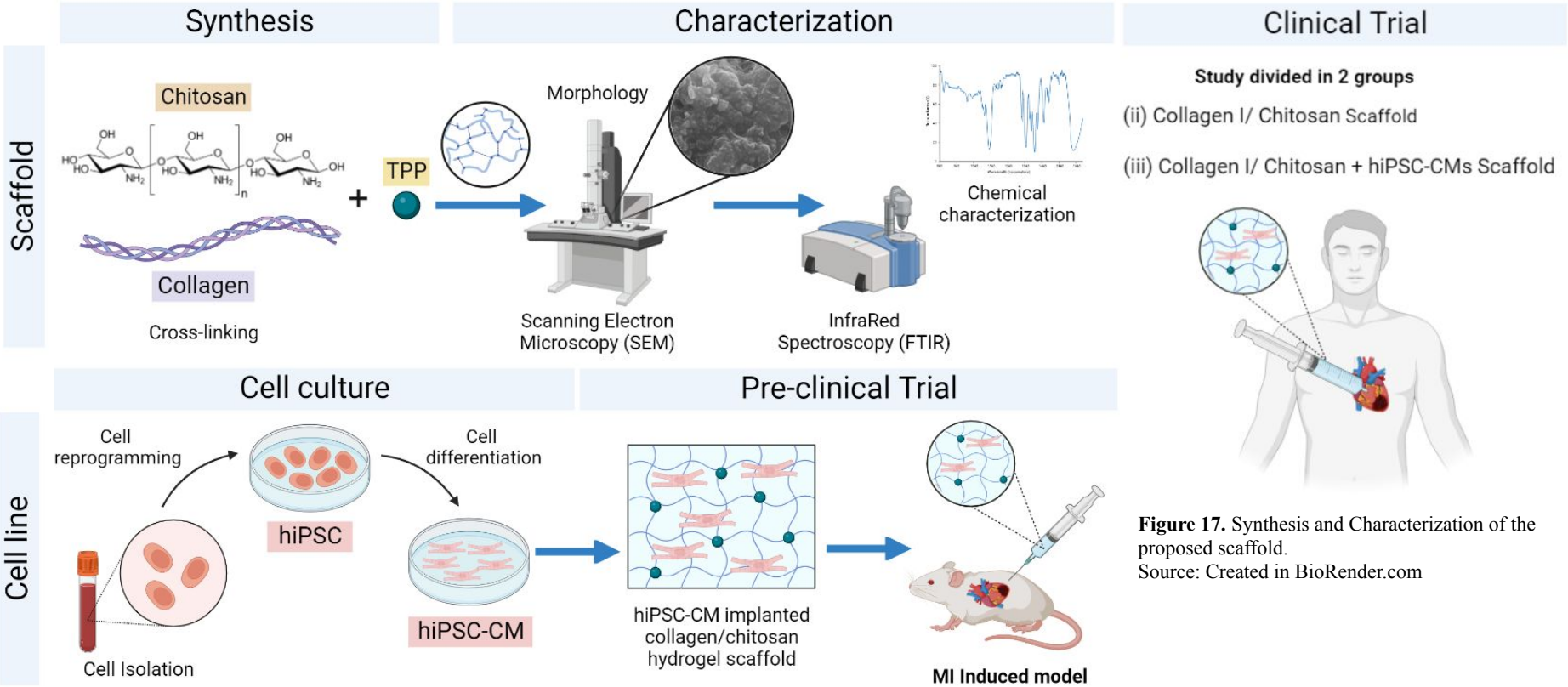


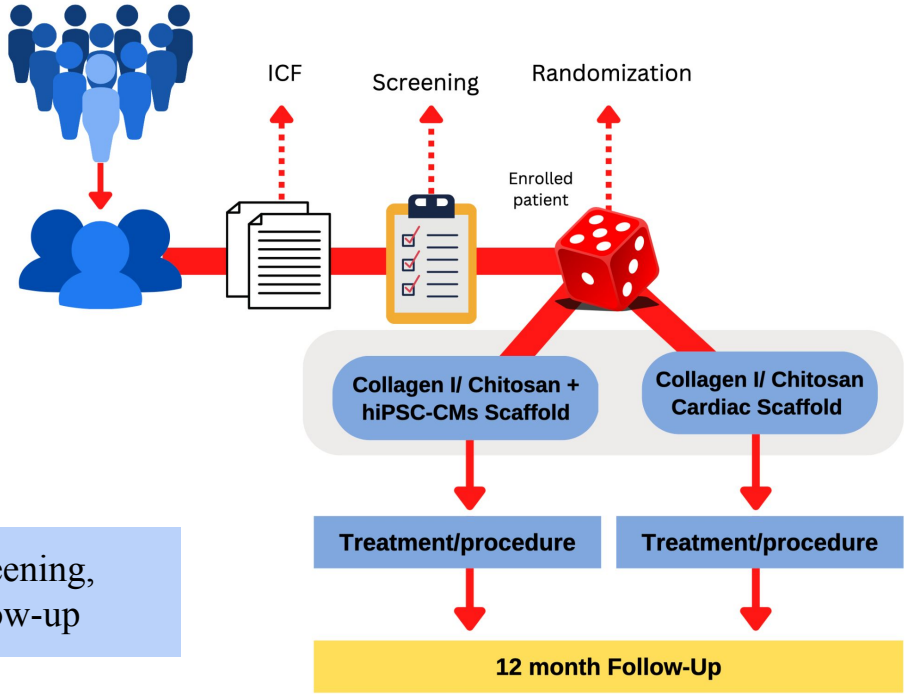
Figure 17. Synthesis and Characterization of the proposed scaffold.
Source: Created in BioRender.com

Clinical Trial

Study Design

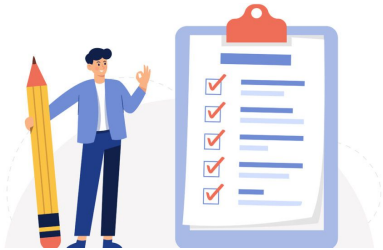
- Randomized
- Phase I/II clinical trial
- Double arm study
- Interventional
- Single blind

The study will consist of the following phases: screening, randomization, single blind treatment, clinical follow-up



Inclusion criteria

1. A history of myocardial infarction with a residual akinetic scar
1. Ejection fraction between 20% and 50%
2. Patients free of angina symptoms
3. Presence of Q wave in the ECG
4. Signed ICF (Informed Consent Form)
5. Ages: over 18 at time of signing ICF

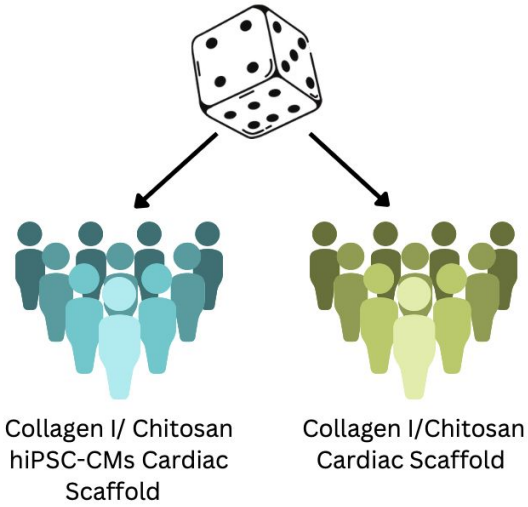


Exclusion criteria

1. Patients who had cardiogenic shock or endstage heart failure
2. History of leukopenia (white blood cells) or thrombocytopenia (platelets)
3. Evidence for terminal disease
4. Patients under treatment with oncology drugs or immunologic suppression
5. Known stroke (in the last month) or major cardiac surgery
6. Female patient who is pregnant, nursing, or of child-bearing potential

Screening: Patients providing informed consent will undergo screening prior to the study treatment. Eligible patients will be randomized through a web based response system.

- Baseline evaluation 1 week before the surgery



Patient selection and group assignment

The two arms of the study will be randomly assigned through a web based response system.

