Journal of Cellular Biochemistry 101:281–294 (2007)

Decorin Synthesized by Arterial Smooth Muscle Cells Is Retained in Fibrin Gels and Modulates Fibrin Contraction

Pamela Y. Johnson, Susan Potter-Perigo, Michel D. Gooden, Robert B. Vernon, and Thomas N. Wight*

Hope Heart Program, Benaroya Research Institute at Virginia Mason, Seattle, Washington 98101-2795

Abstract Fibrin serves as a provisional extracellular matrix (ECM) for arterial smooth muscle cells (ASMC) after vascular injury, yet little is known about the effect of fibrin on ECM remodeling by these cells. To address this question, monkey ASMC were grown on fibrin gels and tissue culture (TC) plastic, and proteoglycan synthesis and accumulation were assessed by radiolabeling. Initial rates of ^{35}S-sulfate incorporation into proteoglycans were identical for both groups, but increased proteoglycan accumulation was observed in cultures grown for 48 h on fibrin. This increased accumulation on fibrin was due to reduced proteoglycan turnover and retention within the fibrin gel. Decorin and biglycan constituted 40 and 14% of the total proteoglycan in the fibrin gels, whereas their combined contribution was only 12% in control matrices. To explore whether the retention of decorin in fibrin had any influence on the properties of the fibrin gel, ASMC-mediated fibrin contraction assays were performed. Both de novo synthesis of decorin as well as decorin added during polymerization inhibited the ability of the cells to contract fibrin. In contrast, decorin added exogenously to mature fibrin matrices had no effect on fibrin gel contraction. This study illustrates that decorin derived from ASMC selectively accumulates in fibrin and modifies fibrin architecture and mechanical properties. Such an accumulation may influence wound healing and the thrombotic properties of this provisional pro-atherosclerotic ECM. J. Cell. Biochem. 101: 281–294, 2007.

Key words: decorin; extracellular matrix; fibrin; contraction; smooth muscle; vascular

Fibrinogen, the precursor of fibrin, is an important risk factor for cardiovascular disease. Epidemiological and biochemical studies suggest that high levels of plasma fibrinogen may promote increased deposition of fibrin in the vasculature [Vasse et al., 1996; Koenig, 1998]. Fibrin has been found in normal arterial intima, and in early and advanced atherosclerotic lesions [Shekhonin et al., 1990; Bini and Kudryk, 1994; Smith, 1994, 1996]. In a balloon-injury model in rabbits, fibrin deposits were detectable in the neointima 2–7 days after injury [Courtman et al., 1998]. As has been described for the progression of wound repair, arterial smooth muscle cells (ASMC) remodel the fibrin matrix to one rich in proteoglycans and hyaluronan, and subsequently, a fibrous, collagen-rich matrix [Bennett and Schultz, 1993; Gailit and Clark, 1994]. However, little is known about progression of events involved in this remodeling. In balloon-injured arteries, a thin layer of fibrin forms at sites of endothelial denudation, and ASMC migrate and proliferate in this provisional matrix between days 4 and 7 after injury [Geary et al., 1998]. However, by day 14, fibrin is replaced by proteoglycans and collagen and fibrosis ensues [Geary et al., 1998]. Thus, ASMC come into contact with fibrin early in the injury response but the effect of fibrin on extracellular matrix (ECM) production by these cells has not been examined.

To investigate this interaction, we have asked whether fibrin directly influences the synthesis and turnover of proteoglycans by ASMC. We found that decorin synthesized by ASMC accumulates in fibrin gels and that this retention modulates ASMC-mediated contraction. We show that mRNA levels for versican, decorin, and biglycan are reduced or unchanged in ASMC grown on fibrin, yet the initial synthetic

Grant sponsor: National Institutes of Health; Grant numbers: 5 P01 HL 18645-27, HL 07312-19, 2 P01 HL 18645-25.

*Correspondence to: Dr. Thomas N. Wight, Benaroya Research Institute at Virginia Mason, Hope Heart Program, 1201Ninth Avenue, Seattle, Washington 98101-2795.
E-mail: twight@benaroyaresearch.org

Received 30 June 2006; Accepted 22 September 2006
DOI 10.1002/jcb.21182

rate for total proteoglycans is not altered. We found that proteoglycan turnover is reduced in fibrin cultures, resulting in increased retention of proteoglycans in the fibrin matrix. Although the retention of both biglycan and decorin in the fibrin matrix increases several fold over the control matrix, decorin is the most abundant of the small proteoglycans in the fibrin gel, and we show that decorin alters the ability of the ASMC to contract the fibrin gel. These observations suggest that ASMC remodel fibrin in such a way as to form a proteoglycan-rich matrix. These proteoglycans in turn impact the volume and biomechanical properties of the fibrin that could potentially influence the development of thrombi, as well as atherosclerotic and restenotic lesions.

MATERIALS AND METHODS

Cell Culture

Monkey ASMC were explanted from the thoracic aortas of pigtail monkeys (*Macaca nemestrina*) as previously described [Ross, 1971]. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with high glucose containing pyruvate, and non-essential amino acids supplemented with 10^5 units/L penicillin, 10^5 units/L streptomycin (Invitrogen, Carlsbad, CA) 5% calf serum (Hyclone, Logan, UT) unless otherwise noted.

To prepare 2-mm thick fibrin gels, 2 mg/ml plasminogen-depleted fibrinogen (Calbiochem, San Diego, CA) in 25 mM 4-(2-Hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES)-DMEM was treated with thrombin (Calbiochem) (0.3125 units/ml final concentration) in HEPES-DMEM containing 70 μM $CaCl_2$ and incubated at 37°C for 90 min. To inactivate residual thrombin, the gels were rinsed twice with PBS and treated with 1 μg/ml of the synthetic irreversible thrombin inhibitor, D-phenylalanyl-L-prolyl-L-arginyl-chloromethyl ketone (PPACK), (Calbiochem) for 30 min at 25°C. Before seeding the cells, the PPACK was removed and the gels were rinsed three times with PBS or DMEM.

Fisher 344 rat ASMC infected with the empty retroviral vector (LXSN) or the retroviral vector (LDSN) containing the complete coding sequence for bovine decorin were prepared as previously described [Fischer et al., 2000]. The LDSN cells were used in some fibrin gel contraction assays and LXSN cells served as controls.

Northern Analysis of Versican, Biglycan, and Decorin

ASMC were cultured in 60-mm dishes for RNA isolation. Total RNA was isolated from ASMC using Trizol reagent (Invitrogen) that is based on the RNA isolation protocol developed by Chomczynski and Sacchi [1987]. Before adding Trizol to cells grown on fibrin matrices, the cell/fibrin layers were snap-frozen in liquid nitrogen and lyophilized to remove water from the gels. To harvest RNA from the cells, 2 ml of Trizol was added to control cultures or prepared fibrin samples. An additional 150 μl of 3 M sodium acetate was added to samples to increase the salt concentration before the isopropanol precipitation. For Northern analysis, 10 μg of total RNA was electrophoresed through 0.9% agarose-formaldehyde gels, transferred to Zetaprobe membranes (Bio-Rad, Richmond, CA) and then UV cross-linked to the membrane. Blots were hybridized with the following proteoglycan cDNA probes: human versican, a mixture of clones F1 and C7 for base-pairs 1–1373 and 2607–6092 specific for the N-terminal and the β-glycosaminoglycan regions respectively [Zimmermann and Ruoslahti, 1989] that recognize both the V0 and V1 splice variants, generously supplied by Erkki Ruoslahti, The Burnham Institute, La Jolla, CA; full-length human biglycan, clone p16 [Fisher et al., 1991] and full-length bovine decorin [Fisher et al., 1989] generously supplied by Larry Fisher and Marian Young, both of the Craniofacial and Skeletal Disease Branch of the National Institute of Health, Bethesda, MD. Hybridization and washing conditions were performed as previously described [Schönherr et al., 1993]. 28S RNA was visualized by staining with ethidium bromide. Autoradiograms were quantitated by scanning and using NIH Image J software. Arbitrary units were normalized to the density of 28S bands.

