A multiphase model of growth factor-regulated atherosclerotic cap formation

Michael G. Watson · Helen M. Byrne · Charlie Macaskill · Mary R. Myerscough

Abstract
Atherosclerosis is characterised by the growth of fatty plaques in the inner artery wall. In mature plaques, vascular smooth muscle cells (SMCs) are recruited from adjacent tissue to deposit a collagenous cap over the fatty plaque core. This cap isolates the thrombogenic plaque content from the bloodstream and prevents the clotting cascade that leads to myocardial infarction or stroke. Despite the protective role of the cap, the mechanisms that regulate cap formation and maintenance are not well understood. It remains unclear why some caps become stable, while others become vulnerable to rupture. We develop a multiphase PDE model with non-standard boundary conditions to investigate collagen cap formation by SMCs in response to diffusible growth factor signals from the endothelium. Platelet-derived growth factor stimulates SMC migration, proliferation and collagen degradation, while transforming growth factor-β stimulates SMC collagen synthesis and inhibits collagen degradation. The model SMCs respond haptotactically to gradients in the collagen phase and have reduced rates of migration and proliferation in dense collagenous tissue. The model, which is parameterised using in vivo and in vitro experimental data, reproduces several observations from plaque growth in mice. Numerical and analytical results demonstrate that a stable cap can be formed by a relatively small SMC population and emphasise the critical role of TGF-β in effective cap formation. These findings provide unique insight into the mechanisms that may lead to plaque destabilisation and rupture. This work represents an important step towards the development of a comprehensive in silico plaque model.

Keywords Atherosclerosis · Multiphase model · Smooth muscle cells · Platelet-derived growth factor · Transforming growth factor-β
Mathematics Subject Classification 92C05 · 92C17 · 92C37

1 Introduction

Cardiovascular diseases are the leading global cause of mortality (World Health Organization 2017). Atherosclerosis, the growth of fat-filled plaques in the walls of arteries, is the primary cause of most cardiovascular disease-related deaths. Unstable atherosclerotic plaques can rupture, and subsequent blood clot formation may occlude blood flow and lead to myocardial infarction or stroke (Hansson and Libby 2006). Smooth muscle cells (SMCs) play an important role in the prevention of these grave clinical outcomes by forming a stabilising cap of fibrous tissue over the lipid-rich core in developing plaques (Alexander and Owens 2012). To understand how plaques become unstable and dangerous, it is important to understand the behaviour of plaque SMCs and the corresponding mechanisms of fibrous cap synthesis or degradation. Despite extensive experimental investigation, the in vivo dynamics of cap formation remain poorly understood.

The absence of a dynamic understanding of cap formation and maintenance can be largely attributed to the slow rate of plaque development. In humans, atherosclerosis can begin in childhood and plaques may take several decades to progress towards a dangerous, unstable state (Lusis 2000). Even in the apolipoprotein-E (ApoE) deficient mouse (the most common animal model for experimental atherosclerosis studies), plaques require a period of several months to grow to an advanced stage. Consequently, observations of plaque tissue resected from experimental mice are typically made at distinct (or even single) time points. Mathematical modelling provides a powerful tool to study the dynamic mechanisms that underlie these patchy experimental observations. In this paper, we use the multiphase framework developed in Watson et al. (2018) and build a new model to study the formation of the protective fibrous cap by atherosclerotic plaque SMCs.

Atherosclerotic plaques develop in the narrow intimal layer of the artery wall (Fig. 1). The intima is located between the endothelium (a thin sheet of endothelial cells that lines the vessel lumen) and the media, which houses several striated layers of quiescent (contractile) SMCs. The intima and the media are separated by a dense tissue membrane known as the internal elastic lamina (IEL). The outermost layer of the artery wall beyond the media is called the adventitia. Plaque formation is initiated by blood-borne low-density lipoproteins (LDL), which accumulate in the intima and become oxidised or modified in different ways. The presence of modified LDL triggers the recruitment of immune cells (monocytes) from the bloodstream by a process of transendothelial migration. These monocytes differentiate into macrophages, which can consume the lipid and remove it from the intima by migration to the adventitial lymphatics (Moore et al. 2013). However, when lipid-filled macrophages (known as foam cells) die within the plaque, they release their lipid content and other cellular debris. This reinforces the immune response and can lead to a cycle of chronic inflammation that promotes plaque growth and, ultimately, the development of necrotic tissue.

At an intermediate stage of plaque formation, medial SMCs adopt an active (synthetic) state and migrate through the IEL into the intima. SMC activation is believed...
Fig. 1 Schematic diagram of a cross-section through the inner artery wall (layer widths not to scale and outer adventitial layer not shown). Atherosclerotic plaques develop in the intima, which is separated from the blood flow in the lumen by a thin layer of cells called the endothelium. Deeper in the wall, the intima is separated from the media by a membrane called the internal elastic lamina.

...to be initiated by an injured endothelium, which may suffer mechanical disruption as the intimal layer expands to accommodate the infiltration of lipid and immune cells (Faggiotto and Ross 1984). Once inside the plaque, SMCs replace the existing intimal extracellular matrix (ECM) with a dense matrix of fibrillar collagens (Adiguzel et al. 2009). SMCs are subject to an abundance of signalling cues in the plaque, but two chemicals known to be particularly critical to cap formation are platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β). PDGF plays an important role as a SMC mitogen and chemoattractant (Rutherford et al. 1997; Sano et al. 2001), while TGF-β enhances plaque stability by stimulating SMC collagen production (Mallat et al. 2001; Lutgens et al. 2002). PDGF and TGF-β can be produced by a variety of plaque cells, but circulating platelets that adhere to sites of endothelial injury are believed to be a significant source of both growth factors (Ross 1999; Toma and McCaffrey 2012). TGF-β is secreted as a latent complex and the active component of TGF-β must be liberated before it can bind to target cells (Singh and Ramji 2006). Liberation of active TGF-β typically requires chemical or mechanical stimulation, and TGF-β derived from platelets is believed to be activated by shear forces in the bloodstream (Ahamed et al. 2008). A schematic diagram of the cap formation process is provided in Fig. 2.

Recent advances in cell lineage tracing techniques have begun to reveal new insights into the behaviour of plaque SMCs. Chappell et al. (2016) and Jacobsen et al. (2017) have performed studies using so-called Confetti transgenic mice, where individual medial SMCs can be labelled by inducing the unique expression of one of four possible fluorescent proteins in each cell. Both of these studies have identified that SMC populations in advanced plaques consist of several large monochromatic patches, indicating that plaque SMCs are oligoclonal and derived from only a limited number of medial progenitor cells. These results are significant because they highlight, in the ApoE mouse at least, that proliferation makes a much larger contribution to SMC accumulation in plaques than previously thought.

These studies provide an exciting new window into the cellular mechanisms of fibrous cap formation, and have significant potential to advance current understanding of atherosclerosis progression. However, much is still unknown about the factors that underlie the formation and maintenance of the collagenous cap and, in particular, the reasons why certain caps remain thick and stable whilst others become thin and
Fig. 2  Schematic diagram of the key processes in atherosclerotic cap formation. Lipoprotein accumulation in the intima triggers an immune response that leads to intimal growth and endothelial disruption. Diffusible growth factors PDGF and TGF-β are released from sites of injury by endothelial cells and adherent platelets. Contractile SMCs in the media are stimulated to adopt a synthetic state and respond chemotactically to PDGF by migrating through the internal elastic lamina. Once inside the plaque, synthetic SMCs remodel the existing ECM and are stimulated by TGF-β to deposit a dense cap of collagenous tissue adjacent to the endothelium.

vulnerable to rupture. Several possible modes of long-term cap degradation have been proposed in the literature. These include an increase in SMC death—possibly due to cellular senescence (Wang et al. 2015)—or an increase in collagen destruction due to either elevated matrix metalloproteinase (MMP) production by inflammatory cells (Hansson et al. 2015) or reduced SMC sensitivity to TGF-β signalling (Chen et al. 2007; Vengrenyuk et al. 2015). The model that we develop in this paper addresses these gaps in biological understanding by establishing a framework to simulate cap formation dynamics and assess the deleterious impact of a variety of ECM degradation mechanisms.

We have recently published a two-phase PDE model that studied the migration and proliferation of media-derived SMCs in response to an endothelium-derived source of diffusible PDGF (Watson et al. 2018). The model domain was taken to be a one-dimensional cross-section through the diseased intima and the flux of SMCs and PDGF into the plaque were captured by a set of non-standard boundary conditions. The model did not explicitly consider the synthesis of a fibrous cap, but the approach did provide several insights into the mechanisms that regulate SMC migration to the cap region. In particular, the model predicted that SMC recruitment from the media is likely to be a rate-limiting factor for SMC accumulation in plaques—an observation that is supported by the recent lineage tracing studies of Chappell et al. (2016) and Jacobsen et al. (2017). In the current paper, we build upon this earlier model by incorporating a profile of diffusible, endothelium-derived TGF-β and an explicit representation of collagenous ECM remodelling. This allows us to perform a detailed investigation of growth factor-stimulated fibrous cap formation by plaque SMCs. Note that the new
model is more than just an elementary extension of the earlier approach. We assume that the SMCs and the nascent ECM are coupled by a nonlinear mechanical feedback that allows us to investigate the influence of factors including haptotactic SMC migration, which may play an important role in the cap formation process (Nelson et al. 1996; Hou et al. 2000; Lopes et al. 2013).

Interest in mathematical modelling of the cell activity in atherosclerosis has grown over recent years. The majority of studies published to date have focussed on modelling the inflammatory response that characterises the early stages of plaque development (El Khatib et al. 2007; Pappalardo et al. 2008; Cohen et al. 2014; Chalmers et al. 2015; Ford et al. 2019), where several groups have coupled their models to detailed representations of blood flow and/or intimal growth (Bulelzai and Dubbeldam 2012; Filipovic et al. 2013; Islam and Johnston 2016; Yang et al. 2016; Bhui and Hayenga 2017). In contrast, only a handful of models have been proposed to study events in the later stages of plaque progression. McKay et al. (2004) performed foundational work in this area by proposing a PDE model that included both plaque SMC recruitment and subsequent collagen deposition. The only other model to consider collagen synthesis by invading SMCs is that of Cilla et al. (2014), who developed a comprehensive 3D model of blood flow, transmural transport and plaque growth. Neither of these modelling approaches, however, provide an adequate description of the cap formation process. Several other models of plaque progression (Poston and Poston 2007; Friedman and Hao 2015)—including studies of PDGF-induced intimal thickening (Fok 2012) and intraplaque haemorrhage (Guo et al. 2018)—have considered SMCs independently of their collagen-synthesising activity. Interested readers are referred to Parton et al. (2016) for a comprehensive review of mathematical and computational approaches to atherosclerosis modelling.

Beyond atherosclerosis, a variety of mathematical models have been developed to study the response of vascular SMCs to other sources of endothelial injury. One area that has attracted significant research interest is in-stent restenosis—the rapid recurrence of a narrowed lumen after surgical deployment of an artery-widening stent. Mechanical stresses imposed by the stent can locally denude the endothelium and elicit an intense healing response that involves rapid proliferation of medial SMCs and significant neointima formation. Discrete and continuous models of SMC behaviour during in-stent restenosis have been developed by several research groups (Lally and Prendergast 2006; Evans et al. 2008; Zahedmanesh et al. 2014; Tahir et al. 2015). Researchers have also developed models of the vascular SMC response to surgical interventions such as vein grafting (Budu-Grajdeanu et al. 2008; Garbey et al. 2017) and blood filter insertion (Nicolas et al. 2015). The tissue repair carried out by artery wall SMCs in response to vascular injury also shares several similarities with the process of dermal wound healing. In dermal wounds, fibroblasts take the role of the SMC and migrate to the wound site to regenerate the damaged collagenous tissue. Wound fibroblasts are known to be stimulated by growth factors including PDGF and TGF-β, and the corresponding implications for healing have been studied in a variety of modelling frameworks (Olsen et al. 1995; Cobbold and Sherratt 2000; Haugh 2006; McDougall et al. 2006; Cumming et al. 2010; Menon et al. 2012).

The model of cap formation that we present in this paper is inspired by the multiphase theory developed in Byrne and Owen (2004), Lemon et al. (2006) and Astanin
and Preziosi (2008). Multiphase models have been widely developed to study articular cartilage (reviewed in Klika et al. 2016), tumour growth (Preziosi and Tosin 2009; Hubbard and Byrne 2013) and tissue engineering applications (O’Dea et al. 2013; Pearson et al. 2014), but our previous study (Watson et al. 2018) was the first application of the approach in atherosclerosis. Other existing studies of plaque development have predominantly utilised reaction–diffusion equations to model the spatio-temporal evolution of cells and tissues in the plaque (McKay et al. 2004; El Khatib et al. 2007; Filipovic et al. 2013; Cilla et al. 2014; Chalmers et al. 2015, 2017). However, multiphase models can provide a more detailed representation of plaque formation dynamics because they provide a natural framework to account for volume exclusion and mechanical interactions between individual plaque constituents. We continue to develop our multiphase approach in this study because we firmly believe that both volume exclusion and mechanical effects can play a significant role in the development of plaque spatial structure.