The study has 2 arms/groups with **10 patients each one**:

1. Collagen I/ Chitosan + hiPSC-CMs Cardiac Scaffold
2. Collagen I/ Chitosan Cardiac Scaffold

Outcome measures/Clinical Follow-Up

Primary endpoint:
[1 month post surgery]

We assess safety on the basis of the following:

- Development of major adverse cardiac events (death, new myocardial infarction, admittance to hospital due to aggravation of ischaemia, or heart failure)
- Clinical status: dyspnea (shortness of breath), chest pain; and detection of ventricular arrhythmias by 24-hour Holter electrocardiographic monitoring study.

Secondary endpoint: Efficacy

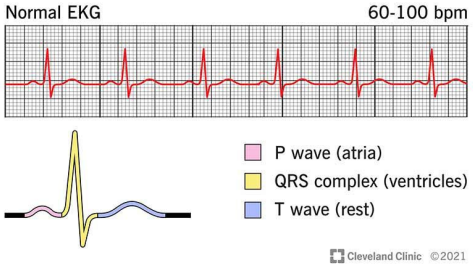
[12 month post surgery follow-up]

		Postoperative week				Postoperative month										
	Surgery	Week 1	Week 2	Week 3	Week 4	2	3	4	5	6	7	8	9	10	11	12
Continuos in-hospital telemetry		■														
ECG holter monitoring			■	■	■											
ECG					■		■			■						■
Echocardiography					■		■			■						■
Radionuclide ventriculography					■		■			■						■
MRI					■		■			■						■
Angiogram					■		■			■						■

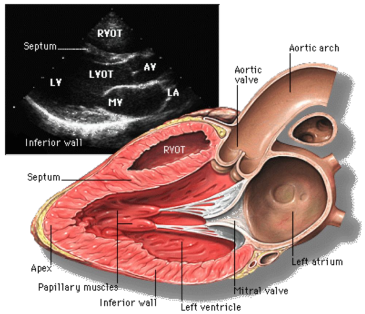
Schedule of Follow-Up activities

Monitoring activities are non-invasive since the patient underwent surgery for the implantation of the scaffold.

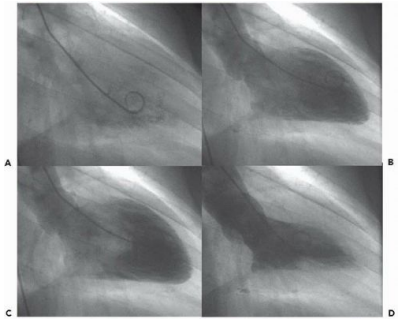
ECG



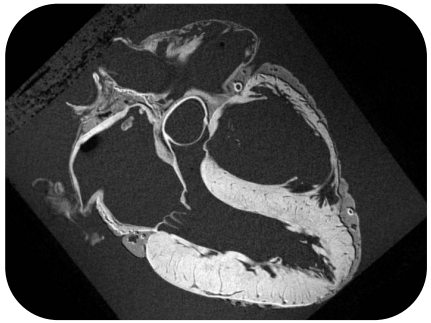
Echocardiography



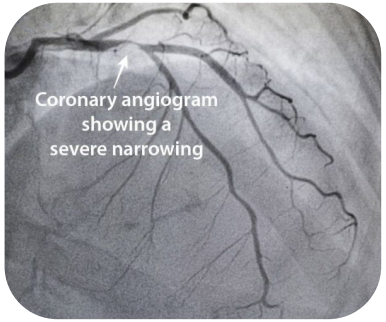
Radionuclide ventriculography



MRI



Angiogram



Conclusions

- An injectable collagen-chitosan hydrogel loaded with hiPSC-CMs was proposed for the regeneration process.
- A phase I clinical trial to test the safety and effectiveness of a tissue engineering based product for the treatment of acute myocardial infarction (MI) was defined.

Potentialities

- The injectable scaffold enables immediate contact with the damaged area.
- Easily adaptable to the contractile motion of the heart.
- The combination of collagen and chitosan enhance the mechanical properties of the scaffold.

Limitations

- Low scalability of the cellular scaffold.
- The lack of electrical conductivity in the scaffold
- Clinical Study: age ranges (over 18) can affect the results. Younger people may have faster regeneration of the damaged tissue. Other factors (lifestyle) that is difficult to track in each patient.

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