Radiolabeling of Proteoglycans

ASMC were seeded in 24-well plates (well diameter 16 mm) at near confluence (4×10^4 cell/cm^2) unless otherwise noted on fibrin or tissue culture (TC) plastic. After 24 h incubation, the culture medium was removed and replaced with medium containing ^{35}S-sulfate or ^{35}S-methionine. The final radioisotopic concentration including the volume of the fibrin gels was 80 μCi/ml ^{35}S-sulfate or 40 μCi/ml

^{35}S-methionine. The cultures were incubated with isotope for 24 h labeling periods unless otherwise noted. After the labeling period, media were removed and the cell-associated ECMs were rinsed one time with PBS. To express interstitial media from fibrin gels, the gels were compressed with a pipette tip or rubber policeman. The media, PBS-rinse, and interstitial media were pooled and designated the secreted fraction. The following complement of protease inhibitors (PIs) was added to all samples: 100 mM 6-aminohexanoic acid, 5 mM *N*-ethylmaleimide, 5 mM benzamidine, and 1 mM phenylmethyl-sulfonyl fluoride (PMSF). To isolate the cell-associated ECM proteoglycans, the cell-associated ECM was extracted with 4 M guanidine HCl, pH 5.8 containing 50 mM sodium acetate, 50 mM EDTA, and 2% Triton X-100 plus PIs at 4°C for 15 h. Alternatively, the cell-associated ECM was extracted with 8 M urea, 50 mM Tris-HCl, pH 7.5, 25 mM NaCl, and 2% Triton X-100 plus PIs.

Proteoglycan Analysis

Total ^{35}S-incorporated radioactivity was determined by cetyl pyridinium chloride (CPC) precipitable radioactivity present in the media or cell-associated ECM fractions. Aliquots (50–200 μl) of media or cell-associated ECM extracts were spotted on 3 MM Whatman paper and washed in 1% CPC as previously described [Wasteson et al., 1973]. Alternatively, sample aliquots were added to 200 μl of 2% CPC and precipitated onto nitrocellulose membranes in a dot-blot vacuum manifold and the membranes were washed four times as previously described [Ågren et al., 1994]. The dried filters were cut, placed in liquid scintillation fluid, and counted in a liquid scintillation counter and CPC precipitable counts were normalized to μg DNA. DNA content per well was assayed by measuring relative fluorescence of cellular DNA using either Hoechst dye 33258 [West et al., 1985] or 3,5-diaminobenzoic acid (DABA) reagent [Gillery et al., 1993].

Pulse Chase Analysis of Proteoglycan Turnover

To determine the kinetics of turnover of proteoglycans cultured on fibrin or TC plastic, ASMC were pulsed with 80 μCi/ml ^{35}S-sulfate for 24 h. After addition of fresh unlabeled chase medium, CPC precipitable counts were monitored in the secreted and cell-associated ECM fraction over 24 h. In order to dilute free isotope within fibrin matrix, fibrin (400 μl) was polymerized in a 14 mm polystyrene well placed within a 35-mm well of a 6-well TC plate. This allowed the cells on fibrin to be exposed to sufficient chase media (10.5 ml) so that any continued labeling would be negligible [Lark and Wight, 1986]. ASMC cultured on plastic were seeded in identical 14-mm wells without fibrin. Radiolabeled proteoglycan in the medium and cell-associated ECM was analyzed at 0, 12, and 24 h after addition of chase medium.

SDS–PAGE

To concentrate the cell-associated ECM and media proteoglycans, samples were applied to DEAE Sephacel anion exchange mini-columns equilibrated with 8 M urea buffer (8 M urea, 50 mM Tris-HCl, 0.25 M NaCl, and 2% Triton X-100, pH 7.5). The columns were washed with at least 40 column volumes and the proteoglycans were eluted with 3 column volumes of 8 M urea buffer plus 3 M NaCl. For each sample, equal dpm were precipitated in 1.3% potassium acetate in 95% ethanol. The ethanol precipitates were allowed to dry and then reconstituted in sample buffer. To reveal core proteins, ethanol precipitates of ^{35}S-methionine labeled proteoglycans were resuspended and digested at 37°C with chondroitinase ABC, in a Tris buffer containing BSA, before the addition of sample buffer. For analysis by SDS–PAGE, samples were electrophoresed through 4–12% gradient resolving gels with a 3% stacking gel.

Autoradiograms were scanned and the density of ^{35}S-sulfate labeled versican, biglycan, decorin, and low molecular weight glycosaminoglycan degradation products from the medium and cell-associated ECM were measured using NIH image J software. The percentage of each proteoglycan per lane was determined.

Assays of Fibrin Gel Contraction

Assays of cell-mediated fibrin gel contraction were performed using a modification of the collagen gel contraction method of Vernon and Gooden [2002]. Monkey ASMC, rat LXSN, or LDSN cells were polymerized in disc-shaped fibrin gels of 2.7-mm diameter. The fibrin gels were polymerized in Teflon rings using 0.14 mg/ml fibrinogen and 0.17 U/ml thrombin in 25 mM HEPES buffered DMEM, pH 7.0. In certain experiments, recombinant human decorin (EMP

Genetech, Denzlingen, Germany) was added before or immediately after polymerization of the fibrin gel. After polymerization for 1 h at 37°C, Teflon rings were removed, and the fibrin gels were floated in 25 mM HEPES buffered DMEM for 24 h at 37°C and subsequently fixed in 1% neutral-buffered formalin. The fixed gels were digitally recorded under darkfield illumination with a Leitz-Wild stereomicroscope. Areas of gels were measured using NIH Image software according to the method of Vernon and Gooden [2002].

Analysis of Fibrin Fiber Structure by Light Scattering

Fibrinogen was polymerized in 96-well plate wells in the presence or absence of increasing molar ratios of decorin or ovalbumin to fibrinogen. To determine the effect of added protein on the fibrin fiber structure, the absorbance (A_{350}) was measured in a Molecular Devices plate reader 30 min after thrombin addition. A_{350} as a measure of turbidity closely correlates with fibrin fiber thickness and network permeability [Carr and Hernandez, 1977].

Statistical Analysis

Statistical significance was determined using a two-tailed Student *t*-test. The number of observations within one experiment was represented by n, and the number of total similar experiments was represented by N. The error bars represent standard error of the mean (SEM) unless otherwise noted. Statistical significance with *P*-values <0.05 or <0.01 were designated with a single "*" or double "**," respectively.

RESULTS

Adhesion, spreading, and cell shape were visually indistinguishable in ASMC grown on TC plastic or fibrin for 3 days. Also, there was no significant difference in proliferation and migration on the two substrata during the same time period (data not shown).

Effect of Fibrin on Proteoglycan Accumulation and Proteoglycan mRNA Expression

To determine whether fibrin influences the synthesis and accumulation of proteoglycans by ASMC, cells were radiolabeled with ^{35}S-sulfate for 2, 4, 8, 12, 24, and 48 h and macromolecular radioactivity present in the media and cell-associated ECM extracts of control and fibrin cultures was determined by CPC precipitation (Fig. 1). No differences were observed in total proteoglycans synthesized and secreted into the media by ASMC cultured on either substrate at any of the time points (Fig. 1A). On the other hand, while the initial rates of proteoglycan

