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Lack of macroscopically evident cardiac regeneration or spontaneous functional recovery in infarcted neonatal pigs

The adult mammalian heart harbors a traceable -albeit limited-capacity for endogenous cardiomyocyte replenishment throughout life. [1-5] Given that heart disease comprises a major cause of mortality, [6-8] the elucidation of the innate cardiac regenerative mechanisms is of paramount therapeutic importance; exogenous stimulation of said mechanisms would potentially enable iatrogenic regeneration in diseased hearts.

In contrast to the adult mammalian heart, the neonatal murine heart exhibits a remarkable capacity for spontaneous regeneration following cardiac injury. [9-11] Whether such robust cardiac regenerative potential is also present in neonatal large mammals has been under-investigated. Recently, two studies reported that 1- and 2-day-old neonatal pigs exhibit a robust cardiac regenerative response post-myocardial infarction (MI), characterized by minimal scarring and spontaneous, complete recovery of left ventricular (LV) function. [12,13] This regenerative capacity is purportedly lost after the first 2 days of life; neonatal pigs infarcted on postnatal day 3 (P3) or later developed mature scars and exhibited persistent systolic dysfunction. [12,13] Here, we also investigated the regenerative potential of neonatal porcine hearts post-MI.

Twelve farm piglets were randomized to undergo MI by permanent ligation of the left anterior descending artery on P1 or P3. Piglets were euthanized at 7 weeks post-MI. Transthoracic echocardiography was performed just prior to euthanasia. Explanted hearts underwent staining with triphenyl-tetrazolium chloride and Masson's Trichrome for scar assessment.

Seven piglets (4 P1 and 3 P3 piglets) successfully completed the protocol. At 7 weeks post-MI, all infarcted hearts exhibited substantial scarring. Representative TTC-stained cardiac slices are depicted in Fig. 1A. Infarct size (P1: 9.5 ± 2.2% vs P3: 8.9 ± 3.6% of LV, $p = 1.000$), infarct circumference (P1: 33.8 ± 7.1% vs P3: 29.8 ± 10.6% of LV, $p = 0.400$), and infarct transmurality (P1: 38.1 ± 4.3% vs P3: 40.4 ± 13.7% of anterior wall, $p = 0.857$) were comparable between infarcted P1 and P3 piglets (Fig. 1B). Masson's Trichrome stain demonstrated dense collagen scars in all infarcted P1 and P3 piglets (Fig. 1C).

No signs of functional recovery were observed; at 7 weeks post-MI all piglets exhibited hypokinesia of the infarcted wall. Representative echocardiographic M-mode tracings are presented in Fig. 1D. Systolic thickening in the anteroseptal (infarcted) LV wall was depressed to a similar degree in infarcted P1 and P3 animals (P1: 31.8 ± 5.3% vs P3: 32.3 ± 8.5%, $p = 0.857$) compared to thickening in the posterior (non-infarcted) wall (P1: 72.5 ± 9.0% vs P3: 69.0 ± 11.4%, $p = 1.000$) (Fig. 1E). Fractional shortening was comparable in infarcted P1 and P3 animals (P1: 25.5 ± 2.9% vs P3: 23.7 ± 4.5%, $p = 0.629$) (Fig. 1E).

Our study has important limitations. First, the sample size is small; however, the results were remarkably consistent across all experimental animals. Second, we did not perform immunohistochemistry for markers of myocyte cell-cycle re-entry. Therefore, we cannot evaluate whether a small (clinically irrelevant and macroscopically undetectable) degree of neomyogenesis occurs at the microscopic level.

In conclusion, contrary to recently published reports, [12,13] we did not observe a robust cardiac regenerative response in neonatal P1 porcine hearts post-MI. Specifically, while previous studies reported minimal scarring and complete recovery of LV function in infarcted P1 and P2 (but not P3) piglets, [12,13] all infarcted hearts of P1 piglets in our study exhibited substantial scarring and persistent hypokinesia of the infarcted myocardium. Scars were similar morphometrically (in terms of infarct size, circumference, and transmurality) and qualitatively (consisting of dense collagen fibers) in P1 and P3 piglets. Furthermore, the degree of systolic dysfunction was comparable in P1 and P3 piglets. While we cannot exclude that increased neomyogenesis may occur in infarcted P1 piglets at a microscopic level, a robust, clinically relevant regenerative response would result in decreased infarct size and improved cardiac regional systolic function in P1 piglets compared to P3 piglets. Because none of these indirect indices of robust neomyogenesis were observed, we conclude that macroscopically evident regeneration does not occur in infarcted P1 porcine hearts.