In the next section, we develop the model equations and introduce the model parameterisation. Where possible, we base our assumptions and our parameter selections on observations of plaque growth in the ApoE mouse, which is the most well-characterised animal model of in vivo atherosclerosis in the experimental literature (Getz and Rar- don 2012). We present the model results in Sect. 3, where we report some interesting analysis before performing a series of numerical simulations. We use these results to investigate the dynamic cellular, biochemical and mechanical mechanisms that regulate cap formation and aim to identify the factors that are most important to the development and maintenance of plaque stability. Finally, we conclude with a discussion of the outcomes of the study in the context of both computational and experimental atherosclerosis research.

2 Model formulation and parameterisation

The plaque tissue in the model is represented as a mixture of three distinct phases: a cellular phase that comprises matrix-synthesising SMCs, an ECM phase that comprises collagen-rich fibrous tissue, and a generic phase that is assumed to comprise the remaining plaque constituents (foam cells, extracellular lipids, interstitial fluid, and so on). We derive mass and momentum balance equations for each phase, and the equations are closed by imposing suitable constitutive assumptions and conditions for the transfer of mass and momentum between each pair of phases. Equations for the concentrations of diffusible PDGF and (active) TGF-β are also included in the model.

We assume that PDGF promotes a migroproliferative SMC phenotype, while TGF-β promotes a matrigenic SMC phenotype (Alexander and Owens 2012). These distinct SMC phenotypes are not explicitly represented in the model. Instead, we assume that the SMC phase contains a continuum of different phenotypes whose average behaviour is regulated by the local growth factor concentrations. We assume that both PDGF and TGF-β reside exclusively in the generic tissue phase, and that they exert influence on the SMC and ECM phases via stress and/or mass transfer terms that are functions of their respective concentrations.
We model the above system on a one-dimensional Cartesian domain that represents a cross-section of diseased arterial intima far from the edges of the plaque. We assume that the domain is bounded by the endothelium at $x = 0$ and by the IEL at $x = L$. The dependent variables are therefore functions of time $t \geq 0$ and position $x \in [0, L]$. We denote the volume fractions of the SMC, ECM and generic tissue phases by $m(x, t), \rho(x, t)$ and $w(x, t)$, and their corresponding intraphase stresses by $\tau_m(x, t), \tau_\rho(x, t)$ and $\tau_w(x, t)$, respectively. We assume that the ECM phase takes the form of a rigid scaffold and the ECM phase therefore has zero velocity for all $x$ and $t$. We denote the (non-zero) velocities of the SMC and generic tissue phases by $v_m(x, t)$ and $v_w(x, t)$. The interstitial fluid pressure is denoted by $p(x, t)$, and the concentrations of PDGF and TGF-$\beta$ in the generic tissue phase are denoted by $P(x, t)$ and $T(x, t)$, respectively.

### 2.1 SMC, ECM and generic tissue phases

In this section we use the principles of mass and momentum conservation to derive equations that describe the SMC, ECM and generic tissue phase dynamics in response to diffusible PDGF and TGF-$\beta$ in the plaque.

#### 2.1.1 Mass balance equations

Assuming that all three phases share the same constant density, the mass balance equations for $m, \rho$ and $w$ can be expressed as follows:

$$\frac{\partial m}{\partial t} + \frac{\partial}{\partial x} (v_m m) = S_m, \tag{1}$$

$$\frac{\partial \rho}{\partial t} = S_\rho, \tag{2}$$

$$\frac{\partial w}{\partial t} + \frac{\partial}{\partial x} (v_w w) = -\left(S_m + S_\rho\right). \tag{3}$$

Here, the functions $S_m$ and $S_\rho$ represent the net rates of SMC and ECM production. We have assumed in Eq. (3) that there is no local source or sink of material and that mass simply transfers from one phase to another as SMCs proliferate or die, and as ECM is synthesised or degraded. We further assume that there are no voids in the plaque tissue, and the three volume-occupying phases therefore satisfy the condition:

$$m + \rho + w = 1. \tag{4}$$

#### 2.1.2 Momentum balance equations and constitutive assumptions

Neglecting inertial effects and assuming that no external forces act on the system, the momentum balance equations for $m, \rho$ and $w$ reduce to a balance between the intraphase and interphase forces that act on each phase:
\[ \frac{\partial}{\partial x} (\tau_{mm}) = F_{\rho m} + F_{wm}, \]
\[ \frac{\partial}{\partial x} (\tau_{\rho \rho}) = F_{m \rho} + F_{w \rho}, \]
\[ \frac{\partial}{\partial x} (\tau_{ww}) = F_{m w} + F_{\rho w}, \]

where \( F_{ij} (= - F_{ji}) \) denotes the force exerted by phase \( j \) on phase \( i \).

Following Lemon et al. (2006), we assume that the \( F_{ij} \) terms in Eqs. (5)–(7) are comprised of interphase pressure and interphase drag components:

\[ F_{m \rho} = (p + \psi) \rho \frac{\partial m}{\partial x} - (p + \psi) m \frac{\partial \rho}{\partial x} - k_{m \rho} m \rho v_m , \]
\[ F_{m w} = p_w \frac{\partial m}{\partial x} - p m \frac{\partial w}{\partial x} + k_{m w} m w (v_w - v_m) , \]
\[ F_{\rho w} = p_w \frac{\partial \rho}{\partial x} - p \rho \frac{\partial w}{\partial x} + k_{\rho w} \rho w v_w . \]

The first term on the right hand side of each of Eqs. (8)–(10) describes the interfacial force exerted by phase \( j \) on phase \( i \). This force is assumed to be proportional to the interphase pressure between the phases and to the degree of contact of phase \( j \) with phase \( i \). The force is assumed to act in the direction of increasing interfacial contact and, on the macroscale, this contributes a further term proportional to the gradient in phase \( i \). The second term on the right hand side of each equation contributes a corresponding reaction force, which is exerted by phase \( i \) on phase \( j \). Note that we have included an additional (contact-dependent) interphase pressure contribution \( \psi \equiv \psi (m, \rho) \) in the equation for \( F_{m \rho} \). This additional pressure is included to account for traction forces that are generated as the SMCs translocate through the ECM. We assume that traction forces between the other pairs of phases in the system are negligible in comparison, so the equations for \( F_{m w} \) and \( F_{\rho w} \) include only the contact-independent pressure \( p \) in their interphase pressure terms. The final term on the right hand side of each equation represents the interphase drag, which we assume to be proportional to the relative velocities of phases \( i \) and \( j \) with constant of proportionality \( k_{ij} (= k_{ji}) \). We further assume that the drag depends linearly on the volume fraction of each phase \( i \) and \( j \) so that no drag can occur in the absence of either phase.

The generic tissue phase contains several active constituents such as foam cells and macrophages, but we shall assume here that it behaves as an inert isotropic fluid. We make this assumption in order to focus on how the SMC phase interacts with other key factors in the plaque environment. We shall assume that the SMC phase displays more complex behaviour than the generic tissue phase and, in particular, that SMCs alter their motility in response to both the local PDGF concentration and the local ECM volume fraction. Neglecting viscous effects, and combining the approaches of Byrne and Owen (2004) and Lemon et al. (2006), we adopt the following models for the intraphase stress terms in Eqs. (5)–(7):

\[ \tau_m = - [ p + \rho \psi (m, \rho) + \Lambda(P) ] , \]
\[\tau_\rho = -\left[ p + m\psi (m, \rho) \right],\]  
\[\tau_w = -p.\] (12) (13)

In Eq. (11), the extra pressures \(\Lambda(P)\) and \(\rho\psi (m, \rho)\) describe the respective influences of the PDGF concentration and the ECM volume fraction on the pressure in the SMC phase. The ECM-derived extra pressure term is proportional to the interphase pressure contribution \(\psi\) and is weighted according to the degree of contact between the SMC and ECM phases. We assume that the SMC phase has a complementary influence on the pressure in the ECM phase, and this is captured by the extra pressure term \(m\psi (m, \rho)\) in Eq. (12). We define the functions \(\Lambda\) and \(\psi\) to be (Byrne and Owen 2004; Lemon et al. 2006):

\[\Lambda(P) = \frac{\chi P}{1 + (\kappa P)^n_P},\]  
(14)

\[\psi (m, \rho) = -\chi_\rho + \frac{\delta m^{n_\rho}}{(1 - m - \rho)^{n_\rho}},\]  
(15)

where \(\chi_P, \kappa, n_P, \chi_\rho, \delta\) and \(n_\rho\) are positive parameters. Equation (14) is deliberately chosen to be a decreasing function of \(P\) as this ensures that chemotactic SMC migration up PDGF gradients will provide stress relief in the SMC phase. The first term in Eq. (15), which ensures that \(\psi\) will be negative for sufficiently small \(m\), reflects an assumption that the SMCs have an affinity for the collagenous ECM. Note that this adhesion mechanism introduces the potential for haptotactic SMC migration up gradients in the ECM phase. The second term in Eq. (15) contributes a net repulsion that forces SMC movement down ECM gradients due to crowding when \(m\) or \(\rho\) become sufficiently large.

### 2.1.3 Mass transfer terms

We assume that the SMC phase source term \(S_m\) in Eq. (1) includes both a linear death term and a proliferation term that requires the uptake of material from the generic tissue phase. We assume that the net SMC proliferation rate is proportional to the sum of a constant baseline mitosis rate \(r_m\) and an additional rate that depends on the local PDGF concentration. PDGF is a well-known mitogen for SMCs, and in vitro evidence suggests that SMC proliferation can be significantly upregulated with saturating kinetics in the presence of increasing PDGF concentrations (Munro et al. 1994). Combining these considerations, we define:

\[S_m = r_m m w \left[ 1 + \frac{A_m P}{c_m + P} \right] - \beta_m m,\]  
(16)

where \(A_m\) quantifies the maximum possible PDGF-stimulated increase in the baseline SMC mitosis rate, \(c_m\) represents the PDGF concentration at which the half-maximal increase in the baseline SMC mitosis rate occurs, and \(\beta_m\) denotes the rate of SMC death. We remark here that, while normal vascular SMCs are known to be growth...
inhibited by TGF-β, in vitro experiments have shown that atherosclerotic plaque-derived SMCs retain their proliferative capacity in the presence of TGF-β (McCaffrey et al. 1995).

We define the ECM phase source term $S_\rho$ in Eq. (2) by assuming that the collagenous matrix is continuously remodelled by the cells that occupy the plaque. Based on a variety of experimental observations (Mallat et al. 2001; Vaday et al. 2001; Lutgens et al. 2002; Kubota et al. 2003; Ogawa et al. 2004; Borrelli et al. 2006; Risinger et al. 2010), we assume that both PDGF and TGF-β regulate the remodelling process. We propose the following form for the ECM phase source term, where we assume that ECM is synthesised by SMCs but is also degraded by both SMCs and plaque immune cells:

$$S_\rho = r_s mw \left[ 1 + \frac{A_s T}{c_s + T} \right] - r_d m \rho \left[ 1 + \frac{A_d P}{(c_d + P) (1 + \gamma_d T)} \right] - \beta_\rho \rho w \left[ 1 + \frac{\epsilon \gamma_\rho T}{1 + \gamma_\rho T} \right].$$  (17)

The first term in Eq. (17) represents the net rate of ECM synthesis by the SMC phase, which we assume to be proportional to the sum of a constant baseline ECM production rate $r_s$ and an additional rate that depends on the local TGF-β concentration. The role of TGF-β as a stimulant for SMC collagen production in plaques has been clearly demonstrated by in vivo studies (Mallat et al. 2001; Lutgens et al. 2002) and, following in vitro observations (Kubota et al. 2003), we assume that the rate of ECM synthesis increases with saturating kinetics in response to increasing $T$. The parameter $A_s$ quantifies the maximum possible TGF-β-stimulated increase in the baseline ECM synthesis rate, while $c_s$ denotes the TGF-β concentration at which the half-maximal increase in the baseline ECM synthesis rate occurs. Note that the ECM synthesis rate is also proportional to $w$ because we assume that ECM synthesis by SMCs again requires the uptake of material from the generic tissue phase.

The second and third terms in Eq. (17) represent the respective net rates of ECM degradation by plaque SMCs and by plaque immune cells, both of which are known to produce MMPs. We assume that the net rate of ECM degradation by SMCs is proportional to the sum of a constant baseline degradation rate $r_d$ and an additional rate that depends on the local concentration of both growth factors. In vitro studies indicate that SMCs upregulate their production of MMP-2 and MMP-9 in response to stimulation with PDGF, but also that co-stimulation with TGF-β can inhibit this response (Borrelli et al. 2006; Risinger et al. 2010). We represent this behaviour with a simple functional form where $A_d$ quantifies the maximum possible PDGF-stimulated increase in the baseline ECM degradation rate, $c_d$ denotes the PDGF concentration at which the half-maximal increase in the baseline ECM degradation rate occurs and $\gamma_d$ quantifies the extent of inhibition of PDGF-stimulated ECM degradation by TGF-β. Experimental observations also indicate that TGF-β can protect the plaque ECM from degradation by MMP-9-producing macrophages (Vaday et al. 2001; Ogawa et al. 2004). Assuming that macrophages and other inflammatory cell types comprise a certain fraction of the generic tissue phase, we capture this behaviour in the final term by assuming that the net rate of immune cell ECM degradation is proportional to both $w$ and a decreasing function of $T$. The parameter $\beta_\rho$ denotes the maximum possible rate of immune cell ECM degradation, which we assume to be related to the extent of inflammation in the plaque tissue. The dimensionless parameter $\epsilon \leq 1$ quantifies the
minimum possible rate of TGF-β-inhibited immune cell ECM degradation (i.e. $\varepsilon_\beta$, while $\gamma_\rho$ quantifies the rate at which immune cell ECM degradation decreases with increasing $T$.

