Temperature-responsive biodegradable injectable hydrogel containing adipose-derived stem cells for myocardial ischemia

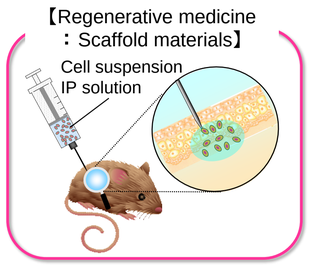
Yuichi Ohya[1,2], Yuta Yoshizaki[3,4] ▪ Nozomi Mayumi[1] ▪ Akinori Kuzuya[1,2]
[1]Faculty of Chemistry and Materials, Bioengineering, Kansai University ▪ [2]KUMP-RC. Kansai University ▪
[3]ORDIST, Kansai University [4]Grauate School of Pharmaceutical Science, Tohoku University

Introduction

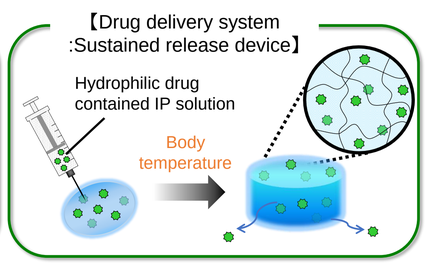
Temperature-responsive injectable polymer (IP)

IP is *in situ* gelling polymer solution

【Regenerative medicine ： Scaffold materials】

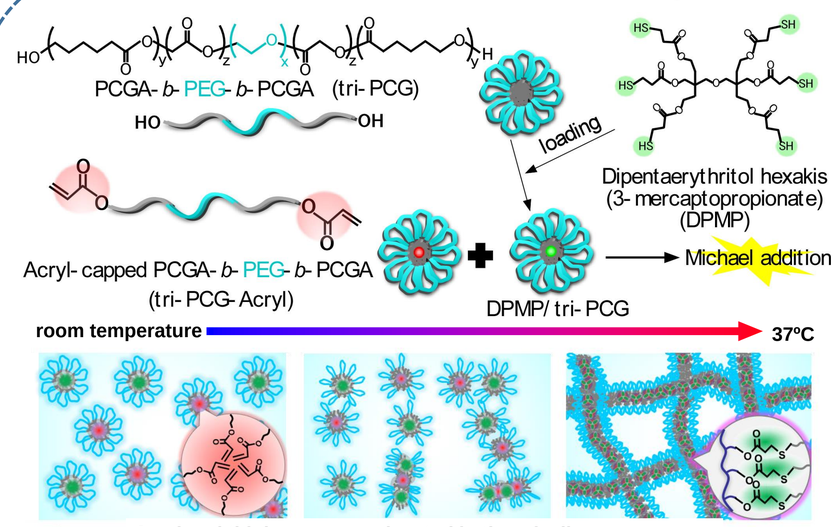


【Drug delivery system :Sustained release device】



- Rapid sol-to-gel transition in response to temperature
- Easy injection with a syringe

Concept of This Study

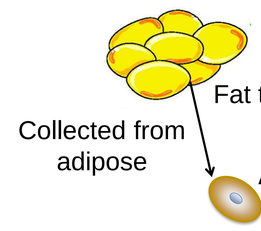


DPMP Acryl and thiol groups are located in the micelle core.

Duration of the hydrogel formed was significantly improved and could be controlled from 1 to 90 days by changing the content of tri-PCG-Acryl.

Figure 1. Schematic illustration for the temperature-responsive irreversible sol-to-gel transition mechanism of IP formulations with increasing temperature from r. t. to body temperature.
Y. Ohya *et al.*, *Biomatar.Sci.*, **2017**, *5*, 1304-1314.

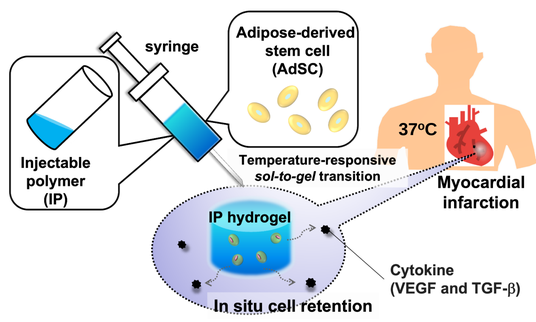
Adipose-derived Stem Cell: AdSC



- *Frequency of malignant transformation*
  adipose < bone marrow << iPS
- *Time required for separation of cells*
  adipose < bone marrow << iPS
- *Examples of clinical application*
  adipose > bone marrow >> iPS

