A Connexin43 Mimetic Peptide Promotes Regenerative Healing and Improves Mechanical Properties in Skin and Heart

Emily L. Ongstad,¹ Michael P. O'Quinn,² Gautam S. Ghatnekar,³ Michael J. Yost,⁴ and Robert G. Gourdie,^{1,2,*}

¹Department of Bioengineering, Clemson University, Clemson, South Carolina.

Departments of ²Regenerative Medicine and Cell Biology and ⁴Surgery, Medical University of South Carolina, Charleston, South Carolina.

³FirstString Research, Inc., Charleston, South Carolina.

Significance: Evidence is building that the gap junction protein connexin43 (Cx43) is an important molecule in regenerative healing of skin and heart. Excess scarring from skin wound healing is a continuing clinical problem. Humans generally lack the ability to regenerate tissue following injury, and some degree of fibrotic repair occurs. In the skin, this results in unsightly scars with inferior mechanical properties. In the heart, scarring causes disruption in the contractility of cardiac muscle and increases the risk of deadly arrhythmia. Therapies that tip the balance of wound healing away from scar tissue and toward regeneration would thus represent a significant medical advance.

Recent Advances: A cell-permeant peptide, α CT1 (alpha connexin carboxyl-terminal peptide), based on the carboxyl-terminus of connexin43, has been shown to elicit changes in gap junction organization and intracellular communication. In the skin, α CT1 applied at acute time points results in decreased inflammatory response, reduced area of scar progenitor tissue, and restoration of more normal dermal structure and mechanical strength. α CT1 application to infarcted hearts improved cardiac contractility, reduced the propensity for arrhythmia, and increased conduction velocity through the injured heart.

Critical Issues: Application of therapies like α CT1 could reduce cutaneous scarring and improve mechanical properties of healed skin and the contractile function and electrical stability of the heart following injury or surgery.

Future Directions: α CT1 is a potential therapy for cutaneous wounds that could lead to reduced scarring and improvements in the mechanical properties of healed skin. For injured myocardial tissues, this Cx43 mimetic peptide may also provide a therapeutic approach for targeting pathological fibrosis and reducing the likelihood of sudden death from cardiac arrhythmias.

Robert G. Gourdie, PhD

Submitted for publication July 7, 2011.

*Correspondence: Department of Regenerative Medicine and Cell Biology, Rm: 601/BSB, 173 Ashley Ave., Medical University of South Carolina, Charleston, SC 29425 (e-mail: gourdie@muscc.edu).

Abbreviations and Acronyms

α CT1 = alpha connexin carboxyl-terminal peptide

Cx43 = connexin43

IBZ = injury border zone

MI = myocardial infarction

ZO-1 = zonula occludens 1

SCOPE

SCAR FORMATION AND FIBROSIS are common outcomes following injury to the skin. The healing process culminates in a new and intact cutaneous barrier, but fails to reestablish the structure and function of unwounded

skin. Damage to the skin sets in motion the reparative process, during which prompt wound closure must be balanced against the need for continuation of function.¹ The earliest step of this progressive sequence is hemostasis, with repair then

proceeding through subsequent inflammatory, granulation tissue deposition, and scar remodeling phases. Each step is orchestrated by intercellular interactions, as well as the production and release of signaling molecules necessary for recruiting the various cells involved in the protection, repair, and maturation of the healing tissue.

The wound healing process has been studied extensively in skin and is being increasingly investigated in other tissues. The heart proceeds through a reparative process similar to skin after injuries such as myocardial infarction (MI) or those resulting from cardiac surgery.² The maintenance of cardiac pumping function and the effective coordination of repair processes is an even more critical balance to strike following injury to the heart. A flawed repair could increase the susceptibility of the beating heart to rupture. Excess scarring of myocardial tissues may lead to disruptions in electrical conductivity and increase the susceptibility of the heart to arrhythmias. Fibrosis is also intimately linked to ventricular dilation, intractable declines in contractile function, and eventual death from heart failure. Unfortunately, humans have a limited capacity to regenerate tissues and organs without fibrosis. As such, much ongoing research is focused on developing strategies to prevent excess scar deposition and to regenerate normal structure and mechanical function of damaged tissues.