### 2.2 Diffusible growth factors

In this section we derive reaction–diffusion equations that describe how the concentrations of diffusible PDGF and TGF-β evolve in the plaque tissue. In addition to the equations presented below, the model also includes a source term for each growth factor at the endothelium ($x = 0$) and sink term for each growth factor at the media ($x = L$). These effects are incorporated via boundary conditions and will be discussed in Sect. 2.4.

#### 2.2.1 Mass balance equations

We assume that PDGF and TGF-β transport occurs pre-dominantly in the generic tissue phase (notionally in the interstitial fluid), and that growth factor diffusion within the ECM and SMC phases is negligible in comparison. We further assume that the characteristic timescales of PDGF and TGF-β diffusion are significantly shorter than the characteristic timescales of SMC migration and ECM remodelling. We therefore neglect advective growth factor transport in the generic tissue phase and assume the following quasi-steady state mass balance equations for $P$ and $T$:

\begin{align}
0 &= D_P \frac{\partial}{\partial x} \left[ w \frac{\partial P}{\partial x} \right] + S_P, \\
0 &= D_T \frac{\partial}{\partial x} \left[ w \frac{\partial T}{\partial x} \right] + S_T.
\end{align}

Here, $D_P$ and $D_T$ are constant chemical diffusion coefficients, while $S_P$ and $S_T$ denote local sink terms for PDGF and TGF-β, respectively. Following Astanin and Preziosi (2008), we have assumed that the diffusive flux of both chemicals is modulated by the factor $w$. This reflects our assumption that PDGF and TGF-β exist only in the generic tissue phase and, hence, that no flux of either chemical can occur in the absence of generic tissue. In practice, this means that SMC and ECM accumulation in the plaque will reduce the capacity for chemical transport through the intima.

#### 2.2.2 Source terms

We assume that both growth factors are taken up by SMCs in the plaque, and also that both growth factors undergo natural decay. These assumptions lead to the sink terms:

\begin{align}
S_P &= -\eta_P m w P - \beta_P w P, \\
S_T &= -\eta_T m w T - \beta_T w T,
\end{align}
where $\eta_P$, $\eta_T$, $\beta_P$ and $\beta_T$ denote the rates of PDGF uptake by SMCs, TGF-\(\beta\) uptake by SMCs, PDGF decay and TGF-\(\beta\) decay, respectively. Note that we include a factor of $w$ in each term to ensure that neither uptake nor decay of either growth factor can occur in the absence of the generic tissue phase.

### 2.3 Model simplification

In this section, we show how the three-phase model developed in Sect. 2.1 can be reduced to a system of two coupled nonlinear equations for the SMC and ECM phases. In all that follows, we shall assume that the volume fractions of all three phases $m$, $\rho$ and $w$ are strictly positive for all $x$ and $t$.

Using Eq. (4) to replace all occurrences of $w$ with the equivalent expression $1 - m - \rho$, the sum of the mass balance equations (1)–(3) reduces to:

$$\frac{\partial}{\partial x} \left[ m \left( v_m m + v_w (1 - m - \rho) \right) \right] = 0. \quad (22)$$

Assuming a zero-flux condition for the SMC phase at the endothelium (i.e. $v_m = v_w = 0$ at $x = 0$), integration of Eq. (22) with respect to $x$ gives the following expression for the velocity of the generic tissue phase:

$$v_w = -v_m \left( \frac{m}{1 - m - \rho} \right). \quad (23)$$

Summing the momentum balance equations (5)–(7), and substituting in the expressions (11)–(13) gives the following equation for the tissue pressure gradient:

$$\frac{\partial p}{\partial x} = -\frac{\partial}{\partial x} \left[ m \left( \Lambda + 2 \rho \psi \right) \right]. \quad (24)$$

A second expression for the tissue pressure gradient is obtained by substituting the expressions (9), (10) and (13) into Eq. (7) [note that Eq. (8) becomes redundant at this juncture]. Cancelling terms, we obtain:

$$\frac{\partial p}{\partial x} = k_{mw} m v_m - v_w \left( k_{mw} m + k_{w\rho} \rho \right). \quad (25)$$

Equating the right hand sides of Eqs. (24) and (25), and then using Eq. (23) to eliminate $v_w$, we derive the following expression for $v_m$ in terms of $m$, $\rho$ and $P$:

$$v_m = -\left[ \frac{1 - m - \rho}{k_{mw} m (1 - \rho) + k_{w\rho} m \rho} \right] \frac{\partial}{\partial x} \left[ m \left( \Lambda + 2 \rho \psi \right) \right]. \quad (26)$$

For simplicity, we shall assume that the drag between the generic tissue phase and the SMC phase and the drag between the generic tissue phase and the ECM phase
are uniform (i.e. $k_{mw} = k_{wp} = k$). Then, substituting Eq. (26) back into Eq. (1), we arrive at the final mass balance relationship for the SMC phase in the intima:

$$\frac{\partial m}{\partial t} = \frac{1}{k} \frac{\partial}{\partial x} \left[ (1 - m - \rho) \frac{\partial}{\partial x} \left[ m (\Lambda + 2\rho \psi) \right] \right] + S_m (m, \rho, P).$$  \hfill (27)

Our reduced model therefore comprises Eq. (27) alongside the following expressions for $\rho$, $P$ and $T$:

$$\frac{\partial \rho}{\partial t} = S_{\rho} (m, \rho, P, T),$$  \hfill (28)

$$0 = D_P \frac{\partial}{\partial x} \left[ (1 - m - \rho) \frac{\partial P}{\partial x} \right] + S_P (m, \rho, P),$$  \hfill (29)

$$0 = D_T \frac{\partial}{\partial x} \left[ (1 - m - \rho) \frac{\partial T}{\partial x} \right] + S_T (m, \rho, T).$$  \hfill (30)

Figure 3 summarises the key interactions involved in fibrous cap formation in the model.

### 2.4 Boundary and initial conditions

In this section we define the boundary conditions required to solve Eqs. (27), (29) and (30), and the initial conditions required to solve Eqs. (27) and (28). The assumptions that we make are largely consistent with those made previously in Watson et al. (2018).
Note that explicit boundary conditions are not required for $\rho$ because Eq. (28) does not contain any spatial flux terms. Equation (28) is valid at all points $x \in [0, L]$ and effectively represents an ODE that must be solved at each spatial position.

Before we introduce the boundary conditions for the SMC phase, it is worthwhile to consider the biological meaning of the complex flux term in Eq. (27). Specifically, the quantity $(1 - m - \rho) \frac{\partial}{\partial x} \left[ m (\Lambda + 2\rho \psi) \right]$ describes the SMC flux in the intima by a combination of nonlinear diffusion, chemotaxis to PDGF and adhesion to (or repulsion by) the ECM phase. This can be verified by writing the flux term in the form:

$$(1 - m - \rho) \frac{\partial}{\partial x} \left[ m (\Lambda + 2\rho \psi) \right] = D_{eff} \frac{\partial m}{\partial x} - C_{eff} m \frac{\partial P}{\partial x} - H_{eff} m \frac{\partial \rho}{\partial x}, \quad (31)$$

where $D_{eff}(m, \rho, P) = (1 - m - \rho) \left[ \Lambda + 2\rho \left( \psi + m \frac{\partial \psi}{\partial m} \right) \right]$ is the effective diffusion coefficient, $C_{eff}(m, \rho, P) = (1 - m - \rho) \left[ -\frac{d\Lambda}{dP} \right]$ is the effective chemotaxis coefficient, and $H_{eff}(m, \rho) = 2 \left( 1 - m - \rho \right) \left[ -\psi - \rho \frac{\partial \psi}{\partial \rho} \right]$ is the effective haptotaxis coefficient. Note that $C_{eff}$ is positive by definition, while both $D_{eff}$ and $H_{eff}$ can be positive, negative or zero. We discuss the consequences of negative SMC diffusion in the context of model well-posedness in Sect. 4.5.

Recall that in the model simplification in Sect. 2.3 we have already assumed a zero-flux condition for SMCs at the endothelium. We therefore have the following boundary condition for the SMC phase:

$$\frac{1}{k} (1 - m - \rho) \frac{\partial}{\partial x} \left[ m (\Lambda + 2\rho \psi) \right] = 0 \text{ at } x = 0$$

$$\Rightarrow \frac{\partial}{\partial x} \left[ m (\Lambda + 2\rho \psi) \right] = 0 \text{ at } x = 0. \quad (32)$$

Note that this zero-flux condition does not stipulate zero gradient in the SMC phase at $x = 0$. Rather, the condition specifies a dynamic flux balance, whereby any SMC flux towards the endothelium (e.g. by chemotaxis) must be matched by an equivalent flux in the opposite direction (e.g. by diffusion).

SMC invasion of the plaque requires the activation of quiescent SMCs in the media by chemical signalling, and subsequent production of progeny that can negotiate the porous IEL (Chappell et al. 2016; Jacobsen et al. 2017). The IEL provides a physical barrier to cell movement, so it is likely that a sustained and directed migratory response is required for synthetic SMCs to enter the plaque. We therefore assume that chemotaxis is the dominant mechanism of SMC migration across the IEL, and that any contribution from passive SMC diffusion or from ECM-mediated haptotaxis is negligible. We introduce the parameter $m_M < 1$ to represent a constant volume fraction of activated medial SMCs, and assume a chemotactic flux of these cells into the intima in response to the PDGF gradient at the medial boundary:
\[
\frac{1}{k} (1 - m - \rho) \frac{\partial}{\partial x} \left[ m (A + 2 \rho \psi) \right] = \frac{1}{k} (1 - m - \rho) \frac{d \Lambda}{d \beta} m_M \frac{\partial \beta}{\partial x} \quad \text{at} \ x = L \\
\Rightarrow \frac{\partial}{\partial x} \left[ m (A + 2 \rho \psi) \right] = \frac{d \Lambda}{d \beta} m_M \frac{\partial \beta}{\partial x} \quad \text{at} \ x = L. \quad (33)
\]

There are several points to note regarding the format of this boundary condition. First, the model assumes that no SMCs will enter the intima in the absence of a PDGF gradient at the medial boundary. Second, the condition does not stipulate continuity of \( m \) across the boundary (i.e. in general, \( m \neq m_M \) at \( x = L \)). Finally, since we are assuming a closed system, the boundary condition implies that any flux of SMCs into the intima must be balanced by an equivalent efflux from the generic tissue phase into the media. While tissue constituents such as macrophages and foam cells are known to be capable of leaving the plaque across the medial boundary (Moore et al. 2013), the assumption that a generic tissue phase efflux will exactly balance the SMC phase influx is an over-simplification of reality. However, we resolve that our modelling assumptions remain reasonable provided that the total influx of plaque SMCs remains relatively small. An extended model that explicitly captures intimal growth could, of course, provide a more comprehensive treatment of the problem. We postpone modelling of domain growth for future work because we believe that the current approach provides significant insight into the cap formation process without the added complexity of tracking a moving boundary.

Stimulated endothelial cells and adherent platelets at the lesion site are believed to be the dominant sources of PDGF in the plaque (Funayama et al. 1998), and we model this influx of endothelium-derived PDGF with the following boundary condition:

\[
D_P (1 - m - \rho) \frac{\partial \beta}{\partial x} = -\alpha_P (1 - m - \rho) \quad \text{at} \ x = 0 \\
\Rightarrow \frac{\partial \beta}{\partial x} = -\frac{\alpha_P}{D_P} \quad \text{at} \ x = 0, \quad (34)
\]

where \( \alpha_P \) quantifies the rate of PDGF flux into the intima, and the term \((1 - m - \rho)\) is included for consistency with the modulated PDGF transport inside the model domain. Note the corresponding implication that the absolute rate of PDGF influx into the plaque will reduce as the SMC and ECM phases accumulate at the luminal boundary. This introduces an implicit self-regulation mechanism in the model whereby SMCs can effectively downregulate the signal that recruits them to the cap region. Note that endothelium PDGF production is known to be regulated by wall shear stress (Hsieh et al. 1991). In a growing plaque that increasingly encroaches on the lumen, the rate of PDGF influx could therefore be expected to change with time. We neglect such a time dependence in the current model because the endothelium remains fixed, but time dependent PDGF influx will be an important consideration in future work that explicitly models plaque growth.

The boundary condition (33) stipulates that SMC migration into the plaque from the media requires a negative PDGF gradient at the medial boundary. We therefore treat the IEL as a permeable membrane and assume that PDGF can diffuse into the media from the intima according to the following boundary condition:
\[
DP (1 - m - \rho) \frac{\partial P}{\partial x} = \sigma_P (PM - P) (1 - m - \rho) \text{ at } x = L
\]
\[
\Rightarrow \frac{\partial P}{\partial x} = \frac{\sigma_P}{DP} (PM - P) \text{ at } x = L.
\]

Here, \( \sigma_P \) denotes the permeability of the IEL to PDGF, \( PM \) represents the PDGF concentration in the media, and the term \( (1 - m - \rho) \) is again included for consistency with the modulated chemical transport inside the domain.

