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Infarct scar as living tissue

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■ **Abstract** Infarct scar tissue has long been considered inert (acellular, composed simply of fibrillar collagen) and whose function is simply to restore structural integrity to infarcted myocardium and to provide tensile strength that prevents tissue rupture. Technologies of cellular and molecular biology have altered this perspective. Infarct scar is now recognized as living tissue: composed of a persistent population of fibroblast-like cells whose ongoing activity includes a regulation of collagen turnover and scar tissue contraction and which are nourished by a neovasculature. Herein we briefly review these various components of the infarct scar that provide for its dynamic nature and which is relevant to today's interest in preventing heart failure through a rebuilding (regrowing) of myocardial tissue at the infarct size.

■ **Key words** Myocardial infarction – infarct scar – scar neovasculature – tissue engineering – myofibroblasts

Introduction

For over a century, the scar that appears at the site of myocardial infarction (MI) has been considered inert: acellular tissue composed simply of woven fibrillar collagen; its purpose to merely provide tensile strength to the infarct site and thereby resist tissue deformation and prevent myocardial rupture. Despite this simplistic viewpoint, earlier reports recognized that connective tissue continued to accumulate at the infarct site for years after MI and scar tissue was vascularized (17–19). How could this be if there were no fibroblasts present and why peruse acellular tissue?

Today, the transmural infarct scar is recognized as living tissue (7, 28). It is composed of a population of fibroblast-like cells termed myofibroblasts (myoFb) because of their expression of α -smooth muscle actin and resultant contractile behavior. These cells are persistent at the infarct site for years; they do not disappear after the scar has formed (35, 28), as is the case in skin where programmed cell death, or apoptosis, accounts for their disappearance (11). Not only are they persistent, but myoFb of the infarct scar continue to turn over type I and III fibrillar collagens long after scar tissue has restored the structural integrity of the infarcted myocardium. In order to carry out these functions, these cells must be nourished. This is accomplished by a neovascu-

lature. Moreover, this structural protein scaffolding is innervated by postganglionic nerve fibers.

MyoFb collagen turnover is regulated by substances they elaborate *de novo*. These include angiotensin (Ang) II and transforming growth factor- β_1 (TGF- β_1). These substances are soluble and therefore able to traverse the heart's common interstitial space, where they can enhance fibroblast collagen synthesis at sites distant to the MI. The resultant fibrosis that appears over time at sites remote to the infarct represents the majority of connective tissue found in ischemic cardiomyopathy. It is considered the major component of the adverse structural remodeling found in the failing human heart of ischemic origin (2).

Herein we briefly review these various components of the infarct scar that provide for its dynamic nature and define it as living tissue and which are relevant to "regrowing" myocardial tissue at the infarct site.

Tissue repair at the site of infarction

A highly orchestrated process of tissue repair follows the necrotic loss of cardiomyocytes. It begins with an activation of latent matrix metalloproteinases (MMPs) that degrade the existing extracellular matrix and coronary vasculature (8). This proteolytic activity declines by the end of week 1 postMI coincident with the increased expression of MMP inhibitors, termed tissue inhibitors of MMPs, or TIMPs (30). Circulating inflammatory cells that include neutrophils and monocytes/macrophages arrive at the infarct site soon after MI. They respectively contribute to the proteolytic digestion and phagocytosis of infarcted tissue. These inflammatory cells home to the site of MI drawn there by adhesion molecules and chemoattractant cytokines (or chemokines) expressed by endothelial cells of coronary vasculature that borders on the infarct site. Their migration into the infarct site is facilitated by MMP proteolytic activity while their ability to gain access to the infarct site is determined by the nature of the collateral circulation. Inflammatory cells disappear from the infarct site within weeks following MI, a consequence of their programmed cell death.

Fibrillar collagen in scar tissue

Scar tissue is composed predominantly of type I and III fibrillar collagens. Whittaker et al. (34) have examined the alignment of collagen fibers forming the infarct scar and found them to course in an essentially circumferential direction concordant with the predominant alignment of cardiac muscle fibers (23). Figure 1 A identifies the dense fibrillar collagen network found in the infarct scar 4 wks after MI in the rat heart.

Scar myofibroblasts

Myofibroblasts are found at the infarct site soon after the arrival of inflammatory cells. Cells which account for the appearance of myoFb are uncertain. They may include the following: interstitial fibroblasts; adventitial fibroblasts; pericytes; a population of circulating fibroblasts known as fibrocytes; or circulating monocytes or circulating bone marrow-derived progenitor cells that transdifferentiate at the infarct site. It is presumed that TGF- β_1 , elaborated by macrophages, governs the appearance of the myoFb phenotype (10).