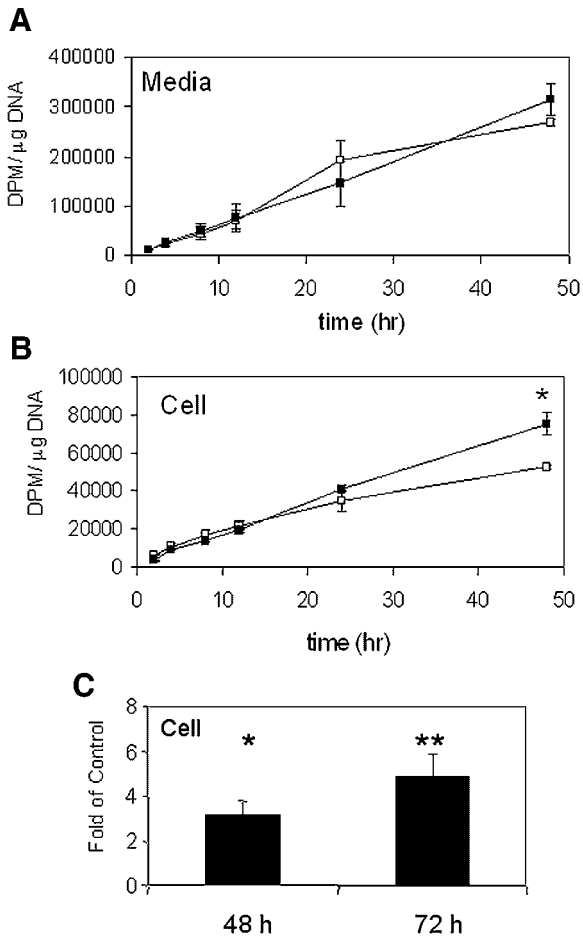

Fig. 1. Proteoglycan synthesis. ASMC proteoglycans were metabolically labeled with ^{35}S-sulfate 24 h after seeding on fibrin or tissue culture (TC) plastic substrata and harvested at times up to 48 h to detect accumulation of ^{35}S-proteoglycan as measured by CPC precipitable radioactivity. Mean total proteoglycan accumulated per well was normalized to µg DNA/well for individual experiments (N = 3). **A**: Proteoglycan accumulated into the media of control (open squares) and fibrin (solid squares) cultures. **B**: Proteoglycan accumulated into the cell-associated ECM of control (open squares) and fibrin (solid squares) cultures. **C**: ASMC were seeded on fibrin or TC plastic substrata for 24 and 48 h, and proteoglycans were subsequently metabolically labeled with ^{35}S-sulfate for 24 h. Radiolabeled material was harvested from the cell-associated ECMs at 48 and 72 h after seeding, and the mean fold increase of five experiments (N = 5) of ^{35}S-sulfate incorporation in fibrin over control cultures was assessed on a per cell basis. Data are expressed as mean ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$.

accumulation in the cell-associated ECM were identical for the first 12 h, significantly more proteoglycans accumulated in the cell-associated ECM of the fibrin cultures by 48 h (Fig. 1B). To further assess proteoglycan accumulation in the fibrin matrix, ASMC were seeded for 24 and 48 h on TC plastic or fibrin before the addition of ^{35}S-sulfate for a further 24 h. Mean proteoglycan accumulation on a per cell basis in the cell-fibrin ECM at 48 and 72 h for five individual experiments were 3.15- and 4.92-fold, respectively, over controls (Fig. 1C). There was no difference in proteoglycan accumulation in the medium between control and fibrin cultures (data not shown).

In order to determine whether fibrin induced changes in mRNA levels of versican, biglycan, and decorin, Northern analysis was performed after 48 h of culture in four individual experiments. The amount of versican and decorin mRNA in ASMC cultured on fibrin gels was reduced to 80 and 30% of control mRNA levels respectively, and these reductions were statistically significant (Fig. 2A,B). Biglycan mRNA was reduced to 50% of control, but this was not statistically significant. These mRNA levels were not accompanied by a reduction in the synthetic rate of proteoglycans and more proteoglycans accumulated in the fibrin cultures than controls by 48 h (Fig. 1A,B).

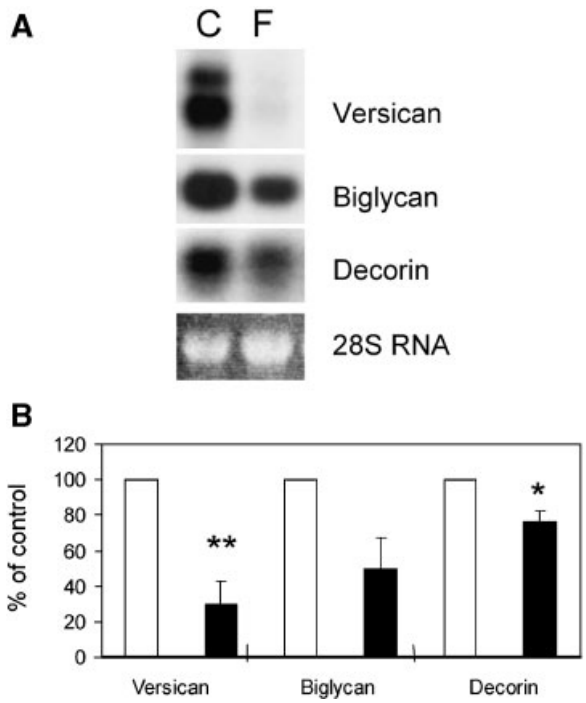

Fig. 2. Northern analysis. **A**: A representative autoradiogram showing the mRNA for versican, biglycan, and decorin in ASMC cultured on plastic or fibrin gels. **B**: Intensity of the bands was quantified and normalized to 28S RNA in four individual experiments (N = 4) and mRNA levels were expressed as % of control where open bars and solid bars represent mRNA from control and fibrin gel cultures respectively. Data are expressed as mean ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$.

Effects of Fibrin on Proteoglycan Turnover

Since neither mRNA expression levels nor initial rates of proteoglycan synthesis were increased in fibrin cultures, the increased accumulation of proteoglycans in fibrin matrices suggests that proteoglycan turnover was altered. To examine the effect of fibrin on proteoglycan turnover, confluent ASMC cultures seeded on plastic or fibrin were pulsed for 24 h with media containing ^{35}S-sulfate and chased with unlabelled media. Media and cell-associated ECM samples were collected at 0, 12, and 24 h. CPC precipitable counts were measured and expressed as percent of radioactivity in the cell-matrix layers at time 0 of the chase. The half-life of the ^{35}S-labeled material deposited in the cell-associated ECM was greater in fibrin gels than on plastic (Fig. 3A). In control cultures, the $t_{1/2}$ was 7.5 h while on fibrin it was greater than 24 h, when 68% of radioactivity remained.

Greater retention of CPC precipitable radiolabeled proteoglycans in the cell-fibrin ECM could be due to reduced degradation or slower release into the medium. To distinguish between these two possibilities, total radioactivity retained in the cell-associated ECM plus the medium was determined (Fig. 3B), and an estimation of degradation was based on a loss of CPC precipitable counts. Significantly more (~100%) CPC precipitable radioactivity was retained in total fibrin cultures than in total cultures grown on plastic. By 24 h after addition of cold chase media, only 40% of the total (medium plus cell-associated ECM) control CPC precipitable ^{35}S-material remains. Therefore, reduced retention of proteoglycans in the cell-associated ECM of control cultures is due to a greater degradation rate and not simply due to release of more proteoglycan into the medium (Fig. 3B).

Accumulation of Versican, Biglycan, and Decorin in Fibrin Matrices

To determine if accumulation of specific types of proteoglycans was altered, equal counts of radiolabeled proteoglycans extracted from the

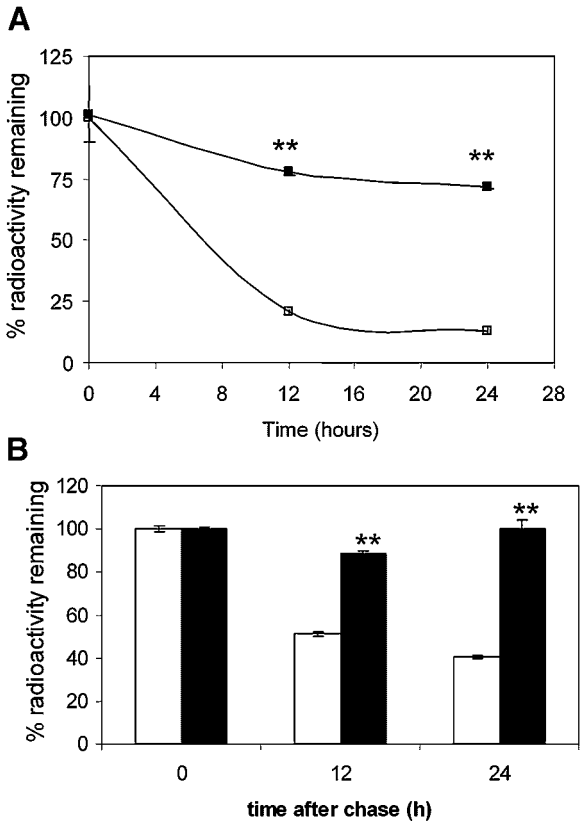

Fig. 3. Proteoglycan turnover. **A**: Pulse-chase analysis. ASMC proteoglycans were metabolically labeled with ^{35}S-sulfate for 24 h. Cold chase medium was added and the percentage of CPC precipitable radioactivity remaining in the cell-associated ECM fractions from cultures grown on plastic (open squares) and fibrin gels (solid squares) was measured at 0, 12, and 24 h. **B**: Loss of proteoglycans from the culture system. Total radioactivity remaining in the medium and cell-associated ECM was greater in fibrin (solid bars) cultures in comparison to TC-plastic (open bars) cultures. Error bars represent standard error of the mean of n = 4 replicates within experiments (N = 2) with similar results. Data are expressed as mean ± SEM. $^{**}P < 0.01$. Error bars in (A) are smaller than the point markers.

