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Systems Analysis of the Role of Bone Morphogenic Protein 4 in Endothelial Inflammation

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Abstract

Shear stress is an important factor in the onset and progression of atherosclerosis. High and unidirectional laminar stress is seen as protective, while low and oscillatory shear stress is considered pro-inflammatory and pro-atherogenic. The mechanosensitive response of endothelial cells is governed by a complex system of genes, proteins, and signals that operate at distinctly different time scales. We propose a dynamic mathematical model that quantitatively describes this mechanosensing system and permits novel insights into its functioning. The model, the first of its kind, is constructed within the guidelines of *Biochemical Systems Theory* and accounts for different time scales by means of approximated delays. Parameter values are obtained directly from biochemical observations in an *ad hoc* fashion. The model reflects most documented observations well and leads to a number of predictions and novel hypotheses. In particular, it demonstrates the crucial role of Bone Morphogenic Protein 4 and p47^{phox}-dependent NADPH oxidases in endothelial inflammation.

Keywords

Atherosclerosis; Bone Morphogenic Protein 4 (BMP4); Biochemical Systems Theory (BST); Inflammation; Mechanosensing; Monocyte adhesion; NADPH oxidase; Shear stress

INTRODUCTION

Atherosclerosis is a chronic inflammatory response that is characterized by the hardening of arteries and loss of their elasticity.^{40,50} Its hallmark is the formation of atheromatous plaques consisting of macrophages, smooth muscle cells, cholesterol crystals, and calcification

within vascular smooth muscle cells. Disturbed blood flow dynamics is believed to be one of the pertinent factors determining the localization of developing plaques, which preferentially occur in the branched or curved regions of arteries.⁶⁸ When blood flows through an artery, endothelial cells are subjected to several mechanical forces. Among these, shear stress is believed to be the most important. This tangential frictional force is caused by blood flowing along the walls of the vessel and constitutes an important signal to the endothelial cells lining the arterial wall.^{17,21} Straight sections of the arteries are exposed to relatively unidirectional, high-level laminar shear stress (ULS) and are typically lesion-free. By contrast, the complex arterial geometry and pulsatile blood flow during the cardiac cycle cause disturbed, turbulent flow conditions with low shear in branched and curved regions of the arteries, and these tend to be lesion-prone.^{17,21} For investigations *in vitro*, these complicated flow patterns at arterial branches are mimicked with experimental set-ups that repeatedly change the direction of blood flow, thus yielding oscillatory shear stress (OSS).^{22,48,57}

Emerging evidence suggests that shear stress is an essential regulator of inflammatory responses in endothelial cells, and thus in the critical early phase of atherogenesis.^{40,46,60} OSS is a potent pro-atherogenic factor that can stimulate monocyte adhesion by inducing one of the earliest measurable markers of atherogenesis, namely expression of the *Inter-Cellular Adhesion Molecule-1* (ICAM1).^{29,60} In contrast, ULS is atheroprotective and has been shown to suppress ICAM1 as well as *Vascular Cell Adhesion Molecule-1* (VCAM1) expression. The mechanisms with which OSS and ULS exert opposite roles in inflammation and atherosclerosis have so far remained unclear, even though they have received intense attention. Some studies suggest that the opposite effects of OSS and ULS are likely mediated through differential expression of several specific genes and proteins in each condition.^{7,19,48,57} Among these, DNA microarray studies and subsequent functional studies have identified the recently discovered mechanosensitive gene product *Bone Morphogenic Protein 4* (BMP4) as a potent inflammatory cytokine.⁵⁷

The role of BMP4 in mechanosensing is complicated, as the protein acts in concert with numerous other known components of the inflammatory response, including reactive oxygen species (ROS), NADPH oxidase, and the signaling molecule NF- κ B. These components interact in a complicated fashion and on different time scales, which renders intuitive cause-and-effect argumentation problematic. To shed light on the systemic nature of the mechanosensitive response to different stresses, we have in this study developed a dynamic mathematical model that allows us to merge all pertinent information into a computational structure that permits easy interrogation, analysis, diagnostics, scenario simulations, and analyses that are different and complementary to experimental approaches. The model is the first of its kind. It is mainly constructed within the framework of *Biochemical System Theory* (BST)^{54,55,62,66,67} but extended to permit the account for time delays. At this point, the model is obviously not complete but captures experimental findings quite well qualitatively and semi-quantitatively.

METHODS

Structure of the Mechanosensitive Response System

The shear stress response system consists of numerous genes, proteins, and biochemical molecules. Those believed to be most important are reviewed in this section.

BMP4—BMP4 is a potent inflammatory cytokine that is synthesized from a 408 amino acid precursor, proteolytically cleaved in the Golgi apparatus, and secreted as active protein.³⁶ Its activity is counterbalanced by secreted antagonists, such as noggin, follistatin, and Matrix Gla Protein (MGP), which have been found to be co-expressed with BMP4 in cultured endothelial cells.^{11,41} Binding of BMP4 to its antagonists prevents it from binding to the cognate receptor and thus blocks BMP4-induced signaling. Cell culture studies have shown that BMP4 expression is stimulated by OSS and inhibited by ULS.^{57,58} Treatment of cultured endothelial cells with BMP4 alone leads to similar responses as OSS, including increased ICAM1 expression and monocyte adhesion.⁵⁷ Furthermore, OSS-induced endothelial inflammatory responses can be completely blocked by treating the cell with sufficient amounts of the BMP4 antagonist noggin or upon administration of BMP4-siRNA.⁵⁷ Opposite to our initial expectation, however, ULS tends to decrease BMP4 antagonist expression, while OSS up-regulates it.¹¹ This up-regulation may be accomplished through both BMP4-dependent and -independent mechanisms, which would reveal a novel negative feedback regulation of the action of BMP4 under shear stress. These and other studies strongly indicate the important, essential, and specific role of BMP4 in shear-dependent inflammation, which is a key feature of atherosclerosis.

Reactive Oxygen Species—ROS have been implicated in many cardiovascular diseases including atherosclerosis and hypertension and play an essential role in many intracellular signaling pathways that ultimately lead to changes in gene transcription, protein synthesis and, consequently, cell function.^{2,14,51,64} Under physiological conditions, ambient production of ROS occurs at low concentrations, which are necessary for endothelial cell growth and proliferation.^{26,53} However, under pathological conditions, large quantities of ROS are produced, resulting in a mismatch between ROS formation and the ability of antioxidants to degrade them, which subsequently leads to a state of oxidative stress.⁶⁴

Two of the most important ROS for the cardiovascular system are superoxide (O_2^-) and hydrogen peroxide (H_2O_2).^{49,59,64} Hydrogen peroxide is mainly derived from superoxide, either spontaneously or through an enzymatic reaction catalyzed by superoxide dismutase (SOD). Outside its role as substrate for the conversion to hydrogen peroxide, superoxide acts as a more or less general reducing agent. For instance, it reacts with nitric oxide with a turnover rate approaching the diffusion limit.⁸ Other sources producing ROS include mitochondria, uncoupled endothelial nitric oxide synthase (eNOS) and cytochrome P450.^{2,64} Unlike O_2^- , which is highly reactive and short-lived, H_2O_2 is more stable and has a longer half-life. The elimination of hydrogen peroxide is tightly regulated by antioxidants and redox buffering, which is accomplished with metabolites such as glutathione, peroxiredoxin, and thioredoxin and the corresponding enzymes, such as glutathione peroxidase.⁵² Recent studies have shown that hydrogen peroxide stimulates ROS production via several self-

propagation mechanisms, including mitochondrial damage, sources of NADPH oxidase, xanthine oxidase, and uncoupled eNOS.⁹

