Collagen Fibre Degradation and Re-synthesis after Infarction: Time-course, Structure, and Regulation

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With 3 Figures

Abstract

In the past, myocardial collagen has been regarded as a passive by-stander in the events that occur during and after myocardial infarction. However, the crucial role that collagen plays, particularly in terms of providing strength and stiffness to the heart, is increasingly appreciated. Much progress has been made in recent years in understanding the molecular basis for collagen degradation and synthesis. Nevertheless, as a structural protein, additional insight into collagen's role and importance can be gained by examining the fibres on a macroscopic level. To perform such evaluation requires the utilization of appropriate histochemical technique combined with quantitative structural and image analysis. The use of picrosirius red staining in conjunction with polarized light microscopy has become a common method to assess myocardial collagen; however, the application of other sulphonated azo dyes can provide additional information and advantages for such architectural assessment. We have employed such approaches to examine collagen fiber degradation and re-synthesis after infarction. In conclusion, collagen is not an inert material, and healing after myocardial infarction is not merely a passive process. These facts provide us with many opportunities to intervene in the degradation and re-synthesis process to improve outcome. Furthermore, in contrast to the minutes available to intervene successfully in muscle necrosis, the window of opportunity for successful intervention in healing remains open for days and perhaps even years. Further study of the architectural changes in collagen fiber structure should enable new therapies to be developed.

Zusammenfassung

In the past, myocardial collagen has been regarded as a passive by-stander in the events that occur during and after myocardial infarction. However, the crucial role that collagen plays, particularly in terms of providing strength and stiffness to the heart, is increasingly appreciated. Much progress has been made in recent years in understanding the molecular basis for collagen degradation and synthesis (Jugdutt 2003). Nevertheless, as a structural protein, additional insight into collagen’s role and importance can be gained by examining the fibers on a macroscopic level. To perform such evaluation requires the utilization of appropriate histochemical technique combined with quantitative structural and image analysis. Picosirius red staining in conjunction with polarized light microscopy has become a common method to assess myocardial collagen and is the approach that we have employed to examine collagen fiber degradation and re-synthesis after infarction (Whittaker et al. 1989, 1991a).

Collagen fibers appear surprisingly susceptible to injury; for example, repetitive brief coronary artery occlusion is sufficient to damage fibers even in the absence of muscle necrosis (Whittaker et al. 1991b). Such damage is exacerbated by prolonged ischemia resulting in infarction, and, within days, the extent of collagen damage is sufficient to compromise structural integrity allowing infarct expansion to occur. For example, one crucial structural component is the collagen struts that are aligned perpendicular to the long-axis of the muscle cells (Fig. 1A). Normally, these struts tether the muscle cells and prevent myocyte slippage; however, because these struts are thin, they are vulnerable to the combined effects of enzyme-mediated degradation and the increased mechanical stresses to which they are subjected in the infarct (Whittaker et al. 1991a). Once broken, myocyte slippage and infarct expansion occur even though thicker interstitial collagen fibers remain (Fig. 1B). The repair process begins immediately, and soon new collagen fibers are produced to replace the necrotic muscle and, as these fibers mature, they are able to resist the distending forces and hence stabilize the infarct.

Fig. 1  Rat myocardium stained with picrosirius red and viewed with circularly polarized light. (A) Normal, non-infarcted myocardium. The muscle cells can be identified by their cross-striations, while collagen appears as bright fibers located between the cells. An arrow (upper left) indicates a region between adjacent muscle cells where collagen struts can be seen. (B) Infarct 4 days after coronary artery occlusion. Degradation of the muscle has resulted in the loss of cross-striations. Although thick collagen fibers can still be seen, the thin struts are no longer present.
This healing phase takes several weeks to complete, and during this time collagen can be affected and manipulated in many different ways; for example, by reperfusion of the occluded artery, by administration of pharmacological agents, and also by laser irradiation. Reperfusion, even at a time too late to salvage any muscle, is known to improve outcome. This benefit appears to derive from acceleration in the rate of healing. Less well appreciated influences on infarct healing include pharmacological agents such as calcium-channels blockers and angiotensin converting enzyme inhibitors; for example, amiodipine and quinapril appear, at least in rat models of infarction, to delay healing (Whittaker et al. 2000, Zdrojewski et al. 2002). Although such agents can act directly on collagen synthesis, they may also influence healing by remodeling blood vessels within the scar; specifically, decreasing lumen area and therefore decreasing blood flow (Whittaker et al. 2000). Figure 2 shows the typical appearance of blood vessels within healed infarcts; even though vascular density increases (as illustrated in the left-hand panel), there is a substantial decrease in the lumen cross-sectional area. Interestingly, such lumen decreases are often eccentric and resemble the pattern frequently seen in restenosis after balloon angioplasty of coronary arteries (Fig. 2B). This post-infarction vascular remodeling represents another facet of infarct healing that has yet to be investigated.

![Blood vessels located within healed rat infarcts stained with picrosirius red and viewed in bright-field. (A) A cluster of three small arteries viewed in cross-section. The lumen area in all of these vessels is very small. (B) Large intramyocardial artery showing eccentric proliferation of smooth muscle cells reducing lumen area.](image)

Even after the healing process is complete, there are opportunities to affect the scar. For example, one well-known property of collagen is that it shrinks when heated to a temperature around 70°C (Vangsness et al. 1997). Figure 3 shows two pieces of Achilles tendon before and after heating using laser irradiation. By targeting the heat delivery to specific locations along the tendon, we were able to “sculpt” the tendon. The composition of tendon is similar to that of healed myocardial scar and so the same procedure can be used to “sculpt” the scar. Four weeks after permanent occlusion of the left coronary artery in rats, the epicardial surface of the heart was exposed and laser energy from a
neodymium: ytterium-aluminium-garnet laser (wavelength 1.32 µm) was delivered via an optic fiber held approximately 5 mm above the surface and swept back and forth until scar shrinkage was seen. In this manner, we were able to immediately reduce left ventricular diameter and also ventricular volume, effectively reversing the infarct expansion that had occurred soon after infarction (Whittaker 1999).

![Image of Achilles tendon before and after laser irradiation](image)

**Fig. 3** Pieces of Achilles tendon before (A) and after (B) laser irradiation.

In conclusion, collagen is not an inert material and healing after myocardial infarction is not merely a passive process. These facts provide us with many opportunities to intervene in the degradation and re-synthesis process to improve outcome. Furthermore, in contrast to the minutes available to intervene successfully in muscle necrosis, the window of opportunity for successful intervention in healing remains open for days and perhaps even years. Understanding of the architectural changes in collagen fiber structure after myocardial infarction will enable new therapies to be developed.

**References**


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