Extracellular Matrix Remodeling After Balloon Angioplasty Injury in a Rabbit Model of Restenosis


Abstract
Remodeling of the vessel wall after balloon angioplasty injury is incompletely understood, and in particular, the role of extracellular matrix synthesis in restenosis has received little attention. The objective of the present study was to determine the sequence of changes in collagen, elastin, and proteoglycan synthesis and content after balloon injury and to relate these changes to growth of the intimal lesions and extent of cell proliferation. In a double-injury non–cholesterol-fed model, right iliac arterial lesions in 43 rabbits were treated with balloon angioplasty, and the rabbits were killed at five time points ranging from immediate to 12 weeks. Vessel wall collagen and elastin content and synthesis were measured after incubation with 4C-proline and separation with a cyanogen bromide extraction procedure. Sulfated glycosaminoglycan synthesis was measured after incubation with 35S-sulfate, papain digestion, and ethanol precipitation. Continuous in vivo infusion of bromodeoxyuridine (96 hours) was used to assess cell proliferation. The intimal area significantly increased from 0.27±0.08 to 0.73±0.11 mm² between 0 and 12 weeks. Intimal and medial cell proliferation were modest and peaked at 1 week (labeling indexes of 4.8% and 3.0%, respectively) and then markedly declined by 2 weeks. Significant increases in collagen, elastin, and proteoglycan synthesis, up to 4 to 10 times above control nondamaged contralateral iliac arteries, were noted at 1, 2, and 4 weeks. These increases in synthesis were accompanied by significant increases in collagen and elastin content (by ~35%) that coincided with the temporal increase in cross-sectional area. Our data suggest that extracellular matrix formation is a major factor in the development of the restenosis lesion. (Circ Res. 1994;75: 650-658.)

Key Words: extracellular matrix • restenosis • balloon angioplasty • collagen • elastin • cell proliferation

Restenosis after an initially successful angioplasty of an atherosclerotic plaque remains the major limitation of coronary angioplasty in humans. Attempts to modify the restenosis process by pharmacologic or mechanical approaches have been uniformly disappointing and reflect a basic lack of understanding of the underlying biological mechanisms involved in the vascular response to injury. Restenosis is characterized by intimal hyperplasia, which consists predominantly of smooth muscle cells embedded in an extracellular matrix consisting of collagen, elastin, and several types of glycoproteins (fibronectin, vitronectin, and proteoglycans). The growth of these lesions appears to be dependent on three properties of these intimal smooth muscle cells: proliferation, migration, and extracellular matrix synthesis. Smooth muscle cell proliferation has been assumed to be the dominant cellular event in the restenosis process. However, studies of restenotic human coronary athrectomy specimens have shown that cell division is infrequent and at low levels from 1 to 1100 days after percutaneous transluminal coronary angioplasty (PTCA). Although the time course of smooth muscle cell proliferation after balloon catheter injury to normal vessels has been well characterized in animal models, remodeling of the vessel wall after balloon angioplasty injury is incompletely understood, and in particular, the role of extracellular matrix synthesis in restenosis has received little attention. To date, limited pathological study of the serial changes occurring after PTCA has suggested the importance of the contribution of the extracellular matrix in the development of the restenosis lesions. Recent work by Schwartz et al has indicated that cells account for only ~11% of neointimal volume in human restenosis specimens, with the remaining volume consisting of extracellular matrix. However, these pathological studies have been limited to morphology and provide no biochemical data to assess the matrix synthetic activity of a lesion at a particular point in time. These issues are pivotal, since experimental and clinical approaches to restenosis therapies have tended to focus on inhibiting smooth muscle cell proliferation while ignoring the potential importance of controlling extracellular matrix synthesis and accumulation.

The objective of the present study was to investigate the pathophysiology of the intimal thickening that occurs in the arterial wall after balloon angioplasty injury of a previously injured artery. We also exploited this restenosis model to examine the temporal sequence of changes in collagen, elastin, and proteoglycan synthesis.
and content and the relation of these changes to growth of the intimal lesions and indexes of cell proliferation. In our double-injury rabbit model of restenosis, the first injury ("primary") creates a lesion in the intima, which 3 weeks later serves as the substrate for the second injury ("angioplasty"). This enables the study of matrix changes in abnormal arteries containing fibromuscular plaques rather than in normal vessels, a situation more analogous to human angioplasty, which is performed on a background of atherosclerotic vascular disease.