NAPH Oxidase—NADPH oxidase has been identified as an important player in the shear-induced inflammation in endothelial cells.^{18,29,31} The enzyme was originally discovered in phagocytes of the innate immune system, where it was found to be responsible for generating large bursts of O_2^- during the process of phagocytosis.³⁴ This phagocyte NADPH oxidase has been well characterized.³ It comprises a membrane-associated complex, flavocytochrome b₅₅₈, composed of one p22^{phox} (for phagocyte oxidase) subunit and one gp91^{phox} (now called nox2) subunit, and several cytosolic regulatory subunits, including p47^{phox}, p67^{phox}, p40^{phox}, and the small GTPase Rac1 or Rac2. In most cases, the enzyme remains inactive in resting cells. However, when exposed to any of a very wide variety of stimuli, p47^{phox} is phosphorylated and translocates from cytosol to the membrane, thereby activating the enzyme.¹

Similar to its phagocytic counterpart, vascular (non-phagocytic) NADPH oxidase comprises membrane compartments and a cytosolic complex.^{6,10,25,35,56} In addition to nox2, other nox proteins (termed nox1, 3, 4, and 5) have been found.^{12,61} Unlike the NADPH oxidase in leukocytes, which is activated only upon stimulation and generates O_2^- in a burst-like manner, recent studies have suggested that some of the vascular NADPH oxidases may exist in a preassembled form and appear to generate low levels of ROS continuously in the absence of extrinsic stimulation.^{27,38} Furthermore, the vascular NADPH oxidase can be regulated by humoral factors, including cytokines, growth factors and vasoactive agents.^{10,35} Physical factors like shear stress and pulsatile stretch can also stimulate NADPH oxidase activation.^{18,31}

Pathologically high shear stress was experimentally shown to be able to stimulate protein kinase C,³³ which is well known for its capability of phosphorylating p47^{phox} on its serine residues during the activation of neutrophils in response to phorbol ester, and in endothelial cells in response to angiotensin II or TNFα.^{37,63} Based on these established, comparable responses to other stimuli, we hypothesize the existence of a pathway for shear stress to regulate the activation of NADPH oxidase through enhanced phosphorylation of p47^{phox}. A recent study *in vitro* furthermore suggests that shear stress can regulate the activity of NADPH oxidase via a BMP4-dependent mechanism.^{29,58} Short-term (1 h) and long-term (18–24 h) exposures to OSS have been shown to increase O_2^- production in cultured endothelial cells to a similar level. Compared with OSS, ULS induces a similar transient increase in the short term but the opposite long-term effect in terms of superoxide production. While both nox1 and nox2 are activated by OSS, only nox1 expression was shown to be induced by BMP4,^{28,58} suggesting that nox1 may be the NADPH oxidase that mediates the OSS- and BMP4-dependent response.

Nuclear Factor κ B—As a downstream signaling target and very important transcription factor, *Nuclear Factor κ B* (NF κ B) has been indicated to participate and play an essential role in OSS-induced monocyte adhesion.^{44,57} Although the mechanism is not completely clear, hydrogen peroxide is believed to be the upstream molecule that initiates the signal to degrade the inhibitors of NF κ B (I κ B) and release active NF κ B, which can then translocate

to the nucleus to mediate expression of a wide array of genes that participate in inflammation and immune processes.⁵⁸ ICAM1 has been shown to be one of these targets.¹⁵

Interactions Within the Mechanosensitive Response System—The collection and interpretation of numerous results and observations, obtained with extensive series of experiments, have suggested the following mechanism for BMP4-triggered mechanotransduction.^{18,29,31,57,58} OSS induces inflammation and early markers of atherosclerosis in endothelial cells by triggering mechanisms that involve the stimulation of BMP4 expression, which in turn enhances the activity and expression of p47^{phox}-dependent NADPH oxidases, including nox1, in an autocrine-like manner. The OSS-induced activation of NADPH oxidase enhances the production of ROS, up-regulates ICAM1 expression and activation of the transcription factor NF κ B, and strengthens monocyte adhesion to endothelial cells, a critical step in atherogenesis. In contrast to OSS, ULS was shown to inhibit BMP4 expression, thus inducing the opposite effect. Intriguingly, both types of shear stress are able to induce an acute increase in superoxide production during an exposure of 1 h, but induce opposite effects on superoxide production for much longer exposure.²⁹ The processes constituting BMP4-triggered mechanotransduction are schematically presented in Fig. 1.

Although this pathway has been well characterized and tested experimentally,^{57,58} several questions remain unanswered. For instance, why do OSS and ULS induce similar short-term effects but different long-term effects with regard to superoxide production? How does the activity of NADPH oxidase dynamically change in response to a specific external stimulus such as shear stress? And how do the various regulatory mechanisms, which govern the expression of all subunits, dynamically cooperate to effect the overall changes in NADPH oxidase activity and the subsequent production of ROS? These types of questions are difficult to answer solely with experimental means.

Modeling Framework

The work presented here consists purely of mathematical and computational modeling. The primary goal of the modeling effort is to translate the BMP4-dependent inflammatory pathway (Fig. 1), triggered by shear stress, into a mathematical structure that permits diagnoses and analyses and yields insights into the cooperation among the different components of the system.

Very few, if any, of the processes governing the mechanotransduction system in Fig. 1 are known in sufficient depth to allow the formulation of mechanistic, mathematical representations. This situation is quite typical for biological phenomena that include gene expression, signaling, and metabolic conversion and suggests the use of concept modeling²³ with canonical models that are well suited as default representations of systems composed of ill-characterized components. Specifically, we use the modeling framework of *Biochemical Systems Theory* (BST), which over the past four decades has been developed, analyzed, improved, extended, and applied in the contexts of a wide variety of biological systems.^{54,55,62,66,67}

A particular advantage of BST is a set of guidelines for setting up models for diagrams as the one shown in Fig. 1. Namely, a differential equation is formulated for each component (dependent variable) that varies over time. This equation consists of all processes or fluxes producing or degrading this component. The processes are modeled as products of power-law functions, which are based on the rigorous foundation provided by Taylor's theory of numerical analysis.³² As a result, each product of power-law functions contains a non-negative *rate constant* as well as every variable that has a *direct* effect on the process, raised to a real-valued power. Each of these powers, called a *kinetic order*, uniquely quantifies the effect that the associated variable X_j has on the process. A positive kinetic order indicates an activating influence; a negative kinetic order expresses inhibition, and a kinetic order with value zero means that the variable has no effect.

BST comes in several variants, of which the generalized mass action (GMA) representation is of primary relevance here.⁶⁷ The GMA format focuses on the processes among the system variables, and every flux or process associated with a dependent variable is separately represented with a product of power-law functions. Thus, the generic GMA formulation of the dependent system variable X_i reads

$$\dot{X}_i = \pm \gamma_{i1} \prod_{j=1}^{n+m} X_j^{f_{ij1}} \pm \gamma_{i2} \prod_{j=1}^{n+m} X_j^{f_{ij2}} \pm \cdots \pm \gamma_{ik} \prod_{j=1}^{n+m} X_j^{f_{ijk}} \pm \cdots, \quad i=1, \dots, n. \quad (1)$$

The products generically run to $n + m$. The first n variables are the dependent variables, whereas the remaining m variables are independent variables, which have an effect on the system but are not affected by the system. A typical example is a constant enzyme activity.