media and cell-associated ECM of ASMC cultured on fibrin or plastic substratum were applied to SDS–PAGE in four individual experiments. We have previously found that monkey ASMC primarily produce versican, biglycan, and decorin that separate into discrete bands on SDS–PAGE [Schönherr et al., 1993; Yao et al., 1994]. A representative autoradiogram showed the radiolabeled bands characteristic for versican, biglycan, and decorin were present in media and cell-associated ECM samples from both culture conditions (Fig. 4A). To determine the relative amount of radioactivity in each band, densitometric scans of SDS–PAGE autoradiograms were taken and analyzed by NIH Image J software. Care was taken to select autoradiograms for quantitation in which proteoglycan bands were not saturated. The prominent band, Band 2, at the beginning of the resolving gel was comprised of a versican complex (unpublished result, Lemire and Wight), and its radioactivity was added to that of the main versican band (Fig. 4A). In four individual experiments, no significant differences in the relative density of bands representing intact versican, biglycan, or decorin were observed in the medium of control and fibrin cultures where versican, biglycan, and decorin comprised 60, 10, and 30%, respectively (Fig. 4B). The cell-associated ECM samples from one experiment did not run cleanly by SDS–PAGE. Because the autoradiogram appeared smeared and sufficient radioactivity did not remain to repeat the SDS–PAGE, cell-associated ECM data from one experiment was omitted from the analysis. However, in the cell-associated ECM fraction of three experiments, relative densities of the decorin and biglycan bands were increased in the cell-fibrin ECM. Versican was not significantly changed as a percentage of the total when compared to controls (Fig. 4A lanes 3,4) and was about 50% (Fig. 4C). Data from size exclusion chromatography support the proteoglycan percentages found using SDS–PAGE. Although biglycan and decorin cannot be resolved with Sepharose CL2B size exclusion chromatography, ^{35}S labeled versican eluted in one peak that represented approximately 55% of the total labeled material from both control and fibrin cultures (data not shown). In control cultures, only $4.5 \pm 1.0\%$ and $7.4 \pm 1.9\%$ of the ^{35}S-sulfate labeled material found in the cell-associated ECM migrated as biglycan and decorin respectively whereas in fibrin cultures, $14 \pm 2.7\%$ and $40 \pm 2.3\%$ of ^{35}S-sulfate labeled bands were biglycan and decorin. Radiolabeled material of $36 \pm 3.6\%$ in the cell-associated ECM of control cultures migrated as a broad band of low molecular weight (100–50 kD) (Fig. 4A, lane 3,4C). Previous studies have shown that this material represents degradation fragments of glycosaminoglycan chains that may contain small protein components [Yeo et al., 1992]. Treatment of these so-called GAG degradation products with protease does not shift their elution profile using size exclusion chromatography [Chang et al., 1983]. On the other hand, there was a negligible amount of material in that

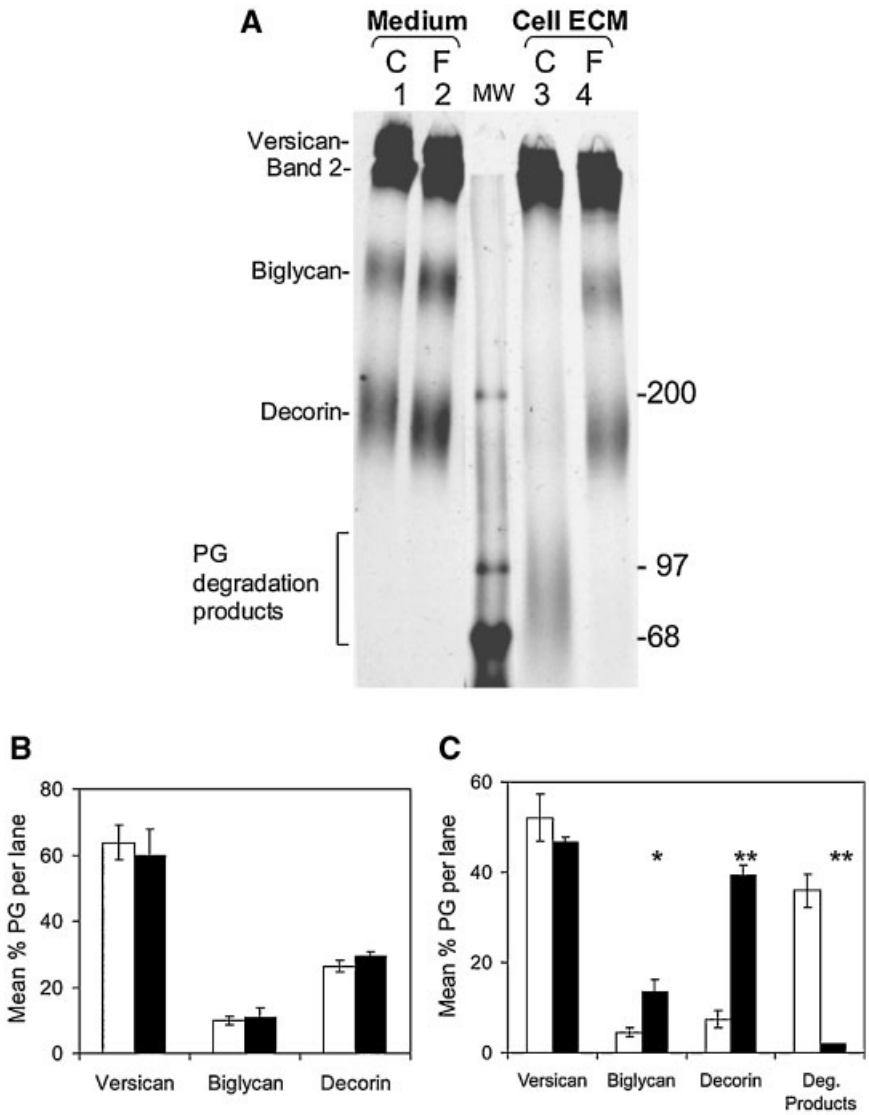

Fig. 4. Redistribution of decorin and biglycan in the cell-associated ECM of fibrin cultures. **A**: ASMC grown on fibrin or TC plastic were radiolabeled from 24 to 48 h after seeding. Equal amounts of ^{35}S-sulfate labeled medium and cell-associated ECM proteoglycans were applied to SDS–PAGE. A representative autoradiogram is presented. **Lanes 1** and **2** medium; **3** and **4** cell-associated ECM. Lanes 1 and 3 control (C); 2 and 4 fibrin (F). The density of ^{35}S-sulfate labeled versican, biglycan, decorin, and low molecular weight glycosaminoglycan degradation products from the (**B**) media (N = 4 experiments) and (**C**) cell-associated ECM (N = 3 experiments) of control (open bars) and fibrin (solid bars) cultures were measured by scanning and NIH image J software and the percentage of each proteoglycan per lane was determined and averaged for N experiments. Data are expressed as mean ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$.

size range in the cell-fibrin ECM. Absence of fragments of degraded glycosaminoglycan from the cell-fibrin ECM is consistent with prolonged turnover times demonstrated in Figure 3A.

Chondroitinase ABC digestion of ^{35}S-methionine labeled proteoglycans from monkey ASMC reveals the protein cores of versican, biglycan, and decorin. Equal counts of ^{35}S-methionine labeled material were applied to SDS–PAGE to confirm that the proteoglycan increases observed in the fibrin matrix are due to increased deposition of protein core and not due to changes in the glycosaminoglycan moiety. Core proteins for versican separate into two bands with the apparent molecular weights of 455 and 350 kD [Schönherr et al., 1991]. An unidentified 230-kD protein core was notably reduced in the cell-associated ECM of fibrin cultures, but this result was not consistent in all experiments. This band may represent a degradation product

of versican. In a study with human ASMC, degradation products of versican in the molecular weight range of 170–260 kD have been identified using a polyclonal antibody specific for human versican Western blotting [Evanko et al., 2001]. Doublet core proteins for biglycan and decorin have overlapping approximate molecular weights of 45 and 50 kD [Schönherr et al., 1993]. Analysis of the proteoglycan core proteins from the media confirmed that there was no difference in the relative proportions of versican, biglycan, and decorin (Fig. 5, lanes 1,2). In the cell-associated ECM, ^{35}S-methionine labeled versican accumulated similarly in fibrin and control cultures. Biglycan and decorin were retained in the fibrin gel, but detection of them in the cell-associated ECM of control cultures was negligible (Fig. 5 lanes 3,4).