MyoFb are first found at the infarct site on day 3 – 4 postMI. They are responsible for formation of the scar via their expression (at mRNA and protein levels) of type I and III fibrillar collagens (9, 28). Their growth and the alignment of fibrillar collagen are spatially regulated by their expression of a tissue polarity gene, *frizzled-2*, also found in *Drosophila* and which accounts for planar polarity, such as alignment of limbs and wings during embryonic development (3).

MyoFb remain at the infarct site and do not undergo apoptosis, as is the case in other injured tissues (e.g., skin) which have the capacity to regenerate parenchyma. In postmortem infarcted human heart tissue, myoFb have been found at the infarct site years after MI (35). In the infarcted rodent heart, we have observed myoFb at the infarct site 6 months postMI (28). Figure 1 B identifies the presence of α -smooth muscle actin-positive myoFb residing in the matrix of the 4-wk-old scar of the infarcted rat heart.

Scar myofibroblast metabolic activity

Scar myoFb have a diverse portfolio of metabolic activities. *In situ* hybridization, *in vitro* autoradiography and immunohistochemistry have shown that these cells express renin, angiotensin converting enzyme (ACE), and angiotensin receptors at the infarct site (16, 21, 25–27, 29).

Studied in culture under serum-deprived conditions, which eliminates circulating renin, angiotensin converting enzyme (ACE) and AngI, myoFb obtained from the 4-wk-old infarct scar are found to express angiotensinogen, cathepsin D, ACE and AngII receptors (15). In the infarcted rat heart these receptors are found to be predominantly of the AT₁ subtype (15, 16, 27). In an autocrine manner, AngII regulates myoFb expression of TGF- β_1 and it is this cytokine that regulates myoFb collagen turnover (4, 15).

In vivo, AngII-induced expression of TGF- β_1 is also involved in the regulation of fibroblast collagen synthesis at the infarct and remote sites and which is abrogated by AT₁ receptor antagonist (31). The continued expres-