The translation of a diagram such as Fig. 1 into model equations is strictly governed by the guidelines of BST. In fact, it is so straightforward that a GMA model can be composed by hand or automatically with corresponding software.²³ While the formulation of equations is easy, the severe difficulty of the model design step is the identification of appropriate numerical values for all rate constants and kinetic orders. This parameter identification heavily depends on the availability and quality of the supporting data, other biological information, and on educated assumptions.

Available Data and Model Assumptions

Data—Few of the available data are time-series or truly quantitative. Instead, most are given as singular values or extents that are relative to a control level, which is usually assumed to be measured under static no-flow conditions. Much information is semi-qualitative and of the type “24 h after onset of OSS variable X increases several fold over control.” Variables, such as mature BMP4, nox1, active NF κ B, and ICAM1, have been characterized through these types of data.^{57,58} Measurements of superoxide and hydrogen peroxide are given by their specific product concentrations, which can be recalculated into ratios over control.^{29,42,58} Additional information extracted from p47 knockout experiments²⁹ and treatments with BMP4 or BMP4 antagonist⁵⁷ is qualitative and of the type “variable X significantly increases/decreases in comparison with the control under this specific

treatment.” Shear stresses with different patterns and intensities are added to the system as external stimuli. Among these, ULS typically has a range of 5–15 dyn/cm², while OSS has been investigated at only one intensity of ± 5 dyn/cm².

Accounting for the primarily semi-qualitative nature of the available data, most model variables are assumed to have their steady state at the “nominal” numerical value of 1 (100%). Phosphorylated p47 is assumed to have a steady state of 0.2, which corresponds to one-fifth of the available nox1 at steady state under control conditions. Obviously, these numerical values are quite crude. However, since essentially all model results are expressed as relative results, the numerical values are not as influential as one might think.

Assumptions and Simplifications—By design, the proposed GMA model, which was constructed under the guidelines of *BST*⁶⁷ includes the following key components: BMP4-mRNA, BMP4-precursor, mature BMP4 protein, BMP4 antagonist, BMP4 and BMP4 receptor (BMP4/R) complex, nox1-mRNA, nox1-protein, the free phosphorylated form of p47, active NADPH oxidase, superoxide, hydrogen peroxide, nitric oxide, active NF κ B, and ICAM1 expression. Shear stress is treated as an independent variable; i.e., it affects the system but is not affected by it. Interactions and connections between variables are entered into the model equations according to the biological observations as they are diagrammed in Fig. 1. Thus, two major assumptions for this prototype model are that Fig. 1 is sufficiently comprehensive to gain interesting insights and that the power-law representation is sufficiently adequate for semi-quantitative explanations and predictions.

An initial simplification is that we do not consider all known subunits of NADPH oxidase individually, but focus on the two most pertinent subunits, namely nox1 as the representative of the transmembrane form and p47 as the representative of the cytosolic form. This assumption appears to be reasonable because other subunits are either not significantly regulated by shear stress or do not strongly affect the pathways of interest here.⁵⁸ Furthermore, the total amount of free unphosphorylated and phosphorylated forms of p47 is assumed to be constant due the fact that the amount of the cytosolic component should be of the same or similar magnitude as the amount of the transmembrane component under normal conditions (without external stimulus).

NADPH oxidase and xanthine oxidase are considered to be the only representatives of sources for superoxide production. This assumption ignores the contributions of other sources,^{2,64} such as mitochondrial, cytochrome P450, and uncoupled eNOS, presuming their expression and contribution to superoxide production to remain unchanged or to change only insignificantly in response to shear stress. Furthermore, we presume that the substrate NADPH is not limiting the enzymatic reaction rate and that the concentration of NADPH is about the same under different shear stress conditions. As a consequence, NADPH can be treated as a constant, independent variable that can be merged mathematically with the appropriate rate constants.

Although some experiments have pointed out that long exposure of endothelial cells to shear stress could possibly affect the expression of various enzymes,^{4,5,18,19,30,45} including the enzymes catalyzing the conversion of superoxide to hydrogen peroxide (e.g., SOD) and the

degradation of hydrogen peroxide (e.g., catalase, glutathione peroxidase, thiol peroxidase), we assume that the expression and activities of all enzymes in the corresponding reactions remain constant in this initial phase of model development. We plan to consider their effect and regulation by shear stress in the future (see also “Discussion” section).

The system under investigation contains several organizational levels, including gene transcription, translation into protein, and enzyme-catalyzed biochemical reactions. These processes operate at distinct time scales, which are at the order of minutes, seconds or less for biochemical reactions, but hours for gene transcription and protein synthesis. As an indication of these differences, the level of BMP4-mRNA was significantly decreased after ~2 h exposure to ULS, but protein expression of BMP4 was first observed ~10 h after oscillatory shear stress applied,⁵⁷ thus suggesting a significant time delay during protein synthesis. At the other end of the spectrum, the reaction rate of nitric oxide and superoxide has been measured to be approaching the diffusion limit.⁶⁴ Since we are primarily interested in the activity of the system as a whole, which occurs at a relatively slow time scale of hours, we initially ignore dynamic changes happening at much faster time scales (of minutes, seconds, or faster). This separation of time scales is a common strategy in modeling⁴⁷ and allows us to consider fast reactions, like conversions between superoxide and nitric oxide, and between superoxide to hydrogen peroxide, always to be in a steady state and therefore constant. To address the relatively slow generation of new proteins, the *de novo* appearance of BMP4 precursor, BMP4 antagonist, nox1, and ICAM1 is modeled with appropriate time delays.

As indicated in the system diagram (Fig. 1), shear stress affects the system simultaneously through two distinct pathways. One is mediated through the delayed BMP4- and BMP4 antagonist-associated pathway, while the other is hypothesized to occur through phosphorylation of p47. Details and specifics of the signaling pathway with which shear stress regulates BMP4-mRNA and BMP4 antagonist are still unclear. Therefore, we simply use a power-law function with the format S^{f_i} to represent the strength of the signal. S represents the shear stress intensity and f_i , $i = 1, 2$ represents the effects of shear stress on BMP4 mRNA and BMP4 antagonist expression separately, namely:

$$f_i = \begin{cases} \text{positive,} & OSS \\ \text{negative,} & ULS \\ 0, & STATIC \end{cases} \quad (2)$$

Moreover, in order to indicate the protein synthesis of BMP4 antagonist, one time delay is added to the corresponding pathway.

The effect of the signal for the second pathway is assumed to decay exponentially, because of two reasons. One is that the amount of phosphorylated p47 was detected to increase significantly at time $t = 2$ min (2 min can be ignored in our slow timescale system) after high fluid shear stress is applied.³³ The other is that among three types of representative functions (constant, exponentially decaying function, burst signal function) that we explored in simulations (results not shown), the simulation results for exponentially decaying function

best matched the experimental observations. Therefore, an exponentially decaying function is used in current simulations until more experimental measurements are available.