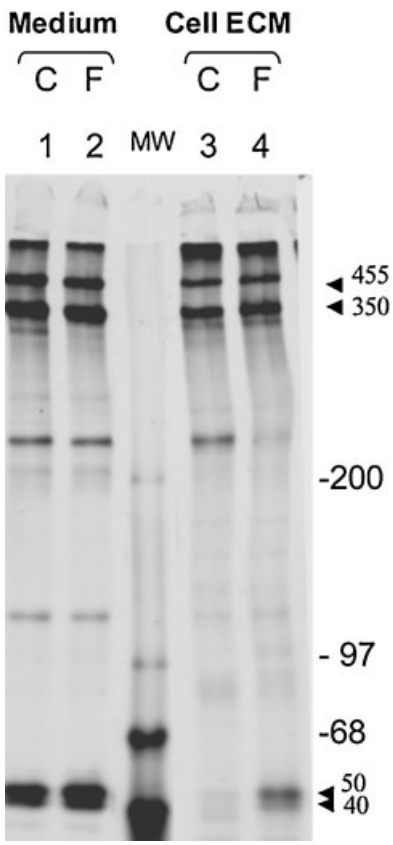

Fig. 5. Analysis of proteoglycan core accumulation in fibrin matrix. ^{35}S-methionine labeled material was digested with chondroitin ABC lyase to reveal core protein bands. Equal amounts of ^{35}S-methionine labeled media and cell-associated ECM proteoglycans isolated from ASMC grown on fibrin or TC plastic were applied to SDS–PAGE. A representative autoradiogram of one of two similar experiments is presented. **Lanes 1** and **2** medium; **3** and **4** cell-associated ECM. Lanes 1 and 3 control (C); 2 and 4 fibrin (F).

To determine the absolute amount of each ^{35}S-sulfate labeled proteoglycan synthesized, densitometric scans of SDS–PAGE gels were used to determine the percent distribution of radioactivity that represented each intact proteoglycan (Fig. 4). The percent distribution of radioactivity was then multiplied by total CPC precipitable counts. Total CPC-precipitable counts did not vary significantly in media samples (data not shown) but were increased threefold in cell-associated matrix of fibrin cultures radiolabeled from 24 to 48 h (Fig. 1C). The versican, biglycan, and decorin secreted into the medium were the same for both treatments (Fig. 6A). The mean absolute amount of radiolabeled versican retained in the fibrin gel for three individual experiments was almost fourfold more than that found in matrices of cells cultured on control plastic (Fig. 6B). The total amount of versican did not differ significantly between control and fibrin cultures although fibrin influences its compartmentalization (Fig. 6C). On the other hand, biglycan and decorin accumulation in the fibrin gel dramatically increased 12-fold and 20-fold respectively when compared to their accumulation in control matrices (Fig. 6B). The increased retention of decorin in total culture did not merely represent a change of distribution between compartments because it represented a statistically significant 2.5-fold increase in total accumulation (Fig. 6C).

Decorin Influences Fibrin Architecture and Contraction

Although both biglycan and decorin accumulation by ASMC in the fibrin matrix increased dramatically, decorin is substantially more abundant than biglycan. In addition, it has been shown that decorin binds directly to fibrinogen [Dugan et al., 2003]. Therefore, we hypothesized that the presence of decorin in the fibrin matrix could have a functional significance for ASMC behavior. About 39% of the proteoglycan produced by ASMC in the fibrin-cell ECM was decorin (Fig. 4C). To study whether decorin could influence ASMC interaction with the fibrin matrix, cell-mediated fibrin gel contraction assays were performed either in the presence or absence of exogenously added human recombinant decorin.

When recombinant human decorin was added after fibrin polymerization, fibrin contraction by

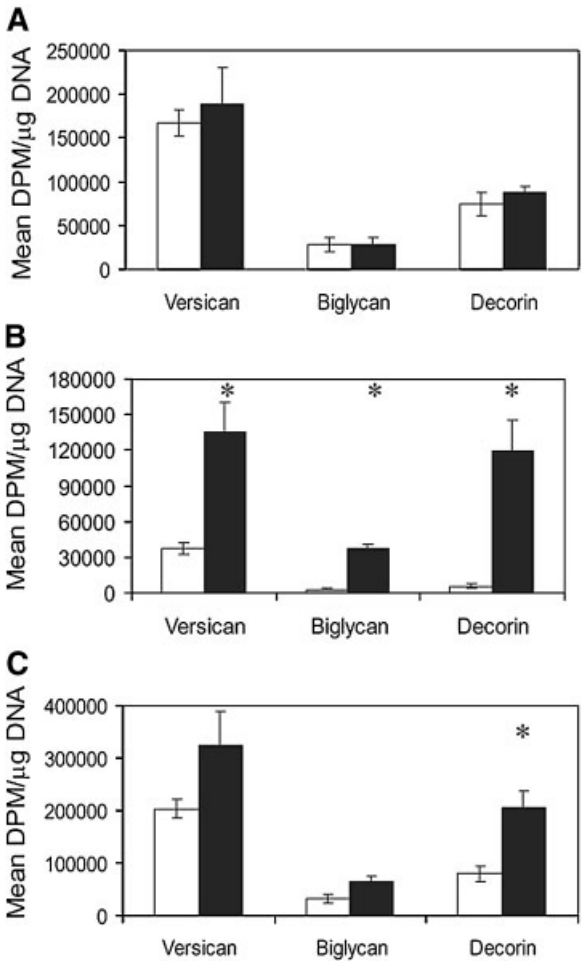

Fig. 6. Increased retention of proteoglycans by fibrin matrix. Mean total accumulation of versican, biglycan, and decorin in the medium and cell-associated ECM per μg DNA by ASMC cultured on TC plastic (open bars) or fibrin gels (solid bars). The absolute amount of each proteoglycan per μg DNA in the medium and cell-associated ECM preparations was calculated by the following steps: (1) Densitometric scans of SDS–PAGE autoradiograms (Fig. 4) were used to determine the percent of the total represented by each band. (2) Total CPC precipitable incorporated ^{35}S-labeled material per μg DNA was determined. (3) The total material was multiplied by the percent incorporation for each molecule to yield the absolute amount of ^{35}S-labeled material per μg DNA for each proteoglycan. **A**: Medium (N = 4). **B**: Cell-associated ECM (N = 3). **C**: Total: Medium plus cell-associated ECM (N = 3).

ASMC was not changed (Fig. 7A). However, when human recombinant decorin was added to fibrinogen before polymerization, ASMC-mediated fibrin contraction was significantly reduced relative to controls lacking decorin (Fig. 7B). This reduction in contraction may be due to a change in fibrin architecture since the addition of decorin to fibrin reduced the turbidity of the fibrin gels in a dose-dependent manner (Fig. 7C). The decrease in turbidity suggests that the interaction of decorin with fibrinogen during polymerization of the fibrin alters the architecture of the fibrin network by forming finer fibrils with a lower mass to length ratio [Carr and Hernandez, 1977; Hayen et al., 1999]. Additional evidence that decorin altered ASMC interactions with fibrin was demonstrated using Fisher 344 rat ASMC that overexpress decorin. In previous studies, rat ASMC were stably transfected with the retroviral vector that contains the bovine decorin gene (LDSN) or control empty vector (LXSN). LDSN-transfected ASMC secreted 30 μg bovine decorin per 10^7 cells over a 24-h period [Fischer et al., 2001]. De novo synthesis of decorin attenuated fibrin gel contraction. LDSN ASMC exhibited a reduced ability to contract the fibrin matrix when compared with control LXSN ASMC (Fig. 7D). In three experiments, 2.2- to 3.8-fold less contraction was observed for LDSN cultures.

