Myocardial regeneration after embryonic cryoinjury influenced by epicardium

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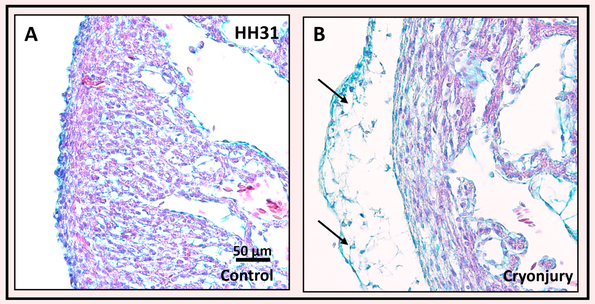
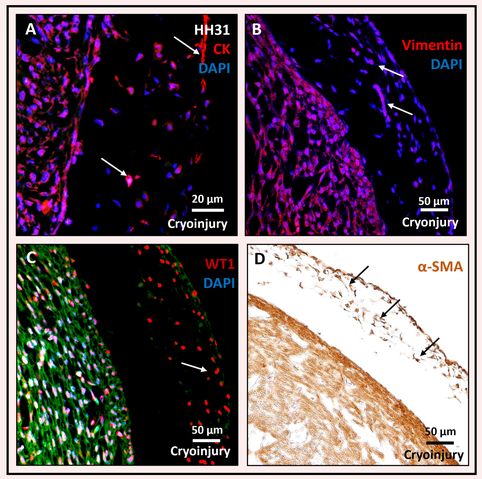
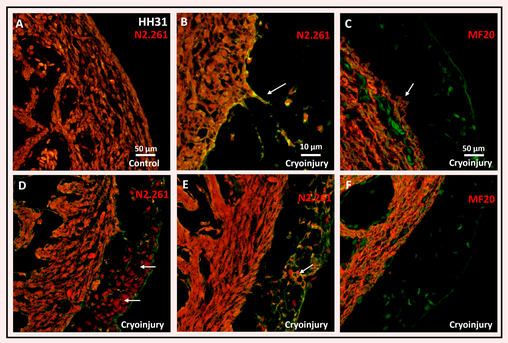
Epicardium



The epicardium after cryoinjury in Figure B is thickened, detached and wrinkled in the affected, but also in the remote area. In the subepicardial region there are migrating cells from the epicardium. Samples are stained with Hematoxylin-Eosin-Alcian Blue.



As a result of cryoinjury, cytokeratin (A) and vimentin (B) positive cells are observed in the subepicardial region, confirming epithelial-mesenchymal transition. Epicardial activation is confirmed by increased expression of WT1 (C). SMA-positive cells are also detected (D).



In the area of damage, there are myocardial protrusions into the subepicardium that are positive for both N2.261 (B) and MF20 (C). In the subepicardium, there are cells that are only positive for N2.261 (E). We believe that these are un-differentiated cardiomyocytes.

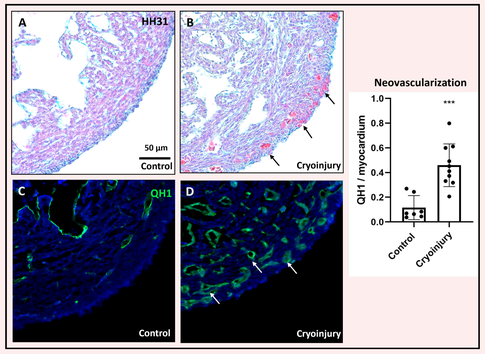
ABSTRACT

The embryonic epicardium is outermost layer of the heart, which undergoes epithelial-to-mesenchymal transformation, contributing cells for formation of coronary vasculature.
Morphological analysis confirmed changes in the epicardial region.

As a result of the cryoinjury, the epicardium was wrinkled, detached, and thus the subepicardial area increased. In the subepicardium we detected many cells participating in the epithelial-mesenchymal transition (α-SMA, WT1, N2.261, CK, Vimentin). Regenerative potential was also confirmed by increased neovascularization (QH1) or proliferation (EdU) after cryoinjury. Functional analysis show temporal loss of epicardial activation, in the infarct zone, but heart activation is restored after time.