TARGET ARTICLES

1. Ghatnekar G, O'Quinn M, Jourdan J, Gurjarpadhye A, Draughn R, Gourdie R: Connexin43 carboxyl-terminal peptides reduce scar progenitor and promote regenerative healing following skin wounding. *Regen Med* 2009; **4**: 205.
2. O'Quinn M, Palatinus J, Harris B, Hewett K, Gourdie R: A peptide mimetic of the connexin43 carboxyl-terminus reduces gap junction remodeling and induced arrhythmia following ventricular injury. *Circ Res* 2011; **108**: 704.

TRANSLATIONAL RELEVANCE

A potential therapy targeting scar fibrosis based on the gap junction protein connexin43 (Cx43) is described. Cx43 has been implicated in the spread of injury signals in skin, heart, and neural tissues, modulation of TGF β signaling, and ischemic preconditioning/cardioprotection.² Studies in healing skin wounds on mice have shown

decreased levels and phosphorylation of Cx43 at wound edges and decreases in gap junction intercellular communication at acute time points after wounding.^{1–4} In mice expressing no or reduced levels of Cx43, skin wounds closed more quickly and MI injuries were smaller than in wild-type animals.

CLINICAL RELEVANCE

Cutaneous injuries resulting in excess scarring and the subsequent reduced function of skin are significant continuing clinical problems. Mechanical strength of these wounds is never completely restored. Studies examining surgical wounds have shown that by 1 year post-surgery, a maximum of 70–80% tensile strength is achieved.⁵ MI occurs in over 1.2 million patients per year in the United States.⁶ MI causes cardiac tissue necrosis and fibrosis, and may eventually lead to heart failure. Deadly cardiac arrhythmias can be also prompted by injury-induced fibrosis, and by the attendant reorganization of gap junctions in the damaged heart tissue.^{2,7–9}

EXPERIMENTAL MODEL OR MATERIAL: ADVANTAGES AND LIMITATIONS

Mouse and swine models have been used extensively to study wound healing; the former due to its affordability and ease of use, the latter due to the similarity of porcine dermal structure to human skin. Mice provide an affordable and simple model for wound healing—though the loose skin and inflammatory response of mice differ somewhat from humans. As noted in Ghatnekar *et al.* 2009, the relative thickness of porcine skin has the advantage of enabling elements of histoarchitecture such as subcutaneous complexity in vascular pattern to be more easily resolved and assessed for regeneration than in rodent models.¹⁰ In our studies, full thickness incisional and excisional wounds of controlled size were created in the dermis of mice and swine. Pluronic gel (20%), with or without α CT1, was applied to the injuries created. Injuries were observed for several days to assess healing.

Our lab developed a mouse model of MI in which a liquid nitrogen-cooled cryo-probe was used to create a non-transmural injury on the left ventricle (LV) of the mouse heart.¹¹ The approach was based on a method described by Van den Bos and co-workers for generating a fully transmural LV cryo-injury in mice.¹² This cryo-injury model of MI provides a domain of necrotic tissue of consistent size and shape surrounded by a uniform injury border zone (IBZ). Our model is able to generate a

survivable and more repeatable injury and IBZ than models of MI generated by coronary arterial ligation.¹¹

The mechanism promoting cell death in our model differs from an MI in humans (and other animal models) in which arterial occlusion (or ligation) leads to ischemic death of myocardial tissue. However, this is weighed against the benefit of a model providing a reproducible wound to the ventricle from which most mice survive. Improved survival increases sample size, and a repeatable wound geometry provides increased confidence in the identification of statistically significant structural and functional endpoints. This experimentally attractive combination of attributes is typically not possible with experimental models such as coronary arterial ligation, which produce injuries that vary widely in size and shape and generally result in high mortality rates following the procedure.