DISCUSSION

Fibrin deposits in the normal intima and in atherosclerotic lesions are associated with lesion development [Smith, 1994], but little is known as to whether fibrin influences remodeling of the ECM during lesion development. This study demonstrates that fibrin alters proteoglycan turnover by ASMC resulting in increased total proteoglycan accumulation and retention of decorin in the cell-fibrin ECM. The retention of decorin in fibrin alters ASMC-mediated fibrin contraction and may impact other aspects of ASMC biology.

Decorin interacts with a number of ECM molecules including several collagens [Bidanset et al., 1992; Pogány and Vogel, 1992; Font et al., 1993], fibronectin [Schmidt et al., 1991], thrombospondin [Winnemöller et al., 1992], and tenascin [Elfterior et al., 2001]. It has recently been demonstrated that decorin binds fibrinogen in a zinc-dependent manner [Dugan et al., 2003]. While it is not known whether biglycan or versican bind directly to fibrin, their retention in fibrin gels may protect them from proteolysis. SDS–PAGE of ^{35}S-sulfate labeled proteoglycan from control cell-associated ECMs revealed low molecular weight (50–100 kD) fragments that do not appear in fibrin cultures. The 230 kD band that is notably reduced in the cell-associated ECM of fibrin cultures may be a versican fragment. Although the appearance of

this band varied between experiments, its reduction in fibrin cultures is consistent with our finding that proteoglycans associated with the fibrin gel are not degraded as rapidly as in control matrices. The mechanism by which fibrin reduces proteoglycan degradation is not known, but other studies suggest that this protection is not due to inhibition of matrix metalloproteinase activity by fibrin. Several metalloproteinases can degrade fibrin and some metalloproteinases enhance cell invasion and migration into fibrin-ECM [Hiller et al., 2000; Lelongt et al., 2001; Hotary et al., 2002]. It would be of interest to examine the cell-associated ECM from control and fibrin cultures for proteolytic activity to identify whether decreased proteolytic activity in fibrin cultures is solely responsible for reduced proteoglycan degradation.

The degradation of proteoglycans primarily occurs intracellularly in a variety of cell types. Studies have demonstrated that decorin and biglycan are most likely degraded via two endocytic pathways during which they are either completely degraded to free ^{35}S-sulfate or glycosaminoglycan degradation products attached to peptides [Yanagishita and Hascall, 1984]. The increased retention of decorin in the cell-fibrin ECMs may in part be due to reduced endocytosis of decorin bound to fibrin. It has been shown that decorin bound to collagen exhibits reduced endocytosis [Bhide et al., 2005]. The persistent presence of decorin makes it available to interact with various ECM components, and these interactions may influence matrix assembly and integrity. Several studies have demonstrated that the presence of decorin stabilizes ECM matrix assembly [Vogel et al., 1984; Danielson et al., 1997; Fischer et al., 2000; Kinsella et al., 2001]. In vivo, the morphology of collagen fibrils formed in the decorin null mouse is irregular and the skin of

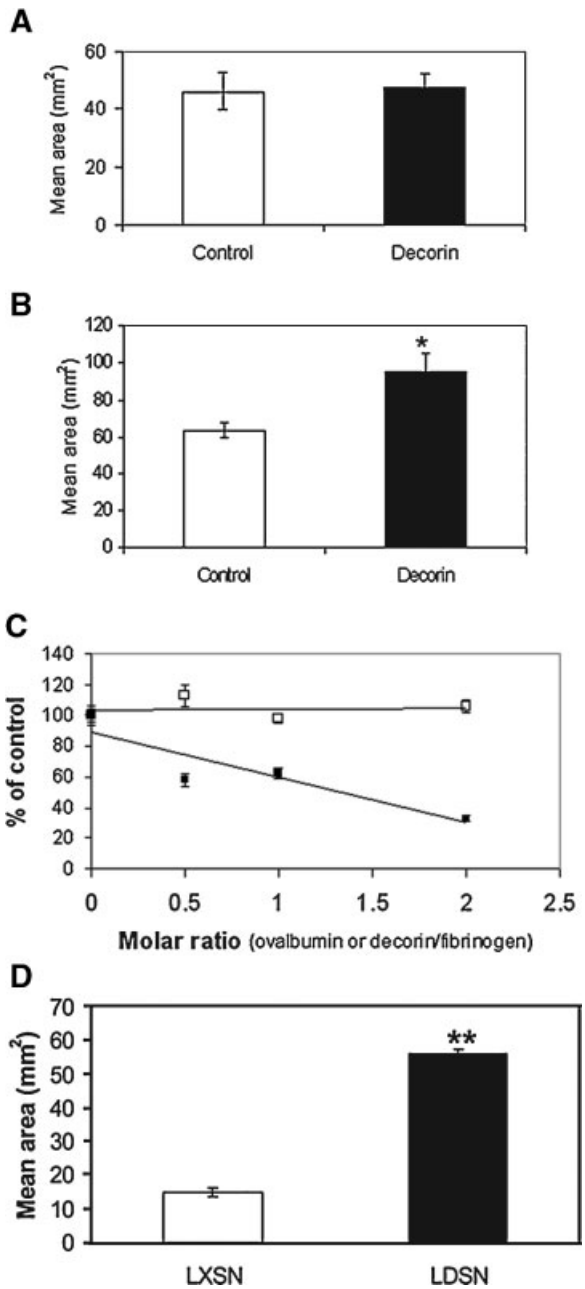

Fig. 7. Decorin attenuates fibrin contraction by ASMC when decorin is present during fibrin polymerization. **A**, **B**: Monkey ASMC in fibrin gels (9×10^5 cells /gel) were supplemented with control medium (HEPES buffered DMEM) or with recombinant human decorin at a 1:3 molar ratio of decorin to fibrinogen, either after (A) or before (B) polymerization of fibrin in Teflon rings with thrombin addition. Subsequently, rings were removed to float gels in medium and gels were assayed for contraction after 24 h of culture. A representative graph from one of two similar experiments of mean gel area for n = 3 replicates of control fibrin gels (open bars) and decorin fibrin gels (solid bars) is presented. **C**: The effect of decorin on fibrin gel turbidity. Decorin (solid squares) or the control protein, ovalbumin (open squares), was added to fibrinogen at the indicated molar ratio, and the absorbance (A_{350}) was measured and expressed as percent of control fibrin gel containing no addition. Data from experiments (N = 2) are expressed as mean % of control ± SEM. **D**: LDSN ASMC that overexpress decorin contract fibrin gels less than cells expressing empty control (LXSN) vector. LDSN or LXSN cells (9.6×10^5 cells/gel) were cultured. A representative graph from one of three similar experiments of mean gel area for n = 4 replicates from control LXSN fibrin gel cultures (open bars) and LDSN fibrin gel cultures (solid bars) is presented. Contraction data are expressed as mean area ± SEM. $^*P < 0.05$ and $^{**}P < 0.01$.

these animals is abnormally fragile [Danielson et al., 1997]. Studies in our laboratory demonstrated that overexpression of decorin in aortic endothelial cells results in increased fibronectin fibrils surrounding the cells [Kinsella et al., 2001]. When the carotids of balloon-injured rats are reseeded with ASMC that overexpress decorin, the resulting neointima contains a highly organized collagen network [Fischer et al., 2000]. In this model, decorin appears to promote a stable collagen network that could profoundly impact the strength, contractility, and degradation of the ECM.

A study of cartilage degradation demonstrated that the collagen fibrillar network protects decorin from extensive proteolysis [Sztrolovics et al., 1999]. It was also found that interaction of decorin and other small leucine-rich proteoglycans protect the collagen network from degradation during early phases of cartilage catabolism [Sztrolovics et al., 1999]. If the incorporation of these small leucine-rich proteoglycans into the fibrin matrix stabilizes the fibrin network in a similar fashion, then proteoglycan-enriched fibrin matrices may resist fibrinolysis. We found that decorin alters fibrin structure and mechanical properties. Because the protease thrombin catalyzes the polymerization of fibrin, it is important to know whether thrombin cleaves decorin directly. The human amino acid sequence of decorin was analyzed using the Web based ExPASy Peptide Cutter tool, and no thrombin cleavage sites were identified. Thus, decorin was probably not altered by thrombin during fibrin polymerization [Gasteiger et al., 2005]. It is possible that decorin retained in fibrin gel may be cleaved by a protease other than thrombin during the course of the 24-h contraction assay. However, radiolabeling experiments with ^{35}S-methionine (Fig. 5) as well as Western analysis (data not shown) do not reveal significant cleavage products of decorin in fibrin cell cultures.