Model Design

Model Equations—Based on Fig. 1 and incorporating the above assumptions, we constructed a set of ordinary differential equations in GMA format and extended it by accounting for four time delays (τ_1 , τ_2 , τ_3 , and τ_4) associated with the synthesis of BMP4 precursor, BMP4 antagonist, nox1, and ICAM1. The resulting equations are shown in Eq. 3.

$$\begin{aligned}
 \dot{X}_1 &= \gamma_1 S^{f_1} - \gamma_2 X_1^{g_1} & X_1 &- \text{BMP4 mRNA} \\
 \dot{X}_2 &= \gamma_3 X_1(t - \tau_1)^{g_2} - \gamma_4 X_2^{g_3} & X_2 &- \text{BMP4 precursor} \\
 \dot{X}_3 &= \gamma_4 X_2^{g_3} - \gamma_5 X_3^{g_4} - \gamma_6 X_3^{g_5} X_5^{g_6} & X_3 &- \text{mature BMP4} \\
 \dot{X}_4 &= \gamma_5 X_3^{g_4} - \gamma_7 X_4^{g_7} & X_4 &- \text{BMP4/R complex} \\
 \dot{X}_5 &= \gamma_8 X_3^{g_4} S(t - \tau_2)^{f_2} - \gamma_6 X_3^{g_5} X_5^{g_6} & X_5 &- \text{BMP4 antagonist} \\
 \dot{X}_6 &= \gamma_9 X_4^{g_7} - \gamma_{10} X_6^{g_{10}} & X_6 &- \text{nox1 mRNA} \\
 \dot{X}_7 &= \gamma_{11} (C - X_7) P - \gamma_{12} X_7^{g_{11}} - \gamma_{13} X_7^{g_{12}} X_8^{g_{13}} & X_7 &- \text{p47}_{\text{phosphorylated}} \\
 \dot{X}_8 &= \gamma_{14} X_6(t - \tau_3)^{g_{14}} - \gamma_{13} X_7^{g_{12}} X_8^{g_{13}} & X_8 &- \text{nox1} \\
 \dot{X}_9 &= \gamma_{13} X_7^{g_{12}} X_8^{g_{13}} - \gamma_{15} X_9^{g_{15}} & X_9 &- \text{NADPH oxidase}_{\text{active}} \\
 \dot{X}_{10} &= \gamma_{16} X_9^{g_{16}} + \gamma_{17} X_{11}^{g_{17}} - \gamma_{18} X_{10}^{g_{18}} X_{12}^{g_{19}} - \gamma_{19} X_{10}^{g_{20}} & X_{10} &- \text{superoxide}(O_2^-) \\
 \dot{X}_{11} &= 0.5 \gamma_{19} X_{10}^{g_{20}} - \gamma_{20} X_{11}^{g_{21}} & X_{11} &- \text{hydrogen peroxide}(H_2O_2) \\
 \dot{X}_{12} &= \gamma_{21} - \gamma_{18} X_{10}^{g_{18}} X_{12}^{g_{19}} - \gamma_{22} X_{12}^{g_{22}} & X_{12} &- \text{nitric oxide}(NO) \\
 \dot{X}_{13} &= \gamma_{23} X_{11}^{g_{23}} - \gamma_{24} X_{13}^{g_{24}} & X_{13} &- \text{NF}\kappa\text{B}_{\text{active}} \\
 \dot{X}_{14} &= \gamma_{25} X_{13}(t - \tau_4)^{g_{25}} - \gamma_{26} X_{14}^{g_{26}} & X_{14} &- \text{ICAM1}
 \end{aligned} \tag{3}$$

where S and P represent the intensity of shear stress and the signal of shear stress acting on the phosphorylation of p47, respectively. The constant C represents the total amount of free unphosphorylated and phosphorylated forms of p47. The parameters γ_i and g_i are rate constants and kinetic orders, respectively. The ultimate output of the system is formulated in the following equation:

$$[\text{monocyte adhesion}] = \kappa \cdot X_{14}^{g_{26}}, \tag{4}$$

where κ is a constant coefficient, representing the binding ratio of free monocyte to ICAM1.

Parameter Estimation—Many methods have been developed for the estimation of parameters in BST systems.^{13,24,65} Some of them are based on computing kinetic orders and rate constants from information on traditional Michaelis-Menten functions while others use techniques of estimation from time-series data. In our case, neither mechanistic information nor time-series data are available, thus forcing us to develop *ad hoc* methods of parameter estimation.

We are primarily interested in system behaviors on a time scale of hours, rather than questions of what exactly happens in the system within seconds or minutes. This strategy implies that most fast processes will be in a steady state, which permits the exploitation of

genuine benefits of the BST format. As an example, consider variable X_4 , which is modeled with the equation

$$\dot{X}_4 = \gamma_5 X_3^{g_4} - \gamma_7 X_4^{g_7}, \quad (5)$$

in which the quantities γ_5 , γ_7 , g_4 , and g_7 are the parameters that need to be estimated. If the variable is in a steady state, its derivative on the left-hand side of the equation is equal to zero by definition, which leads to the simpler algebraic equation

$$\gamma_5 X_3^{g_4} = \gamma_7 X_4^{g_7}. \quad (6)$$

Logarithmic transformation of this nonlinear equation yields a linear equation in terms of the parameters:

$$g_4 \log(X_3) - g_7 \log(x_4) = \log\left(\frac{\gamma_7}{\gamma_5}\right). \quad (7)$$

Suppose three scenarios have been assessed experimentally. The first may be a control condition S_0 with no stimulus, while conditions S_1 and S_2 refer to external stimuli 1 and 2. If so, X_3 may assume three distinct (or similar) steady states, each of which is characterized by the same linear equation (Eq. 7). These three equations

$$\begin{cases} g_4 \log(X_{3S_S}) - g_7 \log(X_{4S_S}) = \log\left(\frac{\lambda_7}{\lambda_5}\right) \\ g_4 \log(X_{3S_O}) - g_7 \log(X_{4S_O}) = \log\left(\frac{\lambda_7}{\lambda_5}\right) \\ g_4 \log(X_{3S_L}) - g_7 \log(X_{4S_L}) = \log\left(\frac{\lambda_7}{\lambda_5}\right) \end{cases} \quad (8)$$

form a linear system where X_{iS_S} and X_{iS_O} and X_{iS_L} represent the steady-state values of variable X_i under the static control, oscillatory (OSS) and laminar (ULS) stress conditions. These equations may be converted into the condition

$$\frac{g_4}{g_7} = \frac{\log(M_{4S_O})}{\log(M_{3S_O})} = \frac{\log(M_{4S_L})}{\log(M_{3S_L})}, \quad (9)$$

where $M_{iS_j} = X_{iS_j} / X_{iS_S}$, $i = 3, 4$ and $j = O, L$ represents the ratio of the steady-state values under OSS and ULS in relation to the control condition. Thus, even if we do not know the absolute values at the steady states, we can still derive a linear relationship between g_4 and g_7 if we have information on how the steady-state value of this variable changes for different stimuli. Consequently, given some positive value for either g_4 or g_7 , we can easily calculate the value of the other kinetic order to satisfy all three scenarios. Therefore, for all equations with only two terms, we can derive constraint equations (like Eq. 9), assign experience-

based default values to some parameters,⁶⁷ and then infer the values of the others through linear constraints. Similarly, Eq. (7) involves a relationship between γ_5 and γ_7 , which is deduced secondarily between g_4 and g_7 . All constraint equations for rate constants and kinetic orders are presented in the Appendix. The free choice of a kinetic order may seem to insert undue degrees of freedom, but experience with BST systems severely limits the likely values of kinetic orders, which are usually in ranges of $[0, 2]$ if they are positive, or $[-1, 0]$ if they are negative. Equations with more than two terms or several modulators require similar, but more extensive effort of tuning and validating parameter values.