The need for procedures enabling normalization of initial wounded areas such as those predicting “myocardial area at risk” is also obviated in our freeze-injury model, as the initial injury size is determined with precision by timed application of a cryo-probe of set dimension. Nonetheless, once confidently identified in the cryo-injury model, the subset of responsive endpoints can be validated in more clinically relevant myocardial injury models such as arterial ligation, radiofrequency ablation, or surgical wounding of the heart.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Gap junctions were first discovered in heart because of their ability to transmit electrical signals between cells.^{2,13} Gap junctions are responsible for the transmission of small (<1000 Da) intercellular signaling molecules and conduct the ion transients responsible for cell-to-cell propagation of the cardiac action potential. They are composed of two hexameric hemichannels of connexins, each from an adjacent cell. Gap junctions in the healthy human ventricle are preferentially localized at the intercalated disc between cardiomyocytes and are composed of the gap junction protein Cx43.

Cx43 in dermal wound healing

Gap junctions in skin are thought to be responsible for intercellular spread of injury signals, inflammation, re-epithelialization, wound closure, and scarring.^{1,2,13–16} Cx43 is expressed in the dermal and epidermal layers of the skin^{3,4,17–19} and has been previously reported as a target in dermal

wound healing.^{4,19} Downregulation of Cx43 mRNA by antisense in mouse led to accelerated healing of skin wounds,⁴ increased TGF β 1 expression, increased fibroblast proliferation and motility *in vitro*, and an increase in granulation tissue formation *in vivo*.¹⁹ Enhanced re-epithelialization of skin wounds was seen with this antisense treatment in a diabetic skin model in mouse.²⁰

The C-terminus of Cx43 is an important regulatory domain.^{1,13,21} We previously reported on a 25 amino acid peptide comprised of an antennapedia cell internalization sequence linked to the CT-most nine amino acids of the full-length Cx43 CT domain.^{22–24} This peptide, which we call α CT1, binds the postsynaptic density 95/discs large/zonula occludens-1 (PDZ) class II-binding domain of zonula occludens-1 (ZO-1), competitively inhibiting the interactions of endogenous Cx43 with ZO-1. ZO-1 is a member of the membrane-associated guanylate kinase protein family and interacts with actin and connexin proteins, among others. More recently, we have demonstrated that α CT1 treatment of injured tissue is associated with upregulation of protein kinase C-mediated phosphorylation of Cx43,^{11,25} which leads to a decrease in gap junction communication and may be protective.

Following incisional wounding of neonatal mice, application of α CT1 peptide led to faster wound closure, reduced inflammation and gaping, and healing with less scarring than control wounds over a 7-day period.¹⁰ The α CT1 peptide also conferred benefit to excisional wounds in adult mice, as wounds closed faster, were less swollen and inflamed, and healed with less discoloration over a 10-day period. Treatment with α CT1 led to a decrease in inflammation compared to controls.^{10,26}

Epidermal histoarchitecture of healed control excisional wounds in adult mice showed little complexity, as rete pegs were absent (Fig. 1). α CT1-treated wounds demonstrated tissue structure closer to unwounded skin with a fourfold increase in rete pegs compared to controls. Swine is a well-accepted model for wound healing since it has skin that is said to resemble that of humans. At 30 days post-wounding, α CT1-treated wounds in swine showed a large number of rete pegs, significantly reduced granulation tissue area, and partial restoration of dermal vascular pattern compared to control wounds.

