Experiments and mechanochemical modeling of smooth muscle contraction: Significance of filament overlap

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ABSTRACT

The main function of smooth muscle is to maintain/regulate the size of different hollow organs through contraction and relaxation. The magnitude of the active force during contraction is dependent on the number of attached cross-bridges, which can be linked to the overlap between the thin and thick filaments. The relevance of filament overlap and the active cross-bridges in smooth muscle is investigated through a mechanical model founded on Hill’s three-element model. The mechanical model describes a sarcomere-equivalent contractile unit supported by structural observations with a distinct filament overlap and a realistic framework for the filament sliding behavior based on force-velocity experiments. The mechanical model is coupled to the four-state latch-model by Hai and Murphy to capture the electromechanical activation from intracellular calcium concentration to load-bearing cross-bridges. The model is fitted to isometric experiments performed on the pig carotid media and on isotonic quick-release experiments found in the literature. The proposed coupled mechanochemical model with the description of the filament overlap, which has a significant influence on the results, is able to predict isometric experimental data performed at different muscle lengths. The relevance of the filament overlap and the load-bearing cross-bridges is investigated through the model by simulating additional scenarios that has been documented in the literature.

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1. Introduction

Smooth muscle has a key role in several physiological processes, where it determines the wall tension and size of different hollow organs. In the cardiovascular system, contractility of smooth muscle in the blood vessels contributes to the mechanical stability of the vessel wall and determines the diameter and flow resistance. Currently, efforts are made to develop computational models of complex physiological events, to increase our understanding of both basic and clinical/pathophysiological processes. These include modeling of focused vascular events, in, e.g., aneurysm formation in large arteries (Humphrey and Taylor, 2008; Zeinali-Davarani et al., 2011), but also of integrative processes at the organ and organism levels, e.g., the ‘Physiome project’ (Hunter et al., 2002; Hunter and Borg, 2003; Hunter and Nielsen, 2005). In this context, well defined mathematical models of smooth muscle mechanical properties are needed. Several aspects of the biomechanics of the vascular wall including interaction between wall layers and the contribution of connective tissue components have been modeled (e.g., Holzapfel et al., 2000; Holzapfel and Ogden, 2010), but the contractile function of smooth muscle is less well explored. In pioneering works, Hai and Murphy (1988a,b), incorporated regulation via Ca²⁺ and myosin phosphorylation and developed a kinetic model describing cross-bridge interaction during contraction. This and similar models have been further explored in subsequent studies and applied to different organ systems (Yu et al., 1997; Bursztyn et al., 2007; Stålhand et al., 2011). The Hai and Murphy models describe the isometric contraction and properties during active shortening. However, they do not include the strain parameter as described by the length-tension relationship which is important for smooth muscle function when integrated in the vascular wall in vivo.

Smooth muscle is able to generate active force over a broad range of muscle lengths. The length active force relationship is bell-shaped with an optimal muscle length larger than the slack length (Herlihy and Murphy, 1973; Uvelius, 1976). The length-active tension relationship of the striated skeletal muscle can be a large part to be explained by variations in filament overlap within the sarcomere units. At present, a corresponding contractile unit (CU), or sarcomere equivalent, has not been clearly identified in smooth muscle. The thin filaments are attached to the dense body...
structures which might act as anchoring points similar to z-lines in the striated muscles (Bond and Somlyo, 1982; Kargacin et al., 1989). The length of such CUs, between dense bodies, has not been defined. An estimate based on the mechanical and in vitro motility data suggest an approximate half-sarcomere (or CU) length in smooth muscle of approximately 1.2 μm (Löfgren et al., 2003), which is similar to that in skeletal muscle and consistent with the estimate of a thick filament length of 2.2 μm (Ashton et al., 1975). It should be noted that the structure of the thick filament in smooth muscle is not fully resolved, although initial studies with electron microscopy suggested a bipolar structure (similar to that in skeletal muscle) (Ashton et al., 1975), more recent data seem to suggest a side-polar arrangement of myosin heads (Xu et al., 1996).