RESULTS

The results fall into two categories. The first group describes simulations representing observed scenarios. These simulations functionally connect known inputs and outputs and have explanatory character by allowing the detailed tracing of events between stress and response. By contrast, the second group of simulations consists of analyses that do not directly correspond to existing experimental data and characterize the roles of some of the model components on the dynamics of the system. These simulations lead to predictions regarding not yet observed scenarios and to novel hypotheses.

Due to the paucity of quantitative data, all results are presented in relative units, which measure the ratio of each variable under different stimulus conditions in comparison to a control scenario. This type of assessment is actually directly in line with most observations.

All simulations and diagnoses were executed in MATLAB 7.4.0.287(R2007a). Parameter values, including γ_i , g_i , and f_i , constants and initial conditions (given in the Appendix) are always the same except if indicated differently in the corresponding text or figure legend.

Simulation of Three Experimentally Investigated Scenarios

In order to elucidate the distinct roles of OSS and ULS, we implemented three scenarios that had been investigated experimentally. The general starting point for the simulations was the mechanosensitive response system at its control steady state, characterized by static blood overlay without shear, and the simulations consisted of applying ULS (15 dyn/cm²) and OSS (± 5 dyn/cm²), which caused the system to assume different (quasi) steady states. The results are shown in the Fig. 2. The panels in the two top rows show model results (lines) for which corresponding experimental data (circles) are available, except for the BMP4/R complex. The panels in the bottom row show model results for which no data are available.

The levels of BMP4 mRNA, BMP4 precursor, mature BMP4, BMP4 antagonist, and noxl-mRNA increase under OSS and decrease under ULS. Simulations are well consistent with time-series measurements for mature BMP4 under ULS and for other measurements at $t = 24$ h.^{11,29,57,58} An exception is noxl-mRNA under ULS: The experimental result indicates a non-significant increase of noxl-mRNA level when ULS is applied, compared with the static control level, while the simulation shows a noticeable decrease. The logic of the model seems to suggest that the singular available data point might not be correct.

The processes leading to the production of superoxide appear to be adequately simulated as well. Although we have no experimental data for direct comparisons, we can infer their performance by investigating the intriguing dynamics of superoxide. After shear stress was applied at time $t = 0$, the amount of superoxide was observed to increase to a similar level for a short-time exposure (~ 1.7 -fold of the static level at time $t = 1 \text{ h}^{29}$) under both shear stresses, but to reach quite different levels for a longer exposures (~ 1.7 -fold under OSS and about one-third under ULS at time $t = 18 \text{ h}^{29}$). The simulation results of superoxide clearly reflect the observed similarity in short-term effect and the subsequent discrepancy in long-term effect for the two shear stresses. The amounts of superoxide between 1 and 18 h were not experimentally measured and are therefore unknown. Nonetheless, the simulation offers a possible explanation, suggesting a quick drop of superoxide after the initial peak, which may be interpreted as the result of fast depletion of the available nox1 protein. The subsequent climb of superoxide is then caused by the increased amount of nox1 protein, which is induced by OSS through a delayed BMP4-dependent pathway. As a consequence, the relative ratio of the initial condition of nox1 to p47 has an effect on the systemic response, and especially on the dynamics of superoxide production. Opposite to the OSS situation, superoxide production under ULS drops below the control level, although the amount of phosphorylated p47 stays high. This finding is primarily due to the suppression of nox1 protein by ULS through the same pathway. By varying the corresponding parameter values, we could easily change the shape of the dynamic transient to some extent. These simulations are, however, futile until more biological observations at intermediate time points become available that would allow us to determine parameter values with greater precision and reliability.

The second ROS, H_2O_2 , is also simulated well. As discussed in the “Methods” section, we assume no self-propagation or complex signaling mechanisms to be involved in the model. Instead, the activity of H_2O_2 is mainly affected by its precursor, superoxide, and its own degradation. The kinetics of H_2O_2 elimination has been investigated in endothelial cells of the human umbilical vein in culture,⁵² where it was shown to be composed of two different types of reactions exhibiting a linear and nonlinear dependence on the H_2O_2 concentration. In our simulation, one single degradation term is used instead of two, and the corresponding model parameter ($g_{21} = 1.615$) is set between 1 and 2 as a compromise between linearity and second-order nonlinearity. Similar to superoxide, hydrogen peroxide in the simulation is up-regulated roughly twofold under OSS, but greatly down-regulated under ULS. Nitric oxide, an important antioxidant component, is regulated by shear stress in the opposite direction compared with superoxide and hydrogen peroxide.

As the downstream signal receiver and target gene, respectively, $\text{NF}\kappa\text{B}$ activity and ICAM1 are also simulated well. Consistent with experimental observations,⁵⁷ a long-term ($\sim 24 \text{ h}$) simulation reflects an increasing trend for both variables under OSS and a decreasing trend under ULS. Experiments seem to indicate no change in ICAM1 expression for the first few hours, while the corresponding simulation result suggests an immediate increase in ICAM1. The reason for this inconsistency can be traced back to the dynamics of hydrogen peroxide. The current model simplifies both the production and degradation processes of hydrogen peroxide, in line with the primary purposes of this preliminary model. Therefore, the level of hydrogen peroxide increases right after superoxide is increased at the very beginning. As a

consequence, the quick increase of hydrogen peroxide leads to the increase of NF κ B activity and ICAM1 expression.

No quantitative data are available for comparisons of results of BMP4/R complex, and the variables in the bottom row of Fig. 2. Active NADPH oxidase and nox1 protein appear to be reasonable, at least at later times. The simulated concentrations of nitric oxide (NO) also reach reasonable levels after some while, although the initial dynamics may not be correct. Experimental results²⁰ seem to indicate that NO production begins to increase quickly upon ULS and remains elevated as long as the stress is maintained, whereas the simulation exhibits a temporary undershoot. The reason for this initial discrepancy is that the current model assumes constant NO production and only a single degradation reaction with superoxide. In reality, NO production is subject to genetic and metabolic regulation, as well as various signaling events, which are presently not modeled due to the lack of specific data. As a consequence of the simplifications, NO initially decreases in the model under ULS, because ULS induces acute O₂⁻ production, which consumes more NO than under static control. In a similar fashion, the phosphorylated form of p47 remains high under ULS, because nox1 is depleted by ULS in the model.

The number of monocytes bound is simulated as the overall output of the system according to Eq. (4). As shown in the right corner in Fig. 2, long exposure to shear stress (for about one day) increases the number of bound monocytes to approximately threefold over control during OSS (approximately threefold according to experimental measurements²⁹) and decreases the number to ~10% of control during ULS (~50% according to experimental measurements⁵⁷). Given the preliminary nature of the model, these results can be considered both qualitatively and semi-quantitatively consistent with the experimental findings.

