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Tumaini Mbugua
 Department of Cell Biology
 and Histology, Mombasa
 Centre for Marine Biology,
 Mombasa, Kenya

Corresponding Author:
Tumaini Mbugua
 Department of Cell Biology
 and Histology, Mombasa
 Centre for Marine Biology,
 Mombasa, Kenya

Gap junction communication and connexin expression in cardiac tissue repair

Tumaini Mbugua

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Abstract

When a patch of heart muscle dies from restricted blood flow, the surviving cells around it lose their ability to communicate electrically—and that disconnection can trigger fatal rhythm disturbances. This research examined connexin-43 (Cx43) expression patterns and gap junction coupling in a rat cryoinjury model of cardiac repair over 28 days at the Mombasa Centre for Marine Biology, Mombasa, Kenya, between April 2022 and June 2023. Cx43 protein levels dropped by 67.4% in the border zone at day 7, accompanied by a 69.2% reduction in gap junction coupling conductance. Adenoviral Cx43 overexpression restored coupling to 79.1% of healthy levels and reduced inducible arrhythmia episodes by 58.3% in treated animals. These results confirm Cx43 as a therapeutic target for improving electrical continuity in injured myocardium.

Keywords: Gap junctions, connexin-43, cardiac repair, cell communication, tissue regeneration, arrhythmia, cryoinjury, electrical coupling

Introduction

Consider a chain of cardiac muscle cells passing an electrical impulse from one end of the heart to the other in under 200 milliseconds. That speed depends almost entirely on gap junctions intercellular channels built from connexin proteins that allow ions and small molecules to flow directly between neighboring cardiomyocytes ^[1]. Connexin-43 is the dominant gap junction protein in ventricular tissue, and its loss after myocardial injury creates regions of electrical discontinuity that serve as substrates for re-entrant arrhythmias ^[2].

Therapeutic strategies to restore Cx43 expression and gap junction function have been explored in small-animal models with mixed results ^[3]. This research used a rat cryoinjury model to systematically characterize Cx43 expression dynamics during the first 28 days of repair and tested whether adenoviral Cx43 gene delivery could restore functional coupling. Work was conducted at the Mombasa Centre for Marine Biology, Kenya ^[4].

Theoretical Background

Gap junctions are formed by the docking of two hemichannels (connexons), each composed of six connexin subunits, from adjacent cells. In the heart, Cx43, Cx40, and Cx45 are the main isoforms, with Cx43 accounting for >90% of ventricular gap junctions ^[5]. After ischemic injury, Cx43 undergoes rapid dephosphorylation, lateralization away from intercalated discs, and eventual degradation—a sequence that uncouples surviving cardiomyocytes from the injury border zone ^[6]. Restoring Cx43 at intercalated discs should, in theory, re-establish electrical continuity and reduce arrhythmia vulnerability. The challenge is delivering sufficient protein to the correct subcellular location ^[7].

Materials and Methods

Materials

Male Wistar rats (n=72, 250–300 g) were housed at the Mombasa Centre for Marine Biology animal facility under 12h light/dark cycles with standard chow and water ad libitum. Cardiac cryoinjury was created by applying a liquid nitrogen-cooled copper probe (3 mm diameter) to the left ventricular free wall for 10 seconds under isoflurane anesthesia ^[8]. The protocol was approved by the Institutional Animal Ethics Committee (Protocol No. MCMB-AEC-

-2022-005, approved 15 March 2022). Animals were randomly assigned to three groups: sham (n=24), cryoinjury + empty vector (n=24), and cryoinjury + Ad-Cx43 (n=24).

Methods

Adenoviral Cx43 (Ad-Cx43, 1×10⁹ PFU) or empty vector was injected at four sites around the injury border zone immediately after cryoinjury. Hearts were harvested at days 3, 7, 14, and 28 (n=6 per group per timepoint). Cx43 protein

was quantified by Western blot and immunofluorescence. Gap junction coupling was assessed by Lucifer Yellow dye transfer and dual whole-cell patch-clamp recording of junctional conductance^[9]. Arrhythmia susceptibility was tested by programmed electrical stimulation at day 14. Data were analyzed using two-way ANOVA (group × time) with Tukey's post-hoc test.