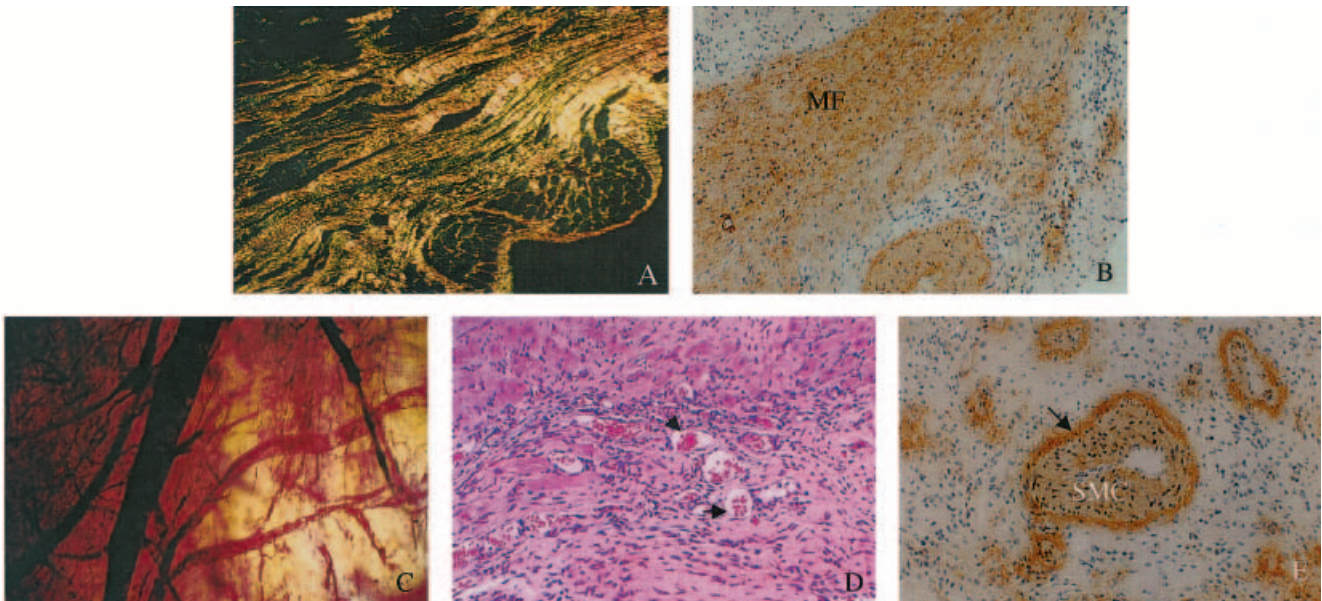


Fig. 1 Collagen deposition, myofibroblasts and blood vessels in a four-week-old infarct scar of the infarcted rat heart. Picosirius red-stained fibrillar collagen, when viewed under polarized light, appears birefringent or yellow in color (A). Alpha-smooth muscle actin-positive myofibroblasts (MF) are found within the infarct scar (B). A carmine red cast of the neovasculature shows vessels of variable dimensions (C). The functionality of these vessels is suggested by the presence of red blood cells (arrowheads) within their lumen (D, hematoxylin & eosin stain). Alpha-smooth muscle actin labeling found in the vascular smooth muscle cells (SMC) and myofibroblasts residing in the adventitia (arrow) of vessels that border on the infarct (E).

sion of ACE and AT_1 receptors and active $TGF-\beta_1$ is observed in the infarcted rodent heart months after MI and underscores their persistent metabolic activity.

Morphology of scar neovasculature

A vascular network begins to form at the infarct site on day 3 postMI. Little is known about its evolution, its ultimate morphologic features and its capacity to deliver and exchange oxygen by conductance and exchange vessels, respectively. Figure 1C provides a carmine red “cast” of the scar neovasculature seen at week 4 postMI. Figure 1D demonstrates the network as seen by light microscopy. Figure 1E identifies α -SMA positive cells found by immunohistochemistry in the neovasculature and which includes vascular smooth muscle cells of arterioles and myoFb present in the perivascular space (or adventitia) of these vessels.

This vascular network is likely derived from several sources: endothelial cell sprouting from neighboring capillaries (angiogenesis); a transdifferentiation of bone

marrow progenitor cells into endothelial cells (vasculogenesis); and an enlargement of neighboring collateral vessels (arteriogenesis).

The scar neovasculature nourishes myoFb and provides for their metabolic activity. The capacity of this network to deliver oxygen and nutrients and the oxygen requirements of myoFb have not been investigated. However, they likely are modest given the ability of fibroblasts to withstand hypoxia (20, 37) and compared to the native coronary circulation it replaced and which formerly had nourished contractile cardiac myocytes, obligate aerobic cells.

Vasomotor reactivity of scar neovasculature

The vasomotor reactivity of the coronary circulation found in the infarct rat heart, including the scar vasculature, was examined by Kalkman et al. (14). Using radio-labeled microspheres and an isolated perfused heart preparation, these investigators found total coronary blood flow to be nearly 10 mL/min with flow to 4-wk-old infarct scar to be approximately 1 mL/min. The low perfusion of scar tissue was evident when coronary flow was expressed per unit tissue weight and when compared to viable, contracting myocardium. These investigators further found an impaired vasodilator reserve to nitroprusside and marked vasoconstriction to vasopressin in the vasculature of scar tissue. In response to vasopressin, flow to the scar was barely detectable. Light microscopy revealed thicker walls of resistance arteries found in scar tissue. Whether this hyperreactivity is related to con-

tractile myoFb of the scar and their presence in the scar neovasculature (Fig. 1 E) has not been addressed.

Scar innervation

Soon after MI and as part of early proteolytic digestion of infarcted tissue, postganglionic neurons are lost at the infarct site. They are regenerated by week 2 and are formed by nerves sprouting from the surrounding non-infarcted myocardium (5, 32). The reinnervation process may even be excessive representing a hyperinnervation of the infarct scar (32). Postganglionic nerve fibers with storage granules are entwined within fibrillar collagen that forms the infarct scar (32). Their function remains unknown, but their presence calls into question a potential contribution of catecholamines to scar tissue contraction and arrhythmogenicity.

