A dynamic model of calcific nodule destabilization in response to monocyte- and oxidized lipid-induced matrix metalloproteinases

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Li R, Mittelstein D, Lee J, Fang K, Majumdar R, Tintut Y, Demer L.L., Hsiai T. A dynamic model of calcific nodule destabilization in response to monocyte- and oxidized lipid-induced matrix metalloproteinases. Am J Physiol Cell Physiol 302: C658–C665, 2012. First published October 26, 2011; doi:10.1152/ajpcell.00313.2011.—Vulnerable plaque remains clinically undetectable, and there is no accepted in vitro model. We characterize the calcific nodules produced by calcifying vascular cells (CVC) in ApoE-null mice, demonstrating increased destabilization of cultured nodules in the presence of oxidized low-density lipoprotein (oxLDL) and monocytes under pulsatile shear stress. CVC implanted in the subcutaneous space of hyperlipidemic mice produced nodules revealing features of calcific atherosclerotic plaque including a fibrous cap, cholesterol clefts, thin shoulder, lipids, and calcium mineral deposits. CVC nodules seeded in the pulsatile flow channel (\( \tau_{avg} = 23 \text{ dyn/cm}^2, \sigma/\dot{\varepsilon} = 71 \text{ dyn cm}^{-2} \text{s}^{-1} \)) underwent deformation and destabilization. Computational fluid dynamics revealed distinct shear force profiles on the nodules. Presence of oxLDL or monocytic THP-1 cells significantly increased the numbers of nodules destabilized from the substrate. Both oxLDL and THP-1 increased matrix metalloproteinase (MMP) activity in CVC. The MMP inhibitor GM6001 significantly reversed oxLDL- and THP-1-induced nodule destabilization, whereas overexpression of MMP-9 increased destabilization. These findings demonstrate that CVC-derived nodules resembled calcific atherosclerotic plaque and were destabilized in the presence of active lipids and monocytes via induction of MMPs.

atherosclerosis; plaque rupture; calcifying vascular cells; calcification

VASCULAR CALCIFICATION develops in arterial regions prone to disturbed flow and oxidative stress. As an actively regulated process, vascular cells differentiate to osteoblast-like phenotype (34). Oxidative stress induces Bmp-Msx2-Wnt signaling pathway to regulate the pathogenesis of vascular calcification (6, 29, 36). In human aortic valve stenosis, dysregulation of antioxidant genes contributes to increased oxidative stress and valvular calcification (28). In the intima, calcification has been identified as a distinct, but relevant process to atherosclerosis (1, 37, 38, 42), beginning as early as the second decade of life, soon after fatty streak formation, and increases with age and lesion progression (39).

The vascular cells that appear to be responsible for producing calcium mineral undergo chondrogenic, leiomyogenic (smooth muscle), and stromogenic (marrow stromal) lineage acquisition in culture (5). In embryogenesis, immature mesenchymal cells aggregate into patterns and differentiate to form mature tissues. Later in life, this process may be recapitulated: raised cellular aggregates form atherosclerotic lesions in a patchy distribution throughout the vascular system and the cardiac valve leaflets (41). In many such lesions, architecturally complete, ectopic bone tissue arises, often even including bone marrow (46). While intimal calcification is clearly associated with atherosclerosis, its role on mechanical plaque stability remains controversial.

Atherosclerotic plaque rupture typically occurs at the shoulder region (24). This phenomenon may be due to concentration of solid tensile stress at a thin edge or due to dissection by fluid shear stress under the edges of lesions. Calcification may contribute by introducing compliance mismatch (9). We previously showed that calcifying vascular cells (CVC, with characteristics of vascular mesenchymal stem cells) form raised nodules when grown on glass or plastic substrates and expressed collagenous extracellular matrix and mineral deposits within 6–10 days (5). These calcifying nodules detached from their substrate in proportion to the magnitude of shear over a range of 4.9 to 400 dyn/cm² (27).