Smooth muscle is activated by increased intracellular Ca^{2+} levels activating the myosin light-chain kinase, which phosphorylates the regulatory light chains (RLC20) on myosin and initiates contraction. Dephosphorylation and deactivation are performed by the myosin light-chain phosphatase (Horowitz et al., 1996). It has been observed that smooth muscle can maintain tension at low levels of phosphorylation (the ‘latch state’), which has lead to the hypothesis that dephosphorylation of attached cross-bridges in smooth muscle results in slowly cycling tension-bearing ‘latch-bridge’ interactions (Dillon et al., 1981), which are included in the Hai and Murphy mathematical models. The excitation–contraction coupling can be divided into two main pathways: ‘electromechanical’ coupling where membrane depolarization results in influx of Ca^{2+} and the ‘pharmacomechanical’ coupling ligand binding to membrane receptors activates contraction via mechanisms not primarily associated with receptor activation have been characterized revealing a hyperbolic relationship between Ca^{2+} and CaM (CaCaM). Previous studies have described a pharmacomechanical and ligand binding to membrane receptors activates contraction via mechanisms not primarily associated with receptor activation have been characterized revealing a hyperbolic relationship between Ca^{2+} and CaM (CaCaM). Previous studies have described a pharmacomechanical and ligand binding to membrane receptors activates contraction via mechanisms not primarily associated with receptor activation have been characterized revealing a hyperbolic relationship between Ca^{2+} and CaM (CaCaM). Previous studies have described a pharmacomechanical and ligand binding to membrane receptors activates contraction via mechanisms not primarily associated with receptor activation have been characterized revealing a hyperbolic relationship between Ca^{2+} and CaM (CaCaM).

2. Methods

2.1. A mathematical model for the smooth muscle contractile unit

In the present work, the influence of the overlap between myosin and actin filaments, effects of phosphorylated/dephosphorylated attached cross-bridges and filament sliding on the active force produced by smooth muscle cells is investigated. The related parameters are studied through a chemomechanical model describing smooth muscle contraction activated via electromechanical excitation–contraction coupling pathways. The proposed chemomechanical model consists of a chemical part, describing the kinetics of myosin phosphorylation and load-bearing cross-bridges, which is triggered by an increase of intracellular calcium transient, and a mechanical part describing the mechanics of the load-bearing cross-bridges with a dependence of the filament overlap and a realistic description of the filament sliding in smooth muscle contractile units.

2.1.1. Intracellular calcium-load bearing cross-bridges

Muscle contraction and relaxation is regulated through phosphorylation/dephosphorylation of the myosin regulatory light chains RLC20. Hence, from a modeling point of view the myosin can initially be divided into two states: dephosphorylated and phosphorylated. The rate of myosin phosphorylation is controlled by the myosin light-chain kinase (MLCK) activity $k_{MLCK}$, which is triggered when Ca^{2+} binds to calmodulin (CaM) forming calcium–calmodulin (CaCaM). Previous studies have described a hyperbolic relationship between CaCaM, Ca^{2+} and MLCK activity $k_{MLCK}$ (Blumenthal and Stull, 1980). Based on previous findings a relationship between $k_{MLCK}$ and [Ca^{2+}] is can be set up in the form

$$k_{MLCK} = n_\eta \frac{[Ca^{2+}]^{h}}{[Ca^{2+}]^{h} + [ED50]^{h}}.$$  (1)

where $n_\eta$ is a fitting parameter, describing the maximal MLCK activity, $h$ is a parameter related to the steepness of the relationship and ED50 is the half-activation constant for [Ca^{2+}] to MLCK for a constant value of CaM. The dephosphorylation is regulated by the myosin light-chain phosphatase (MLCP). No calcium sensitzation via MLCP modulation is considered in this study and thus the rate of MLCP activity, say $k_{MLCP}$, was set to a constant value. The Ca^{2+} and myosin phosphorylation kinetics are summarized as

$$\begin{align*}
\dot{M} & = -k_{MLCK} M + k_{MLCP} M' \\
\dot{M}' & = k_{MLCP} M - k_{MLCK} M'.
\end{align*}$$

where $M$ and $M'$ are their respective time derivatives. Note that $n_{M} + n_{M'} = 1$. At steady-state the phosphorylated and dephosphorylated myosin have reached an equilibrium ($\dot{M} = \dot{M}' = 0$) so that a relationship for $k_{MLCP}$ can be derived as

$$k_{MLCP} = k_{MLCK} \bigg|_{t \to \infty} \frac{n_{M'}}{n_{M}} = k_{MLCK} \bigg|_{t \to \infty} \frac{1 - n_{M'}}{n_{M}}.$$  (3)

The generated active force in smooth muscle can be maintained at low phosphorylation levels and thus, when simulating the cross-bridge kinetics, two load-bearing states have been suggested: phosphorylated-attached and dephosphorylated-attached (latch) states (Hai and Murphy, 1988a).