To further elucidate the relevance of the shear stress and the BMP4-dependant mechanosensing system, we applied external shear stress with different intensities and patterns to the targeting system. As Fig. 3 shows, the simulation results for BMP4 mRNA, BMP4 precursor, mature BMP4, and nox1 mRNA are well consistent with both the available time-series and experimental measurements at one time point ($t = 24$ h).^{16,57,58} Although the simulated results for mature BMP4 under ULS with different intensities are a little bit higher than the experimental measurements (comparing the model results and experimental measurements for ULS at 5 and 15 dyn/cm²), we still can clearly see the trend that ULS with higher intensity further suppresses the BMP4 expression.

Validation Simulations Characterizing the Role of BMP4 Under OSS

Experiments *in vitro*⁵⁷ have shown that BMP4 alone stimulates monocyte binding in a dose-dependent manner with a maximal activation of fourfold to sevenfold over control. In order to test our model by investigating the role of BMP4, we added at time $t = 0$ a bolus of BMP4 to the system, which was initiated at the static control steady state. In a series of simulation experiments, the size of the BMP4 bolus was varied. The combined results are shown in Fig. 4. They suggest that the number of bound monocytes increases in a dose-dependent manner compared with static control level. The maximal activation achieved in the simulation is ~2.5-fold, which is somewhat smaller than indicated by the experiments. Nonetheless, the qualitative trend in this positive correlation is consistent and supports the hypothesis of a

strong effect of BMP4 on the regulation of monocyte adhesion in endothelial cells. Retro-fitting of the model parameter values would make the numerical values more similar, but it is not pursued here in light of the preliminary nature of the model.

To elucidate the role of BMP4 in OSS-induced monocyte adhesion further, we added at time $t = 0$ a bolus of BMP4 antagonist to the system, which was initiated under static or OSS condition. Experimental results⁵⁷ have shown that treating cells with a sufficient amount of noggin (as a BMP4 antagonist) will completely block OSS-induced monocyte adhesion. As shown in Fig. 5, BMP4 antagonist treatment in the model simulation with OSS greatly reduces the number of monocytes bound at time $t = 24$ h. Even in the static control scenario, treating the system with enough BMP4 antagonist reduces monocyte adhesion, which from a different angle illustrates the important role of BMP4 in OSS-induced monocyte adhesion.

Role of p47^{phox} in the Regulation of NADPH Oxidase Activation by Shear Stress

As a requisite subunit for nox1- and nox2-based NADPH oxidase activation, p47 plays an important role in the regulation of enzyme activity in response to various agonists.^{33,39} In order to clarify the specific role of p47 in shear stress-induced NADPH oxidase activation, we artificially blocked the signaling pathway through which shear stress stimulates the phosphorylation of p47 and compared superoxide production with the control case. As shown in Fig. 6 (dashed lines), the normally quick increase of superoxide production during the first few hours after induction by ULS or OSS is totally blocked, while the production over a longer time horizon remains similar. This finding indicates the potential role of p47 in the quick increase of superoxide production for the first few hours and also provides a possible explanation for why both shear stresses seem capable of stimulating superoxide production during acute exposure.

Finally, the system model represents a p47 knockout quite well. When the p47 gene is knocked out, the amount of its corresponding protein becomes extremely small and undetectable by experimental measurements.²⁹ Obviously, if the available p47 is entirely depleted, the amount of active p47-dependent NADPH oxidase approaches zero, and all subsequent reactions will stop. However, if the amount of p47 is not completely zero, but drastically reduced (e.g., 30% of the control case), the simulation demonstrates that both superoxide production and monocyte adhesion become very low with an indistinguishable amount for OSS, ULS and static control conditions (shown in Fig. 7), even for exactly the same set of parameters. Although OSS and ULS can, respectively, up-regulate or down-regulate the nox1 subunit through the BMP4-dependent pathway, the integrative effect of shear stress acting on the activity of NADPH oxidase is limited, or even blocked, if not enough p47 is available, and this strongly implies the mandatory role of p47 in the activation of NADPH oxidase. Experimental observations²⁹ indicate that superoxide produced in endothelial cells obtained from p47^{phox}-deficient mouse aortas (p47^{-/-} system) is about 50% or less of the amount produced by the corresponding p47^{+/+} system under static condition. Our simulation results (Fig. 7, left) point in the same direction but indicate a greater reduction (~20% or less of the amount produced by the p47^{+/+} system) in superoxide. This difference in extent is possibly due to the omission of other contributing sources for superoxide production in the current model.

DISCUSSION

Shear stress has been identified as one of the most important factors for the location of inflammation and subsequent atherosclerosis.^{31,40,46,60} *In vitro* experiments have identified an important cytokine, BMP4, which is involved in shear-induced inflammation in endothelial cells and capable of activating endothelial NADPH oxidase, and thus inducing ICAM1 expression.^{31,57,58} In order to investigate the role of BMP4 in the mechanotransduction system in greater detail, we have in this study developed a prototype mathematical model. The model was constructed in the GMA format of BST.^{54,55,62,66,67}

As an important initial step, we focused on the key components and processes of the targeted pathway as they are described in Fig. 1. Due to the paucity (or entire lack) of available time-series data and kinetic characterizations of the processes within the system, we resorted to a novel *ad hoc* method of identifying suitable parameter values that was consistent with the nature of most of the available data, which report responses in relation to a nominal state. A few assumptions and simplifications (see details in “Methods” section) were made to permit a first grasp of the complexity of the mechanosensing system and to allow numerical implementation. Time delays accounting for the protein synthesis were added to the GMA model as a means of handling different time scales. Indeed, it turned out that these delays were crucial because their removal led to irreconcilable inconsistencies between model and data (results not shown).

Three scenarios, namely static control, OSS, and ULS, were simulated with the model. Most simulation results were found to be consistent with experimental measurements and permitted additional dynamical predictions that have not yet been assessed experimentally. The analysis led to two slight discrepancies between simulation results and experimental observations. One is associated with the steady-state level of nox1-mRNA under ULS condition, which seemed not to change significantly over static control in experiments, but exhibited a significant reduction in the simulation. A possible explanation may be that the static flow control is not a physiologically relevant condition for comparison, because the complete lack of blood flow only occurs in extreme pathological conditions. Excluding the static condition, one could simply compare nox1 mRNA levels under ULS and OSS. In this comparison, the simulation result indicates a significant decrease, which is consistent with the experimental observation. A second discrepancy is the increased ICAM1 expression in the first few hours under ULS, which was not observed *in vitro*. One possible reason for this difference may be that the degradation processes of hydrogen peroxide are extremely simplified at this step, which leads to a direct correlation between the concentration of hydrogen peroxide and ICAM expression. Besides, it is possible that ICAM1 increases as a part of the adaptation response to a sudden change from static to shear conditions (either ULS or OSS). However, it is important to note that ULS inhibits, whereas OSS stimulates, monocyte adhesion to endothelial cells,^{57,58} suggesting that there are additional shear-sensitive changes, including an increase in ROS, that regulate the inflammatory response.

Although it is generally agreed that OSS is a critical pro-inflammatory factor that increases ROS production during long exposure (~1 day), different research groups generated distinct transient dynamics and quantitative measurements. For example, studies conducted by

Griending and her colleagues¹⁸ suggested that endothelial cells gradually increase their superoxide production in response to OSS, which is different from our experimental findings.⁵⁸ This discrepancy in short-time effect may be caused by the different experimental set-up between the two groups. The model is presently too coarse to identify the “correct” response because changes in parameter values permit reproducing both types of observations (results not shown).