Materials and Methods

The Model

The animal experiments were performed in accordance with guidelines set out by the University of Toronto and local hospital ethics review committees. We used an adaptation of the double-injury model of Moore and colleagues.19,20 Under general anesthetic (1% to 4% halothane, nitrous oxide, and oxygen), 43 male New Zealand White rabbits, weighing 3.6 to 3.8 kg, underwent surgical cutdown, after which a 4F sheath (Cordis Corporation) was placed in the right common carotid artery. Hemostasis was achieved by using 1000 IU of heparin (Cordis, Miami, Florida), and the catheter was left in place for the duration of experiment (n=8), 2 weeks (n=8), 4 weeks (n=9), and 12 weeks (n=11). These time periods were selected to evaluate the sequence of cell proliferation and matrix formation based on data from single arterial injury experiments in the literature.10,21

Histology

Representative sections of the intima and media were fixed in 4% paraformaldehyde and embedded in paraffin, and 4-μm sections were cut, stained with hematoxylin/eosin, Movat pentachrome, and Alcian blue stain, and examined under light microscopy for evidence of intimal hyperplasia, and extent of fibrous tissue. Cell types were identified in deparaffinized sections by immunostaining performed with monoclonal antibodies directed against smooth muscle cells (α-smooth muscle cell actin, Sigma Chemical Co) and macrophages (RAM-11, Dakopatts) by using an indirect conjugated-peroxidase procedure (AutoProbe III, Biomedica).

Morphometry

Cross-sectional areas of the intima and media were measured by an observer blinded to the timing of the samples. These studies were performed in Movat-pentachrome-stained sections by a computerized morphometric system (IBAS, Kontron Elektronik) using an Axioplan microscope (Zeiss) and an MTF camera (Dage-MTI Inc). A reticle was used to calibrate the programs for each magnification. The images were taken of a low-power (×2.5) view of each Movat-stained vessel. The Movat staining was visually enhanced by delineation and normalization, and dynamic thresholding discriminated the intima and the media. At sites of breaks in the internal elastic lamina, the user could interact with the system to define intima from media.

To determine intimal cell number, nuclear counting was performed by IBAS using Alcian blue-stained sections. These studies require high-power (×20) images, which are enhanced by normalization and dynamic thresholding. Nuclear counts were then made in at least four random fields of known area (comprising 30% to 50% of the total intimal cross-sectional area), and overall cell density was calculated from these measurements. Total intimal cell number was corrected for, by multiplying the intimal cross-sectional area (from the Movat-stained sections) by the cell density in the adjacent Alcian blue sections. This method has previously been validated.6

Indexes of Cell Proliferation

Alzet osmotic pumps (Alza) containing 330 mg bromodeoxyuridine (BrdU) (Sigma), a thymidine analogue, were implanted subcutaneously 96 hours before death in four to six animals from each group. An additional 330-mg bolus was injected subcutaneously at the time of pump insertion and again 24 hours before death to ensure adequate availability. Immunostaining against BrdU was subsequently determined in tissue sections fixed in paraformaldehyde for 2 hours and stained by use of Biomedica's AutoProbe III detection system using a monoclonal antibody (Dakopatts). Endogenous peroxidase was blocked with 1.5% hydrogen peroxide, and nonspecific binding was inhibited with 10% goat serum. BrdU antibody (1:300 dilution) was added for 45 minutes at 37°C, followed by a secondary antibody for 45 minutes. Sections were counterstained with aqueous hematoxylin. The small intestine was used as a positive control and the liver as a negative control.

Collagen and Elastin Content and Synthesis

Collagen and elastin content and synthesis were determined in 1-cm segments obtained from both the treated (angioplasty) and control (nondilated) common iliac arteries immediately distal to the aortic bifurcation, according to the methods of Keeley and colleagues.10 After the separation of adventitia by careful dissection, the vascular segments were incubated with [3H]-proline (0.5 μCi/mL medium, Du Pont–New England Nuclear), ascorbic acid (0.25 mg/mL medium, ICN Biomedical), and DMEM medium (Sigma) enriched with 10% calf serum (GIBCO Laboratories) for 6 hours. Collagen and elastin were separated overnight by a cyanogen bromide treatment (50 mg/mL in 70% formic acid, 1.0 mL per 50 mg tissue, under N2), which solubilizes all proteins except elastin by cleaving methionine bonds. The supernatant, containing fragments of collagen and other proteins, was dried and hydrolyzed in 6N HCl at 110°C for 24 hours. Since collagen is the only protein in the supernatant fraction containing significant amounts of hydroxyproline, collagen content was measured by determining total hydroxyproline of the hydrolyzate by use of a modification of the method of Blumenkrantz and Asboe-Hansen.24 Radioactive hydroxyproline in this fraction was taken as a measure of the rate of collagen synthesis by the tissue. Total DNA per segment was determined by a fluorometric technique in an aliquot removed from the supernatant after cyanogen bromide treatment.