These nodules have also been likened to atherosclerotic plaque. Gimbrone and Cotran (14) first reported that advanced smooth muscle cell cultures exhibit focal proliferation and secrete excess extracellular matrix, giving rise to “nodular protrusions resembling atherosclerotic lesions” in vitro. In this context, detachment of calcified nodules in response to shear forces provides an entry point to characterize mechanical destabilization of calcific atherosclerotic plaque in the presence of oxidative stress and inflammatory responses.

In the current study, we hypothesized that CVC nodules resemble calcific atherosclerotic plaque and are destabilized in the presence of monocytes and oxidized low-density lipoprotein (oxLDL) via increasing matrix metalloproteinase (MMP) activity under mechanical shear force. We characterized calcific nodules implanted in ApoE-null mice and quantified in vitro nodule destabilization from the substrate or extracellular matrix in response to metabolically active lipids and monocytes under a well-defined hemodynamic shear stress simulating that found in the common carotid arteries (2). Implanted CVC nodules resembled calcified atherosclerotic plaque, harboring thin fibrous cap, collagen type II, smooth muscle cells, and lipids. Calcific nodules were destabilized in the presence of oxLDL or coculture with monocytic THP-1 cells. Both oxLDL and monocytes activated MMP, rendering the calcific nodules prone to destabilization.
MATERIALS AND METHODS

Materials and reagents. THP-1 cells were obtained from American Type Culture Collection. DMEM and RPMI-1640 were purchased from Invitrogen. Fetal bovine serum (FBS) was purchased from Phoenix Research. GM6001 was obtained from Santa Cruz Biotechnology. Real-time PCR reagents were obtained from Applied Biological Materials. Human LDL was kindly provided by Dr. Judith Berliner (Atherosclerosis Research Unit, Univ. of California, Los Angeles). Preparation of oxLDL was performed as previously described (40). Control and recombinant human MMP-9 adenoviruses were kindly provided by Dr. Jack Gauldie (McMaster Univ., Hamilton, ON, Canada).

Cell culture. CVC, derived by dilutional cloning from bovine aortic smooth muscle cell cultures (5), were maintained in DMEM with 15% FBS. CVC were seeded onto glass slides at 100,000 cells/cm² and cultured for 3–6 days to allow nodule formation in preparation for exposure to dynamic flow conditions. Monocytic THP-1 cells were cultured in RPMI with 10% FBS supplemented with 50 μM β-mercaptoethanol.

CVC implantation in hyperlipidemic ApoE-null mice. CVC (1 × 10⁶ cells) were injected into individual 0.2 μm pore-size diffusion chambers, which selectively allowed for interstitial fluid exchange without cellular ingress or egress. Chambers were sealed with methacrylate and implanted into the subcutaneous space of four control (C57BL6 mice) and four hyperlipidemic ApoE-null recipient mice using sterile technique. After 8 wk of regular chow diet, the chambers were removed and specimens were embedded in OCT.

Frozen sections were examined by standard histochemical and immunohistochemical staining. All of the protocols were approved by the Institutional Animal Care and Use Committee and done in accordance with the Guiding Principles in the Care and Use of Animals of the American Physiological Society.

CVC nodules in the pulsatile shear stress system. A LabView-driven stepper motor was programmed to expose CVC to fluid shear in well-defined flow patterns simulating the shear stress profile found in human common carotid arteries (2, 20). The system provides precise, reproduducible flow profiles across the width of the chamber with physiological temporal variations in shear stress (σ̇/ṫ), frequency, and amplitude (35). The pulsatile flow system is composed of a NEMA 34 stepper motor (model N32HRLG-LEK-M2–00, Pacific Scientific), stepper drive (model P70530, National Instruments), universal motion interface (model UMI-7772, National Instruments), and peristaltic pumps (model HV-77200–60, Cole-Parmer). Time-averaged flow rate was monitored downstream by a rotoclip flow meter (model MASS 2100 DI 1.5, Siemens).