In order to illustrate the specific roles of BMP4 and p47, additional simulations were implemented to mimic special experimental treatments, such as BMP4/BMP4-antagonist treatments and a p47 knockout model. The results of these simulations unequivocally pointed to the necessity of BMP4 and p47 in OSS-induced inflammation, suggesting that BMP4 might indeed be a crucial and potent component in the regulation of distinct long-term effects of OSS and ULS, and that p47 might be a critical factor inducing similar short-effects for OSS and ULS.

Of course, the mechanosensing system *in vivo* is much more complicated than our current model. It consists not only of the single pathway targeted here, but it is also tied to uncounted other pathways that could have direct or indirect, weak or strong influences on its genomic or metabolic regulation, on important intra- or extra-cellular signals, and on the production or degradation of some of the key compounds that govern our coarse model system. For instance, a recent study suggests that a cAMP/PKA-dependent pathway plays an essential role in the down-regulation of BMP4 under ULS.¹⁶ Furthermore, as stated in the “Methods” section, the activities and expressions of some critical antioxidant enzymes, such as SOD, eNOS, and peroxiredoxin 1, have been shown to be regulated by long exposure (~1 day) to shear stress, thereby providing a cellular defense mechanism counteracting oxidative stress.^{4,5,30} These regulations were not incorporated in the current model. However, the performed sensitivity analyses of the corresponding parameters (like γ_{19} , γ_{21} , g_{20}) pointed toward reduced quantities of ROS and increased availability of nitric oxide (data not shown). These results may be interpreted as a self-regulatory defense, which further supports the reasonableness of the current model structure.

To grasp the overall response of endothelial cells to shear stress, these potentially important pathways and mechanisms must ultimately be integrated into a single conceptual framework. It is clear that the increasing scope of such extensions and their explorations will overwhelm the linear cause-and-effect thinking that our human mind likes best, and that the only possible remedy will be a sequence of ever more detailed and fine-tuned systematic computational representations. As it stands, the model proposed here is coarse and quite primitive. However, once this prototype model is validated, it can be expanded toward the investigation of other external stimuli like angiotensin II and TNF- α , which also stimulate NADPH oxidase activation and mediate endothelial function in inflammation.^{10,43} The step-wise extension of the model will permit investigations of a growing network of reactions and processes, which might eventually cover the entire dynamic stress response of the endothelial cell.

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APPENDIX A

Parameter Estimation

Due to the paucity of available time-series and quantitative data, the rate constant and kinetic order parameter values (γ_i and g_i) were estimated in an *ad hoc* fashion as described in “Methods” section (“Parameter Estimation” section). All constraints (both for kinetic orders and rate constants) derived from equations with two terms (see Eq. 3) are presented in Table A1.

Given these constraints and assigning a default value (such as 0.5 for substrate dependency or activation and -0.5 for inhibition) to one of the two relevant kinetic orders yields the value for the other one. This procedure requires knowledge of the ratio M_{iS_O} or M_{iS_L} . Either one of the ratios of experimental measurements to the control can be used for the computation. When both ratios are experimentally available, the ratio measured under OSS condition was used. The available ratios that were recalculated from experimental measurements are listed in Table A2.

TABLE A1

Parameter constraint equations.

Kinetic orders	Constraint equations
g_2 and g_3	$\frac{g_2}{g_3} = \frac{\log(M_{2S_O})}{\log(M_{1S_O})} = \frac{\log(M_{2S_L})}{\log(M_{1S_L})}$
g_4 and g_7	$\frac{g_4}{g_7} = \frac{\log(M_{4S_O})}{\log(M_{3S_O})} = \frac{\log(M_{4S_L})}{\log(M_{3S_L})}$
g_9 and g_{10}	$\frac{g_9}{g_{10}} = \frac{\log(M_{5S_O})}{\log(M_{4S_O})} = \frac{\log(M_{5S_L})}{\log(M_{4S_L})}$
g_{12} , g_{13} , and g_{14}	$\begin{cases} g_{14}\log(M_{6S_O}) = g_{12}\log(M_{7S_O}) + g_{13}\log(M_{8S_O}) \\ g_{14}\log(M_{6S_L}) = g_{12}\log(M_{7S_L}) + g_{13}\log(M_{8S_L}) \end{cases}$
g_{12} , g_{13} and g_{15}	$\begin{cases} g_{15}\log(M_{9S_O}) = g_{12}\log(M_{7S_O}) + g_{13}\log(M_{8S_O}) \\ g_{15}\log(M_{9S_L}) = g_{12}\log(M_{7S_L}) + g_{13}\log(M_{8S_L}) \end{cases}$
g_{20} and g_{21}	$\frac{g_{20}}{g_{21}} = \frac{\log(M_{11S_O})}{\log(M_{10S_O})} = \frac{\log(M_{11S_L})}{\log(M_{10S_L})}$

Kinetic orders	Constraint equations
g_{23} and g_{24}	$\frac{g_{23}}{g_{24}} = \frac{\log(M_{13S_O})}{\log(M_{11S_O})} = \frac{\log(M_{13S_L})}{\log(M_{11S_L})}$
g_{25} and g_{26}	$\frac{g_{25}}{g_{26}} = \frac{\log(M_{14S_O})}{\log(M_{13S_O})} = \frac{\log(M_{14S_L})}{\log(M_{13S_L})}$
Rate constants	Constraint equations
γ_3 and γ_4	$\frac{\gamma_3}{\gamma_4} = \frac{X_{2S_S}^{g_3}}{X_{1S_S}^{g_2}}$
γ_5 and γ_7	$\frac{\gamma_5}{\gamma_7} = \frac{X_{4S_S}^{g_7}}{X_{3S_S}^{g_4}}$
γ_9 and γ_{10}	$\frac{\gamma_9}{\gamma_{10}} = \frac{X_{5S_S}^{g_{10}}}{X_{4S_S}^{g_9}}$
γ_{13} and γ_{14}	$\frac{\gamma_{13}}{\gamma_{14}} = \frac{X_{7S_S}^{g_{12}} X_{8S_S}^{g_{13}}}{X_{6S_S}^{g_{14}}}$
γ_{13} and γ_{15}	$\frac{\gamma_{13}}{\gamma_{15}} = \frac{X_{7S_S}^{g_{12}} X_{8S_S}^{g_{13}}}{X_{9S_S}^{g_{15}}}$
γ_{19} and γ_{20}	$\frac{\gamma_{19}}{\gamma_{20}} = \frac{X_{11S_S}^{g_{21}}}{X_{10S_S}^{g_{20}}}$
γ_{23} and γ_{24}	$\frac{\gamma_{23}}{\gamma_{24}} = \frac{X_{13S_S}^{g_{24}}}{X_{11S_S}^{g_{23}}}$
γ_{25} and γ_{26}	$\frac{\gamma_{25}}{\gamma_{26}} = \frac{X_{14S_S}^{g_{26}}}{X_{13S_S}^{g_{25}}}$

X_{iS_S} , X_{iS_O} , X_{iS_L} represent the steady state of variable X_i under Static control, OSS and ULS conditions.
 $M_{iS_O} = X_{iS_O} / X_{iS_S}$, $M_{iS_L} = X_{iS_L} / X_{iS_S}$ represent the ratios of the steady-state values under OSS and ULS in relation to the static control condition.