The latch-state model (Hai and Murphy, 1988a) describes the kinetics of the cross-bridges through four different states: dephosphorylated and unattached cross-bridges (M), phosphorylated and unattached cross-bridges (Mp), phosphorylated and attached cross-bridges (Am) and dephosphorylated and attached cross-bridges (Am'), also referred to as ‘latch’ state. Hai and Murphy (1988a) couple fractions of each state through a system of ordinary differential equations. Thus,

$$\begin{pmatrix}
\dot{M} \\
\dot{M}' \\
\dot{M}_A \\
\dot{M}_A'
\end{pmatrix} =
\begin{pmatrix}
-k_{MLCK} & k_{MLCP} & 0 & k_3 \\
k_{MLCP} & -k(M + k_3) & k_4 & 0 \\
0 & k_3 & -(k_4 + k_{MLCP}) & k_{MLCP} \\
0 & 0 & k_{MLCP} & -(k_{MLCP} + k_3)
\end{pmatrix}
\begin{pmatrix}
M \\
M' \\
M_A \\
M_A'
\end{pmatrix}.$$  (4)

where $k_3$ and $k_4$ describe the rates for the cross-bridge attachment and detachment, and $k_2$ is the rate of detachment of latch cross-bridges (nomenclature: $k_3$, $k_4$ and $k_2$ are taken from Hai and Murphy, 1988a). The rate of myosin phosphorylation for unattached and attached cross-bridges is assumed to have the same value $k_{MLCK}$, and equivalently for the rate of myosin dephosphorylation $k_{MLCP}$. 
2.1.2. Contractile units with constant filament overlap \( L_0 \)

This section provides a brief review of the mecanochemical model presented in Murtada et al. (2010a). The contractile apparatus in smooth muscles consists of a network of contractile units (CUs). We have assumed that the myosin motors in the CU are arranged into myosin filament with the length \( L_m \). Actin filaments with the length \( L_a \) are organized with respect to their polarity on each side of the myosin filament from which the filament overlap \( L_o \) can be distinguished. The cross-bridges on the myosin filament can attach to binding sites on the actin filaments, which are located with a distance \( \delta \) apart. The length change of an activated CU is described through the relative filament sliding, caused by the myosin power-stroke or the external deformation, which is denoted by \( u_t \), and the average elastic elongation of the attached cross-bridges (only in the activated state), which is denoted by \( u_e \). Note that both \( u_t \) and \( u_e \) are taken to be positive for extension.

The stretch \( \lambda \) of a CU can then be expressed as

\[
\lambda = \frac{L_a}{L_a} = \frac{L_{CU} + u_t + u_e}{L_{CU}},
\]

where \( L_{CU} \) is the reference length and \( L_a \) is the current length of the contractile unit, respectively, see Fig. 1. Note that the filaments are modeled as rigid and that any deformation is associated with the cross-bridges and/or with the passive element.

The average elastic elongation of the cross-bridges \( u_e \) is described similar to an elastic spring as the fraction between the force applied on the CU and the average total stiffness of all attached cross-bridges. The force over a CU is expressed as \( P_a/N_{CU} \), where \( P_a \) is the (averaged) first Piola–Kirchhoff stress (engineering stress) and \( N_{CU} \) is the number of CU per unit area in the reference configuration. Thus, when looking at a half CU, the average elastic elongation \( u_e \) of the attached cross-bridges in the activated state can be expressed as

\[
u_e = \frac{P_a}{N_{CU}} \left[ n_{AM} + n_{AM} L_{CU} E_{cb} \right],
\]

where \( n_{AM} + n_{AM} L_{CU} E_{cb} \) is the total number of the attached cross-bridges and \( E_{cb} \) is the elastic stiffness of a single phosphorylated/diphosphorylated cross-bridge with the unit force per length. From Eqs. (5) and (6), the active stress \( P_a \) can be derived as

\[
P_a = \frac{u_t + u_e}{2} \left( \frac{L_{CU} + n_{AM} L_{CU} E_{cb}}{\delta} - 1 \right),
\]

where \( \mu_s = \frac{U_{CB}}{L_{CU}} \left( n_{AM} + n_{AM} L_{CU} E_{cb} \right) \) is a stiffness constant and \( \bar{\pi}_{CB} = u_t / L_{CU} \) is an internal variable describing the relative filament sliding.