The insoluble residue after cyanogen bromide treatment contained essentially pure elastin on the basis of amino acid analysis, which was consistent with other studies.25,26 The dry weight of the residue was therefore taken as the content of total insoluble elastin in the tissue. The dried residue was then hydrolyzed in 6N HCl at 110°C for 24 hours and reconstituted in distilled water. The radioactivity of the residue was taken as
a measure of the rate of insoluble elastin synthesis by the tissue during the incubation period.

Results of collagen and elastin synthesis were expressed per centimeter segment of artery and per microgram DNA.

\[ ^{35} \text{S-Glycosaminoglycan Synthesis}\]

\(^{35}\text{S-Glycosaminoglycan (GAG) synthesis was determined in the 1-cm segment distal to the arterial segment used for the collagen and elastin studies. The treated and control vessels were incubated for 24 hours in 5 \mu\text{Ci}^{[35]}\text{Sulfate (Dupont–New England Nuclear) and medium enriched with 10\% calf serum. The tissue was then treated overnight with papain digestion (Sigma), and GAGs were precipitated with 80\% ethanol. After removal of the supernatant, the residue was dissolved in 70\% formic acid (Fisher Scientific), and GAG synthesis was determined by scintillation counting. Results of GAG synthesis were expressed per centimeter segment of artery and per microgram DNA.\] Statistics

Data are expressed as mean±SEM. To determine overall differences between PTCA and control groups, a two-way ANOVA with repeated measures was performed for each study. To determine specific differences between the PTCA and control curves, paired \(t\) tests were performed at each time point. To find any temporal differences within either the PTCA or control group, a one-way ANOVA was performed. If significant differences were found within a group, Tukey's studentized range test was performed to determine which time points were significantly different. All statistics were computed by using SAS PC V6.04 (SAS Institute Inc). Statistical significance was defined as \(P<.05\).

\[ \text{Results}\]

\[ \text{Histopathology}\]

The fibrocellular intimal thickening that formed after the primary injury (ie, injury 1) contained smooth muscle cells embedded in matrix and served as the substrate for the angioplasty (injury 2). Immediately after the angioplasty, there was obvious evidence of trauma, including platelet-fibrin deposition along the intimal surface, extensive breaks in the internal elastic laminae, focal intramural hemorrhage, and focal intimal dissections that in some cases extended into the media. Smooth muscle cells showed focal necrosis. At all time points of the study, breaks in the internal elastic laminae were evident in some specimens. The intimal lesions usually were circumferential and concentric, although in some specimens the lesions were quite eccentric (Figs 1 through 4). At later time points, scarring was often present in the media. No histological evidence of foam cells was present. The rare presence of calcium in the media was probably due to focal calcification seen on occasion in the normal media of rabbit arteries.\(^{27}\) At all stages, the intima almost entirely contained smooth muscle cells, with rare macrophages (<1\%) identified by immunohistochemistry.

\[ \text{Morphometry}\]

Control iliac arteries essentially contained no measurable neointima. There was a significant and progressive increase in intimal cross-sectional area of the iliac arteries that underwent angioplasty (Fig 5). Immediately after angioplasty, the intimal area was 0.27±0.08 \text{mm}^2 (which was due to intimal formation after the primary injury). At 12 weeks, the intimal cross-sectional area significantly increased to 0.73±0.11 \text{mm}^2 (\(P<.05\)). Medial cross-sectional areas were unchanged during the course of the study.
Proliferation and Intimal Cell Nuclei Studies