P. A. Zuk *et al.*, *Tissue Eng.*, **2001**, *7*, 211-228.

Aim of this study

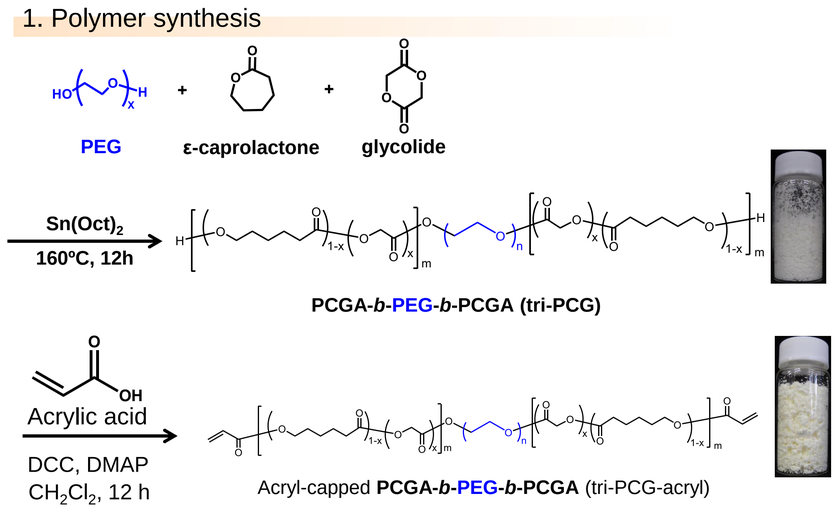


Application of temperature-responsive covalent- bond-forming biodegradable IP system containing AdSCs for myocardial infarction treatment.

Longer retention of AdSCs at the injected site and continuous release of cytokines form the AdSCs.

Results and Discussion

1. Polymer synthesis



Scheme 1. Synthetic route of tri-PCG and tri-PCG-acryl.

2. Preparation of IP formulation

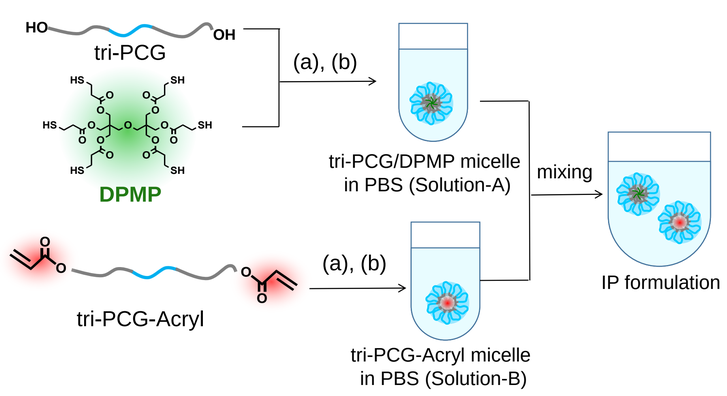
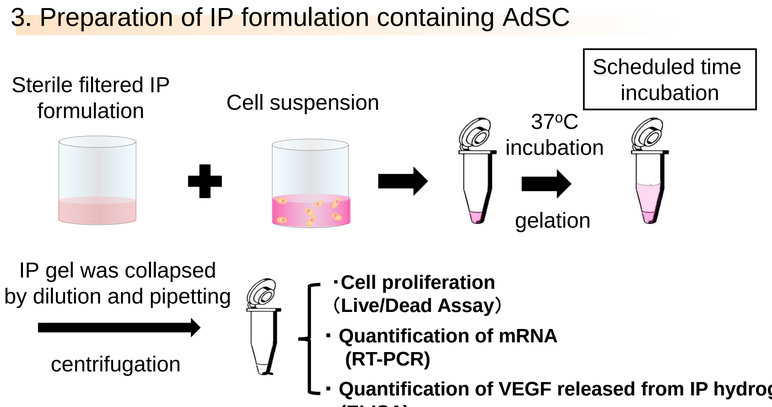


Figure 2. Preparation of IP formulations. (a) Solvent evaporation: i) Dissolved in acetone, ii) poured into water and evaporation to remove acetone. (b) i) Freeze-dry, ii) dissolved in PBS repeating heat at 60 ºC and cool at 0 ºC.