The boundary conditions that we impose for TGF-\( \beta \) are identical in form to those for PDGF. Like PDGF, TGF-\( \beta \) is released by degranulating platelets and activated endothelial cells at the lesion site (Toma and McCaffrey 2012). We therefore define the following condition at the endothelium:

\[
DT (1 - m - \rho) \frac{\partial T}{\partial x} = -\alpha_T (1 - m - \rho) \text{ at } x = 0
\]
\[
\Rightarrow \frac{\partial T}{\partial x} = -\frac{\alpha_T}{DT} \text{ at } x = 0,
\]

where \( \alpha_T \) quantifies the rate of TGF-\( \beta \) flux into the intima. At the medial boundary, we assume:

\[
DT (1 - m - \rho) \frac{\partial T}{\partial x} = \sigma_T (TM - T) (1 - m - \rho) \text{ at } x = L
\]
\[
\Rightarrow \frac{\partial T}{\partial x} = \frac{\sigma_T}{DT} (TM - T) \text{ at } x = L,
\]

where \( \sigma_T \) denotes the permeability of the IEL to TGF-\( \beta \), and \( TM \) represents the TGF-\( \beta \) concentration in the media. Note that the decision to allow TGF-\( \beta \) to diffuse out of the domain is made simply for consistency with the boundary condition (35) for PDGF. The presence of an explicit TGF-\( \beta \) gradient at the medial boundary is not required to initiate cap formation in the model.

Finally, we must define initial profiles for the SMC and collagenous ECM phases in the plaque. Observations of plaque development in mice indicate that the SMC population in the intima is essentially negligible prior to cap formation (Bennett et al. 2016). We therefore initiate the plaque with a small and uniform initial SMC volume fraction:

\[
m(x, 0) = m_i, \quad \text{where } 0 < m_i \ll 1.
\]

We also assume a small and uniform initial profile for the collagenous ECM phase in the plaque:

\[
\rho(x, 0) = \rho_i, \quad \text{where } 0 < \rho_i \ll 1.
\]

This initial collagen is assumed to represent a fraction of the ECM that exists in the intima prior to SMC invasion. The remaining constituents of the initial plaque ECM, whose quantities we do not explicitly track, are assumed to reside in the generic tissue phase and are notionally replaced by the deposition of new collagen over the course of a simulation.
2.5 Model non-dimensionalisation

Using tildes to denote dimensionless quantities, we rescale space $x$, time $t$, PDGF concentration $P$ and TGF-$\beta$ concentration $T$ as follows (note that the SMC volume fraction $m$ and the ECM volume fraction $\rho$ do not require rescaling):

$$\tilde{x} = \frac{x}{L}, \quad \tilde{t} = \frac{t}{t_0}, \quad \tilde{P} = \frac{P}{P_0}, \quad \tilde{T} = \frac{T}{T_0}, \quad \tilde{m} = m, \quad \tilde{\rho} = \rho,$$

where $t_0$, $P_0$ and $T_0$ represent characteristic time, PDGF concentration and TGF-$\beta$ concentration values, respectively. The model parameters can now be non-dimensionalised in the following way:

$$\tilde{\chi}_P = \frac{\chi_P t_0}{kL^2}, \quad \tilde{\kappa} = \kappa P_0, \quad \tilde{\chi}_\rho = \frac{2\chi_\rho t_0}{kL^2}, \quad \tilde{\delta} = \frac{2\delta t_0}{kL^2}, \quad \tilde{r}_m = r_m t_0,$$

$$\tilde{c}_m = \frac{c_m}{P_0}, \quad \tilde{\beta}_m = \beta_m t_0, \quad \tilde{r}_s = r_s t_0, \quad \tilde{\zeta}_s = \frac{c_s}{T_0}, \quad \tilde{r}_d = r_d t_0,$$

$$\tilde{\eta}_d = \frac{c_d}{P_0}, \quad \tilde{\gamma}_d = \gamma_d T_0, \quad \tilde{\beta}_\rho = \beta_\rho t_0, \quad \tilde{\gamma}_\rho = \gamma_\rho T_0, \quad \tilde{\eta}_P = \frac{\eta_P L^2}{D_P},$$

$$\tilde{\beta}_L = \frac{\beta_L L^2}{D_P}, \quad \tilde{\eta}_T = \frac{\eta_T L^2}{D_T}, \quad \tilde{\beta}_T = \frac{\beta_T L^2}{D_T}, \quad \tilde{\alpha}_P = \frac{\alpha_L}{D_T T_0}, \quad \tilde{\alpha}_T = \frac{\alpha_T}{D_T T_0}, \quad \tilde{P}_M = \frac{P_M}{P_0}, \quad \tilde{T}_M = \frac{T_M}{T_0}.$$

Dropping tildes for clarity, the corresponding dimensionless model equations, boundary conditions and initial conditions are:

$$\frac{\partial m}{\partial t} = \frac{\partial}{\partial x} \left[ (1 - m - \rho) \frac{\partial}{\partial x} \left[ m (\Lambda + \rho \psi) \right] \right] + r_m m (1 - m - \rho) \left[ 1 + \frac{A_m P}{c_m + P} \right] - \beta_m m, \quad (40)$$

$$\frac{\partial \rho}{\partial t} = r_s m (1 - m - \rho) \left[ 1 + \frac{A_s T}{c_s + T} \right] - r_d m \rho \left[ 1 + \frac{A_d P}{(c_d + P) (1 + \gamma_d T)} \right] - \beta_\rho \rho (1 - m - \rho) \left[ \frac{1 + \varepsilon \gamma_\rho T}{1 + \gamma_\rho T} \right], \quad (41)$$

$$\frac{\partial}{\partial x} \left[ (1 - m - \rho) \frac{\partial P}{\partial x} \right] = \eta_P m P (1 - m - \rho) + \beta_P P (1 - m - \rho), \quad (42)$$

$$\frac{\partial}{\partial x} \left[ (1 - m - \rho) \frac{\partial T}{\partial x} \right] = \eta_T m T (1 - m - \rho) + \beta_T T (1 - m - \rho), \quad (43)$$

$$\frac{\partial}{\partial x} \left[ m (\Lambda + \rho \psi) \right] = 0 \text{ at } x = 0, \quad \frac{\partial}{\partial x} \left[ m (\Lambda + \rho \psi) \right] = \frac{dA}{dP} \frac{m M}{M} \frac{\partial P}{\partial x} \text{ at } x = L, \quad (44)$$

$$\frac{\partial P}{\partial x} = -\alpha_P \text{ at } x = 0, \quad \frac{\partial P}{\partial x} = \sigma_P (P_M - P) \text{ at } x = L, \quad (45)$$
\[ \frac{\partial T}{\partial x} = -\alpha_T \text{ at } x = 0, \quad \frac{\partial T}{\partial x} = \sigma_T (T_M - T) \text{ at } x = L, \quad \text{ (46)} \]

\[ m(x,0) = m_i, \quad \text{ (47)} \]

\[ \rho(x,0) = \rho_i, \quad \text{ (48)} \]

wherein

\[ \Lambda(P) = \frac{\chi_P}{1 + (\kappa P)^n_P} \text{ and } \psi(m,\rho) = -\chi_{\rho} + \frac{\delta m^{n_{\rho}}}{(1 - m - \rho)^{n_{\rho}}}. \]

2.6 Model parameterisation

A summary of the base case parameter values is provided in Table 1. Our parameter selections have mostly been informed by data and observations from relevant in vitro and in vivo experimental studies. When appropriate data could not be found, we have chosen values that ensure biologically realistic results. Several important parameters (e.g. \( A_m, c_m, \chi_P, \kappa, n_P \)) have been assigned values that are dimensionally the same (or very similar) to those in our earlier model of cap formation. We provided detailed justifications for these parameter values in Watson et al. (2018), so below we focus on explaining the parameter selections that are unique to the current study.

In vivo studies in the ApoE-deficient mouse have shown that plaque fibrous caps typically form over the course of several months (Kozaki et al. 2002). We therefore assume a characteristic timescale of approximately 1 month and set \( t_0 = 2.5 \times 10^6 \text{ s.} \) For the domain length, we set \( L = 120 \mu\text{m.} \) This value is based on data from Reifenberg et al. (2012), who measured intimal thickness in the diseased aortic arches of mice around the time of initial plaque SMC infiltration. For PDGF and TGF-\( \beta \), we assume the characteristic concentrations \( P_0 = 10 \text{ ng ml}^{-1} \) and \( T_0 = 1 \text{ ng ml}^{-1}. \) These values reflect reported concentrations of PDGF in platelets (15–50 ng ml\(^{-1}\); Huang et al. 1988) and TGF-\( \beta \) in blood plasma (2–12 ng ml\(^{-1}\); Wakefield et al. 1995).

In a detailed model of the fibroblast response to PDGF in dermal wound healing, Haugh (2006) estimated PDGF diffusion coefficient and decay rate values that suggest a PDGF diffusion distance of approximately 300 \( \mu\text{m.} \) Based on these estimates, we set our dimensionless PDGF decay rate to be \( \beta_P = 0.2. \) As TGF-\( \beta \) has an almost identical molecular weight to PDGF, we expect that TGF-\( \beta \) will diffuse at a similar rate to PDGF in the plaque tissue. However, active TGF-\( \beta \) has been reported to decay around two orders of magnitude faster than PDGF (Wakefield et al. 1990), and we therefore set \( \beta_T = 20. \) The other parameters that determine the plaque growth factor profiles are more difficult to estimate. We assume, for consistency, that \( \sigma_P = \sigma_T \) and we choose corresponding values for \( \alpha_P \) and \( \alpha_T \) that give reasonable growth factor concentration ranges in the intima. The response of the model to changes in the values of \( \alpha_P \) and \( \alpha_T \) will be an important focus of our numerical simulation studies in Sect. 3.2. Note that we also set \( P_M = T_M = 0 \) because neither growth factor is known to have a prominent source in the medial tissue.

ECM remodelling in the model is governed by a range of concentration-dependent stimulatory and inhibitory effects of PDGF and TGF-\( \beta \). The parameter values in these relationships have all been informed by data from in vitro experiments. Kubota et al. (2003) used SMCs derived from human atherosclerotic plaques to show that TGF-\( \beta \)
Table 1  Dimensionless base case parameter values

| Parameter | Description                                      | Value |
|-----------|--------------------------------------------------|-------|
| $\nu$     | Exponent in SMC phase extra pressure function $A$| 1.8   |
| $\kappa$  | Reciprocal of reference PDGF concentration in SMC phase extra pressure function $A$ | 5.5   |
| $\chi$    | Exponent in SMC phase extra pressure function $\psi$ | 0.45  |
| $T$       | SMC phase motility coefficient                   | 0.3   |
| $\gamma$  | Baseline rate of SMC phase proliferation          | 0.25  |
| $\Delta n$| Maximal factor of PDGF-stimulated increase in rate of SMC proliferation | 14    |
| $\rho$    | PDGF concentration for half-maximal increase in rate of SMC proliferation | 1.5   |
| $\phi$    | Rate of SMC phase loss                           | 0.6   |
| $\beta$   | TGF-$\beta$ concentration for half-maximal increase in rate of ECM synthesis | 0.3   |
| $\mu$     | Maximal factor of TGF-$\beta$-stimulated increase in rate of ECM synthesis | 0.3   |
| $\beta_d$ | Rate of ECM phase degradation by SMCs            | 1.5   |
| $\Delta m$| Maximal factor of PDGF-stimulated increase in rate of ECM degradation by SMCs | 4     |
| $\rho_d$  | PDGF concentration for half-maximal increase in rate of ECM degradation by SMCs | 2.5   |

References:
- Schachter (1997)
- Cai et al. (2007)
- Breton et al. (1986)
- Chapp et al. (1994)
- Munro et al. (1994)
- Iinokura et al. (1999)
- Reifenberg et al. (2003)
- Kubota et al. (2003)
- Borrelli et al. (2006)
Table 1 continued

| Parameter | Description                                                                 | Value | References                  |
|-----------|-----------------------------------------------------------------------------|-------|-----------------------------|
| $\gamma_d$ | Reciprocal of reference TGF-$\beta$ concentration for inhibition of PDGF-stimulated ECM degradation by SMCs | 0.5   | Borrelli et al. (2006)      |
| $\beta_\rho$ | Baseline rate of ECM phase degradation by immune cells                     | 0.75  |                            |
| $\varepsilon$ | Maximal factor of TGF-$\beta$-stimulated decrease in rate of ECM degradation by immune cells | 0.25  | Vaday et al. (2001)         |
| $\gamma_\rho$ | Reciprocal of reference TGF-$\beta$ concentration for inhibition of ECM degradation by immune cells | 10    | Vaday et al. (2001)         |
| $\eta_P$ | Rate of PDGF uptake by SMC phase                                           | 2.5   |                            |
| $\beta_P$ | Rate of PDGF decay                                                         | 0.2   | Haugh (2006)                |
| $\eta_T$ | Rate of TGF-$\beta$ uptake by SMC phase                                     | 2.5   |                            |
| $\beta_T$ | Rate of TGF-$\beta$ decay                                                   | 20    | Wakefield et al. (1990)     |
| $m_M$     | Volume fraction of synthetic SMCs in media                                  | 0.01  |                            |
| $\alpha_P$ | Rate of PDGF influx from endothelium                                        | 0.7   |                            |
| $\sigma_P$ | Permeability of IEL to PDGF                                                 | 4     |                            |
| $P_M$     | PDGF concentration in media                                                 | 0     |                            |
| $\alpha_T$ | Rate of TGF-$\beta$ influx from endothelium                                | 2.5   |                            |
| $\sigma_T$ | Permeability of IEL to TGF-$\beta$                                         | 4     |                            |
| $T_M$     | TGF-$\beta$ concentration in media                                          | 0     |                            |
| $m_i$     | Initial SMC volume fraction in intima                                        | $10^{-4}$ | Bennett et al. (2016)          |
| $\rho_i$ | Initial ECM volume fraction in intima                                        | 0.02  | Reifenberg et al. (2012)     |

