Extracellular matrix microparticles promote heart regeneration in post-myocardial infarction mice

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INTRODUCTION

A heart attack can progress to heart failure in adult mammals in part because low cardiac regenerative capacity. Lack of beating tissue replacement from damage leads to a permanent scar that can impair cardiac function. However, the damaged heart can fully regenerate within 3 weeks in neonatal rodents and lower vertebrates such as zebrafish[1-2]. We hypothesize that the biological features of fetal microenvironment can reduce ventricular wall thinning and fibrosis, and improve heart recovery.

Injectable biomaterials are a promising methodology for improving heart post-injury responses[3]. We have shown in a previous study that delivering cardiac extracellular matrix (dECM) hydrogel into the injured non-regenerative heart reduces wall thinning and preserves cardiac output[4]. In this study, we processed the liquid dECM hydrogel precursor to injectable microparticles that retains the bioactive macromolecules and exhibits increased stability and retention in tissue. The therapeutic efficacy of the slide dECM microparticles were tested in a mice myocardial infarction model. The results demonstrate that dECM microparticles improve heart recovery.

METHODS

Extracellular matrix microparticle preparation

Fetal porcine ventricles were decellularized in SDS solution and Triton X-100 solution and washed in water. Acellular tissue was lyophilized and pulverized in liquid nitrogen to improve enzymatic digestion. dECM powder was digested in pepsin for 4 to 36 hours to generate homogenous dECM solution. Digestion was terminated by adjusting pH to 8. Solubilized dECM was processed to microparticles by electrospray and heat-induced polymerization.

Myocardial infarction (MI) on mice

MI induced by permanent ligation of coronary artery. dECM hydrogel precursor and microparticles were injected to myocardium immediately after ligation. Echocardiography conducted on week3 post-MI.

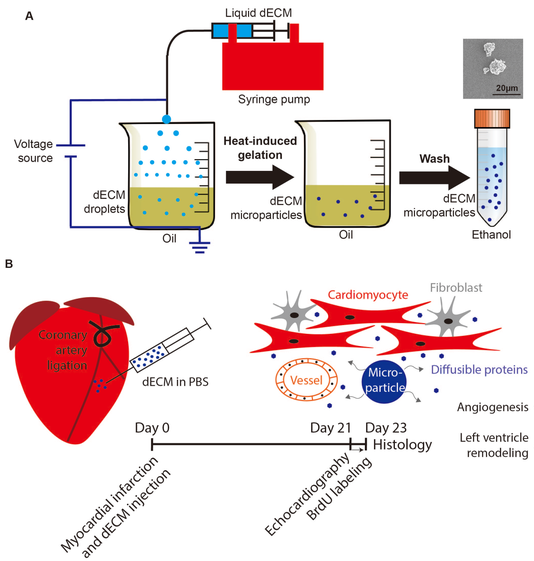

Figure 1. (A) Schematic of dECM microparticles preparation. (B) Experimental setup.

RESULTS

dECM microparticles preserve cardiac function at week 3 post-MI

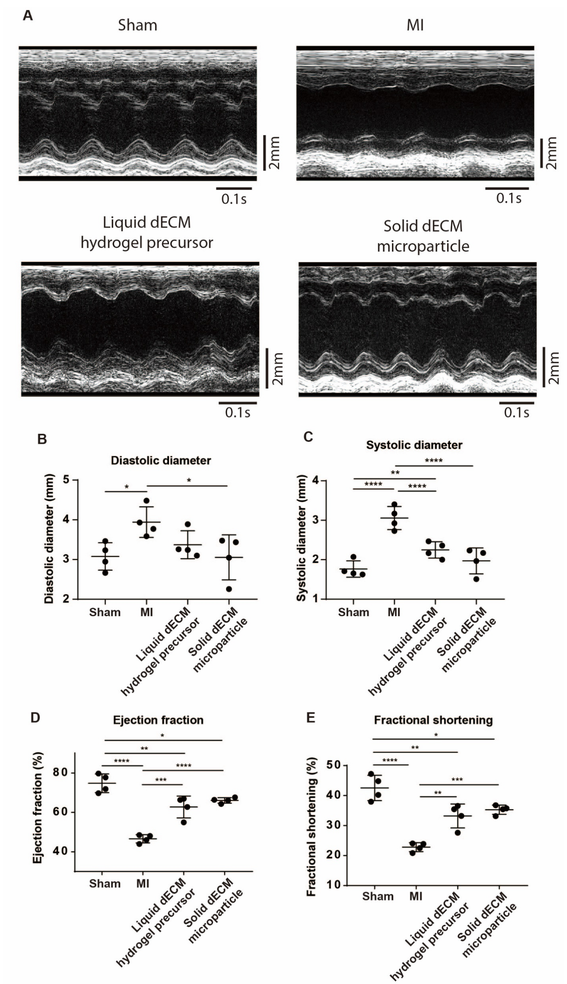

Figure 2. (A) Cardiac function and dimension was examined by echocardiography at week 3 post-MI in a mice MI model. dECM microparticles treated hearts exhibited significantly lowered (B) diastolic diameter and (C) systolic diameter compared to the MI control. Liquid dECM hydrogel precursor treatment showed a trend of reducing ventricular dilation. Both liquid dECM hydrogel precursor and solid dECM microparticles increased (D) ejection fraction and (E) fractional shortening compared to the MI control. (n=4, one-way ANOVA and tukey's test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.)