CVC grown on glass slides (2.5 cm × 1 cm) for 3 days to allow for nodule formation were then preincubated in the presence or absence of 5 × 10⁻⁵/ml of monocytic THP-1 cells or 50 μg/ml of oxLDL for 3 days. The CVC-derived nodules were exposed to pulsatile shear stress (PSS) at a time-averaged shear stress (σ̇avg) of 23 dyn/cm², a temporal gradient (σ̇/ṫ) of 71 dyn·cm⁻¹·s⁻¹, and a pulse pressure (ΔP) of 10 psi for 2.5 h. The flow system was placed in a tissue culture incubator providing 5% CO₂ as well as physiological temperature (37°C) and pH (7.4). Nodule deformation and destabilization in response to shear stress were captured with a JENOPTIK Progess C3 digital camera under an Olympus IX70 microscope. The total numbers of nodules on the glass slides were quantified under microscope before and after PSS exposure. Nodule destabilization is expressed in terms of percentage of nodules detached from the glass slides for quantitative purposes.

Computational fluid dynamic simulation. Computational fluid dynamic (CFD) code was developed to recapitulate shear force distribution (3) on the calcific nodules. Generation of three-dimensional (3-D) geometries and meshes was reconstructed in Solidworks (Concord, MA) based on the measured dimensions. To characterize the fluid velocity gradients near the substrate and nodules, we constructed fine-mesh sizes immediately adjacent to the channel wall and nodular contours. The flow field was modeled by applying the 3-D Navier-Stokes equations. The governing equations, including mass and momentum equations, were solved for laminar, unsteady, incompressible, and non-Newtonian flow. The mass flux, calculated based on the steady flow rates, was applied as the inlet boundary condition and implemented in Solidworks Flow Simulation. The inlet Reynolds number (Re) was calculated from the mean velocity at the inlet.

MMP expression and activity assays. CVC nodules grown in six-well plates were incubated in the presence or absence of monocytic THP-1 cells (5 × 10⁵/ml) or oxLDL (50 μg/ml) for 1–3 days in DMEM-5% FBS. Media supernatants were collected for MMP activity assays and zymogram. The cells were lysed in RNA isolation lysis buffer for measurement of MMP expression by quantitative RT-PCR. MMP activity was measured using the Sensolyte Generic MMP Assay Kit (AnaSpec), and the arbitrary units of MMP activity were calculated, per the manufacturer’s instructions, using reference positive controls.

Zymogram. The supernatant of medium (15 μl) was mixed with 2× loading buffer (Bio-Rad) and loaded into 4–15% Blue Casem zymogram gel (Invitrogen). After running at 125 V for 1.5 h at room temperature, the gel was incubated with zymogram renaturing buffer and rinsed with zymogram developing buffer. The gel was then incubated at 37°C for 36 h. After drying, the gel was scanned into image and the band intensity as indication of MMP activity was quantified with ImageJ software (National Institutes of Health, Bethesda, MD).

Overexpression of MMP-9. CVC were grown for 3 days on glass slides and then infected with control (Adv-DL) or recombinant MMP-9 adenoviruses (Adv-MMP-9) at multiplicity of infection (MOI) of 1:100. The cells were allowed to grow for additional 3 days before shear stress exposure as described above.

Measurement of MMP expression. MMP expression was measured by quantitative RT-PCR as previously described (26). Total RNA was isolated using the Aurum Total RNA Isolation Kit (Bio-Rad) following the manufacturer’s instructions. Potential genomic DNA contamination was removed with on-column DNase I digestion. Approximately 0.5–1 μg of total RNA was reverse-transcribed with Bio-Rad’s iScript cDNA synthesis kit. The primers were designed on the basis of bovine MMP sequences, and thus preferentially detected MMP expression in CVC. The primer sequences were as follows: bMMP1: forward, ATGGGGAAGAAAGTGTTAGGCAAGAGAACG and reverse, GGGTGCTTGCTTCATCACACATCGGTTTTC; bMMP2: forward, CGCCGTGCCCACTATCAAATTT and reverse, TTGGGCGACGCTGAAAGGTTT; bMMP3: forward, CTCGCCAGCTTCTCCTCGTGTGATATGTTG and reverse, TGGTTCCTCCTGTCCCTGGCAGC; bMMP9: forward, GCACACCAACTACATCTACTGACTC and reverse, ATGTACAGTCTGCCGAGCACTAC; and bGAPDH: forward, ATGGTGAAGGTCGGAGTGAACGGATT and reverse, GCGGCGATGCACCTTGCAGAA.