The final column reports any references that have been used to calculate individual parameter values. Values that do not have references have been chosen to ensure biologically reasonable results. Unless otherwise stated, all reported simulations use these values.
can increase baseline collagen synthesis up to twofold, with a half-maximal response occurring for a TGF-β concentration in the interval 0.2–0.5 ng ml\(^{-1}\). We therefore set \(A_s = 1\) and \(c_s = 0.35\). Values for \(\varepsilon\) and \(\gamma_\rho\) are based on results from Vaday et al. (2001), who studied the impact of TGF-β on tumour necrosis factor (TNF)-α-induced expression of MMP-9 in monocytes. Note that TNF-α is known to be an important inflammatory cytokine in atherosclerosis progression (Urschel and Cicha 2015). Vaday et al. (2001) showed that, in the presence of 1 ng ml\(^{-1}\) TNF-α, MMP-9 activity decreased with increasing TGF-β concentration and was reduced by around 50–75% for TGF-β concentrations in the range 0.1–1 ng ml\(^{-1}\). We therefore estimate \(\varepsilon = 0.25\) and \(\gamma_\rho = 10\). The parameter values that quantify the competing effects of PDGF and TGF-β on ECM remodelling by SMCs have been calculated using data from Borrelli et al. (2006). Borrelli et al. (2006) showed that SMCs demonstrate very similar qualitative patterns of MMP-2 and MMP-9 release in the presence of varying concentrations of PDGF and TGF-β. We therefore base our quantitative estimates on the averaged responses of these two MMPs to PDGF and TGF-β. In the absence of TGF-β, the data suggest an average 2.5-fold increase in basal MMP release at 20 ng ml\(^{-1}\) PDGF and an average fourfold increase at 50 ng ml\(^{-1}\) PDGF. When 5 ng ml\(^{-1}\) TGF-β is introduced at 50 ng ml\(^{-1}\) PDGF, the release of MMPs is cut, on average, by around 50%. Based on these observations, we select \(A_d = 4\), \(c_d = 2.5\) and \(\gamma_d = 0.5\).

Of course, the parameters discussed above determine only how ECM remodelling is modified in the presence of growth factors. The underlying baseline rates of ECM synthesis and degradation (\(r_s\), \(r_d\) and \(\beta_\rho\)) must be determined separately. It is difficult to ascertain typical values for these parameters during in vivo plaque progression, so we base our parameter selections on the following considerations. First, we assume that, as invasive plaque SMCs undertake a process of continual ECM remodelling, the values of \(r_s\) and \(r_d\) should be of a similar order. Furthermore, we assume that the value of \(r_s\) must be sufficiently large to allow plaque collagen to accumulate on a timescale similar to that reported in Reifenberg et al. (2012). Second, we assume that the value of \(\beta_\rho\) should be smaller than the value of \(r_d\). We make this assumption because, while inflammatory cells such as macrophages may degrade the plaque ECM more aggressively than SMCs, these cells make up only a fraction of the content of the generic tissue phase. Based on these assumptions, we estimate \(r_s = 1.8\), \(r_d = 1.5\) and \(\beta_\rho = 0.75\).

In Watson et al. (2018), we assumed a dimensionless baseline SMC proliferation rate \(r_m = 0.02\). This value was estimated based on previous experimental observations that suggested a proliferative index of around 1% for plaque SMCs (i.e. 1% of plaque SMCs displaying evidence of mitotic activity at a given point in time). However, as discussed in Sect. 1, recent SMC lineage tracing studies have indicated that plaque SMC proliferation may be more prominent than previously appreciated. For example, Chappell et al. (2016) have recently reported a plaque SMC proliferative index of approximately 4%. Furthermore, this value was determined at an advanced stage of plaque progression, where a significant SMC population had already assembled. Hence, accounting for likely effects such as cell–cell and cell–ECM contact inhibition, we anticipate that the plaque SMC proliferative index could be even larger in early cap formation. We therefore assume a significant increase in our previous estimate and set \(r_m = 0.25\). To our knowledge, no recent lineage tracing study has reported an
equivalent calculation for the SMC apoptotic index. Hence, for the SMC death rate in the model, we use the apoptotic index of 1% that was calculated by Lutgens et al. (1999) and set $\beta_m = 0.6$. This value corresponds to a typical SMC apoptosis time of approximately 8 hours.

Finally, we briefly comment on the rationale for our parameterisation of the SMC affinity for the ECM phase $\chi_\rho$. Expansion of the flux term in Eq. (40) shows that $\chi_\rho$ represents an effective haptotaxis coefficient for SMC movement up gradients in the collagen-rich ECM. The extent to which haptotactic SMC migration contributes to in vivo cap formation is not well known. We therefore make a conservative initial estimate of $\chi_\rho = 0.3$ and will investigate the sensitivity of the model to changes in this value in Sect. 3.2. Note that this conservative estimate for $\chi_\rho$ also arises due to practical considerations. Numerical simulations indicate that the model can become ill-posed if the value of $\chi_\rho$ is chosen to be too large. This issue appears to be related to the onset of negative SMC diffusion, which is an occurrence that we envisage to be unlikely during in vivo plaque growth. We therefore avoid such parameter regimes in the simulations that are presented in this paper. In Sect. 4, we will discuss this issue in greater detail and propose amendments that can be made to the model to reduce or eliminate the ill-posed region of the parameter space.

3 Results

We begin this section by performing a simplified steady state analysis that provides useful insight into the relationship between the SMC phase and the collagenous ECM phase in the model plaque. We then present numerical solutions of the model system (40)–(48).

3.1 Analytical results

The mathematical model derived in Sect. 2 presents a significant challenge for analytical studies. The model does not admit any non-trivial spatially homogeneous steady state solutions, and, given the highly nonlinear nature of the equations, it seems unlikely that closed-form expressions could be derived for any spatially inhomogeneous steady states. We therefore seek a more tractable analytical approach to support the interpretation of the numerical simulation results in Sect. 3.2.

The approach that we adopt exploits the fact that the ECM phase Eq. (41) does not contain a flux term. Consequently, at each position in the model plaque, the value of $\rho$ depends only on the local values of $m$, $P$ and $T$. As noted above, it is intractable to determine general expressions for steady state values of the model variables. Therefore, to simplify the steady state problem, we neglect Eqs. (40), (42) and (43) and simply assume that each of $m$, $P$ and $T$ takes some fixed (steady state) value at each position. We can then derive a steady state expression for $\rho$ that holds at any position in the plaque for any combination of local $m$, $P$ and $T$ values. As will be seen below, this approach allows us to show that for any pair of steady state growth factor concentrations, there exists a steady state SMC volume fraction that maximises the steady state ECM volume.
fraction. For the numerical simulations to be presented in Sect. 3.2, the results of this steady state analysis will allow us to determine: (1) why $\rho$ takes a particular steady state value at each position; and (2) the proximity of each steady state $\rho$ value to its theoretical maximum. The absolute difference between simulated steady state $\rho$ values and their corresponding theoretical maxima is of particular interest in the region near the endothelium. By comparing steady state $\rho$ values with their theoretical maxima in this region, we can assess the efficacy of cap formation in a given simulation and determine how an increase or decrease in SMC recruitment is likely to impact overall cap stability.

To begin, consider a fixed point inside the model domain, and assume that, at that point, the PDGF and TGF-$\beta$ concentrations take the fixed values $P^*$ and $T^*$. Assuming that the SMC volume fraction has the fixed value $m^*$ at the same point, Eq. (41) gives the following expression for the steady state ECM volume fraction $\rho^*$:

$$R_s m^* (1 - m^* - \rho^*) - R_d m^* \rho^* - B_\rho \rho^* (1 - m^* - \rho^*) = 0,$$

(49)

where $\rho^* + m^* < 1$. Note that we have introduced the positive constants $R_s (T^*) = r_s \left[ 1 + \frac{A_s T^*}{c_s + T^*} \right]$, $R_d (P^*, T^*) = r_d \left[ 1 + \frac{A_d P^*}{c_d + P^*} \right]$, and $B_\rho (T^*) = \frac{\beta_\rho}{\frac{1 + \gamma_\rho T^*}{1 + \gamma_\rho T^*}}$, which represent the local steady state rates of ECM synthesis by SMCs, ECM degradation by SMCs, and ECM degradation by immune cells, respectively. Rearranging Eq. (49) gives the following quadratic in $\rho^*$:

$$\rho^{*2} + \left[ m^* \left( 1 - \frac{R_s}{B_\rho} - \frac{R_d}{B_\rho} \right) - 1 \right] \rho^* + \frac{R_s}{B_\rho} m^* (1 - m^*) = 0.$$

(50)

Setting $\mu (T^*) = \frac{R_s}{B_\rho}$ and $\lambda (P^*, T^*) = \frac{R_d}{B_\rho}$, this quadratic gives two possible solutions $\rho^*_\pm$ for the steady state ECM volume fraction in terms of $m^*$:

$$\rho^*_\pm (m^*) = \frac{1}{2} \left[ 1 - m^* (1 - \mu - \lambda) \pm \sqrt{\left[ 1 - m^* (1 - \mu - \lambda) \right]^2 - 4 \mu m^* (1 - m^*)} \right],$$

(51)

where $0 < m^* < 1$. It is trivial to show that the two solutions are real-valued, positive and distinct. However, a physically meaningful solution must satisfy the condition $0 < \rho^*_\pm < 1 - m^*$. The only admissible steady state solution is therefore $\rho^*_+$, which is always stable. From here, we drop the subscript on $\rho^*_+$ and use $\rho^*$ to refer to the negative root in Eq. (51).

For fixed $\mu$ and $\lambda$, plots of $\rho^* (m^*)$ for $m^* \in (0, 1)$ demonstrate a biphasic behaviour where $\rho^*$ increases from zero with increasing $m^*$ but then diminishes towards zero as $m^*$ approaches one (see Fig. 8 in Sect. 3.2.1 for some plotted examples in the context of the base case simulation). The region of decreasing $\rho^*$ is due to the fact that as $m^*$ increases, ECM synthesis is increasingly inhibited by the reduced availability of generic tissue material. An interesting consequence of this biphasic relationship is that, for each pair of growth factor concentrations $P^*$ and $T^*$ (or parameter values $\mu$ and $\lambda$), there exists a unique SMC volume fraction $\tilde{m}^*$ that produces a maximum steady state ECM volume fraction $\tilde{\rho}^*$.
Fig. 4 Heatmaps that show how the maximum steady state ECM volume fraction $\hat{\rho}^*$ and the corresponding SMC volume fraction $\hat{m}^*$ at a fixed point in the plaque vary with the local growth factor concentrations $P^*$ and $T^*$ for $(P^*, T^*) \in [0, 1] \times [0, 1]$. Note that the parameters required to define $\hat{\rho}^*$ and $\hat{m}^*$ ($r_s, A_s, c_s, r_d, A_d, c_d, \gamma_d, \beta_p, \varepsilon, \gamma_p$) have been assigned their base case values (see Table 1).

Explicit expressions for $\hat{m}^*$ and $\hat{\rho}^*$ can be derived by seeking stationary points of the function $\rho^*(m^*)$. By differentiating $\rho^*(m^*)$, setting the result to zero and solving the resulting quadratic in $m^*$, it is possible to show that:

$$\hat{m}^*(\mu, \lambda) = \frac{1 + \sqrt{\lambda}}{1 + \mu + \lambda + 2\sqrt{\lambda}},$$

(52)

where we have selected the physically meaningful root, which satisfies $0 < \hat{m}^* < 1$. Substituting this expression for $\hat{m}^*$ into the negative root in Eq. (51) then gives:

$$\hat{\rho}^*(\mu, \lambda) = \frac{\mu}{1 + \mu + \lambda + 2\sqrt{\lambda}}.$$

(53)

Equations (52) and (53) can now be used to explore the conditions that lead to maximal ECM deposition at a given point in the model plaque.