The average first Piola–Kirchhoff stress \( P_a \) is related to the strain energy \( P_a \) stored in the contractile units. This is obtained by integrating \( P_a \) with respect to \( \lambda \), thus (Holzapfel, 2000)

\[
\Psi_a = \frac{\mu_s L_{CU}}{2L_{CU}} \left( n_{AM} + n_{AM} L_{CU} E_{cb} \right) \left( \lambda - \bar{\pi}_{CB} - 1 \right)^2.
\]

2.1.3. Consideration of varying filament overlap \( L_o(\bar{\pi}_{CB}) \)

The filament overlap \( L_o \) is the length which the thick and thin filaments overlap, and it defines the number of maximum possible attached cross-bridges in a half CU. The filament overlap \( L_o \) depends on the lengths of the actin and myosin filaments \( L_a \) and \( L_m \), and how these filaments move with respect to each other, which can be described through the filament sliding \( u_t \). By assuming that \( L_o > L_m \) and an initial filament overlap of \( x_0 \) in the reference configuration, \( L_o \) can be described as \( L_o = x_0 + u_t/2 \), according to Fig. 2. This relationship would be valid as long as \( u_t < 2(l_m - x_0) \).

The optimal overlap would then occur when \( 2(l_m - x_0) < u_t < 2(l_a - x_0) \) and due to \( L_a > L_m \) the optimal filament overlap \( L_o^{opt} \) would be \( L_o = L_o^{opt} = L_m \), see Fig. 2. When filament sliding increases above \( u_t > 2(l_m - x_0) \), the overlap would decrease linearly according to \( L_o = L_m + L_a - u_t/2 - x_0 \). Note that these equations are based on the condition \( L_o > 0 \). With this description of \( L_o \), the optimal filament sliding, which gives optimal filament overlap \( L_o^{opt} \), is dependent on the length of the actin and myosin filaments \( l_a \) and \( l_m \), respectively.

By introducing an average optimal filament sliding \( u_t^{opt} \), for which optimal filament overlap is reached, we have

\[
L_o^{opt} = L_o(u_t = u_t^{opt}) = \frac{u_t^{opt}}{2} + x_0.
\]

Together with the boundary conditions \( L_a(u_t = 0) = x_0 \) and \( \partial L_a/\partial u_t |_{u_t = 0} = u_t^{opt}/L_{CU} = 0 \), a continuous parabolic function of the filament overlap \( L_o(\bar{\pi}_{CB}) \) can be expressed as

\[
L_o = u_t - \frac{u_t^2}{2L_{CU}^2} + x_0 = \left( \bar{\pi}_{CB} - \frac{u_t^2}{2L_{CU}^2} + x_0 \right) L_{CU},
\]

where \( x_0 = x_0/L_{CU} \) and \( \bar{\pi}_{CB} = u_t^{opt}/L_{CU} \), see Fig. 3.

Next step is to define the normalized optimal filament sliding \( \bar{\pi}_{CB}^{opt} \) and the normalized initial overlap \( \bar{\pi}_{CB}^{opt} \). From Eq. (5), the relationship between the (normalized) optimal muscle length \( \lambda^{opt} \) and the (normalized) optimal filament sliding \( \bar{\pi}_{CB}^{opt} \) can be expressed as

\[
\lambda^{opt} = 1 + \bar{\pi}_{CB}^{opt} + \bar{\pi}_{CB}^{opt},
\]

where \( \bar{\pi}_{CB}^{opt} = u_t^{opt}/L_{CU} \). The optimal stretch \( \lambda^{opt} \) is the stretch at which maximal active isometric force is produced.