Strips of skin excised from mice at 1 and 3 months post-injury were extended to breaking to examine the mechanical properties of wounded tissue. In this type of test, failure of the tissue occurs at the weakest point—usually within the healed wound or at the interface between scar and

Figure 1. (A) Model of Cx43 (blue) in the lipid bilayer (red) with its CT (green) bound to ZO-1 (orange). A model of α CT1 is to the right. (B) Histology of healed excisional wounds on adult mice 10 days post-wounding following treatment with α CT1 or vehicle control. α CT1-treated wounds show increased complexity (i.e., rete pegs), which were absent from control wounds. Scale: left images = 0.5 mm; right images = 2 mm. (C) Healing of excisional wounds on adult mice over 10 days following wounding and α CT1 treatment versus vehicle control. α CT1-treated wounds are significantly smaller at 24 hours and 4, 7, and 10 days as compared to controls. Wound redness and scarring are improved in α CT1 treated wounds. Scale = 1 mm. (D) Representative "dog bone"-shaped skin strips stretched to breaking and corresponding force-extension curves of skin from a healed wound (green) versus unwounded skin (black) in α CT1 and vehicle control groups. α CT1-treated wounds required more force to break. Solid arrows indicate breaking point of skin. Open arrows indicate direction of force applied on "dog bone" skin samples. Adapted from Ghatnekar *et al.* (2009),¹⁰ with permission of Future Medicine Ltd. Color images available online at www.liebertpub.com/wound

normal tissue. At 1 month, α CT1-treated wounds were not significantly stronger or more extensible than control wounds (Fig. 1). However, with ongoing scar remodeling by 3 months, significant improvements in the tensile strength and extensi-

bility of healed wounds were found. Indeed, the strain-to-failure of α CT1-treated wounds approximated 90% that of unwounded skin, while the strain-to-failure of control wounds was only 60% of normal skin at failure.

Cx43 in cardiac injury repair

Cx43 is abundant in the heart and is responsible for electrical conduction between cardiomyocytes. When the heart sustains injury, as occurs during myocardial infarction (MI), some hallmarks of the dermal wound-healing response occur, including fibrosis. Since fibrosis in the IBZ disrupts normal tissue and gap junction organization, this can lead to re-entrant arrhythmias. Another important change that occurs after MI is the lateralization of gap junctions in cardiomyocytes of the IBZ.²⁷ While it remains to be definitively established, gap junction lateralization is a strong candidate as a causal factor in the development of re-entrant arrhythmias.^{28,29}

Since we know from *in vitro* data that α CT1 inhibited remodeling of gap junction size and cellular distribution, cryoinjured hearts were treated with α CT1 in order to establish whether the peptide could inhibit junctional remodeling and reduce the occurrence of re-entrant arrhythmias. It was found that α CT1-treated hearts were less likely to develop arrhythmias in response to programmed stimulation and had increased conduction velocity through the injured myocardium. These changes were found hand-in-hand with stabilization of Cx43 at the intercalated discs in the IBZ, as op-

posed to being laterally distributed between cardiomyocytes bordering the scar.

Injury-induced fibrosis is intimately linked to ventricular dilation, decline in cardiac contractile function, and eventual death from heart failure. To track the contractile function of the injured hearts, echocardiography was performed on mice just prior to surgery (baseline) and then again at 1, 4, 6, and 8 weeks following cryo-injury. Figure 2 illustrates a representative M-mode echocardiogram from a heart 8-weeks post-injury and treatment with α CT1 (Fig. 2). Mean diastolic and systolic volumes were significantly decreased in the α CT1-treated group compared to control groups over an 8-week duration, with the most pronounced divergence in LV systolic and diastolic volumes between α CT1-treated and control groups occurring at 1 week. This reduction in ventricular dilation is indicative of improved mechanical function of peptide-treated hearts and may be prognostic that these animals were less likely to progress to heart failure as a consequence of their myocardial injuries.

INNOVATION

Scarring and lack of regeneration in cutaneous wounds continues to be a problem. α CT1 appears to

Figure 2. (A) Left ventricular dilation that occurs after MI leads to changes in shape and thickness of the heart wall and decreases in mechanical function of hearts. (B) M-mode echocardiogram 1-week post-injury. (C) Bar graph of LV diastolic and systolic volume normalized to baseline over 8 weeks post cryo-injury, comparing treatment and control groups ($n = 7$ α CT1 mice; $n = 8$ Rev; and $n = 6$ Veh). (D) Graphs of LV systolic and diastolic volume at 1, 4, 6, and 8 weeks post cryo-injury (** $p < 0.01$, * $p < 0.1$).