Statistical analysis. Data are expressed as means ± SD. Multiple comparisons were made by one-way analysis of variance (ANOVA), and statistical significance for pairwise comparison was determined using the Tukey test. Values of P < 0.05 were considered statistically significant.

RESULTS

CVC-derived calcifying nodules resembled calcified atherosclerotic plaque in vivo. CVC is a subpopulation of smooth muscle cells from the medial layer of the artery wall producing raised nodules in tissue culture over a 1- to 3-wk period. These nodules ranged from 20 to 2,000 μm in diameter (with average size in the 100- to 200-μm range) and were positively stained by the von Kossa technique demonstrating calcification in the shoulder regions (27). When CVC, within diffusion chambers,
were subcutaneously implanted in ApoE-null mice, they produced atherosclerotic plaque-like structures (Fig. 1, A and B). These CVC-derived structures included fibrous caps and cholesterol clefts (Fig. 1B). The friable shoulder regions revealed an osteoblast-like differentiation of CVC (Fig. 1C) as demonstrated by von Kossa staining (Fig. 1D). These structures were positively stained for collagen type II (Fig. 2A), alkaline phosphatase (Fig. 2B), smooth muscle α-actin (Fig. 2C), and lipids (Fig. 2D). Thus, the CVC-derived calcific nodules harbored histological features of calcific atherosclerotic plaque.

**CVC-derived nodules deformed and destabilized under shear stress.** CVC nodules deformed in response to PSS (mean \( \tau_{ave} = 23 \text{ dyn/cm}^2 \); temporal gradient \( \tau_{it} = 71 \text{ dyn-cm}^{-2} \text{s}^{-1} \) at 1 Hz) (Supplemental Video S1; Supplemental Material for this article is available online at the Journal website). Time-lapse microvideography showed nodules undergoing deformation over 0.5-s to 260-s periods (Fig. 3). Extended exposure of shear stress led to destabilization of the nodules, culminating in detachment from the substrate (Supplemental Video S2).

Computational fluid dynamic reconstruction of the pulsatile flow field illustrated a stream of velocity vectors with a range of magnitudes and directions (Fig. 4A). Mean shear forces were accentuated upstream of the nodules corresponding with the shoulder regions where nodular deformation and detachment were initialized (Fig. 4B, Supplemental Video S2). Instantaneous shear stress profiles for an individual nodule further revealed shear stress mismatch at the interface between the shoulders and substrates (Fig. 4C). A plot depicting the magnitude of shear stress over a given nodule showed the variations in stress in relation to the aspect ratio of the nodule (Fig. 4D). This analysis provides an in silico model of calcific atherosclerotic plaque disruption under pulsatile flow.

**OxLDL and monocytic THP-1 cells promoted the destabilization of CVC-derived nodules.** Metabolically active components of thin cap fibrous atheroma include biologically active lipoprotein particles and monocytes/macrophages, both of which impart oxidative stress in rupture-prone lesions (4, 15, 32). Here, we sought to quantify the destabilization of calcified CVC nodules by a well-defined PSS in the presence of oxLDL or THP-1 monocytes. Coculture of CVC nodules with THP-1 cells significantly increased the numbers of nodules detached by pulsatile flow from 27 ± 6% to 56 ± 6%, \( P < 0.05, n = 3 \) (see Fig. 7A). Similarly, oxLDL treatment also increased nodule detachment from 17 ± 4% to 28 ± 2%, \( P < 0.05, n = 3 \) (see Fig. 7B). These findings suggest that active lipids and monocytes/macrophages promote destabilization of calcified nodules.