Turbidity studies suggest that decorin addition to fibrinogen during polymerization generates thin fibrils with a lower mass-to-length ratio. A fibrin matrix composed of thin fibrils that are tightly packed exhibit reduced fibrinolysis [Collet et al., 2000]. The potential role of decorin in reducing fibrinolysis could be important for maintaining the structure of the provisional matrix during remodeling by ASMC.

The presence of decorin during fibrin gel formation and de novo synthesis of decorin decreased ASMC-mediated fibrin contraction (Fig. 7A,B,D). Reduction of fibrin contraction by decorin may play a role in maintaining the provisional matrix. If the fibrin architecture is not maintained appropriately, processes such as wound healing may be disturbed. For example, although overt wound closure rates were the same as control mice in cutaneous wound healing studies of fibrinogen-deficient mice, histological observations revealed that granulation tissue integrity was poor, epithelial cell migration was altered, and tensile strength of the wound was reduced [Drew et al., 2001]. Although a fibrous scar formed, it is clear that the lack of a well-maintained provisional fibrin matrix interferes with wound healing.

Although decorin attenuates fibrin contraction, de novo synthesis of decorin by ASMC cultured in a collagen matrix enhanced the cell-mediated contraction of collagen gels [Järveläinen et al., 2004]. A recent study of dermal wound healing in decorin deficient (Dcn−/−) mice demonstrated that wound closure was delayed [Järveläinen et al., 2006]. As the authors suggest, delayed wound closure in Dcn−/− can be partially attributed to reduced collagen contraction during the healing process, but the absence of decorin accumulation during the fibrin provisional matrix formation and maintenance may also contribute to the delayed wound closure observed. Without the presence of decorin in the fibrin provisional matrix of the Dcn−/− mice, fibrin may be contracted prematurely and thus lead to poor cell recruitment and ECM remodeling. The dermal wounds of Dcn−/− mice are less cellular [Järveläinen et al., 2006]. Thus, decorin may influence cell migration into the fibrin matrix. This concept is consistent with the effect of other matrix molecules on cell migration into fibrin. For example, platelet-derived growth factor stimulated fibroblasts to migrate from a collagenous stroma to a fibrin clot via a fibronectin conduit [Greiling and Clark, 1997]. In addition, the accumulation of decorin in the fibrin matrix may also be important for the survival of cells at the site of injury. It has been shown that decorin improves cell survival [Xaus et al., 2001] and may be involved in inhibiting apoptosis [Schönherr et al., 1999].

The remodeling of the fibrin matrix in vivo to a decorin-enriched matrix may influence cells other than ASMC. Vascular endothelial cells migrate into fibrin matrices where

neovascularization occurs [Dvorak et al., 1987]. Both the fibrin and matrix-associated growth factors were implicated in angiogenesis [Woodward et al., 1998]. For example, basic fibroblast growth factor, which is angiogenic, binds to fibrin and fibrinogen with high affinity [Sahni et al., 1998]. Decorin is expressed by sprouting vascular endothelial cells and appears to be important for vessel formation [Järveläinen et al., 1992]. Decorin bound to fibrin may affect the structure of the fibrin network, and thus, may promote an extracellular environment that is more permissive for angiogenesis.

From this study, it is clear that fibrin matrices influence the turnover, retention, and localization of proteoglycans by ASMC. The levels of mRNA transcripts of proteoglycans were reduced in fibrin cultures. This reduction of transcript may be due to altered mRNA stability or post-transcriptional modification. The mechanism of fibrin inhibition of decorin degradation remains to be determined. However, deposition and retention of decorin in the fibrin matrix clearly modulates ASMC-mediated fibrin contraction. Future studies are planned to examine the influence of fibrin on the transcription of proteoglycan mRNA and the effect of small proteoglycans on the integrity and biomechanical properties of the fibrin ECM.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (5 P01 HL 18645-27). PYJ was supported in part by a Cardiovascular Training Grant (HL 07312-19) and a NHLBI Postdoctoral Research Supplement for Underrepresented Minorites from the National Institutes of Health (2 P01 HL 18645-25).

REFERENCES

Ågren UM, Tammi R, Tammi M. 1994. A Dot-blot assay of metabolically radiolabeled hyaluronan. Anal Biochem 217:311–315.

Bennett NT, Schultz GS. 1993. Growth factors and wound healing: Part II. Role in normal and chronic wound healing. Am J Surg 166:74–81.

Bhide VM, Laschinger CA, Arora PD, Lee W, Hakkinen L, Larjava H, Sodek J, McCulloch CA. 2005. Collagen phagocytosis by fibroblasts is regulated by decorin. J Biol Chem 280:23103–23113.

Bidanset DJ, Guidry C, Rosenberg LC, Choi HU, Timpl R, Höök M. 1992. Binding of the proteoglycan decorin to collagen type VI. J Biol Chem 267:5250–5256.

Bini A, Kudryk BJ. 1994. Fibrinogen and fibrin in the arterial wall. Thromb Res 75:337–341.

Carr ME, Hernandez H. 1977. Mass-length ratio of fibrin fibres from gel permeation and light scattering. Biopolymers 16:1–15.

Chang Y, Yanagishita M, Hascall VC, Wight TN. 1983. Proteoglycans synthesized by smooth muscle cells derived from monkey (Macaca nemestrina) aorta. J Biol Chem 258:5679–5688.

Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Collet JP, Park D, Lesty C, Soria J, Soria C, Montalescot G, Weisel JW. 2000. Influence of fibrin network conformation and fibrin fiber diameter on fibrinolysis speed: Dynamic and structural approaches by confocal microscopy. Arterioscler Thromb Vasc Biol 20:1354–1361.

Courtman DW, Schwartz SM, Hart CE. 1998. Sequential injury of the rabbit abdominal aorta induces intramural coagualtion and luminal narrowing independent of intimal mass. Circ Res 82:996–1006.

Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo RV. 1997. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. J Cell Biol 136:729–743.

Drew AF, Liu H, Davidson JM, Daugherty CC, Degen JL. 2001. Wound-healing defects in mice lacking fibrinogen. Blood 97:3691–3698.

Dugan TA, Yang VW, McQuillan DJ, Hook M. 2003. Decorin binds fibrinogen in a Zn2+-dependent interaction. J Biol Chem 278:13655–13662.

Dvorak HF, Harvey VS, Estrella P, Brown LF, McDonagh J, Dvorak AM. 1987. Fibrin containing gels induce angiogenesis: Implications for tumor stroma generation and wound healing. Lab Invest 57:673–686.

Elfterior F, Exposito JY, Garrone R, Lethias C. 2001. Binding of tenacin X to decorin. FEBS Letters 495:44–47.

Evanko SP, Johnson PY, Braun KR, Underhill CB, Dudhia J, Wight TN. 2001. Platelet-derived growth factor stimulates the formation of versican-hyaluronan aggregates and pericellular matrix expansion in arterial smooth muscle cells. Arch Biochem Biophys 394:29–38.

Fischer JW, Kinsella MG, Clowes MM, Lara S, Clowes AW, Wight TN. 2000. Local expression of bovine decorin by cell-mediated gene transfer reduces neointima formation after balloon injury in rats. Circ Res 86:676–683.

Fischer JW, Kinsella MG, Levkau B, Clowes AW, Wight TN. 2001. Retroviral overexpression of bovine decorin differentially affects the response of arterial smooth muscle cells to growth factors. Arterioscler Thromb Vasc Biol 21:777–784.

Fisher LW, Termine JD, Young MF. 1989. Deduced protein sequence of bone small proteoglycan I (biglycan) shows homology with proteoglycan II (decorin) and several nonconnective tissue proteins in a variety of species. J Biol Chem 264:4571–4576.

Fisher LW, Heegaard A-M, Vetter U, Vogel W, Just W, Termine JD, Young MF. 1991. Human biglycan gene. J Biol Chem 266:14371–14377.

Font B, Aubert-Foucher E, Goldschmidt D, Eichenberger D, Van der Rest M. 1993. Binding of collagen XIV with the dermatan sulfate side chain of decorin. J Biol Chem 268:25015–25018.