One approach to estimate the normalized initial overlap \( \bar{\pi}_{CB} \) is by comparing the steady-state active stress \( P_a \) at the reference length, and \( P_{opt} \) at the optimal length with the filament overlap at the reference length and the optimal length. By assuming that the majority of the series elasticity \( u_e \) in a CU comes from the cross-bridges and that it is \( L_a \), the filament overlap would not change significantly during isometric contraction \( \Delta u_t = \Delta u_e \approx \epsilon \approx x_0 \) and can be approximated as \( L_a(u_t = 0) \approx x_0 \) at the reference length and \( L_a(u_t = u_t^{opt} + \epsilon) \approx u_t^{opt}/L_{CU}^2 + x_0 \) at the optimal length in the fully contracted state. Assuming that the active stress \( P_a \) in the fully

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**Fig. 1.** Schematic image of a (half) contractile unit (CU), where \( L_{CU} \) is the reference length of CU, \( L_a \) is the current length, \( u_t \) is the filament sliding and \( u_e \) is the average elongation of the cross-bridges. Note that \( u_t \) is negative in contraction and that \( u_e \) only exists when there are load-bearing cross-bridges.
contracted state is proportional to the number of attached cross-bridges, the fraction of the active stress at reference length \( P_0 \) and at the optimal length \( P_{\text{opt}} \) can be expressed through the filament overlaps at the different lengths as

\[
\frac{P_0}{P_{\text{opt}}} = \frac{x_0}{u_{\text{fs}}^{\text{opt}}/2 + x_0} = \frac{x_0}{u_{\text{fs}}^{\text{opt}}/2 + x_0}.
\]

From this relationship \( x_0 \) can be extracted, hence

\[
x_0 = \frac{P_0}{2(P_{\text{opt}} - P_0)} u_{\text{fs}}^{\text{opt}}.
\]

The active stress of a contractile unit with varying filament overlap can be expressed as

\[
P_a = \mu_p L_o(\tilde{\pi}_{\text{fs}})(n_{\text{AMP}} + n_{\text{AM}})(\lambda - \tilde{\pi}_{\text{fs}} - 1),
\]

where now \( L_o(\tilde{\pi}_{\text{fs}}) = L_o(\tilde{\pi}_{\text{fs}})/L_{\text{CL}}. \)

We also include a parallel passive element, residing in the elastin and collagen fibers, which is modeled through a strain energy function \( \Psi_p \) consisting of an isotropic part \( \Psi_{\text{iso}} \) and an anisotropic part \( \Psi_{\text{aniso}} \) (Holzapfel et al., 2000). The isotropic part represents the elastin and is modeled through a classical neo-Hookean model, while the anisotropic part represents families of collagen fibers and is modeled by an exponential function. Thus, the passive stress \( P_p = \partial \Psi_p / \partial \lambda \) of an incompressible thin sheet (media) under simple tension may be derived as (see, e.g., Murtada et al., 2010b)

\[
P_p = \mu_p \left( \frac{1 - \lambda^2}{\lambda^2} \right) + 2C_1 \lambda \exp(C_2(\lambda^2 - 1)^4(\lambda^2 - 1)),
\]

where \( \mu_p, C_1 \) and \( C_2 \) are material parameters.

### 2.1.4. Evolution law for the filament sliding \( \tilde{\pi}_{\text{fs}} \)

The sliding of the actin and myosin filaments during contraction is regulated by the cycling cross-bridges. The behavior of the cycling cross-bridges has been investigated extensively in smooth muscle tissues (Hellstrand and Arner, 1980; Arner, 1982; Kamm and Stull, 1985). One common way to investigate the contractile behavior of smooth muscle is to perform quick-release experiments from which the shortening velocity of the muscle tissue for a certain afterload is obtained, which can be coupled to the behavior of the sliding filaments. The relationship between the shortening velocity and the afterload is described by a hyperbolic function, better known as Hill’s equation (cf. Woledge et al., 1985)

\[
(F + a)(v + b) = (F_0 + a)b,
\]

where \( F \) is the isotonic afterload, \( F_0 \) is the isometric force at which the quick-release is performed, \( v \) is the muscle shortening velocity and \( a, b \) are fitting parameters.
The measured muscle velocity $v$, which takes place at the tissue level, reflects somewhat the behavior of the filament sliding due to the cycling cross-bridges at the protein level which, in the present model, is described by the variable $\Pi_{fs}$. This theory has been supported by other work such as Guilford and Warshaw (1998). Based on this assumption, a similar hyperbolic function is used to describe the behavior of the relative filament sliding $\Pi_{fs}$, thus