The number of BrdU-labeled cells in the intima (107±24 cells per cross section) and media (52±16 cells per cross section) was maximal at 1 week, corresponding to labeling indexes of 4.8% and 3.0%, respectively (Fig 6). However, this increase was transient and declined by week 2. At week 12, there were very few labeled cells (<3 cells per cross section) in the intima and media. BrdU-labeled cells tended to be localized along the luminal surface of the neointima and often appeared in clusters (Fig 7). Isolated cells were also evident deeper in the neointima and the media. At 1 week, prominent staining was also seen in adventitial cells. Immunohistochemistry showed that the vast majority of the intimal cells were smooth muscle cells on the basis of smooth muscle cell-specific actin staining. The number of intimal cell nuclei had a nonsignificant increase from immediately after angioplasty (1421±156 cells per cross section) to 2 weeks (2433±453 cells per cross section) (Fig 8). No further increase in number was found at 4 and 12 weeks despite the progressive increase in intimal cross-sectional area.

Collagen Synthesis and Content

Although synthesis immediately after angioplasty (injury 2) was similar to control levels, marked increases in synthesis, about five times above baseline values, were noted at 1, 2, and 4 weeks and declined at 12 weeks. Within the PTCA group, there was a significant increase in collagen synthesis between immediately after angioplasty (414±130 disintegrations per minute C14OH-proline per segment) and at 2 weeks (1870±392 dpm C14OH-proline per segment) (Fig 9, top left). Expression of collagen synthesis relative to DNA levels to correct for changes in cell number showed significant differences between the PTCA and control vessels at 1 and 2 weeks (Fig 9, top right). Significant differences in collagen content between PTCA and control arteries were present at all times (Fig 9, bottom left). Within the PTCA group, collagen content was essentially un-
but then increased less but then increased immediately after angioplasty (injury 2) to 2 weeks after angioplasty and then stabilized.

changed from immediately after angioplasty to 4 weeks but then increased by 33% from 4 to 12 weeks, at a time that the absolute collagen synthesis, although still above control values, was actually declining. In control iliac arteries, collagen synthesis and content did not change significantly throughout the study period.

Elastin Synthesis and Content

The synthesis profile of elastin was similar to collagen but less marked. A peak increase in elastin synthesis was measured at 1 week, ~350% above control values, and synthesis remained elevated at 2 and 4 weeks (Fig 10, top left). Expression of elastin synthesis relative to DNA levels still showed increases in the PTCA group immediately and at 1 and 2 weeks, but the increases were not statistically significant (Fig 10, top right). Elastin content was significantly increased in the PTCA vessels compared with control vessels at 1, 2, 4, and 12 weeks (Fig 10, bottom left). Within the PTCA group, there was a significant increase (by 35%) in elastin content at 12 weeks versus immediately after angioplasty. Again, a discrepancy was evident between early increases in elastin synthesis and the later increases in elastin content.

35S-Sulfated GAG Synthesis

The synthesis profile of sulfated GAGs was also similar to collagen (Fig 11, left). There was a small increase in GAG synthesis immediately after angioplasty compared with the control side. Marked increases in synthesis, up to 10 times above control levels, were present at weeks 1, 2, and 4 (peaking at 2 weeks), with a reduction at week 12. When GAG synthesis was corrected for DNA, differences in synthesis between the PTCA and control groups were significant immediately and at 2 weeks and were of borderline significance at 1 week (P<.09) and 4 weeks (P<.07) (Fig 11, right). Within the PTCA group, there were significant increases in the 1- and 2-week arteries compared with 4- and 12-week vessels.

Discussion

Our studies are the first to provide quantitative data on the relation between lesion formation and matrix synthesis and content after balloon angioplasty. Marked and significant increases in synthesis of extracellular matrix proteins (collagen, elastin, and sulfated GAGs) occur in defined segments of the arterial wall after vascular injury. Increases in extracellular matrix protein synthesis (two to four times above immediate levels, depending on the matrix protein) peaked at 1 and 2 weeks.
weeks and remained increased at 4 weeks after the second balloon injury and then gradually decreased toward baseline by 12 weeks compared with control nondamaged contralateral iliac arteries. Enhanced matrix protein synthesis persisted in the injured vessel after corrections for DNA levels. Stimulation of matrix protein synthesis was associated with a delayed accumulation of collagen and elastin in the vessel wall and a progressive, almost threefold, increase in intimal cross-sectional area over the 12 weeks of the study.