It is difficult to reconcile our findings with those of Zhu et al. [12] and Ye et al. [13]. Murine studies have demonstrated that the severity of myocardial injury severely impacts upon the regenerative capacity of the neonatal murine heart. [14,15] However, the ischemic insult (as assessed by the anatomic level of coronary artery ligation) appears comparable between our study and those of Zhu et al. [12] and Ye et al. [13]. We therefore propose that additional research is warranted to assess the regenerative potential of neonatal porcine hearts.

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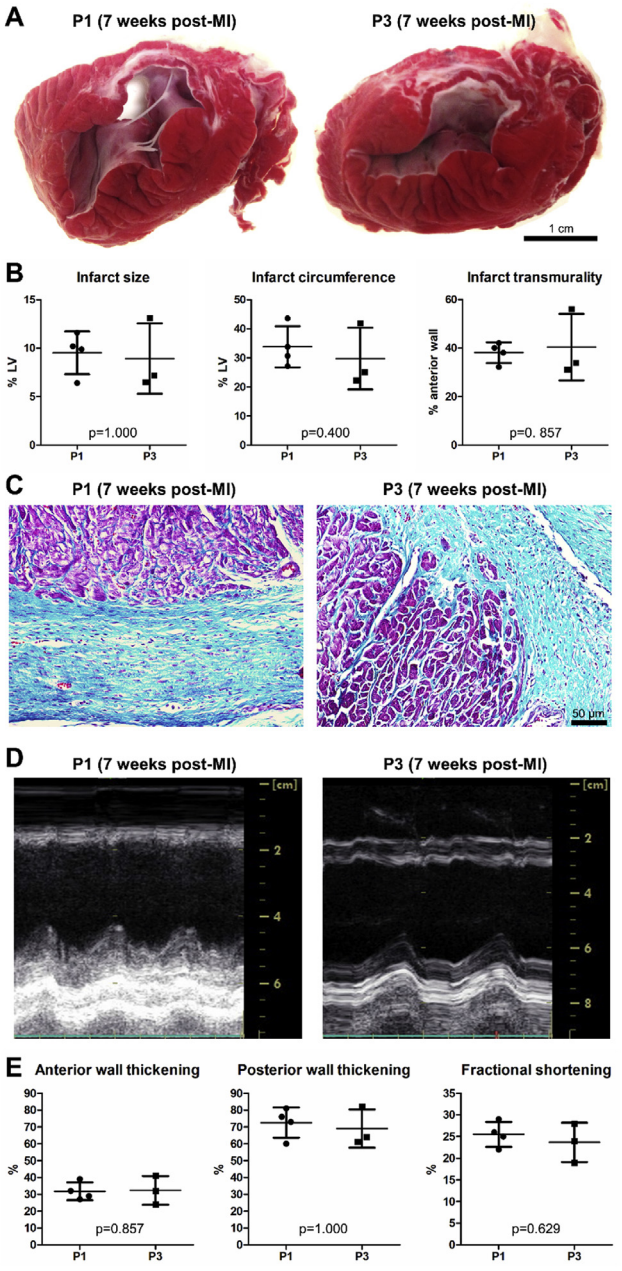

Fig. 1. Infarcted neonatal piglets exhibit substantial scarring and persistent hypokinesia of the infarcted left ventricular wall. A) Representative triphenyltetrazolium chloride (TTC)-stained short-axis cardiac slices of an infarcted 1-day-old (P1) and an infarcted 3-day-old (P3) piglet at 7 weeks post-myocardial infarction (MI). TTC stains viable myocardium brick red, while scar tissue remains unstained (white). Substantial scarring of the anteroseptal left ventricular wall is present in both infarcted P1 and P3 piglets. **B)** Quantitative assessment of infarct size, infarct circumference, and infarct transmurality in infarcted P1 and P3 porcine hearts at 7 weeks post-MI. **C)** Representative photomicrographs of myocardial sections obtained from the infarct border zone after staining with Masson's trichrome. Dense collagen scars (stained blue/green) were detected in both infarcted P1 and P3 piglets at 7 weeks post-MI. **D)** Representative M-Mode tracings of an infarcted P1 and an infarcted P3 piglet at 7 weeks post-MI. M-mode tracings were derived from parasternal short-axis views, obtained at a level below the left ventricular papillary muscles. The infarcted anterior wall is hypokinetic in both infarcted P1 and P3 piglets. **E)** Quantitative assessment of anterior wall thickening, posterior wall thickening, and fractional shortening.

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Conflict of interest

The authors declare that there are no relevant conflicts of interest.

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