The dependence of $\hat{\rho}^*$ and $\hat{m}^*$ on $\mu$ and $\lambda$ can be inspected visually by plotting 2D heatmaps of the right hand sides of Eqs. (52) and (53). However, these plots have limited utility for the interpretation of numerical simulation results because, at steady state in a given simulation, the values of $\mu$ and $\lambda$ at each location in the plaque are not immediately obvious. This is because $\mu$ and $\lambda$ depend not only on the local growth factor concentrations $P^*$ and $T^*$, but also on the ten parameters ($r_s, A_s, c_s, r_d, A_d, c_d, \gamma_d, \beta_p, \varepsilon, \gamma_p$) that appear in the definitions of $R_s, R_d$ and $B_p$. Therefore, to support the interpretation of the numerical results that will be presented in Sect. 3.2, we instead plot heatmaps of $\hat{\rho}^*$ and $\hat{m}^*$ as functions of $P^*$ and $T^*$ where the above parameters have been assigned their base case values (Fig. 4). Note that expressions for $\hat{\rho}^*(P^*, T^*)$ and $\hat{m}^*(P^*, T^*)$ can be written down explicitly (see the relevant definitions above), but we omit these here for brevity.

Figure 4a, b present respective plots of $\hat{\rho}^*(P^*, T^*)$ and $\hat{m}^*(P^*, T^*)$ for $(P^*, T^*) \in [0, 1] \times [0, 1]$. These intervals for $P^*$ and $T^*$ reflect the typical ranges of...
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dimensionless growth factor concentrations that will be considered in our numerical simulations in Sect. 3.2. Intuitively, $\hat{\rho}^*$ takes its largest values when $T^*$ is large and $P^*$ is small (i.e. high net rate of ECM synthesis and low net rate of ECM degradation), and its smallest values when $T^*$ is small and $P^*$ is large (i.e. low net rate of ECM synthesis and high net rate of ECM degradation). The corresponding behaviour of $\hat{m}^*$, on the other hand, is less intuitive. Figure 4b indicates that, over the majority of the plotted ($P^*, T^*$) parameter space, the maximum ECM volume fraction can be generated by a relatively small volume fraction (0.1–0.2) of SMCs.

Figure 4a indicates that, while both growth factors can have a significant impact on plaque ECM levels, $\hat{\rho}^*$ is much more sensitive to $T^*$ than to $P^*$ over the relevant concentration range. This is because TGF-$\beta$ plays a role not just in synthesising the collagenous ECM, but also in preventing its degradation. Of course, it is also important to consider the SMC levels that are required to attain these maximum ECM volume fractions at different plaque growth factor levels. For $T^*$ close to zero, an SMC volume fraction of around 25–30% is required to maximise the steady state ECM volume fraction at around 20–30%. However, for $T^*$ close to one, a maximum steady state ECM volume fraction of 40–50% can be generated by an SMC volume fraction of less than 15%. The model therefore suggests that, in the presence of favourable TGF-$\beta$ levels, it is possible to synthesise around twice as much collagenous ECM with about half as many tissue-synthesising SMCs.

The above results provide a useful tool with which to interpret the final outcome of cap formation in a given model simulation. However, it is important to emphasise that this simplified analysis does not allow us to readily predict cap formation efficacy in a particular case. We have focussed on a fixed spatial position in the plaque and have disregarded dynamic interactions between the model variables, including the role of PDGF in SMC recruitment and the mechanical feedback between the SMCs and ECM. In the next section, we investigate the importance of these factors, and others, by performing numerical simulations with the full system of model equations.

3.2 Numerical results

We begin this section by presenting results from a simulation with the base case parameter values. This is followed by a sensitivity analysis that investigates the impact of key parameters on cap formation. In each case, the model equations (40)–(46) were solved in C++ using the Crank–Nicolson method with fixed spatial ($\Delta x = 10^{-3}$) and temporal ($\Delta t = 10^{-3}$) discretisations. Several checks were made to ensure the reliability of our calculations. Adequacy of time-stepping was assessed by refining the spatial and temporal discretisations with the ratio $\Delta t / (\Delta x)^2$ held fixed. Doubling the spatial resolution ($\Delta x = 5 \times 10^{-4}$) and dividing the timestep by four ($\Delta t = 2.5 \times 10^{-4}$), for example, gave a relative change in each variable of less than $2 \times 10^{-4}$ at steady state in the base case simulation. Several of the reported cases were tested in MATLAB using a method of lines approach. Spatial derivatives were approximated with second order differences and the resulting ODEs were solved with the variable time-stepping routine ode15s. This provided an independent check on the accuracy of our numerical solutions.
3.2.1 Base case simulation

Figure 5 presents the initial profile for each variable in the base case simulation. Both $m$ and $\rho$ are initialised with small, spatially uniform volume fractions. Initial concentration profiles for $P$ and $T$ are obtained by solving Eqs. (42) and (43) subject to the boundary conditions (45) and (46). Noting that $1 - m - \rho = \text{const}$ and $m \approx 0$ at $t = 0$, Eqs. (42) and (43) can be reduced to the simpler form $\frac{\partial^2 G}{\partial x^2} = \beta G G$ (where $G = P, T$). The $P(x, 0)$ and $T(x, 0)$ profiles in Fig. 5 represent the exact solutions of these simplified equations. The PDGF profile, which is diffusion-dominated ($\beta_P \ll 1$), shows an approximately linear decrease across the intima, while TGF-$\beta$, which is degradation-dominated ($\beta_T \gg 1$), shows a rapid exponential decline. Consequently, TGF-$\beta$ is more strongly localised to the endothelium and, of course, to the desired region of collagen cap formation.

The time evolution of the model variables until $t = 4$ in the base case simulation is presented in Fig. 6. The results demonstrate significant accumulations of SMCs (Fig. 6a) and ECM in the cap region over the course of the simulation and a concurrent reduction in the overall concentrations of PDGF (Fig. 6c) and TGF-$\beta$ (Fig. 6d). The reduction in plaque PDGF and TGF-$\beta$ levels is partly due to uptake by the invading SMCs, but can mostly be attributed to reduced chemical diffusion through the increasingly populated cap region. Note that the PDGF profile undergoes a more dramatic change than the TGF-$\beta$ profile as a consequence of this modulated diffusivity.

Figure 6a shows that the media-derived model SMCs respond to PDGF by migrating towards the endothelium and proliferating rapidly over the first 2 months of simulation time. Due to the ongoing increase in ECM deposition and concurrent reduction in PDGF levels, the SMCs divide more slowly after this time point and the plaque SMC population peaks at around 4 months. Interestingly, Fig. 6b demonstrates that cap formation occurs on a longer timescale. After 2 months of simulation time, only 50% of
the final ECM content has been deposited, and the approximate steady state ECM profile is not attained until after about 6 months. The results in Fig. 6a, b are qualitatively and quantitatively consistent with the experimental observations of Reifenberg et al. (2012). For ApoE knockout mice fed a high-fat diet for 24 weeks, Reifenberg et al. (2012) found that plaque SMC content increased from around 3% at 8 weeks to around 7% at both 16 and 24 weeks, while plaque collagen content increased from around 3% at 8 weeks to around 17% at 16 weeks and then to around 24% at 24 weeks. These values show evidence of early saturation in SMC numbers and delayed saturation in collagen levels, both of which occur on similar timescales to those depicted in our simulation data.

Figure 7 presents the profile of each model variable in the plaque at the later time $t = 8$ (solid lines). The simulation is close to steady state at this time point, and the only notable change from the results at the latest time points in Fig. 6 is a small reduction in the SMC volume fraction throughout the domain. This reduction in SMC numbers appears to be due to the delayed accumulation of ECM, which causes increased inhibition of SMC migration and SMC proliferation at a late stage of cap formation. At $t = 8$, the total volume fractions of SMCs and collagenous ECM in the plaque are approximately 8.6% and 21.4%, respectively. These values are consistent with obser-
Fig. 7  Base case simulation results that show approximate steady state profiles (solid lines) for the PDGF (blue) and TGF-\(\beta\) (green) concentrations, and the ECM (magenta) and SMC (red) volume fractions. Dotted lines represent the analytically-derived maximum steady state ECM volume fraction \(\hat{\rho}^*\) (pink) and corresponding steady state SMC volume fraction \(\hat{m}^*\) (red) at each \(x\). The \(\hat{m}^*\) and \(\hat{\rho}^*\) values are functions of the local PDGF and TGF-\(\beta\) concentrations and have been calculated at each \(x\) by Eqs. (52) and (53), respectively. All plots taken at time \(t = 8\) (color figure online).

vations from several studies of plaque growth in the ApoE knockout mouse (Mallat et al. 2001; Clarke et al. 2006; Reifenberg et al. 2012).

In Fig. 7, we have also plotted \(\hat{m}^*\) and \(\hat{\rho}^*\) at each spatial position in the plaque (dotted lines). These plots, which have been constructed using Eqs. (52) and (53), represent the maximum ECM volume fraction \(\hat{\rho}^*\) that could be attained at each position \(x\), and the corresponding SMC volume fraction \(\hat{m}^*\) that would be required to achieve this maximum. For each \(x\), the values of \(\hat{m}^*\) and \(\hat{\rho}^*\) are given by a unique pair of values \(\mu\) and \(\lambda\), which depend on the local PDGF and TGF-\(\beta\) concentrations \(P(x, 8)\) and \(T(x, 8)\). The \(\hat{m}^*\) and \(\hat{\rho}^*\) curves intersect their corresponding \(m(x, 8)\) and \(\rho(x, 8)\) profiles at \(x \approx 0.08\), showing that ECM deposition is maximised at this position. At all other positions in the plaque, \(\rho(x, 8)\) is smaller than \(\hat{\rho}^*\) due to the presence of either too few \((x \gtrsim 0.08)\) or too many SMCs \((x \lesssim 0.08)\). Interestingly, \(\rho(x, 8)\) remains close to \(\hat{\rho}^*\) throughout the region proximal to the endothelium \((x \lesssim 0.2)\). This is despite the fact that \(m(x, 8)\) is considerably larger than \(\hat{m}^*\) for \(x \approx 0\) and considerably smaller than \(\hat{m}^*\) for \(x \approx 0.2\).

Figure 8 presents additional data that helps to explain why near-maximal ECM deposition can be attained for a range of SMC volume fractions near the endothelium. For a series of positions in the model plaque \((x = 0, 0.1, 0.2, 0.3, 0.45, 1)\), Fig. 8 plots the analytical expression for the steady state ECM volume fraction \(\rho^*(m^*)\) [negative root in Eq. (51); solid lines]. Each \(\rho^*(m^*)\) curve uses the local growth factor concentrations \(P(x, 8)\) and \(T(x, 8)\). In addition, we plot individual data points that show the simulated steady state \((m, \rho)\) values at each \(x\) at \(t = 8\) (open circles), and the maximum \((\hat{m}^*, \hat{\rho}^*)\) of each \(\rho^*(m^*)\) curve (asterisks). Note that each \(\rho^*(m^*)\) curve is relatively flat for a wide range of \(m^*\) values either side of its maximum at \(\hat{m}^*\). Therefore, when the simulated steady state value of \(m\) lies inside the shallow region of the \(\rho^*(m^*)\) curve—as is the case for \(x \lesssim 0.2\) in the base case simulation—
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Fig. 8 Plot that compares near-steady state $m$ and $\rho$ values for several values of $x$ in the base case simulation with the corresponding analytical steady state results derived in Sect. 3.1. Solid lines represent $\rho^\ast (m^\ast)$ [negative root in Eq. (51)] at the spatial positions $x = 0$ (red), $x = 0.1$ (green), $x = 0.2$ (blue), $x = 0.3$ (magenta), $x = 0.45$ (orange) and $x = 1$ (purple). Each $\rho^\ast (m^\ast)$ curve is a function of the local PDGF and TGF-β concentrations. Data points represent near-steady state $(m, \rho)$ values from the base case simulation (open circles) and the maximum $(\hat{m}^\ast, \hat{\rho}^\ast)$ of each individual $\rho^\ast (m^\ast)$ curve (asterisks) at each $x$. All curves and data points taken at time $t = 8$ (color figure online).

the simulated steady state value of $\rho$ will differ only slightly from its theoretical maximum. For example, the simulated SMC volume fraction at $x = 0$ is much larger ($m \approx 0.202$) than $\hat{m}^\ast \approx 0.155$, but the corresponding simulated ECM volume fraction ($\rho \approx 0.408$) remains very close to $\hat{\rho}^\ast \approx 0.415$. Indeed, Fig. 8 indicates that, at $x = 0$, any steady state $m$ in the range $[0.102, 0.233]$ would give a steady state $\rho > 0.4$ for the same local growth factor concentrations. This suggests that the fibrous cap generated in the base case simulation would be robust to a relatively large reduction in cap region SMC numbers.

3.2.2 Sensitivity analysis

In this section, we perform sensitivity analyses to investigate how certain parameter values determine the extent of collagen deposition in the cap region. In particular, we investigate both the impact of SMC adhesion to the nascent collagenous matrix and the role of the relative rates of growth factor influx. Figure 9 presents near-steady state SMC and ECM profiles for a range of simulated cases.