improve the ability of skin to heal regeneratively. Current clinical approaches to reduce scarring and improve wound healing typically include the use of wound dressings with a mix of growth factors that require repeated application. When applied at a single acute time point, α CT1 is able to speed up the wound healing process, reduce scar tissue area, and improve mechanical properties of the scar out as far as 3 months post-injury.¹⁰

Our more recent studies in heart show that acute application of α CT1 to an experimental MI limits gap junctional remodeling in the IBZ and reduces arrhythmias. Peptide treatment is also associated with reduced ventricular dilation, a predictive indicator of heart failure. In heart, there presently are no other therapies in clinical practice that effectively reduce re-entrant arrhythmias or inhibit the progression to heart failure.

Technical innovations in the target articles include approaches to whole-mount assessment of initial inflammatory response in skin and a novel protocol for generating a repeatable non-transmural cryoinjury on the mouse left ventricle. Additionally, methodological approaches for delivery of the cell-permeant peptide α CT1 *in vivo* are provided, including formulation of the peptide in F127 pluronic thermogel and methylcellulose patches. The slight detergent properties of pluronic gel make it a good candidate for delivery of α CT1. The thermosensitive gel is applied as a liquid and easily conforms to the skin wound defect. It sets at body temperature and remains intact for several days after application. Methylcellulose can be made into adherent patches that remain immobilized on the heart surface and localize peptide to the injury for up to 48 hours after application.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

While wound healing and scar mitigation therapies have seen significant advances over the years, we are some way from attaining perfectly regenerated tissue after wounding. So, there is still a need for the development of therapies that will have a higher clinical impact. Translation of new wound healing therapeutics to the clinic follows a long time course and approval of novel therapies is necessarily subject to numerous regulatory steps. In our animal pre-clinical models, α CT1 is deliv-

TAKE-HOME MESSAGE

Basic science advances

- Wound healing in skin and heart in pre-clinical animal models can be improved through modulation of the gap junction protein connexin43.
- Gap junction organization and associated electrical properties can be improved in the injury border zone of the heart post-MI in animal models.

Clinical science advances

- Scar size can be reduced and mechanical properties of skin can be improved with application of α CT1 after skin wounding.
- Arrhythmias and left ventricular dilation in an experimental model of MI can be reduced by acute application of α CT1 peptide.

Relevance to clinical care

- Healing of cutaneous wounds, including scar mitigation and diabetic or venous ulcers, may be improved by therapies based on Cx43 C-terminus mimetic peptides.
- Ongoing research on these mimetic peptides may also eventually lead to novel treatment approaches of the presently intractable problem of heart failure.

ered in a pluronic gel. To bring this therapy to the clinic, a clinically acceptable delivery vehicle for the peptide has been developed in compliance with good manufacturing practice. This formulation was developed and tested in an external application on skin wounds in a small phase I clinical trial of 48 human subjects by FirstString Research, Inc. and no safety issues were found. Larger phase II clinical trials for indications in skin are now set to proceed. While efficacy will be a focus of the phase II trials, safety monitoring is paramount. This is imperative since the pre-clinical work indicates efficacy as an anti-arrhythmic drug in the injured heart. Internal application of the peptide in the human heart would require a level of attention to potential safety issues that exceeds those of a less obligate organ or tissue.

FUTURE DEVELOPMENT OF INTEREST

Application of α CT1 to skin and heart has proven effective at reducing time to wound closure, reducing scar tissue area, and improving mechanical properties in the former, and reducing fibrosis and incidence of arrhythmias in the latter. Since this peptide primarily affects gap junctional organization in the cell, and gap junctional connexins are present in almost every tissue of the body, application of this peptide to other injured tissues may be warranted. When foreign materials are implanted into the body, significant amounts of fibrous encapsulation typically occurs, which can lead to failure of the implant or its extrusion from the body. Reducing the amount of fibrosis that oc-

curs in these situations is a noteworthy goal. Cx43 has also been shown to play an important role in skeletal muscle regeneration, as it is expressed early in regeneration of injured muscle, but its expression is decreased at later time points. Aiding the regeneration of skeletal muscle may be possible through modulation of connexins in this tissue. As fibrosis is such a ubiquitous feature of the wound healing response in humans, targeting Cx43 function following damage to brain, spinal cord, and cornea are additional important injury scenarios that warrant further investigation.

