Differentiation 91 (2016) 72–77

Contents lists available at ScienceDirect

Differentiation

journal homepage: www.elsevier.com/locate/diff

A chick embryo cryoinjury model for the study of embryonic organ development and repair

Paul Palmquist-Gomes [a,b], Juan Antonio Guadix [a,b,*], José M. Pérez-Pomares [a,b,*]

[a] Department of Animal Biology, Institute of Biomedicine of Málaga (IBIMA), Faculty of Sciences, University of Málaga, Málaga, Spain
[b] Area of Biotechnology, Andalusian Centre for Nanomedicine and Biotechnology (BIONAND), Campanillas, Málaga, Spain

ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form
17 October 2015
Accepted 27 October 2015
Available online 11 November 2015

Keywords:
Cryocauterization
Chick embryo
Developmental patterning
Tissue repair/regeneration

ABSTRACT

Tissue ablation is a classic experimental approach to study early embryo patterning. However, ablation methods are less frequently used to assess the reparative or regenerative properties of embryonic tissues during organogenesis. Surgical procedures based on the removal of a significant amount of tissue during organ formation very much depend on the skills of the researcher, are difficult to reproduce, and often result in extensive tissue disruption leading to embryonic death. In this paper, we present a new protocol to generate discrete, locally-restricted and highly reproducible wounds in the developing chick embryo using a liquid N_2-cooled metallic probe. This *in ovo* procedure allows for the study of organ-specific tissue responses to damage, such as compensatory cell growth, cell differentiation, and reparative/regenerative mechanisms throughout the embryonic lifespan.

1. Introduction

Loss-of-function experiments are key to the understanding of a variety of developmental processes (Gilbert, 2013). In some animal models, transgenesis allows for time-specific and space-dependent disruption of gene function. However, classical developmental biology also deals with the primarily epigenetic tissue regulatory mechanisms that take place during embryogenesis (Forgacs and Newman, 2005). These include characteristic phenomena as tissue fusion, cell intercalation or critical cell density-dependent differentiation and growth (e.g. cell number requirements during muscle differentiation as determined by the *community effect* (Gurdon et al., 1993).

Microsurgery has been used to subtract a relevant group of cells or tissues in various animal model embryos (e.g. *Xenopus laevis*, *Danio rerio*) (Kuwada, 1993; Lynch and Fraser, 1990), but is not a suitable procedure for the study of mammalian embryos. On the contrary, avian embryos, sharing with mammals the amniotic condition, can be easily manipulated *in ovo* without affecting the viability of the organism. In this context, ablation experiments in the chick embryo can be regarded as a reasonable complement to the experimentation in other vertebrate animal models in which this approach is not feasible.

A well-known alternative to the classic surgical excision of tissue is the local removal of cells by chemical or physical methods. Among the latter, cauterization procedures using heat or ice-cooled metallic probes to 'burn' adult tissues have been extensively reported in the literature (Chablais et al., 2011; Taylor et al., 1951). Experimental studies on vertebrate embryonic tissue damage are, however, far less frequent, and they mostly focus on the ablation of exposed ectodermal tissues (epidermis, neural plate or neural crest) (Degen and Gourdie, 2012). This has prevented developmental biologists from applying this simple and fast method to the study of embryonic morphogenesis, which requires the coordinated interplay between cell proliferation, migration and differentiation leading *de novo* formation of tissue patterns.

Cauterization methods can also be used to study of the unique 'regenerative' properties of embryonic tissues. Remarkably, the concept of tissue regeneration is not frequently associated with embryonic tissues, mostly because cell proliferation, which is regarded as the local dynamic force driving the restoration of lost body parts in adult tissues undergoing epimorphic regeneration (Sánchez-Alvarado, 2000) is ubiquitous in the embryo. Instead, compensatory growth of tissues seems to be the mechanism used by vertebrate embryos to regenerate lost body parts. On the other hand, classic adult reparative mechanisms like fibrosis, which is a frequent response to pathologic stimuli in postmitotic organs, are considered to be a rare event in embryonic tissues (Degen and Gourdie, 2012; Gurtner et al., 2008).