SMC affinity for collagen We investigate the role of SMC haptotaxis by examining the sensitivity of the model to the parameter $\chi_{\rho}$, which quantifies the affinity of the SMCs for the ECM phase. A simulation with $\chi_{\rho} = 0$ produces results that are only marginally different to those from the base case simulation. This highlights that the base case value $\chi_{\rho} = 0.3$ was particularly conservative, and we therefore consider a considerably larger $\chi_{\rho}$ value. Setting $\chi_{\rho} = 0.8$, we find that the solution dynamics are practically identical to the base case simulation for the first 5–6 weeks of simulation time. Beyond this point, however, the emerging ECM phase exerts an increasing influence on the
SMC behaviour and the model solution diverges from the base case dynamics (Fig. 9a). As expected, the increase in $\chi_\rho$ results in increased SMC recruitment to the cap region. More surprising, however, is that the total plaque SMC content is reduced from 8.6 to 7.1%, and there is no overall increase in ECM deposition in the cap region. The reduction in SMC numbers appears to be due to an increased squeeze on the PDGF influx, which reduces both SMC recruitment from the media and SMC mitosis inside the intima. The lack of increase in cap ECM deposition, on the other hand, can be attributed to the fact that the SMC volume fraction near the endothelium has increased well beyond the level required to maximise ECM synthesis (c.f. Fig. 4). Of course, a potential benefit of the increased haptotaxis simulated here is that less ECM is deposited beyond the cap region (due to the drop in SMC numbers), which may contribute to reduced hardening of the diseased arterial tissue.

**Growth factor influx** We investigate the importance of the relative rates of PDGF and TGF-β influx from the endothelium by examining the sensitivity of the model to the parameters $\alpha_T$ and $\alpha_P$. To facilitate the comparison of cap formation dynamics in the simulations in this section, we introduce a simple metric to measure the SMC
and ECM levels proximal to the endothelium at a given point in time. Specifically, we define the quantity $V_i(t; X)$, which denotes the average volume fraction of phase $i$ ($i = m, \rho$) in the interval $x \in [0, X]$ at time $t$:

$$V_i(t; X) = \frac{1}{X} \int_0^X i(x, t) \, dx,$$

(54)

where $0 < X \ll 1$. In all that follows, we choose (arbitrarily) to set $X = 0.2$ and assume that the interval $x \in [0, 0.2]$ represents what we shall refer to as the cap region. We remark that we have calculated $V_i$ values for several different choices of cap region width, but (within reasonable bounds) the precise choice of $X$ does not alter our qualitative findings in any of the below sensitivity studies.

A key target of this study is to identify factors that lead to reduced plaque stability and increased likelihood of plaque rupture. Given that collagen cap deposition enhances plaque tensile strength and inhibits the leakage of plaque material, we surmise that the cap region collagen content provides a reasonable metric for assessing rupture potential. In the context of the model, we therefore define the “stability” of a simulated plaque on the basis of its long-term $V_\rho$ value. As cap SMCs are unlikely to contribute substantially to improving plaque tensile strength or inhibiting plaque leakage, we do not explicitly account for cap SMC content when defining plaque stability. Having said that, $V_m$ values remain useful to calculate because they provide insight into how changes in the plaque environment and/or SMC dynamics feed into cap deposition.

Figure 9b presents near-steady state SMC and ECM profiles for a simulation with no TGF-\(\beta\) influx ($\alpha_T = 0$). The results show that the absence of TGF-\(\beta\) has reduced the total collagen deposition in the plaque by approximately 15%. This reduction in collagen deposition is smaller than expected, but it is clear that collagen levels have been rescued to some degree by an increase in SMC numbers. This increase in SMC numbers is directly attributable to the reduced rate of collagen deposition because contact inhibition of SMC proliferation remains lower, and PDGF influx from the endothelium remains higher throughout the simulation. Having said that, the simulation results still demonstrate that the absence of TGF-\(\beta\) can decimate the fibrous cap. Calculations show that, near steady state, the average ECM volume fraction in the cap region $V_\rho$ ($8; 0.2$) has decreased by approximately 40% from 0.381 to 0.235. This result is consistent with the findings of Mallat et al. (2001) and Lutgens et al. (2002), who report that inhibition of TGF-\(\beta\) signalling in the ApoE knockout mouse can lead to an unstable plaque phenotype.

For PDGF, we consider the impact of both a decrease ($\alpha_P = 0.3$) and an increase ($\alpha_P = 1.1$) in the rate of PDGF influx (we cannot simulate a case with $\alpha_P = 0$ because the model requires PDGF to stimulate SMC recruitment from the media). To interpret the results of these simulations, it is helpful to note that increasing $\alpha_P$ increases both the PDGF gradient across the plaque and the PDGF concentration throughout the plaque tissue. Near-steady state SMC and ECM profiles for $\alpha_P = 0.3$, $\alpha_P = 1.1$ and the base case simulation ($\alpha_P = 0.7$) are shown in Fig. 9c, d, respectively. The SMC profiles show a large disparity in their volume fractions, particularly near the endothelium. This reflects the significant differences in the relative rates of chemotaxis...
and proliferation in each case. The ECM profiles, on the other hand, show relatively little variation, but do still demonstrate some interesting features. For example, the case with $\alpha_P = 1.1$ produces a slightly lower cap collagen content compared to the base case simulation despite recruiting over 60% more cap SMCs. This result can mostly be attributed to the fact that the cap SMC volume fraction has significantly exceeded the level required to attain maximum ECM synthesis. However, note that the elevated plaque PDGF levels also contribute to an enhanced rate of ECM degradation by the invading SMCs. In contrast, in the simulation with $\alpha_P = 0.3$, the sparse SMC population makes a remarkably strong attempt at cap synthesis. In this case, the cap region collagen content is only 20% less than that in the base case simulation despite a cap SMC content that has been depleted by over 60%.

Of course, examining these SMC and ECM profiles at only one time point gives no real indication of the overall simulation dynamics. Hence, in Fig. 10, we present the average cap SMC volume fraction $V_m(t; 0.2)$ and the average cap ECM volume fraction $V_\rho(t; 0.2)$ as functions of time $t$. For $\alpha_P = 1.1$, Fig. 10a shows a slow initial rate of cap SMC recruitment, followed by a period of rapid population growth that saturates after 3–4 months. However, despite a cap SMC population that significantly exceeds the base case levels from 2 months onwards, the temporal accumulation of cap ECM follows a similar pattern to the base case simulation and fails to exceed the base case $V_\rho$ value at any time (Fig. 10b). For $\alpha_P = 0.3$, we observe completely different dynamics. The reduced PDGF levels in this case inhibit both the rate and the extent of cap SMC recruitment (Fig. 10a), which, in turn, creates a lag in the rate of cap ECM accumulation (Fig. 10b). Hence, although these SMCs have the capacity to generate large quantities of ECM, their limited numbers ensure that this process takes an extended period of time.

We conclude this study by briefly considering the consequences of simultaneously changing the rates of influx of both PDGF and TGF-β. Figure 11 presents bar charts of $V_\rho(8; 0.2)$ and $V_m(8; 0.2)$ for simulations with each unique pair of values from $\alpha_T = 0, 1.25, 2.5, 3.75, 5$ and $\alpha_P = 0.3, 0.5, 0.7, 0.9, 1.1$. Figure 11a, which plots the $V_m$ value for each simulation, shows two clear trends: (1) increasing $V_m$ with increasing

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**Fig. 10** Plots that show how **a** $V_m(t; 0.2)$ and **b** $V_\rho(t; 0.2)$ vary with time for the base case simulation (solid lines) and simulations with a smaller ($\alpha_P = 0.3$; dot-dash lines) or larger ($\alpha_P = 1.1$; dashed lines) rate of PDGF influx.
Fig. 11  Charts that show how the rates of PDGF influx $\alpha_P$ and TGF-$\beta$ influx $\alpha_T$ influence $a$ $V_m(t; 0.2)$ and $b$ $V_\rho(t; 0.2)$ after 8 months of simulation time. Simulations were performed for each unique pair of values from the lists $\alpha_P = 0.3, 0.5, 0.7, 0.9, 1.1$ and $\alpha_T = 0, 1.25, 2.5, 3.75, 5$. The central bar on each chart represents the outcome of the base case simulation.

$\alpha_P$ (mainly due to increased SMC proliferation) and (2) decreasing $V_m$ with increasing $\alpha_T$ (due to the inhibitory effects of increased ECM deposition). Interestingly, the plot for $V_\rho$ indicates a relatively large region of the $(\alpha_T, \alpha_P)$ parameter space where the model predicts a robust cap formation response (Fig. 11b). This region corresponds to parameter regimes with large TGF-$\beta$ levels, moderate PDGF levels and, consequently, moderate volume fractions of cap SMCs.

4 Discussion

Mature atherosclerotic plaques contain a core of necrotic material and release of this material into the circulation can cause myocardial infarction or stroke. Fibrous cap formation protects against these outcomes by stabilising the plaque and sequestering dangerous content from the bloodstream. However, the mechanisms that regulate cap formation, and the factors that may lead the cap to fail, are poorly understood. In this paper, we have developed a multiphase model of collagen cap synthesis by growth factor-stimulated SMCs. We have studied how diffusible PDGF and TGF-$\beta$ co-operate (and compete) in the recruitment of vascular SMCs to remodel the plaque ECM.

4.1 Applicability of the model to human atherosclerosis

The model in this study is designed to investigate cap formation in the ApoE-deficient mouse. We choose to focus on this transgenic mouse model of atherosclerosis because there exists an extensive experimental literature with which to inform and validate our modelling assumptions. While it may be tempting to extrapolate the model to human disease (e.g. by solving on a wider domain), such an approach would be naive because human plaque SMC behaviour remains poorly understood (Bennett et al. 2016). Unlike mice, healthy human arteries contain a resident population of intimal SMCs and the extent of medial SMC recruitment to human plaques has yet to be established.
4.2 Comparison with previous work and limitations of the model

The research in this paper has some similarities and several key differences to the earlier work of Watson et al. (2018). In Watson et al. (2018), we established a novel modelling framework to study the chemotactic recruitment of plaque SMCs in response to endothelium-derived PDGF. We did not explicitly model the deposition of a fibrous cap. Instead, we assumed that the plaque ECM profile would evolve to more-or-less reflect the plaque SMC profile and, hence, that an increase in SMC recruitment to the cap would generally improve cap stability. The current work uses the framework of Watson et al. (2018) as a foundation, but builds a substantial new model that focusses on ECM remodelling by plaque cells in response to both PDGF and TGF-β. The model also includes haptotactic SMC migration in response to dynamically varying ECM gradients and, hence, to the best of our knowledge, this paper presents the first attempt to include both chemotactic and haptotactic cell movement in a multiphase model. The model results demonstrate that the plaque SMC profile does not necessarily provide a reliable indication of the corresponding ECM profile, nor of the overall likelihood of fibrous cap stability. This is in contrast to the assumptions we made in the simpler model of Watson et al. (2018).

By retaining the modelling framework from our previous study, we have also retained some of its limitations. In particular, the model remains on a fixed domain and does not explicitly account for the influx of LDL and immune cells that may contribute to plaque growth during cap formation. The current work can therefore be interpreted as a model of cap formation in a plaque that otherwise exists in a dynamic equilibrium (i.e. where any influx of LDL and immune cells from the bloodstream is exactly balanced by an efflux of lipid-loaded foam cells to the adventitial lymphatics). Of course, even with this interpretation, it is arguable that domain growth should be included to account for the accumulation of SMCs and ECM in the plaque. Without domain growth, increasing SMC and ECM levels are balanced by a loss of material from the generic tissue phase. This loss of generic tissue phase material can be interpreted as a loss of the initial non-collagenous ECM, which is degraded and subsequently recycled, and as a loss of interstitial fluid, which is squeezed out of the plaque or absorbed by SMCs to produce new material. Note, however, that this interpretation becomes less reasonable if the total SMC and ECM volume fractions in the plaque become large because significant quantities of other generic tissue phase constituents (e.g. immune cells, lipids) are also effectively lost from the tissue. Despite these limitations, we believe that the fixed domain model provides a useful and computationally straightforward tool for modelling atherosclerotic cap growth. In future, we will develop a comprehensive moving boundary approach to explore how expansion of the intima by immune cell and SMC activity influences cap formation efficacy.

In the biomedical literature, fibrous caps are most commonly defined in terms of their thickness (the width of the collagenous region between the endothelium and the fatty plaque core) and there is a broad understanding that plaques with thicker caps are less vulnerable to rupture. We believe, however, that any definition of a cap should consider both its thickness and collagen content. One would expect, for example, that a thick cap with high collagen content would provide greater stability than a thick cap.
with low collagen content. In this paper, we have characterised our simulated caps based solely on their collagen content. This is because, in the absence of an explicit representation of the plaque core, the concept of cap thickness cannot be defined in a meaningful way. In future work, we will develop new models to simulate coupled cap and core formation. This will enable us to explicitly investigate which parameters are responsible for determining cap thickness.

4.3 Analytical results explain the (local) SMC–ECM relationship

In Sect. 3.1, we exploited the absence of an ECM phase flux term to investigate what determines the steady state ECM volume fraction at a given position in the plaque. For fixed PDGF and TGF-β concentrations, we showed that the steady state ECM volume fraction $\rho^*$ has a biphasic dependence on the steady state SMC volume fraction $m^*$, and, correspondingly, that there exists a unique steady state SMC volume fraction $\tilde{m}^*$ that gives a maximum steady state ECM volume fraction $\tilde{\rho}^*$. Moreover, we derived expressions for $\tilde{m}^*$ and $\tilde{\rho}^*$ in terms of the dimensionless parameter groupings $\mu$ and $\lambda$, where $\mu$ represents the ratio of the net rate of ECM synthesis by SMCs to the net rate of ECM degradation by immune cells, and $\lambda$ represents the ratio of the net rate of ECM degradation by SMCs to the net rate of ECM degradation by immune cells. The most significant finding of this analysis is that, for a realistic set of parameter values, the model predicts that the maximum ECM volume fraction can generally be achieved by a relatively small SMC volume fraction ($< 20\%$ even at moderate TGF-β levels).