dECM microparticles preserve ventricular wall thickness and reduce fibrosis

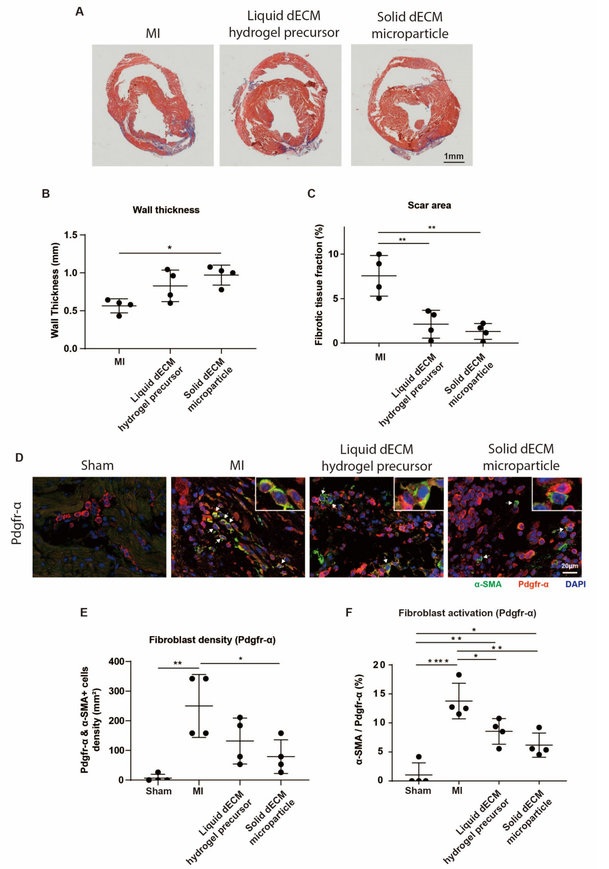

Figure 3. (A) Ventricular wall thickness and fibrosis were examined by Masson's Trichrome staining at week 3 post-MI. (B) Solid dECM microparticles treated hearts exhibited significantly increased wall thickness in the infarct zone compared to the MI control. Liquid dECM hydrogel precursor treatment resulted in a trend of increased wall thickness though not significantly higher than the MI control. (C) Both dECM microparticles and hydrogel precursor reduced fibrosis compared to the MI control. (D) Fibroblast activation was examined by immunostaining for α-smooth muscle actin (α-SMA) and platelet derived growth factor receptor-α (Pdgfr-α). (E) The density of activated fibroblasts was significantly lowered by dECM microparticles but not by liquid dECM hydrogel precursor compared to the MI control. (F) The ratio of α-SMA+ fibroblasts to total fibroblasts was lowered by both dECM microparticles and hydrogel precursor compared to the MI control. (n=4, one-way ANOVA and tukey's test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.)

dECM microparticles promote angiogenesis

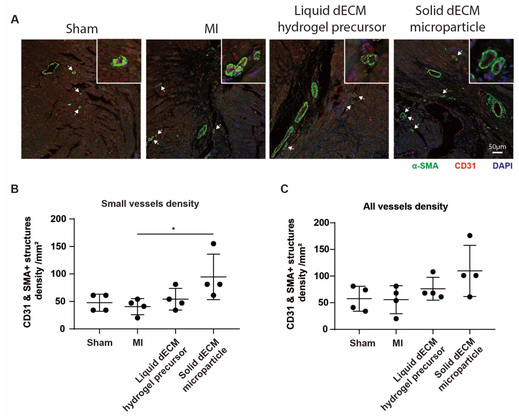

Figure 4. (A) Angiogenesis was examined by immunostaining at week 3 post-MI. (B) The density of small vessels was increased only in dECM microparticles treated hearts. (C) The density of all vessels was not significantly modulated by the treatments though dECM microparticles treated hearts showed a trend of increased vessel density. (n=4, one-way ANOVA and tukey's test, *p<0.05.)

dECM microparticles exhibit increased collagenase resistance

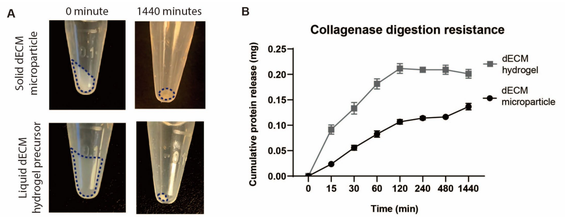

Figure 5. (A) dECM microparticles and hydrogels were digested in collagenase digestion buffer for 24h. (B) dECM microparticles exhibit increased collagenase digestion resistance compared to hydrogels.

CONCLUSION

Solubilized extracellular matrix can be processed to microparticles. The solid dECM microparticles exhibit increased stability compared to dECM hydrogel. Delivering dECM microparticles into post-MI mice hearts preserves cardiac function and ventricular wall thickness, lowers fibrosis and fibroblast activation, and stimulates angiogenesis. Together, the results demonstrate that solid dECM microparticles can be developed as a therapy for cardiac injury.

REFERENCES

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