The synthetic capacity of activated smooth muscle cells, although recognized in in vitro studies, has not been well studied after balloon injury. In a single-injury model performed in normal rabbit aortas, Helin et al. demonstrated an early increase in hyaluronic acid and water, followed by an increase in chondroitin sulfate content and a later increase in heparan sulfate, dermatan sulfate, and collagen contents. Clowes et al. had previously noted in a single-injury rat carotid model that matrix production contributes to intimal thickening predomi-

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**Fig 10.** Top, Graphs show elastin synthesis per segment (left) and per microgram DNA (right). PTCA indicates balloon angioplasty. In the PTCA group, when data were expressed per segment, significant increases in elastin synthesis were present at 1, 2, and 4 weeks. \( +P < .05 \) vs control. When collagen synthesis was corrected for DNA, the increases in synthesis immediately and at 1 and 2 weeks after angioplasty were not significant. Bottom, Graph shows that elastin content was significantly increased in the PTCA vessels at 1, 2, 4, and 12 weeks. Within the PTCA group, there was a significant increase in elastin content at 12 weeks vs immediately after angioplasty; \( +P < .05 \) vs immediately after angioplasty; \( +P < .05 \) vs control.

**Fig 11.** Graphs show \( ^{35} \)S-glycosaminoglycan (GAG) synthesis per segment (left) and per microgram DNA (right). PTCA indicates balloon angioplasty. Left, Significant differences in synthesis between PTCA and control iliac arteries were noted at 1, 2, and 4 weeks, with a reduction by week 12. In the PTCA group, there was a significant increase in GAG synthesis between 1 and 12 weeks. \( *P < .05 \) vs 12 weeks after angioplasty; \( +P < .05 \) vs control. Right, Increased synthesis in the PTCA group compared with the control group was significant immediately and at 2 weeks after angioplasty and borderline at 1 week \( (P < .09) \) and 4 weeks \( (P < .07) \) after angioplasty. Within the PTCA group, synthesis in 1- and 2-week arteries was significantly higher than in 4- and 12-week arteries. \( *P < .05 \) vs 4 and 12 weeks after angioplasty; \( +P < .05 \) vs control.
nantly after the second week following injury, although these were qualitative observations. The important contribution of extracellular matrix to overall lesion development suggested in the present study is supported by Schwartz et al., who recently calculated that cells account for only ~11% of neointimal volume in human restenosis specimens.

Although the overall effect, based on biochemical and histologic data, of balloon angioplasty was net accumulation of collagen, elastin, and proteoglycans, important and unexpected discrepancies were noted between the changes in synthesis and content of collagen and elastin during the study period. Despite marked increases in synthesis at 1 and 2 weeks, total collagen content was essentially unchanged up to 4 weeks and subsequently increased between 4 and 12 weeks while synthesis rates were declining (although still above control levels). Elastin showed smaller increases in synthesis than did collagen, but content also increased at a time of declining synthesis rates. The most likely explanation for these discrepancies between rates of synthesis and extracellular matrix accumulation is a variable rate of protein degradation during the growth of the lesion, which appears to be an important mechanism in the regulation of matrix protein content. In a rat model of hypertension, similar patterns of collagen and elastin synthesis and content were demonstrated. Evidence from several sources has indicated that insoluble elastin turns over very slowly if at all. However, Johnson et al. showed, in young growing pigs, that despite comparable rates of elastin synthesis in segments of aorta and pulmonary arteries, the pulmonary artery accumulated only 33% of the elastin deposited in the aorta, suggesting enhanced degradation. Similar discrepancies between collagen synthesis and accumulation were also seen between the different vessels. Our data suggest that degradation of newly synthesized proteins is particularly prominent in the first weeks after balloon injury. Accumulation of matrix proteins after balloon injury at later time periods could only have occurred as a result of an imbalance between synthesis and degradation. Enzymes such as metalloproteinases (MMPs) and elastases may be involved in these degradative processes. However, specific MMP and elastase activity in the vessel wall after balloon injury has not yet been characterized. It is also possible that the lack of early accumulation of collagen and elastin in vivo may be due to a true lack of early synthesis and that the ex vivo experiments may represent the synthetic properties of cells in a deregulated state. However, previous work has suggested that the ex vivo and in vivo rates of synthesis of elastin under these conditions are comparable.