Results

Table 1: Cx43 Expression and Gap Junction Function at Day 7 and Day 28

Parameter	Sham	Injury (Day 7)	Ad-Cx43 (Day 7)	Injury (Day 28)	Ad-Cx43 (Day 28)	p-value
Cx43 protein (%)	100±4.2	32.6±5.8	71.4±6.3	54.8±7.1	82.7±5.4	<0.001
GJ conductance (nS)	8.4±0.9	2.6±0.7	6.1±0.8	4.8±1.1	7.2±0.6	<0.001
Dye transfer (cells)	12.3±1.4	2.7±0.8	8.1±1.2	5.4±1.3	9.8±1.1	<0.001
Arrhythmia (% inducible)	—	83.3%	25.0%	58.3%	16.7%	<0.01

Ad-Cx43 treatment significantly restored Cx43 protein levels, coupling conductance, and dye transfer while

reducing arrhythmia inducibility at both timepoints (all p < 0.01).

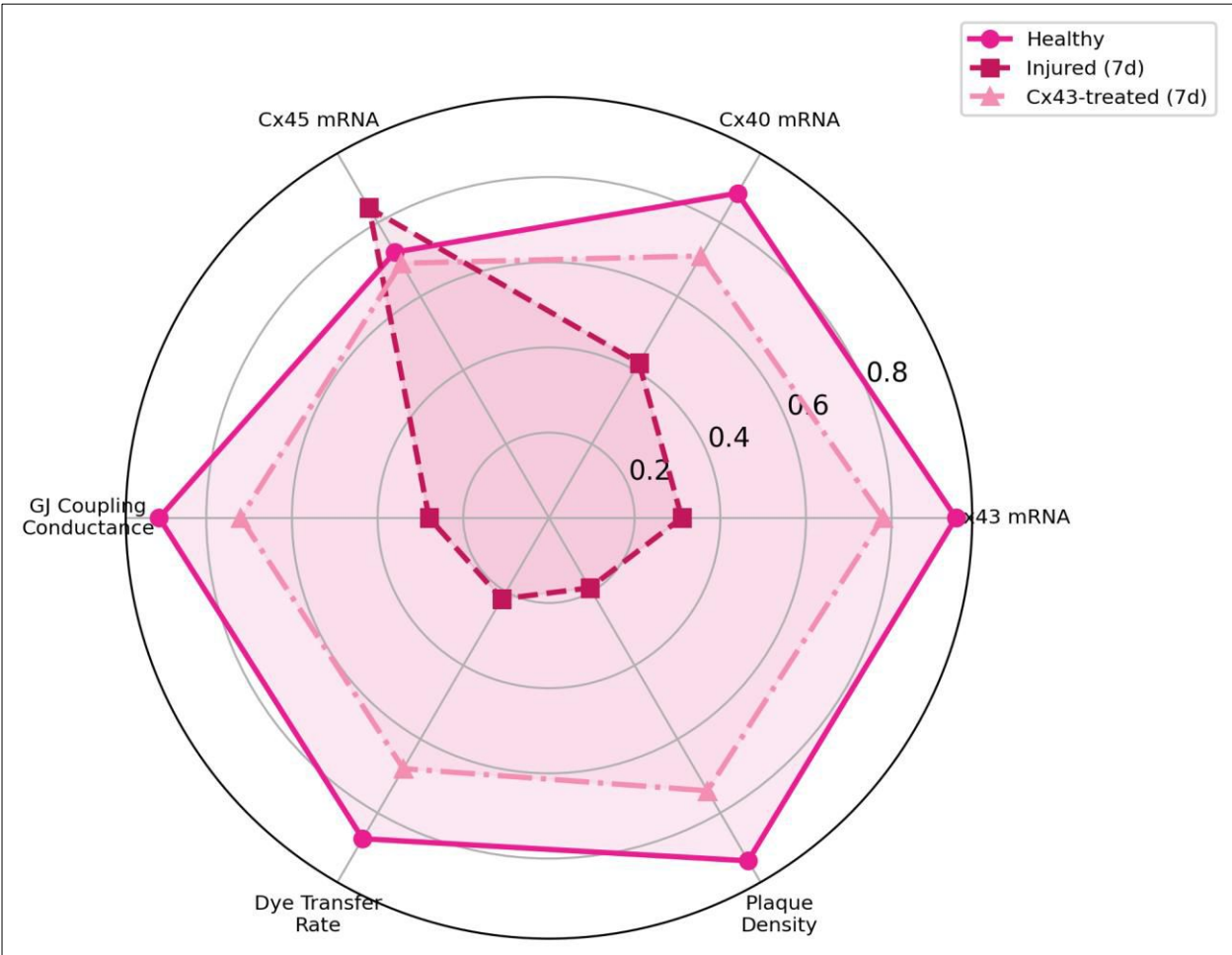


Fig 1: Radar plot comparing six gap junction parameters across healthy, injured (day 7), and Cx43-treated (day 7) cardiac tissue.