3. Preparation of IP formulation containing AdSC



Sample condition :
(**1**) Physically cross-linked IP hydrogel (Phys-IP) **(tri-PCG)**
(**2**) Chemically cross-linked IP hydrogel (Chem-IP) **(tri-PCG/DPMP + tri-PCG-Acryl)**

Figure 3. Schematic illustration of IP formulation containing AdSC.

4. Proliferation behavior of AdSC in IP hydrogel

: gel state : sol state

Physically cross-linked IP hydrogel (Phys-IP): tri-PCG
Chemically cross-linked IP hydrogel (Chem-IP): (tri-PCG/DPMP + tri-PCG-Acryl)
Polymer weight: 15wt%
Cells : **AdSC**

tripPCG-Acryl content **25%**
SH : Acryl = 1:1

Total cell
Living cell
Dead cell

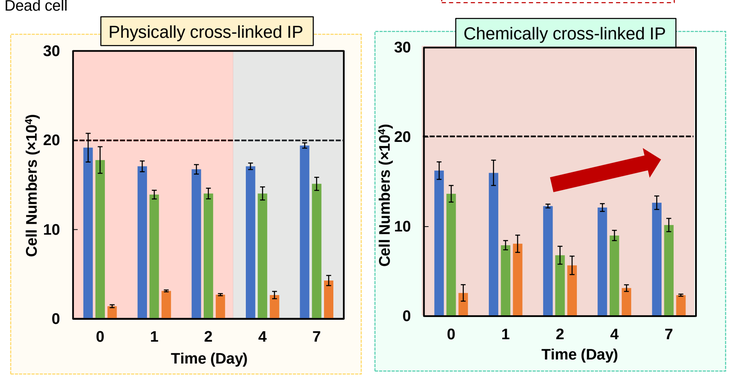


Figure 4. Proliferation of AdSC cultured in tri-PCG, [tri-PCG/DPMP + tri-PCG-Acryl] hydrogel incubated at 37ºC for 0, 1, 2, 4, 7days.

Certain amount of AdSCs could survive in the chemically cross-linked IP hydrogel.

5. mRNA expression of AdSC in IP hydrogel

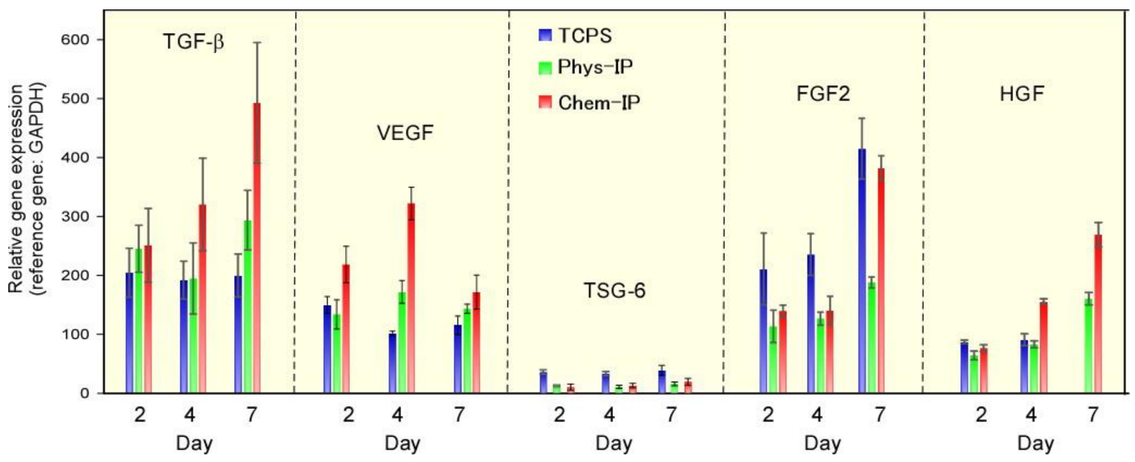


Figure 5. Relative mRNA expression of VEGF normalized by GAPDH) of AdSC were analyzed by RT-PCR. Cells were cultured in various hydrogels.

Gene expressions of major cytokines, such as VEGF, was accelerated in the chemically cross-linked IP hydrogel, and the cytokines were actually released from the hydrogel.