Scar contractile behavior

MyoFb and their α -SMA microfilaments are joined to one another through gap junctions and to the scar's fibrillar collagen network via fibronexin. This creates a contractile scar tissue assembly. The now classic study of Gabbiani et al. (13) demonstrated the contractile behavior of scar tissue. Others have confirmed these findings (1, 12). Given the presence of α -SMA in myoFb, present also in vascular smooth muscle cells, it is not surprising that AngII, catecholamines, endothelin-1, serotonin and

vasopressin promote scar tissue contraction while papaverine induces its relaxation. As noted earlier, AngII receptors are present in myoFb of the infarct scar (16, 27) and one would presume other receptors for these ligands are as well, as is the case for myoFb, found in heart valve leaflets (6, 22, 24, 36). The contribution of infarct scar tonus to diastolic dysfunction and scar tissue contraction to the appearance of sudden pulmonary edema has been suggested (33), but remains unknown.

Summary and future directions

Scar tissue that appears following MI is a living tissue. It consists of persistent, metabolically active and contractile myoFb that reside within its fibrillar collagen scaffolding nourished by a neovascular network. Understanding the evolution and maturation of this tissue, including the architecture of its fibrillar collagen scaffolding and the composition and behavior of its vascular network, will be essential if today's interest in rebuilding the infarcted heart is to prove successful. Whether by the grafting of exogenous cardiomyocytes, derived from fetal heart muscle cells or stem cells, or by the homing and transdifferentiation of bone marrow-derived progenitor cells into cardiomyocytes, rebuilt myocardium must ultimately consist of anatomically appropriate, viable and functional myocardial tissue. Such "tissue engineering" will take place in the interstices of the living infarct scar. When and how best to accomplish this goal should prove interesting challenges.