$$ (P_a + x)(-\Pi_{fs} + \beta) = (P_c + x)\beta, $$

which can be rewritten as

$$ \Pi_{fs} = \beta \frac{P_c - P_a}{P_c + x}. $$

Therein, $F$ has been replaced by $P_a$, the active measurable stress which in an isotonic experiment is equivalent to the constant afterload, and $P_0$ has been replaced by a new driving stress $P_c$, which is related to the driving force from the attached cross-bridges. The fitting parameters $x$ and $\beta$ are equivalent to $a$ and $b$ in Hill’s equation. The velocity $\Pi_{fs}$ denotes the rate of the normalized relative filament sliding $\Pi_{fs}$. Note that the negative sign in front of $\Pi_{fs}$ is due to the fact that $\Pi_{fs}$ is defined as positive in extension.

Due to its hyperbolic form Hill’s equation is only valid for muscle shortening and does not predict the correct behavior for sudden muscle extension. To include the filament sliding behavior during sudden muscle extension, Eq. (18) can be extended to

$$ \Pi_{fs} = \beta \frac{P_c - P_a}{P_c + x} - \beta \frac{P_s - P_a}{P_s + x}, $$

where $P_{vec}$ is the maximal load-bearing capacity of the contractile fibers, i.e., the maximal stress carried by a CU at optimal length (yield stress), and $\beta_1$ and $\beta_2$ are fitting parameters. Due to the mathematical shape of the extended Hill’s equation, the first part is dominant for muscle shortening and the second part is dominant for muscle extension. If only muscle shortening is relevant, the extended part of the filament sliding behavior can be excluded.

The driving stress $P_c$ depends on the mechanical state (contraction/extension) of the smooth muscle. During muscle contraction, the driving stress, say $P_{vec}$, is quantified by the power-strokes of the cycling cross-bridges ($n_{AMP}$) and can be quantified as

$$ P_c = P_{vec} = n_{AMP} \cdot T_{fs} \cdot \eta_{AMP}, $$

where $\eta_{AMP}$ is a parameter related to the force of a power-stroke of a single cross-bridge and $T_{fs}(\Pi_{fs})n_{AMP}$ is related to the total number of cycling cross-bridges. Muscle contraction will only occur as long as $P_a < P_{vec}$, see Eq. (18).

During muscle extension all attached cross-bridges, both cycling (phosphorylated) and latch (dephosphorylated) are considered to carry load and the driving stress, say $P_{vec}$, is quantified as

$$ P_c = P_{vec} = n_{AMP} \cdot T_{fs} \cdot \eta_{AMP} + n_{AM} \cdot T_{fs} \cdot \eta_{AM}, $$

where $\eta_{AM}$ is related to the force-bearing capacity of a dephosphorylated cross-bridge during muscle extension and $T_{fs}(\Pi_{fs})n_{AM}$ is related to the total number of latch cross-bridges. Muscle extension will only occur as long as $P_a > P_{vec}$, see Eq. (18).

When $P_{vec} < P_a \leq P_{vec}$ no filament sliding will occur and

$$ P_c = P_a. $$

2.2. Isometric contraction experiments of pig carotid arteries

To estimate the material parameters in the proposed model, length-tension experiments were conducted on medial strips extracted from pig common carotid arteries. Common carotid arteries from pig were obtained from the local slaughterhouse and transported at 4°C in physiological Krebs–Ringer bicarbonate buffer with 2.5 mM CaCl$\text{$_2$}$ solution (PSS). The arteries were dissected and medial strips were cut out in the circumferential direction.

The muscle strips were tied with a silk thread at the one end to a steel rod and at the other end to a Grass FT03 force-transducer (Grass Medical Instruments, Quincy, MA, USA) attached to a micrometer screw. The muscle strips were held in a 25 ml open organ bath at 37°C in PSS oxygenated with 95% O$_2$ and 5% CO$_2$. The muscle strips were stretched to ~10% of their resting length and allowed to equilibrate for 30 min in PSS. To confirm that the muscle strips were alive and to precondition the strips, a high K$^+$ contraction was induced by adding 80 mM KCl and this was once repeated after 25 min. The protocol of the active length-tension experiment was first to relax the muscle strips in Ca$^{2+}$-free PSS for 10 min and then stretch the muscle strip to a specific length. Calcium was added to the solution bath (2.5 mM CaCl$_2$), and after 5 min the muscle strips were contracted by adding 80 mM KCl. The contraction was recorded for 5 min. The protocol was repeated for different muscle lengths. After a complete length-tension experiment, the muscle strips were allowed to relax in a Ca$^{2+}$-free PSS and EGTA (1 mM) for 10 min. An additional passive length-tension experiment was then performed on the muscle strips to confirm that the muscle strips were completely relaxed in the Ca$^{2+}$-free PSS during the active experiment. After each experiment, the length and the weight were measured for each muscle strip.