Another important hypothesis generated from our data is that smooth muscle cell proliferation itself may be overestimated as the dominant mechanism in intimal hyperplasia and restenosis. In this model, only modest and early smooth muscle cell proliferation was evident. Intimal cell number, which reflects both proliferation and migration, increased during only the first 2 weeks after balloon angioplasty, while neointimal area continued to progressively increase up to 12 weeks. However, the relative contributions of cellular and matrix components to the intimal lesion requires the measurement of intimal volumes, which were not determined in the present study. The importance of cell proliferation in the restenosis process following coronary and peripheral angioplasty in humans has been disputed. Two studies of restenotic human coronary atherectomy specimens using antibodies directed against PCNA (another cell marker for proliferation) have indicated that proliferation is infrequent and at low levels from 1 to 1100 days after PTCA (<1%), which is consistent with our results in the rabbit artery. However, higher levels of proliferation (20.6%) in atherectomy restenosis specimens have been reported by one group.

Neointimal formation has been studied in a variety of animal species and models. Much of the interest in proliferation as a mechanism for remodeling after vascular injury is based on data from normal rat carotid arteries, which have demonstrated an intense proliferative response to balloon denudation and more rapid development of intimal lesions. In previously undamaged vessels in small rodents, thymidine indexes of 66% and >30% at 1 week after vascular injury have been measured in mice and rat carotid arteries, respectively. Although proliferation was assessed with radioactive thymidine labeling in these particular studies, both thymidine and BrdU are incorporated into DNA during the S phase, and Zeymer et al. have shown that there are no differences between the two methods for detecting proliferation. These proliferation studies have stimulated most current approaches to restenosis therapy (ie, block cell proliferation). Our results suggest that the vessel wall response to injury depends on many factors, including the species and underlying substrate (preexisting intimal lesion). Although the rat carotid injury model has made important contributions to our understanding of mechanisms involved in cell proliferation, it may not reflect events occurring in diseased atherosclerotic human arteries that are treated with balloon angioplasty and subsequently restenosis. Similar to our results, Hanke et al. have noted more modest intimal smooth muscle cell proliferation in a rabbit carotid model in which a preexisting plaque is formed by electrical stimulation rather than balloon injury. The double-injury rabbit iliac model used in the present study has several features that mimic human coronary restenosis. First, the absence of cholesterol in the neointima appears to be an advantage over animal models that rely on high cholesterol diets for lesion formation. These lesions typically contain large number of foam cells and are unlike human restenosis lesions. The overall histologic appearance of the neointimal lesions (including the predominance of smooth muscle cells) in our noncholesterol-fed rabbit model resembles human fibrous coronary plaques and restenosis lesions and is distinctive from the cholesterol-fed rabbit model, which is characterized by a large number of macrophages (~50% of intima). Our model also reproduced histologic features of acute angioplasty injury (dissection, endothelial denudation, and intramural hematomas) and the typical appearance of intimal hyperplasia evident in human restenosis lesions. However, the inflammatory component is mild. This differs from atherosclerotic plaques, in which more marked inflammation is present and is often associated with the necrotic area of the plaque. Second, our data on BrdU labeling show low proliferation rates ~14 days after angioplasty, which
seems to simulate the human situation alluded to above. Almost no information is available concerning cell proliferation in the first 2 weeks following coronary angioplasty in humans. Moreover, the progression of lesion severity in the first 12 weeks after angioplasty in our recent study approximates angiographic progression documented in two human coronary angioplasty follow-up studies.49,50

In summary, the present study has indicated that extracellular matrix formation is a major factor in the growth of the restenosis lesion. Matrix synthesis, protein accumulation, and progressive neointimal formation continued for weeks after cellular proliferation (which was quite modest in this model) had returned to low baseline levels. Furthermore, our data have shown that significant amounts of newly synthesized extracellular matrix proteins such as collagen and elastin may not accumulate in the vessel wall, particularly in the early weeks after balloon angioplasty, suggesting that degradation and turnover of extracellular matrix proteins are important vascular events in remodeling and have an impact on the subsequent development of the neointimal lesion. Further efforts are required to delineate the mechanisms controlling the synthesis and turnover of matrix proteins after arterial balloon injury. Ultimately, understanding the processes regulating extracellular matrix deposition should create new opportunities for therapeutic manipulation. Novel pharmacologic approaches that promote or accelerate either the decreased synthesis or increased turnover of these extracellular matrix components could substantially affect restenosis independent of cell proliferation.

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