References

1. Appleton I, Tomlinson A, Chander CL, Willoughby DA (1992) Effect of endothelin-1 on croton oil-induced granulation tissue in the rat. A pharmacologic and immunohistochemical study. *Lab Invest* 67: 703–710
2. Beltrami CA, Finato N, Rocco M, Feruglio GA, Puricelli C, Cigola E, Quaini F, Sonnenblick EH, Olivetti G, Anversa P (1994) Structural basis of end-stage failure in ischemic cardiomyopathy in humans. *Circulation* 89: 151–163
3. Blankesteyn WM, Essers-Janssen YPG, Verluyten MJA, Daemen MJAP, Smits JFM (1997) A homologue of *Drosophila* tissue polarity gene *frizzled* is expressed in migrating myofibroblasts in the infarcted rat heart. *Nat Med* 3: 541–544
4. Campbell SE, Katwa LC (1997) Angiotensin II stimulated expression of transforming growth factor- β_1 in cardiac fibroblasts and myofibroblasts. *J Mol Cell Cardiol* 29: 1947–1958
5. Chen PS, Chen LS, Cao JM, Sharifi B, Karagueuzian HS, Fishbein MC (2001) Sympathetic nerve sprouting, electrical remodeling and the mechanisms of sudden cardiac death. *Cardiovasc Res* 50: 409–416
6. Chester AH, Misfeld M, Yacoub MH (2000) Receptor-mediated contraction of aortic valve leaflets. *J Heart Valve Dis* 9: 250–254
7. Cleutjens JP, Blankesteyn WM, Daemen MJ, Smits JF (1999) The infarcted myocardium: simply dead tissue, or a lively target for therapeutic interventions. *Cardiovasc Res* 44: 232–241
8. Cleutjens JPM, Kandala JC, Guarda E, Guntaka RV, Weber KT (1995) Regulation of collagen degradation in the rat myocardium after infarction. *J Mol Cell Cardiol* 27: 1281–1292
9. Cleutjens JPM, Verluyten MJA, Smits JFM, Daemen MJAP (1995) Collagen remodeling after myocardial infarction in the rat heart. *Am J Pathol* 147: 325–338
10. Desmoulière A, Geinoz A, Gabbiani G, Gabbiani G (1993) Transforming growth factor- β_1 induces α -smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol* 122: 103–111
11. Desmoulière A, Redard M, Darby I, Gabbiani G (1995) Apoptosis mediates the decrease in cellularity during the transition between granulation tissue and scar. *Am J Pathol* 146: 56–66
12. Gabbiani G (1998) Evolution and clinical implications of the myofibroblast concept. *Cardiovasc Res* 38: 545–548
13. Gabbiani G, Hirschel BJ, Ryan GB, Statkov PR, Majno G (1972) Granulation tissue as a contractile organ. A study of structure and function. *J Exp Med* 135: 719–734
14. Kalkman EA, van Haren P, Saxena PR, Schoemaker RG (1997) Regionally different vascular response to vasoactive substances in the remodelled infarcted rat heart; aberrant vasculature in the infarct scar. *J Mol Cell Cardiol* 29: 1487–1497
15. Katwa LC, Campbell SE, Tyagi SC, Lee SJ, Cicila GT, Weber KT (1997) Cultured myofibroblasts generate angiotensin peptides *de novo*. *J Mol Cell Cardiol* 29: 1375–1386
16. Lefroy DC, Wharton J, Crake T, Knock GA, Rutherford RAD, Suzuki T, Morgan K, Polak JM, Poole-Wilson PA (1996) Regional changes in angiotensin II receptor density after experimental myocardial infarction. *J Mol Cell Cardiol* 28: 429–440
17. Levine SA (1929) Coronary thrombosis: its various clinical features. *Medicine* 8: 245–418
18. Lodge-Patch I (1951) The ageing of cardiac infarcts, and its influence on cardiac rupture. *Br Heart J* 13: 37–42
19. Mallory GK, White PD, Salcedo-Salgar J (1939) The speed of healing of myocardial infarction. A study of the pathologic anatomy in seventy-two cases. *Am Heart J* 18: 647–671
20. Packer L, Fuehr K (1977) Low oxygen concentration extends the lifespan of cultured human diploid cells. *Nature* 267: 423–425
21. Passier RC, Smits JF, Verluyten MJ, Daemen MJ (1996) Expression and localization of renin and angiotensinogen in rat heart after myocardial infarction. *Am J Physiol* 271: H1040–H1048
22. Pinto JE, Vigiore P, Saavedra JM (1991) Autoradiographic localization and quantification of rat heart angiotensin converting enzyme. *Am J Hypertens* 4: 321–326
23. Streeter DD, Spotnitz HM, Patel DP, Ross J, Sonnenblick EH (1969) Fiber orientation in the canine left ventricle during diastole and systole. *Circ Res* 24: 339–347
24. Sun Y, Diaz-Arias AA, Weber KT (1994) Angiotensin-converting enzyme, bradykinin and angiotensin II receptor binding in rat skin, tendon and heart valves: an *in vitro* quantitative autoradiographic study. *J Lab Clin Med* 123: 372–377
25. Sun Y, Weber KT (1996) Angiotensin converting enzyme and myofibroblasts during tissue repair in the rat heart. *J Mol Cell Cardiol* 28: 851–858
26. Sun Y, Weber KT (1996) Angiotensin-converting enzyme and wound healing in diverse tissues of the rat. *J Lab Clin Med* 127: 94–101
27. Sun Y, Weber KT (1996) Cells expressing angiotensin II receptors in fibrous tissue of rat heart. *Cardiovasc Res* 31: 518–525
28. Sun Y, Weber KT (2000) Infarct scar: a dynamic tissue. *Cardiovasc Res* 46: 250–256
29. Sun Y, Zhang J, Zhang JQ, Weber KT (2001) Renin expression at sites of repair in the infarcted rat heart. *J Mol Cell Cardiol* 33: 995–1003
30. Sun Y, Zhang JQ, Zhang J, Lamparter S (2000) Cardiac remodeling by fibrous tissue after infarction in rats. *Journal of Laboratory & Clinical Medicine* 135: 316–323
31. Sun Y, Zhang JQ, Zhang J, Ramirez FJA (1998) Angiotensin II, transforming growth factor- β_1 and repair in the infarcted heart. *J Mol Cell Cardiol* 30: 1559–1569
32. Vracko R, Thorning D, Frederickson RG (1990) Fate of nerve fibers in necrotic, healing, and healed rat myocardium. *Lab Invest* 63: 490–501
33. Weber KT (1997) Extracellular matrix remodeling in heart failure. A role for *de novo* angiotensin II generation. *Circulation* 96: 4065–4082
34. Whittaker P, Boughner DR, Kloner RA (1989) Analysis of healing after myocardial infarction using polarized light microscopy. *Am J Pathol* 134: 879–893
35. Willems IEMG, Havenith MG, De Mey JGR, Daemen MJAP (1994) The α -smooth muscle actin-positive cells in healing human myocardial scars. *Am J Pathol* 145: 868–875
36. Yamada H, Fabris B, Allen AM, Jackson B, Johnston CI, Mendelsohn FAO (1991) Localization of angiotensin converting enzyme in rat heart. *Circ Res* 68: 141–149
37. Zhang S, Azhar G, Nagano K, Wei JY (2001) Differential vulnerability to oxidative stress in rat cardiac myocytes versus fibroblasts. *J Am Coll Cardiol* 38: 2055–2062