6. Cytokine release from IP hydrogel

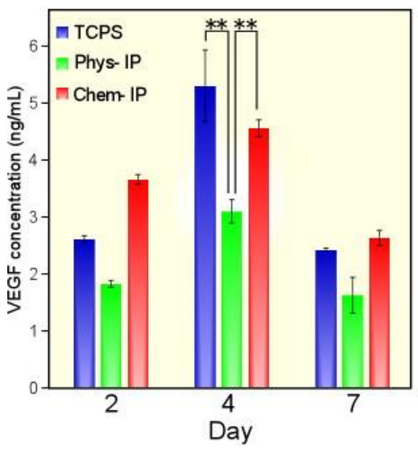
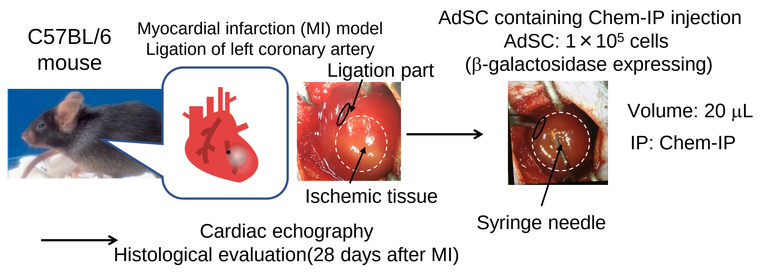


Figure 6. VEGF production from AdSCs cultured on TCPS or in IP hydrogels for 2, 4, 7 days. VEGF concentration in cell media was measured by ELISA.

7. Treatment effect of myocardial infarction



7-1. Evaluation of heart function

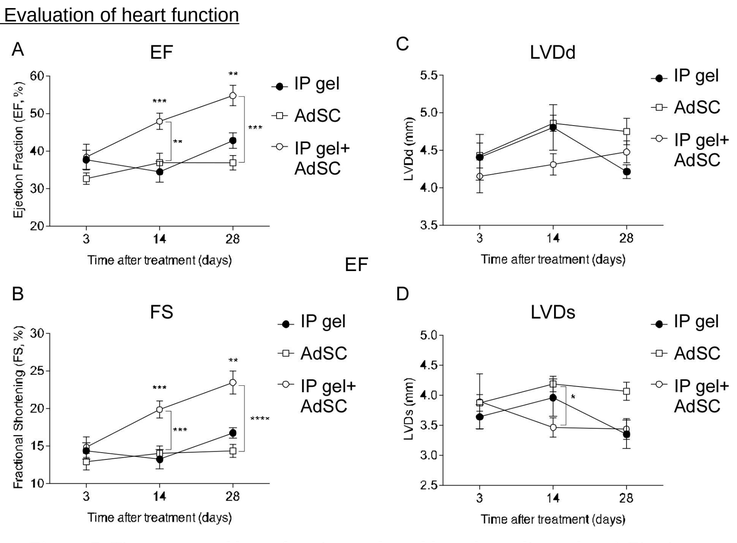


Figure 7. Time course of heart function evaluated by echocardiography. a) Ejection fraction (EF). b) Fractional shortening (FS). C) Left ventricular end-diastolic dimension (LVDd). D) Left ventricular end-systolic dimension (LVDs).

7-2. Angiogenesis and AdSC retention

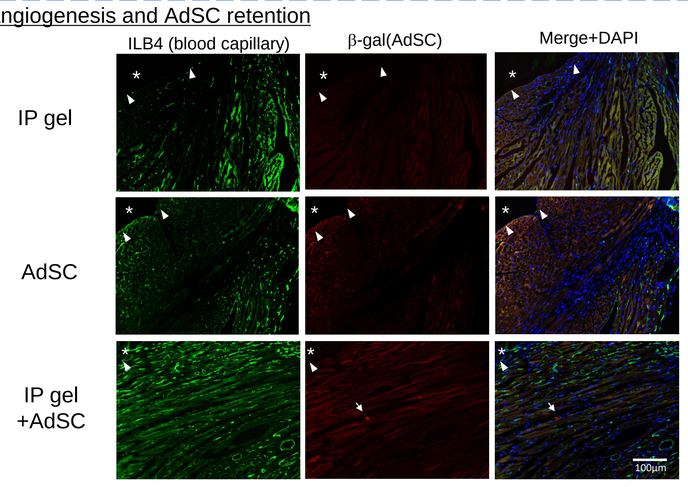


Figure 8. Immunofluorescent staining of mice heart section. The asterisk represents left ventricular cavity, the triangle represents endocardium, the arrow represents injected AdSC.