Our study confirmed the importance of the epicardium in the healing of myocardial infarction, increased neovascularization, and cell proliferation, which led to the restoration of the electrical conduction. Myocardial healing was complete and without fibrotic scar. This could be potentially used for devising novel regenerative strategies in humans, where activated epicardium, may repair myocardial infraction.

Neovascularization



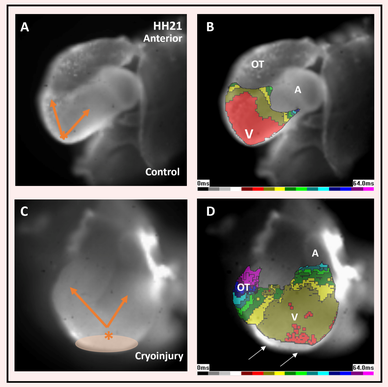
This plate demostrates the formation of the new coronary vasculature in heart affected by cryo probe (B and D) compared to control hearts (A and C). A and B: Cross section from a HH31 quail heart stained with Hematoxylin-Eosin-Alcian Blue. B: New vessels are seen on the surface of the left ventricular myocardium. C and D Cross section stained with QH1. D: QH1 staining representing the formation of new blood vessels in the area affected by cryo probe. Coronary vessel density was markedly increased after the cryoinjury (E), n=10 (cryoinjury), n=8 (control).

CONCLUSION

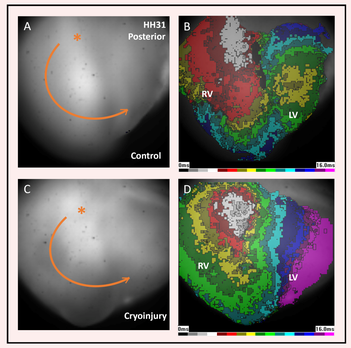
Cryoinjury of quail hearts results in:

- changes in the epicardium in the affected, but also in the remote area
- enlarged subepicardial region with edema
- formation of the new undifferentitated cardiomyocytes
- increased cell proliferation
- formation of the new coronary vessels
- absence of the electical activity in the injured area

Optical mapping

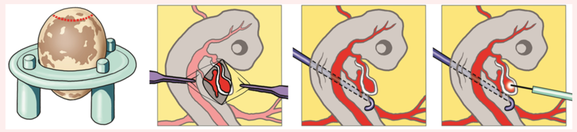


Optical mapping was used for the analysis of electrophysiological changes. The heart was analyzed 2 hours after cryoinjury at stage HH21. The area injured after cryoinjury is not electrically active (C-D).

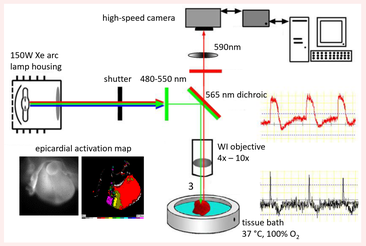


There were no changes in electrical activity in embryo 3 days after cryoinjury compared to controls.

METHODS



Fertilized eggs were incubated at 38 °C in a 40-60% relative humidity atmosphere. Stage HH21 (ED3 quail, ED4 chicken) embryos were selected according to the Hamburger-Hamilton classification. Cryoinjury was performed with a hand-made metal probe soaked in liquid nitrogen. Probe touched the left ventricle for 2s, until the ventricle bleached. After re-incubation to stage HH31 (ED7), embryos were removed and the heart was dissected and fixed in 4% PFA/PBS.



Set-up for optical mapping. Samples were stained with di-4-ANNEPS.

Proliferation and Apoptosis

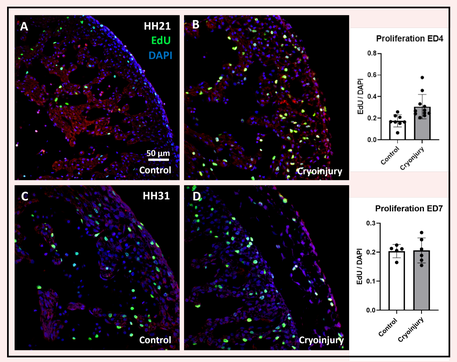


Figure shows the different levels of proligerative activity in controls (A,C) and subjects after cryoinjury (B, D). Immediately after cryoinjury, EdU was administered in vivo for 2 hours The number of labelled nuclei in the ventricular area after cryoinjury was significantly increased, n = 6 (cryoinjury), n=5 (control). There was no difference in proliferation after three days.

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