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**Fig. 1.** Calcific atherosclerotic nodules. A: gross pathology of human calcific aortic valve stenosis, showing numerous calcific nodules (arrows) on the aortic face of the valve leaflets (kindly provided by Michael Fishbein, UCLA School of Medicine). B: histochemical staining of calcifying vascular cell (CVC) nodule produced in vivo within a subcutaneous diffusion chamber implanted in a hyperlipidemic ApoE-deficient mouse. C and D: high-magnification images of serial sections from the shoulder region revealing calcium mineral deposits by hematoxylin and eosin (C) and von Kossa staining (D). Nodules measure approximately 0.5–2 mm in diameter.

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**Fig. 2.** Characterization of CVC-derived calcific nodules. A–D: immunohistochemical and histochemical staining of cryosections of in vivo CVC nodules using antibodies for collagen type II (A), alkaline phosphatase (B), smooth muscle α-actin (C), and Oil Red O for lipids (D).
OxLDL and THP-1 upregulated MMP activity in CVC. To test whether induction of MMPs contribute to the mechanism of destabilization, we analyzed MMP activity. Both oxLDL treatment and THP-1 cell treatment significantly induced MMP activity compared with control (CVC = 4.9 ± 0.5, THP-1 = 4.5 ± 2.1, CVC + THP-1 = 13.8 ± 4.1, P < 0.01 vs. CVC; CVC + oxLDL = 9.7 ± 2.6, *P < 0.05 vs. CVC, n = 4) (Fig. 5A). To verify changes in MMP activity by THP-1 and oxLDL treatment, we performed casein zymogram assay as a sensitive measurement for stromelysin such as MMP-3 (stromelysin 1) by using the media supernatants of treatments. A single band of 50- to 60-kDa range, presumably MMP-3, was shown in the zymogram. In agreement with the MMP activity assay, THP-1 and oxLDL increased MMP-3 activity in the zymogram (Fig. 5B). Furthermore, coculture of CVC with THP-1 cells significantly increased MMP-1, MMP-3, and MMP-9, but decreased MMP-2 mRNA expression in a time-dependent manner (Fig. 6A, P < 0.05, n = 3). OxLDL treatment also regulated MMP expression to a similar extent (Fig. 6B, P < 0.05, n = 3), suggesting that active lipids and monocytes destabilize calcific nodules via MMPs.

OxLDL- and THP-1-induced MMP activity mediated the destabilization of CVC nodules. To corroborate the role of MMPs in nodule destabilization, we cocultured CVC nodules with THP-1 cells or treated the nodules with oxLDL in the presence or absence of an MMP inhibitor, GM6001 (10 μM), and quantified nodule destabilization under PSS. MMP inhibition completely abrogated THP-1-induced CVC nodule destabilization (Fig. 7A; nodules detached: control = 27 ± 6%, THP-1 = 56 ± 6%, P < 0.05 vs. control; THP-1 + GM6001 = 24 ± 3%, P = 0.42 vs. control, n = 3). MMP inhibitors also reversed oxLDL-mediated nodule destabilization to a similar extent (control = 17 ± 4%, oxLDL = 28 ± 2%, P < 0.05 vs. control, oxLDL + GM6001 = 18 ± 1%, P = 0.80 vs. control, n = 3) (Fig. 7B). Hence, monocytes and oxLDL promoted destabilization of calcified nodules via an elevated MMP activity. To further demonstrate the destabilizing role of MMP, CVC nodules were infected with MMP-9 adenovirus (Adv-MMP-9) for overexpression of MMP-9 or with control adenovirus (Adv-DL). MMP-9 overexpression significantly increased nodule destabilization as compared with control (Fig. 7C, nodule detached: Adv-DL = 24 ± 24.5%, Adv-MMP-9 = 59.1 ± 19.9%, P < 0.05, n = 5).