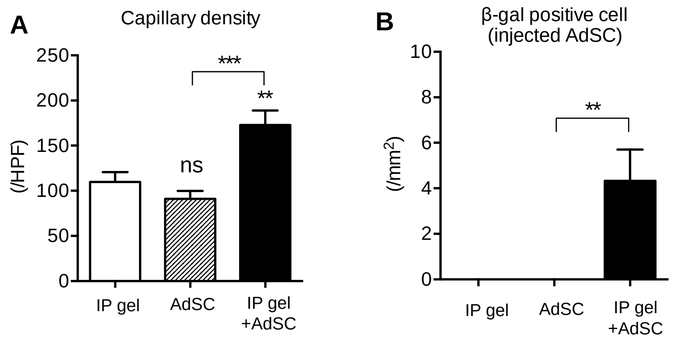


Figure 9. Quantification of capillary density (A) and the number of β-galactosidase positive cells detected around the ligated part (B).

7-3. AdSC delivery using IP hydrogel reduces fibrosis

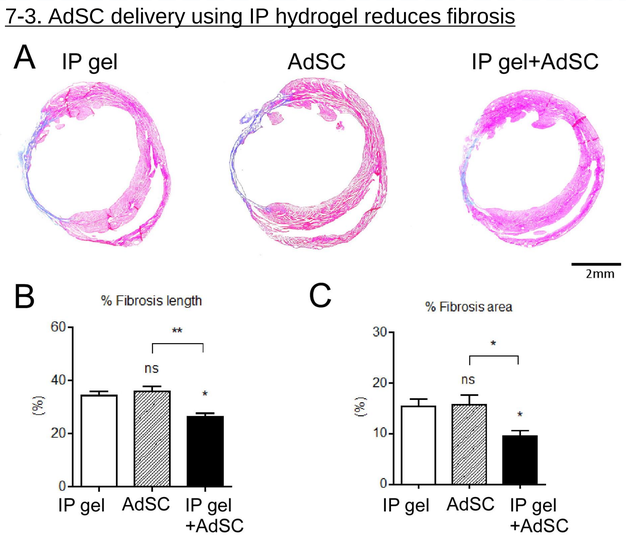


Figure 10. (A) Masson's trichrome staining images of mice heart. (B) Quantification of fibrosis length and (C) %of fibrosis area.

Therapeutic effects in myocardial infarction model mice

7-1. Better recovery in heart function (EF, FS,) was observed in IP/AdSC treated groups
7-2. Retention of AdSCs at the injected tissue and significant production of blood capillary was observed for IP/AdSC treated groups.
7-3. Fibrosis level of the IP/AdSC treated groups was lower than those in control.

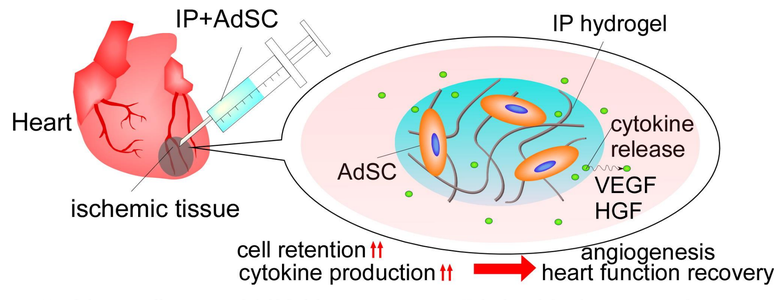


Figure 11. Schematic illustration of AdSC delivery system using IP hydrogel for the treatment of ischemic heart diseases. IP hydrogel improve *in situ* cell retention and cytokine production.

Conclusions

IP hydrogel retained AdSCs and kept cells alive. AdSC cultured in chemically cross-linked IP hydrogel showed higher VEGF mRNA expression than those of physically cross-linked hydrogel and TCPS. IP/AdSC system improved the retention of AdSC at ischemic heart tissue and recovery of heart function.

References

For more information, see the publication:
Y. Yoshizaki, Y. Ohya *et al., Sci. Technol. Adv. Mater.* **2021**, *22*, 627-642.