ACKNOWLEDGMENTS AND FUNDING SOURCES

This work was supported in part by grants from the NIH (R41AR053798, 2R42AR053798-02A2, HL056728, and HL082802, HL007260, HL095320) and AHA (051283U, 87651). R.G.G. is also sup-

ported as a Medical University of South Carolina (MUSC) Board of Trustees Eminent Scholar.

AUTHOR DISCLOSURE AND GHOSTWRITING

R.G.G. is a member of the Scientific Advisory Board of FirstString Research, Inc., a biotechnology startup company spun off from the Gourdie laboratory (Medical University of South Carolina) that has taken α CT1 to clinical trials for indications in skin wound healing. R.G.G. has modest equity ($\leq 5\%$ of total) in FirstString Research, Inc. G.S.G. is a founder, stockholder, and CEO of FirstString Research, Inc. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was used in the production of this manuscript.

REFERENCES

- Rhett JM, Ghatnekar GS, Palatinus JA, O'Quinn M, Yost MJ, and Gourdie RG: Novel therapies for scar reduction and regenerative healing of skin wounds. *Trends Biotechnol* 2008; **26**: 173.
- Palatinus JA, Rhett JM, and Gourdie RG: Translational lessons from scarless healing of cutaneous wounds and regenerative repair of the myocardium. *J Mol Cell Cardiol* 2010; **48**: 550.
- Richards TS, Dunn CA, Carter WG, Usui ML, Olerud JE, and Lampe PD: Protein kinase C spatially and temporally regulates gap junctional communication during human wound repair via phosphorylation of connexin43 on serine368. *J Cell Biol* 2004; **167**: 555.
- Qiu C, Coutinho P, Frank S, Franke S, Law LY, Martin P, *et al.*: Targeting connexin43 expression accelerates the rate of wound repair. *Curr Biol* 2003; **13**: 1697.
- Jones V, Bale S, and Harding KG: Acute and chronic wound healing. In: *Wound Care Essentials Practice*, edited by Baranowski S and Ayello EA. Lippincott Williams and Wilkins, Ambler, PA. 2006.
- American Heart Association: *Statistical Update: Heart Disease and Stroke Statistics*. AHA, 2011.
- Spach MS, Heidlage JF, Barr RC, and Dolber PC: Cell size and communication: Role in structural and electrical development and remodeling of the heart. *Heart Rhythm* 2004; **1**: 500.
- Cabo C, Yao J, Boyden PA, Chen S, Hussain W, Duffy HS, *et al.*: Heterogeneous gap junction remodeling in reentrant circuits in the epicardial border zone of the healing canine infarct. *Cardiovasc Res* 2006; **72**: 241.
- Kieken F, Mutsaers N, Dolmatova E, Virgil K, Wit AL, Kellezi A, *et al.*: Structural and molecular mechanisms of gap junction remodeling in epicardial border zone myocytes following myocardial infarction. *Circ Res* 2009; **104**: 1103.
- Ghatnekar GS, O'Quinn MP, Jourdan LJ, Gurjarpadhye AA, Draughn RL, and Gourdie RG: Connexin43 carboxyl-terminal peptides reduce scar progenitor and promote regenerative healing following skin wounding. *Regen Med* 2009; **4**: 205.
- O'Quinn MP, Palatinus JA, Harris BS, Hewett KW, and Gourdie RG: A peptide mimetic of the connexin43 carboxyl terminus reduces gap junction remodeling and induced arrhythmia following ventricular injury. *Circ Res* 2011; **108**: 704.
- van den Bos EJ, Mees BM, de Waard MC, de Crom R, and Duncker DJ: A novel model of cryoinjury-induced myocardial infarction in the mouse: a comparison with coronary artery ligation. *Am J Physiol Heart Circ Physiol* 2005; **289**: H1291.
- Palatinus JA, Rhett JM, and Gourdie RG: The connexin43 carboxyl terminus and cardiac gap junction organization. *BBA—Biomembranes* 2012; **1818**: 1831.
- Chanson M, Derouette JP, Roth I, Foglia B, Scerri I, Dudez T, *et al.*: Gap junctional communication in tissue inflammation and repair. *Biochim Biophys Acta* 2005; **1711**: 197.
- De Maio A, Vega VL, and Contreras JE: Gap junctions, homeostasis, and injury. *J Cell Physiol* 2002; **191**: 269.
- Lin JH, Weigel H, Cotrina ML, Liu S, Bueno E, Hansen AJ, *et al.*: Gap-junction-mediated propagation and amplification of cell injury. *Nat Neurosci* 1998; **1**: 494.
- Kretz M, Maass K, and Willecke K: Expression and function of connexins in the epidermis, analyzed with transgenic mouse mutants. *Eur J Cell Biol* 2004; **83**: 647.
- Risek B, Klier FG, and Gilula NB: Multiple gap junction genes are utilized during rat skin and hair development. *Development* 1992; **116**: 639.
- Mori R, Power KT, Wang CM, Martin P, and Becker DL: Acute downregulation of connexin43 at wound sites leads to a reduced inflammatory response, enhanced keratinocyte proliferation and wound fibroblast migration. *J Cell Sci* 2006; **119**: 5193.
- Wang CM, Lincoln J, Cook JE, and Becker DL: Abnormal connexin expression underlies delayed wound healing in diabetic skin. *Diabetes* 2007; **56**: 2809.
- Herve JC: The connexins. *Biochim Biophys Acta* 2004; **1662**: 1.