DISCUSSION

This study provides a novel in vitro model of calcific nodule destabilization and characterizes its mechanical vulnerability to programmed fluid shear stress in response to metabolically active lipids and macrophages. This model, consisting of CVC-derived nodules, when grown in vivo, demonstrated histological features characteristic of calcific atherosclerotic plaque, including a fibrous cap, cholesterol clefts, foam cells, thin shoulder, lipids, collagen II, alkaline phosphatase, and calcium mineral deposits. Computational fluid dynamic (CFD) reconstruction further provided hemodynamic profiles for the individual nodules, demonstrating shear stress mismatch at the interface between the nodules and substrates. While both THP-1 cells and oxLDL upregulated MMP expression and activity in CVC nodules, blocking of MMP activity with
GM6001 completely reversed nodule destabilization. Artificially overexpressing MMP-9 in CVC further accentuated nodule destabilization. Taken together, these findings provide new biomechanical insights into the interplay between active metabolic factors and calcification in a dynamic environment leading to calcific plaque destabilization.

Vascular cells implanted into mice via the diffusion chamber technique engendered organized mesenchymal tissue including bone and cartilage (10). Our in vitro model of atherosclerotic plaque was generated by CVC, also known as vascular mesenchymal cells, a subpopulation of smooth muscle cells from the medial layer of the aortic wall (5, 43). In addition to showing the histological features of atherosclerotic plaques, CVC-derived nodules also expressed a wide range of genes and gene products typical of calcified atherosclerotic plaque, including osteopontin, fibronectin, α-actin, caldesmon, bone morphogenetic protein-2, osteonectin, osteocalcin, collagen types I and III, laminin, matrix GLA protein, and cytokines. Previously, the cells were termed calcifying vascular cells (CVC) because of their ability to calcify spontaneously, a process requiring about 6–10 days and accelerated by treatment with β-glycerophosphate (5 mM).

It has been suggested that plaque instability is caused by a dynamic imbalance in oxidative stress (17), inflammation (18, 45), and proteolytic activity (11). Fluid shear stress promotes mechanical failure when plaque is raised, focal, and eccentric (16, 18). We previously showed that CVC-derived calcific nodules detach in response to a ramping magnitude in shear stress (27). In the present study, we used videomicroscopy to capture real-time nodule deformation and destabilization in response to PSS at a constant time-averaged shear stress. We applied CFD to demonstrate shear force distribution, highlighting the shear mismatch between the shoulder regions of the nodule and the substrate [extracellular matrix (ECM)].

These vascular mesenchymal cells, originally termed calcifying vascular cells (CVC), undergo osteoblastic differentiation, express bone-specific proteins, and produce ECM that incorporates hydroxyapatite mineral under regulation of developmental genes. We also demonstrated that these cells are also multipotent, with the capacity for chondrogenic, leiomyogenic (smooth muscle), and stromogenic (marrow stromal) lineage.
acquisition. Given their substantial capacity for self-renewal, we proposed that they are closely related to mesenchymal stem cells (7, 33). Interestingly, these cells self-organize into patterns of regularly spaced nodular aggregates or ridges in a reaction-diffusion process governed by morphogens: bone morphogenetic protein-2 and its inhibitor (12). These findings suggest that the artery wall contains mesenchymal stem cells with lineage plasticity and the capacity for self-organization, thus accounting for both ectopic tissues in atherosclerotic arteries as well as the long-known patchy distribution of atherosclerosis. In the present study, we also provide evidence that CVC-derived nodules are a relevant model for calcified atherosclerotic plaque.

Oxidized LDL and monocyte-macrophages are metabolically active components of mechanically unstable plaque (4, 15, 32). Here, we showed that monocytes/macrophages and oxLDL promote nodule destabilization under physiological shear stress. Despite interexperimental variations due to passage number or degree of calcification, both THP-1 mononuclear cells and oxLDL consistently destabilized the calcified nodules. Thus, the CVC nodules responded to the metabolically active factors in a similar manner as in vivo atherosclerotic plaque would.