TABLE A2

Experimental ratios of steady-state values under OSS and LSS in relation to static control.

Variable index (i)	M_{iS_O}	M_{iS_L}
1 (BMP4 mRNA)	1.5	0.3

Variable index (i)	M_{iS_O}	M_{iS_L}
2 (BMP4 precursor)	2	0.25
3 (mature BMP4)	1.2	0.15
5 (BMP4 antagonist)	1.25	0.1
6 (nox1 mRNA)	3.5	1.5
10 (superoxide)	1.7	0.3
11 (hydrogen peroxide)	2	–
13 (NF κ B _{active})	3.4	–
14 (ICAM1)	3.0	–

The steady states for the above variables in static control are assumed to be 1 (100%). ULS 15 dyn/cm², OSS $\pm$ 5 dyn/cm². “–” indicates that no experimental measurement are presently available.

TABLE A3

Numerical values of initial conditions (ICs) and independent variables.

Dependent variables	Numerical value (IC)	Dependent variables	Numerical value (IC)	Independent variable	Numerical value
BMP4 mRNA	1	nox1	1	$S(U_{LS})$	5–15 dyn/cm ²
BMP4 precursor	1	NADPH oxidase _{active}	1	$S(OSS)$	$\pm$ 5 dyn/cm ²
Mature BMP4	1	Superoxide	1	$S(STATIC)$	1
BMP4/R complex	1	Hydrogen peroxide	1		
				Constant	Numerical value
				C	1
				κ	1
BMP4 antagonist	1	Nitric oxide	1	ρ	5
nox1 mRNA	1	NF κ B _{active}	1	σ	0.1
p47 _{phosphorylated}	0.2	ICAM1	1	offset	1

Once the kinetic orders are set, the rate constants are secondarily deduced with the constraint equations in a similar fashion. While these methods determine a good portion of the needed parameter values, other parameters require default assumptions, experience, and additional efforts for fine tuning and validating, based on available biological observations. Examples are the parameters in the system equations in Eq. (3) that contain external inputs or more than two terms.

Simulation Settings

All simulations (with results displayed in Figs. 2 to 7 in the text) use the same numerical sets of initial conditions (Table A3) and parameter values (Table A4), with the exception of parameters under investigation in a specific simulation, as described in the text and the corresponding figure legends.

The signal of shear stress acting on the phosphorylation of p47 is represented by an exponentially decaying function of the form

$$p = \begin{cases} \rho \cdot \exp(-\sigma \cdot (t - t_0)) + offset, & t \geq t_0 \\ 1, & t < t_0 \end{cases},$$

where ρ represents the intensity of the phosphorylation signal after shear stress is added to the system at time $t = t_0$. The numerical values of ρ , σ , and *offset* are listed in Table A3.

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FIGURE 1.

Simplified schematic representation of the BMP4-triggered system of shear-dependent inflammation in endothelial cells. Numbers in parentheses refer to pertinent references. *Solid lines* indicate material flow through the pathway, while *dashed lines* indicate signals without the flow of material. τ indicate time delays.

FIGURE 2.

Simulation results of three scenarios. *Blue line*: static control; *Red line*: OSS (± 5 dyn/cm²); *green line*: ULS (15 dyn/cm²). Lines indicate simulation results; dots are experimental measurements.

FIGURE 3.

Simulation results of shear stress with different intensities and modalities. Lines indicate simulation results; dots are experimental measurements. *Black:* static control; *red:* ULS (5 dyn/cm²); *green:* ULS (10 dyn/cm²); *blue:* ULS (15 dyn/cm²); *magenta:* ULS (30 dyn/cm²); *dashed black line.* OSS (±5 dyn/cm²).

FIGURE 4.

Simulated number of bound monocytes under different BMP4 treatments. *Solid black line:* static control; *solid red line:* OSS (± 5 dyn/cm²); *dashed red line:* treatment with BMP4 bolus of 1 at time $t = 0$; *dashed green line:* treatment with BMP4 bolus of 2 at time $t = 0$; *dashed blue line:* treatment with BMP4 bolus of 4 at time $t = 0$; *dashed magenta line:* treatment with BMP4 bolus of 9 at time $t = 0$; *dashed cyan line:* treatment with BMP4 bolus of 19 at time $t = 0$; *dashed black line:* experimentally measured maximum increase (in relation to control with BMP4 treatment).

FIGURE 5.

Simulated number of bound monocytes with and without BMP4 antagonist treatment. *Solid black line*: static control; *solid red line*: OSS (± 5 dyn/cm²); *dashed black line*: static control with a BMP4 antagonist bolus of 2 at time $t = 0$; *dashed red line*: OSS (± 5 dyn/cm²) with a BMP4 antagonist bolus of 9 at time $t = 0$.

FIGURE 6.

Simulated superoxide production under different conditions. Lines indicate simulation results; dots are experimental measurements. *Black line*: static control; *solid red line*: OSS (± 5 dyn/cm²); *dashed red line*: OSS (± 5 dyn/cm²) with blocked signal pathway through which oss otherwise stimulates the phosphorylation rate of p47^{phox}; *solid green line*: ULS (15 dyn/cm²); *dashed green line*: ULS (15 dyn/cm²) with blocked signal pathway through which OSS otherwise stimulates the phosphorylation rate of p47^{phox}.

FIGURE 7.

Comparison of selected simulation results investigating the p47^{+/+} and p47^{-/-} systems under different shear stress conditions. In the p47^{+/+} system, $C = 1$ and initial condition of p47_{phosphorylated} = 0.2; in the p47^{-/-} system, $C = 0.3$ and initial condition of p47_{phosphorylated} = 0.06.

TABLE A4

Numerical values of rate constants, kinetic orders, and time delays.

Kinetic order	Numerical value	Kinetic order	Numerical value	Rate constant	Numerical value	Rate constant	Numerical value
g_{17}	0.786	g_{17}	0.52	r_1	0.94	r_{17}	40
g_{18}	2.289	g_{18}	1	r_2	0.94	r_{18}	60
g_{19}	1.433	g_{19}	1	r_3	0.42	r_{19}	20
g_{20}	2.5	g_{20}	2	r_4	0.42	r_{20}	10
g_{21}	3.5	g_{21}	1.615	r_5	0.286	r_{21}	70
g_{22}	3.5	g_{22}	1	r_6	0.134	r_{22}	10
g_{23}	4.782	g_{23}	1	r_7	0.286	r_{23}	100
g_{24}	0.8	g_{24}	0.631	r_8	0.134	r_{24}	100
g_{25}	4.593	g_{25}	1	r_9	100	r_{25}	100
g_{26}	0.5	g_{26}	1.067	r_{10}	100	r_{26}	100
g_{11}	1	$f_1(OSS)$	0.2	r_{11}	0.6		
g_{12}	1	$f_1(ULS)$	-0.35	r_{12}	0.4	Time delay	Numerical value
g_{13}	1	$f_1(STATIC)$	0	r_{13}	2	τ_1	10 h
g_{14}	0.8	$f_2(OSS)$	0.72	r_{14}	0.4	τ_2	11 h
g_{15}	1	$f_2(ULS)$	-2	r_{15}	0.4	τ_3	0.1 h
g_{16}	1	$f_2(STATIC)$	0	r_{16}	40	τ_4	0.1 h