Interestingly, for several of the simulations in Sect. 3.2, the model predicts steady state SMC volume fractions near the endothelium that are above the level required to maximise ECM deposition. Consequently, the steady state cap region ECM levels in these simulations are typically below their maximum possible level. With regard to plaque stability, this outcome appears to be undesirable. However, note that a slight excess in SMC numbers above the optimum level may be beneficial because it affords the plaque greater robustness to destabilisation mechanisms such as SMC loss or diminished TGF-β signalling. When the typical steady state SMC volume fraction in the cap region is below the level required for maximum ECM deposition, an equivalent drop in SMC numbers or loss of TGF-β signalling would have far more pronounced consequences for plaque stability.

4.4 Numerical results agree with experimental observations

The base case simulation reproduced several observations from experimental studies of plaque growth in the ApoE-deficient mouse. For example, temporal changes in the model plaque SMC and ECM levels were consistent with the qualitative pattern reported in Reifenberg et al. (2012). Plaque SMCs accumulated rapidly over the first 2 months of simulation time and maintained a relatively stable level thereafter. Plaque ECM, on the other hand, accumulated more slowly and reached a stable level after 4–6 months. The total SMC and ECM content in the model plaque after 4–8 months were in good quantitative agreement with values reported in several experimental studies (Mallat et al. 2001; Clarke et al. 2006; Reifenberg et al. 2012). An interesting
aspect of the base case simulation was that the total plaque SMC content peaked at 9.2% after 4 months of simulation time and then dropped to 8.6% as the ECM levels increased. This is a subtle effect, but for simulations where the plaque ECM content reaches higher levels (e.g. for larger $r_s$, smaller $r_d$ or smaller $\beta_r$), we observe a more pronounced suppression of SMC numbers late in cap formation (results not shown). These findings are consistent with observations of plaque growth in ApoE mice resistant to type I collagen degradation (Fukumoto et al. 2004). Fukumoto et al. (2004) found that, relative to standard ApoE mice, these compound-mutant mice had significantly more collagen and significantly fewer SMCs in their plaques. These observations suggest that excessive accumulation of plaque collagen can tip the balance towards a net reduction in plaque SMC levels. The model presented here makes a similar prediction and suggests that SMC loss from collagen-rich plaques can be explained by an increase in ECM-mediated contact inhibition of SMC proliferation and a decrease in the capacity for PDGF transport in the dense intimal tissue.

A consistent finding throughout this study is that TGF-\(\beta\) plays a critical role in the ability of plaque SMCs to deposit a stable, collagen-rich fibrous cap. The analysis in Sect. 3.1, for example, indicates that the presence of TGF-\(\beta\) can enable significantly fewer SMCs to deposit significantly more ECM than would otherwise be possible. These analytical results are further supported by numerical simulations. Compared to the base case, a sensitivity simulation with no TGF-\(\beta\) (\(\alpha_T = 0\)) showed an overall decline in steady state plaque ECM levels, which included a substantial reduction in cap region ECM deposition. These observations are qualitatively consistent with those from equivalent experiments in ApoE mice (Mallat et al. 2001; Lutgens et al. 2002). However, we also note some inconsistencies between the in vivo and in silico results. For example, the model predicted a 15% decrease in plaque ECM content coupled to a 55% increase in plaque SMC content, whereas the above experimental studies showed a 50% decrease in plaque collagen and no overall change in plaque SMC content after TGF-\(\beta\) blockade. The reasons for these differences are not clear, but the experimental results also showed that TGF-\(\beta\) blockade led to increased inflammatory cell content and larger lipid cores in the murine plaques. The SMC response in these in vivo plaques may therefore have been blunted by increased competition for space or by increased SMC death in the inflammatory plaque environment. The absence of these confounding factors in the model could potentially explain why the plaque SMC and ECM content are over-predicted in the current in silico approach.

For TGF-\(\beta\) to play its role in cap formation, PDGF is first required to recruit SMCs to the cap region. PDGF may contribute both positively and negatively to cap formation. Experiments suggest that PDGF can increase SMC production of matrix-degrading MMPs, in addition to stimulating SMC migration and mitosis. These factors were included in the model, and simulations with different rates of PDGF influx \(\alpha_P\) showed interesting results. For \(\alpha_P\) values in the range 0.3–1.1, SMC recruitment to the cap region increased significantly with increasing \(\alpha_P\). Cap region ECM levels, on the other hand, were relatively insensitive to \(\alpha_P\) and showed a biphasic response, with maximal steady-state ECM deposition at the base case value (\(\alpha_P = 0.7\)). These qualitative patterns of cap SMC recruitment and ECM deposition have been shown to be independent of the corresponding rate of TGF-\(\beta\) influx (for \(\alpha_T\) in the range 0–5).
The reported simulation with $\alpha_P = 0.3$ (at its base case value) is interesting because it shows that even a small amount of SMCs can generate a substantial amount of ECM. From a total plaque SMC volume fraction of only 4.0% (vs. 8.6% in the base case simulation), the total plaque ECM volume fraction reaches 15.0% (vs. 21.4% in the base case simulation). This result is qualitatively consistent with the observations of Clarke et al. (2006), who studied the impact of diphtheria toxin (DT)-induced SMC apoptosis in the plaques of ApoE mice. Plaque SMC content in DT treated mice was reduced more than fourfold relative to untreated mice (2.5% vs. 10.2%), but plaque collagen content fell just over twofold (8.0% vs. 18.1%). Of course, despite the relatively small drop in the long-term plaque collagen content, the simulation with $\alpha_P = 0.3$ also showed significantly delayed cap formation relative to the base case. This simulation therefore provides a plausible explanation for the observations of Kozaki et al. (2002), who studied the influence of PDGF blockade on cap formation in ApoE mice. In this study, PDGF signalling was inhibited either by using transgenic ApoE mice with no PDGF expression in their circulating cells or by treating standard ApoE mice with a PDGF receptor antagonist. The authors suggest that PDGF signalling may only have been partially disrupted in each case, and both methods were shown to delay rather than prevent cap formation. Kozaki et al. (2002) did not report the SMC content of the plaques in their study, but the reported influence of PDGF inhibition on in vivo cap formation is entirely consistent with the modelling results in this paper.

4.5 SMC haptotaxis and the problem of model ill-posedness

SMCs are known to respond haptotactically to gradients in collagen substrates and we have included this phenomenon in the model by assuming that the SMC phase has an affinity for the ECM phase (quantified by $\chi_\rho$). Simulations for different $\chi_\rho$ values emphasise the important role of SMC chemotaxis in initiating cap formation because, even with a relatively large $\chi_\rho$, it took over a month of simulation time for the nascent ECM profile to have a discernible influence on the SMC phase. In the long term, an increase in $\chi_\rho$ led to steeper SMC and ECM profiles but did not increase ECM deposition in the cap region. It would be interesting to comprehensively investigate how the relative contributions of SMC haptotaxis and SMC chemotaxis influence cap formation by simultaneously varying the values of $\chi_\rho$ and $\chi_P$ (or $\alpha_P$). Such an investigation was not the primary intention of the current work, but the model presented herein creates the opportunity for a more focussed future study.

One reason why a thorough study of SMC haptotaxis is challenging is that the model can become ill-posed for large $\chi_\rho$. To understand why this is the case, recall the form of the effective SMC diffusion coefficient $D_{\text{eff}}$ in Eq. (31) and note that the sign of $D_{\text{eff}}$ is determined by the expression:

$$
\Lambda + \rho \left( \psi + m \frac{\partial \psi}{\partial m} \right) = \frac{\chi_P}{1 + (\kappa_P)^{n_P}} - \rho \chi_\rho + \frac{\delta \rho m^{n_\rho} \left[ (1 + n_\rho) (1 - \rho) - m \right]}{(1 - m - \rho)^{n_\rho + 1}},
$$

(55)
where the first and third terms on the RHS are strictly positive. Recall, also, that we assume zero flux for SMCs at the endothelium [Eq. (32)]. In practice, the chemotactic and haptotactic SMC fluxes almost always act towards the endothelium, creating a negative gradient in the SMC phase at $x = 0$. To satisfy the zero flux boundary condition, the diffusive SMC flux must therefore be positive and act down the SMC gradient away from the endothelium. Hence, if $D_{\text{eff}}$ becomes negative at any stage, the boundary condition cannot be satisfied and the problem becomes ill-posed.

Equation (55) indicates that negative $D_{\text{eff}}$ can occur if SMC adhesion to the ECM is strong enough to counteract the other mechanisms of cell spreading (i.e. if the second term on the RHS is dominant). Note that this is most likely if $P$ is large (first term on RHS negligible) and/or if $m$ is small (third term on RHS negligible). In general, it is difficult to remove the problem of ill-posedness entirely. However, there exist several possible strategies to reduce the problematic region of parameter space. First, we could regularise the problem by including viscous effects in Eq. (11) (Lemone et al. 2006). Second, we could introduce a background SMC diffusion by adding a constant SMC phase pressure to the function $\Lambda$ (Byrne and Owen 2004). Third, we could assume that $\chi_\rho$ decreases with increasing PDGF concentration (i.e. $\chi_\rho \equiv \chi_\rho (P)$). As SMC proliferation increases with increasing $P$, this latter case would effectively introduce a “go-or-grow” mechanism in the SMC phase. Note, however, that this would also add an ECM phase-dependent term to the effective chemotaxis coefficient $C_{\text{eff}}$. Of course, the downside of these potential solutions is that each would introduce additional model parameters whose values may be difficult to determine in practice. See El Khatib et al. (2009), Silva et al. (2016) and Mel’nyk (2019) for related work on the well-posedness of atherosclerosis models in the context of reaction–diffusion systems.

### 4.6 Implications for plaque destabilisation mechanisms

So, what conclusions can we draw from the model about factors that have been reported to contribute to plaque instability? Results suggest that, under conditions where initial SMC recruitment is strong and plaque TGF-β levels remain favourable, the fibrous cap should be robust to relatively significant SMC loss. However, if, as suggested by Wang et al. (2015), that replicative senescence can lead to long-term loss of SMCs, the model would predict rapid immune cell degradation of the cap as the SMC population falls to critically low levels. In conditions where the plaque SMC numbers and TGF-β concentration remain at moderate levels, the model suggests that an increase in ECM degradation by immune cells alone is unlikely to destabilise the plaque. In results not reported in this paper, a sensitivity simulation with a twofold increase in the rate of immune cell ECM degradation ($\beta_\rho = 1.5$) predicts only a relatively small change in the cap region ECM volume fraction (32% vs. 38% in the base case simulation). This makes sense because, based on our modelling assumptions, the majority of the plaque immune cell content resides deep in the plaque beneath the cap region. Of course, the current model is unable to predict the consequences of immune cell ECM degradation at the plaque shoulders, where rupture events are commonly reported to occur (Bennett et al. 2016). Investigation of this phenomenon will require the development of cap formation models in two or three spatial dimensions.
The most critical factor in maintenance of plaque stability, as predicted by the model, is the continued presence of TGF-β and its continued capacity to stimulate SMCs (and immune cells). Our analysis and simulations have indicated that TGF-β can play a critical role not only in the formation of a stable cap, but also in ensuring that cap formation is efficient and does not require excessive SMC recruitment. Interestingly, recent in vivo and in vitro experiments have shown that cholesterol loading in several cell types, including SMCs, can significantly attenuate responsiveness to TGF-β (Chen et al. 2007; Vengrenyuk et al. 2015). Given the extent of cholesterol accumulation in the intima during atherosclerotic plaque growth, it is therefore plausible that modified LDL consumption by SMCs (and macrophages) indirectly contributes to the breakdown of plaque ECM by inhibiting the protective actions of TGF-β. Investigation of this hypothesised cap destabilisation mechanism is another important target for future modelling studies.

5 Conclusions

In this paper, we have developed a novel three-phase model of fibrous cap formation in the atherosclerosis-prone mouse. The model investigates the roles of endothelium-derived PDGF and TGF-β in the regulation of collagen cap deposition by synthetic vascular SMCs, which migrate both chemotactically and haptotactically in the arterial intima. We have parameterised the model using data from a wide range of in vitro and in vivo studies and our numerical simulations reproduce a number of experimental observations. Our results provide insight into potential mechanisms of plaque instability and long-term cap degradation. The model presented in this paper can be taken in several new directions, including by introducing domain growth, extending to higher dimensions and incorporating phenomena such as plaque core formation and SMC cholesterol accumulation. This work therefore represents an important step in the development of a dynamic and mechanistic understanding of atherosclerotic plaque formation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Affiliations

Michael G. Watson1 · Helen M. Byrne2 · Charlie Macaskill1 · Mary R. Myerscough1

1 School of Mathematics and Statistics, University of Sydney, Sydney, Australia
2 Wolfson Centre for Mathematical Biology, Mathematical Institute, University of Oxford, Oxford, UK