Abbreviations: HH, Hamburger and Hamilton stages of chick embryonic development

* Corresponding authors at: Department of Animal Biology, Faculty of Sciences, University of Málaga, Campus de Teatinos s/n, 29071 Málaga, Spain.
E-mail address: jmperezp@uma.es (J.M. Pérez-Pomares).

http://dx.doi.org/10.1016/j.diff.2015.10.011
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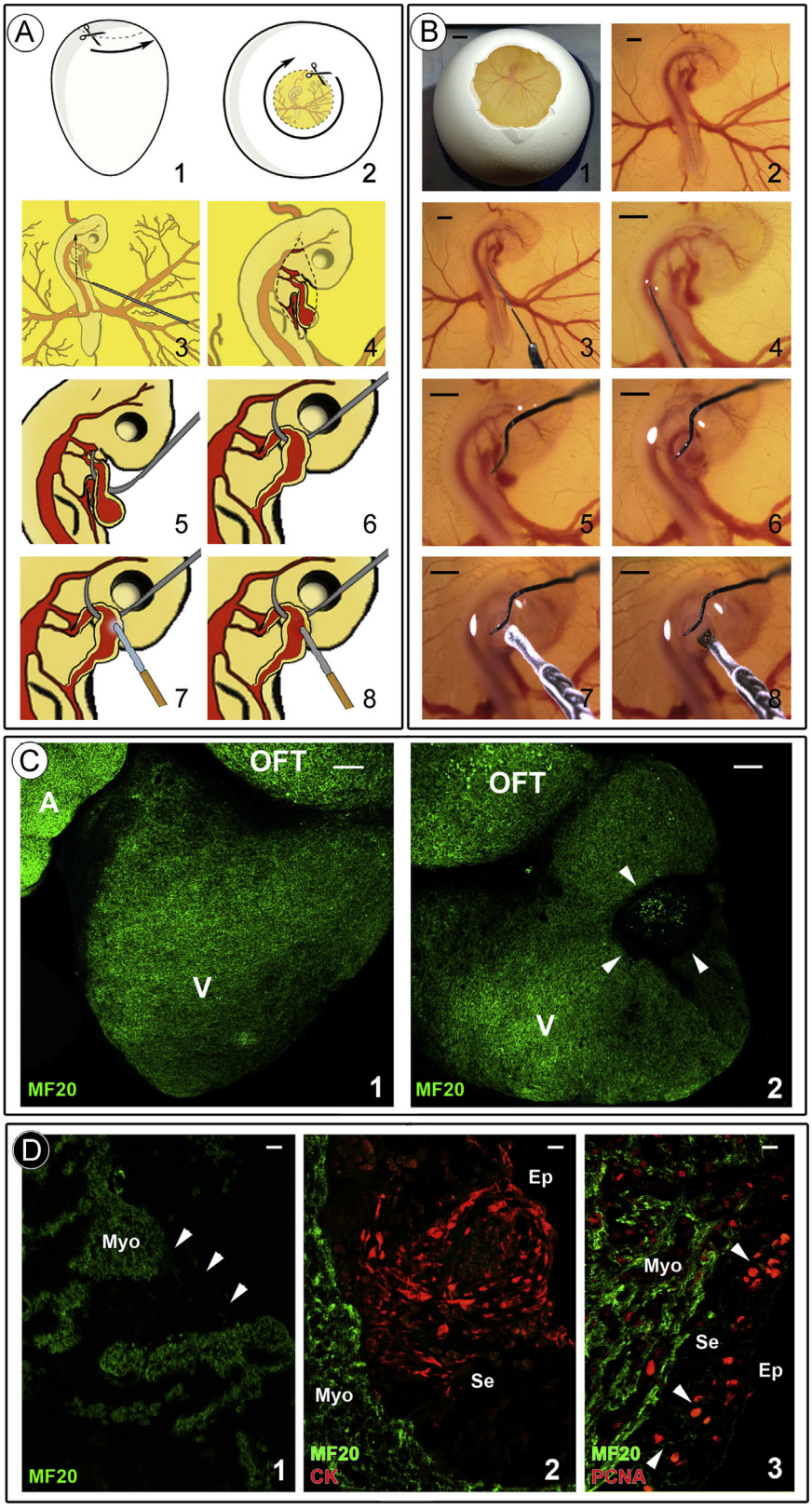

Fig. 1. Cryocauterization of the chick embryonic heart. The procedure, which is detailed in the text, is illustrated with a series of cartoons (A, 1–8) and photographs (B, 1–8). C shows whole mount myosin immunohistochemichal stainings (MF20 antibody, green) of control (1) and cryoinjured (2) hearts; arrowheads in 2 point to the damage area. D. Significant loss of myocardial tissue 24–48 h after cryocauterization is evident in tissue sections stained with the MF20 antibody (green) (D1, arrowheads). cytokeratin-positive cells accumulate at the damage site (D2, red). Mitosis occurs in most cardiac muscle ($PCNA^+$, red cell nuclei; $MF20^+$ cytoplasm) and non-muscle cells ($PCNA^+$ red cell nuclei, MF20-negative cytoplasm, arrowheads). Abbreviations: A, atrium; Ep, epicardium; Myo, myocardium; OFT, cardiac outflow tract; Se, subepicardium; V, ventricle. Scale bars: B1 = 5 mm; B2–8 = 1 mm; C1,2 = 120 μm; D1–3 = 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)