MMPs, proteolytic enzymes that break down extracellular matrix, such as that produced by CVC nodules on a glass substrate (27), are implicated in tissue remodeling, angiogenesis, and tumor metastasis. They are produced by CVC and play an important role in plaque stability (22, 31). Dual roles of MMP in initial thickening and late-stage rupture of atherosclerotic plaque have been documented (23, 30, 31). Increased MMP activity in the advanced plaque may be responsible for the thinning of the fibrous cap that renders the necrotic core prone to rupture (13, 15, 25). In our in vitro model, oxLDL and monocyte treatment increased overall MMP activity and increased the expression of MMP-1 (collagenase 1), MMP-3 (stromelysin 1), and MMP-9 (gelatinase B), but decreased MMP-2 (gelatinase A) expression. The latter is consistent with the finding of Wilson et al. (44) that mildly oxidized LDL decreased MMP-2 expression. However, Haug et al. (19) found the opposite with oxidized LDL in vascular smooth muscle cells. In macrophages derived from a U937 cell line, oxLDL was reported to upregulate MMP-2 expression, suggesting that the effects of oxLDL on MMP-2 expression may be cell-type and cell-stage specific. Despite a decrease in MMP-2 expression, the total MMP activity increased in response to oxLDL and THP-1, at least in part because of increased expression by CVC. The mechanism of how THP-1 and oxLDL regulate the expression of MMP in CVC remains an interesting question for

Fig. 6. Effects of THP-1 cells and oxLDL on MMP expression in CVC. CVC grown in 6-well plates were cocultured with or without THP-1 cells for 1, 2, or 3 days (A) or treated with or without 50 μg/ml of oxLDL for 3 days (B). Expression of MMPs was measured by quantitative RT-PCR (*P < 0.05, vs. control; n = 3).

Fig. 7. MMPs mediated THP-1 cells and oxLDL-induced CVC nodule instability. CVC nodules grown on glass slides were cocultured with or without THP-1 cells (A) or treated with or without 50 μg/ml of oxLDL for 3 days in the presence or absence of an MMP inhibitor, GM6001 (GM) (B), or infected with recombinant MMP-9 adenovirus (Adv-MMP-9) or control adenovirus (Adv-DL) at multiplicity of 100 for 3 days (C). The nodules were then exposed to pulsatile shear stress. Numbers of detached nodules were calculated as percentage of total nodules (*P < 0.05, n = 3; **P < 0.05, n = 5; #P = 0.42 vs. control, n = 3; ##P = 0.80 vs. control, n = 3).
future study. While MMP traditionally have been thought to function primarily in the degradation of extracellular matrix molecules, recent data suggest that MMPs may also function as important regulators of matrix biology, inflammation, and osteogenesis including in the control of arterial calcification (21). Mechanical interactions of cells with their matrix and environment, including cell-generated traction and matrix stiffness, strongly influence vascular pathology, including the development of calcification. Thus, MMP effects on the mechanical strength of extracellular matrix may influence vascular cell calcification, and conversely, calcification may alter cell-generated traction and MMP expression (8).

While numerous in vivo studies have revealed a dual role for MMP in atherosclerosis and plaque rupture, preclinical and clinical studies of plaque stability have demonstrated mixed results for modulating MMP expression and activities (30). In our studies, inhibition of MMP activities completely blocked oxLDL- and monocyte-induced nodule instability, whereas overexpression of MMP-9 accentuated its instability. Thus, MMP may predominately destabilize advanced calcific plaques. In sum, the phenotypic and biomechanical similarities between CVC-derived nodules and calcific atherosclerotic plaque provide a novel in vitro model to assess the interplay between metabolically active stressors and calcific atherosclerotic plaque. This well-defined hemodynamic system further reveals that oxLDL and monocytes may simulate clinical plaque instability via increased expression of matrix remodeling metalloproteinases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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