Gailit J, Clark RAF. 1994. Wound repair in the context of the extracellular matrix. Curr Opin Cell Biol 6:717–725.

Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A. 2005. Protein identification and analysis tools on the ExPASy server. In: Walker JM, editor. The proteomics protocols handbook. Totowa, NJ: Humana Press.

Geary RL, Nikkari ST, Wagner WD, Williams JK, Adams MR, Dean RH. 1998. Wound healing: A paradigm for lumen narrowing after arterial reconstruction. J Vasc Surg 27:96–106.

Gillery P, Bonnet A, Borel JP. 1993. A fluorometric deoxyribonucleic acid assay for tridimensional lattice cultures of fibroblasts. Anal Biochem 210:374–377.

Greiling D, Clark RAF. 1997. Fibronectin provides a conduit for fibroblast transmigration from collagenous stroma into fibrin clot provisional matrix. J Cell Sci 110: 861–870.

Hayen W, Goebeler M, Kumar S, Riessen R, Nehls V. 1999. Hyaluronan stimulates tumor cell migration by modulating the fibrin fiber architecture. J Cell Sci 112:2241–2251.

Hiller O, Lichte A, Oberpichler A, Kocourek A, Tschesche H. 2000. Matrix metalloproteinases collagenase-2, macrophage elastase, collagenase-3, and membrane type 1-matrix metalloproteinase impair clotting by degradation of fibrinogen and factor XII. J Biol Chem 275:33008–33013.

Hotary KB, Yana I, Sabeh F, Li XY, Holmbeck K, Birkedal-Hansen H, Allen ED, Hiraoka N, Weiss SJ. 2002. Matrix metalloproteinases (MMPs) regulate fibrin-invasive activity via MT1-MMP-dependent and -independent processes. J Exp Med 195:295–308.

Järveläinen HT, Iruela-Arispe ML, Kinsella MG, Sandell LJ, Sage EH, Wight TN. 1992. Expression of decorin by sprouting bovine aortic endothelial cells exhibiting angiogenesis *in vitro*. Exp Cell Res 203:395–401.

Järveläinen H, Vernon RB, Gooden MD, Francki A, Lara S, Johnson PY, Kinsella MG, Sage EH, Wight TN. 2004. Overexpression of decorin by rat arterial smooth muscle cells enhances contraction of type I collagen in vitro. Arterioscler Thromb Vasc Biol 24:67–72.

Järveläinen H, Puolakkainen P, Pakkanen S, Brown EL, Höök M, Iozzo RV, Sage EH, Wight TN. 2006. A role for decorin in cutaneous wound healing and angiogenesis. Wound Repair Regen 14:443–452.

Kinsella MG, Fischer JW, Mason DP, Wight TN. 2001. Retrovirally mediated expression of decorin by macrovascular endothelial cells. J Biol Chem 275:13924–13932.

Koenig W. 1998. Haemostatic risk factors for cardiovascular diseases. Eur Heart J 19(Suppl C):C39–C43.

Lark MW, Wight TN. 1986. Modulation of proteoglycan metabolism by aortic smooth muscle cells grown on collagen gels. Arteriosclerosis 6:638–650.

Lelongt B, Bengatta S, Delauche M, Lund LR, Werb Z, Ronco PM. 2001. Matrix metalloproteinase 9 protects mice from anti-glomerular basement membrane nephritis through its fibrinolytic activity. J Exp Med 193:793–802.

Pogány G, Vogel KG. 1992. The interaction of decorin core protein fragments with type I collagen. Biochem Biophys Res Comm 189:165–172.

Ross R. 1971. The smooth muscle cell. II. Growth of smooth muscle in culture and formation of elastic fibers. J Cell Biol 10:172–186.

Sahni A, Odrljin TM, Francis CW. 1998. Binding of basic fibroblast growth factor to fibrinogen and fibrin. J Biol Chem 273:7554–7559.

Schmidt G, Hausser H, Kresse H. 1991. Interaction of the small proteoglycan decorin with fibronectin. Involvement of the sequence NKISK of the core protein. Biochem J 280:411–414.

Schönherr E, O'Connell BC, Schittny J, Robenek H, Fastermann D, Fisher LW, Plenz G, Vischer P, Young MF, Kresse H. 1999. Paracrine or virus-mediated induction of decorin expression by endothelial cells contributes to tube formation and prevention of apoptosis in collagen lattices. Eur J Cell Biol 78:44–55.

Schönherr E, Jarvelainen HT, Sandell LJ, Wight TN. 1991. Effects of platelet-derived growth factor and transforming growth factor-beta 1 on the synthesis of a large versican-like chondroitin sulfate proteoglycan by arterial smooth muscle cells. J Biol Chem 266:17640–17647.

Schönherr E, Järveläinen HT, Kinsella MG, Sandell LJ, Wight TN. 1993. Platelet-derived growth factor and transforming growth factor-beta1 differentially affect the synthesis of biglycan and decorin by monkey arterial smooth muscle cells. Arterioscler Thromb 13:1026–1036.

Shekhonin BV, Tararak EM, Samokhin GP, Mitkevich OV, Mazurov AV, Vinogradov DV, Vlasik TN, Kalantarov GF, Koteliansky VE. 1990. Visualization of apo B, fibrinogen/fibrin, and fibronectin in the intima of normal human aorta and large arteries and during atherosclerosis. Atherosclerosis 82:213–226.

Smith EB. 1994. Fibrin deposition and fibrin degradation products in atherosclerotic plaques. Thromb Res 75:329–335.

Smith EB. 1996. Haemostatic factors and atherogenesis. Atherosclerosis 124:137–143.

Sztrolovics R, White RJ, Poole AR, Mort JS, Roughley PJ. 1999. Resistance of small-leucine-rich repeat proteoglycan to proteolytic degradation during interleukin 1 stimulted cartilage catabolism. Biochem J 339:571–577.

Vasse M, Paysant J, Soria J, Collet JP, Vannier JP, Soria C. 1996. Regulation of fibrinogen biosynthesis by cytokines consequences on the vascular risk. Haemostasis 26(Suppl 4):331–339.

Vernon RB, Gooden MD. 2002. An improved method for the collagen gel contraction assay. In Vitro Cell Dev Biol Anim 38:97–101.

Vogel KG, Paulsson M, Heinegård D. 1984. Specific inhibition of type I and type II collagen fibrillogenesis by the small proteoglycan of tendon. Biochem J 223:587–597.

Wasteson Å, Uthne K, Westermark B. 1973. A novel assay for the biosynthesis of sulphated polysaccharide and its application to studies on the effects of somatomedin on cultured cells. Biochem J 136:1069–1074.

West DC, Sattar A, Kumar S. 1985. A simplified in situ solubilization procedure for the determination of DNA and cell number in tissue culture mammalian cells. Anal Biochem 147:289–295.

Winnemöller M, Schön P, Vischer P, Kresse H. 1992. Interactions between thrombospondin and the small proteoglycan decorin: Interference with cell attachment. Eur J Cell Biol 59:47–55.

Woodward AS, García-Cardeña G, Leong M, Madri JA, Sessa WC, Languino LR. 1998. The synergistic activity of

avb3 integrin and PDGF receptor increases migration. J Cell Sci 111:469–478.

Xaus J, Comalada M, Cardo M, Valledor AF, Celada A. 2001. Decorin inhibits macrophage colony-stimulating factor proliferation of macrophages and enhances cell survival through induction of p27(Kip1) and p21(Waf1). Blood 98:2124–2133.

Yanagishita M, Hascall VC. 1984. Proteoglycans synthesized by rat ovarian granulosa cells in culture. Isolation, fractionation, and characterization of proteoglycans associated with the cell layer. J Biol Chem 259:10260–10269.

Yao LY, Moody C, Schönherr E, Wight TN, Sandell LJ. 1994. Identification of the proteoglycan versican in aorta and smooth muscle cells by DNA sequence analysis, in situ hybridization and immunohistochemistry. Matrix Biol 14:213–225.

Yeo T-K, Yeo K-T, Wight TN. 1992. Differential transport kinetics of chondroitin sulfate and dermatan sulfate proteoglycan by monkey aorta smooth muscle cells. Arch Biochem Biophys 294:9–16.

Zimmermann DR, Ruoslahti E. 1989. Multiple domains of the large fibroblast proteoglycan, versican. EMBO J 8: 2975–2981.