22. Hunter AW, Jourdan J, and Gourdie RG: Fusion of GFP to the carboxyl terminus of connexin43 increases gap junction size in HeLa cells. *Cell Commun Adhes* 2003; **10**: 211.
23. Rhett JM, Jourdan LJ, and Gourdie RG: Connexin43 connexon to gap junction transition is regulated by zonula occludens-1. *Mol Biol Cell* 2011; **22**: 1516.
24. Hunter AW, Barker RJ, Zhu C, and Gourdie RG: Zonula occludens-1 alters Connexin43 gap junction size and organization by influencing channel accretion. *Mol Biol Cell* 2005; **16**: 5686.
25. Palatinus JA, Rhett JM, and Gourdie RG: Enhanced PKCepsilon mediated phosphorylation of connexin43 at serine 368 by a carboxyl-terminal mimetic peptide is dependent on injury. *Channels (Austin)* 2011; **5**: 236.
26. Soder BL, Propst JT, Brooks TM, Goodwin RL, Friedman HI, Yost MJ, *et al.*: The connexin43 carboxyl-terminal peptide ACT1 modulates the biological response to silicone implants. *Plast Reconstr Surg* 2009; **123**: 1440.
27. Smith JH, Green CR, Peters NS, Rothery S, and Severs NJ: Altered patterns of gap junction distribution in ischemic heart disease. An immunohistochemical study of human myocardium using laser scanning confocal microscopy. *Am J Pathol* 1991; **139**: 801.
28. Peters NS, Coromilas J, Severs NJ, and Wit AL: Disturbed connexin43 gap junction distribution correlates with the location of reentrant circuits in the epicardial border zone of healing canine infarcts that cause ventricular tachycardia. *Circulation* 1997; **95**: 988.
29. Cabo C and Boyden PA: Heterogeneous gap junction remodeling stabilizes reentrant circuits in the epicardial border zone of the healing canine infarct: a computational study. *Am J Physiol Heart Circ Physiol* 2006; **291**: H2606.