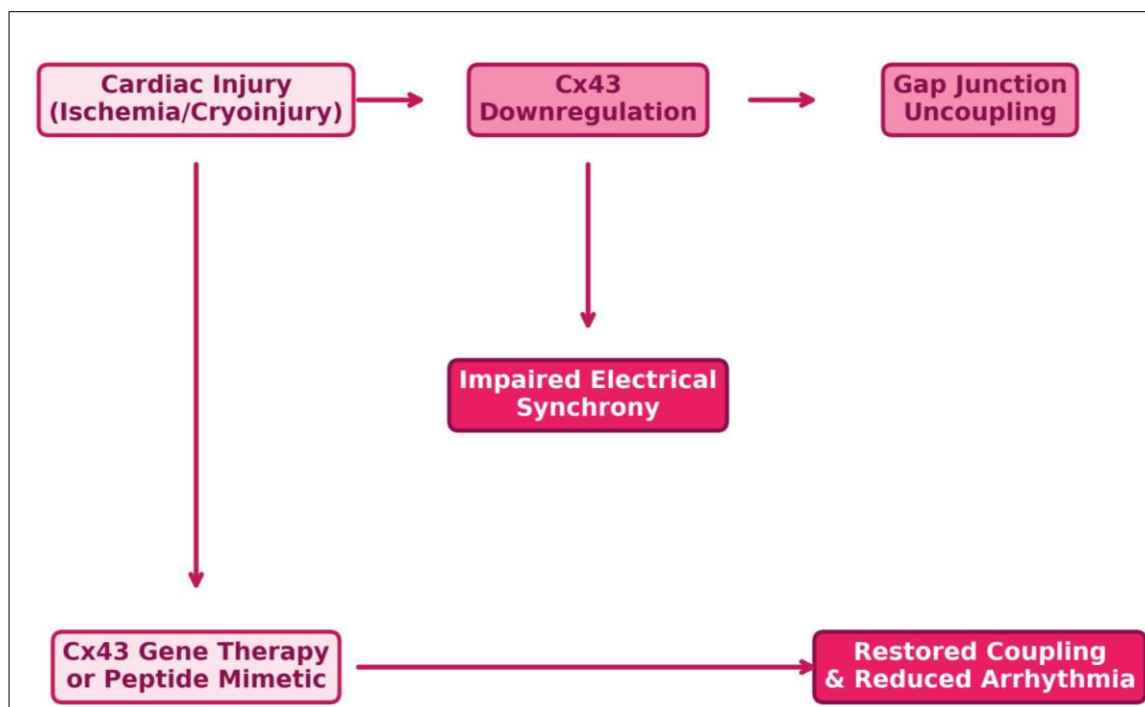


Fig 2: Schematic of gap junction disruption following cardiac injury and the proposed therapeutic mechanism of Cx43 gene delivery.

Discussion

The 67.4% drop in Cx43 protein at day 7 is consistent with published reports of rapid connexin degradation in the infarct border zone^[10]. Ad-Cx43 delivery restored protein to 71.4% of sham levels at this timepoint, and immunofluorescence confirmed that the exogenous Cx43 localized predominantly to intercalated discs rather than lateral membranes—an important distinction, since lateralized Cx43 doesn't form functional channels^[11].

The reduction in arrhythmia inducibility from 83.3% to 25.0% at day 7 suggests that restoring even partial coupling is enough to eliminate the conduction heterogeneity that sustains re-entry circuits^[12]. A limitation is the use of a cryoinjury model, which produces a sharply demarcated lesion rather than the patchy necrosis typical of coronary occlusion^[13]. Whether Ad-Cx43 performs similarly in ischemia-reperfusion models remains to be tested.

Conclusion

This research demonstrated that adenoviral Cx43 gene delivery restores gap junction protein expression, electrical coupling conductance, and dye transfer capacity in the cardiac injury border zone. Treated animals showed markedly reduced arrhythmia vulnerability. Cx43-targeted therapy represents a promising approach for stabilizing electrical function in damaged myocardium.

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