2.3. Statistics

All simulations were conducted with the mathematical software MATLAB. The model parameters were fitted using a least-square method.

3. Results

3.1. Active stress development for different initial stretches

The isometric force behavior at different stretches were determined on 15 strips obtained from 10 pigs. The first Piola–Kirchhoff stress $P_a$ was calculated from the isometric force data by measuring the weight and length from each muscle strip and by assuming a smooth muscle mass density of $\rho_{SM} = 1.050$ g/cm$^3$. The muscle stretch was obtained by normalizing the muscle strip lengths with their slack length. From these data, passive and active stress behaviors for a certain (normalized) muscle length were extracted. The length-tension behavior at 5 min after activation is presented in Fig. 4. The complete active responses for different (normalized) muscle lengths ($\lambda = 1–2$) from the time of activation up to 5 min are presented in Fig. 5.

3.2. Fitting the chemical part

The intracellular calcium transient used for the simulations was based on data taken from the literature (Rembold and Murphy, 1988), see Fig. 6(a). The myosin RLC$_{20}$ phosphorylation at steady-state was set to $n_{RLC}^{t-\infty} = 0.41$ (Rembold and Murphy, 1988, 1990), which together with Eq. (3) allowed the estimation of the MLCP activity to $K_{MLCP} = K_{MLCP}^{t-\infty} = 1.44$. By setting $h = 4$ and $D_{SO} = 3.7 \times 10^{-7}$ M (Rembold, 1990), the parameters $\eta$ and $K_{MLCP}$ were numerically estimated to $\eta = 21.55$ min$^{-1}$ and $K_{MLCP} = 9.76$ min$^{-1}$ by comparing $n_{MLCP}$ from Eq. (2) with the experimental myosin phosphorylation data (Rembold and Murphy, 1988, 1990), see Fig. 6(b).
The rate constants $k_3$, $k_4$ and $k_7$ in the four state model were set to $k_3 = 4.0 \text{ min}^{-1}$, $k_4 = 0.05 \text{ min}^{-1}$ and $k_7 = 0.002 \text{ min}^{-1}$. The rate constants are loosely based on values used in Hai and Murphy (1988a) but are adjusted to fit the present experimental data. The behavior of the fractions of phosphorylated, dephosphorylated, attached and detached cross-bridges for a specific intracellular calcium transient (see Fig. 6(a)) can be seen in Fig. 7. There are initially more phosphorylated attached cross-bridges (AMp) up to 1.5 min after activation, but then the process is dominated by dephosphorylated cross-bridges (AM).

3.3. Fitting the mechanical part

The parameters $x_0$ and $\pi^{opt}_{fs}$ in the filament overlap function $L_0(\pi_0)$ were estimated from the active length-tension experiment and from the optimal elastic elongation of the cross-bridges. From
the active length-tension experiment $\lambda_{\text{opt}} = 1.5$, $P_0 = 44.5$ kPa and $P_{\text{opt}} = 69.6$ kPa can be extracted from Fig. 4. The elastic elongation of the cross-bridges at optimal muscle length was set to $\pi_{\text{e, opt}} = 0.02$ (Arner, 1982). Through the description of the contractile unit (Eq. (5)) the normalized filament sliding at optimal muscle length $\pi_{\text{fs, opt}}$ was calculated as

$$\pi_{\text{fs, opt}} = \lambda_{\text{opt}} - \pi_{\text{e, opt}} = 0.48,$$

and together with Eq. (13), the value for $\pi_0$ was obtained as 0.8544.

The parameter $\mu_2$ was estimated to $\mu_2 = P_{\text{opt}}/[(\pi_{\text{fs, opt}}/2 + \pi_0)$ ($n_{\text{AMP}} + n_{\text{AM, latched}}$)] = 5.3 MPa, obtained from Eq. (14) at optimal muscle length $\lambda_{\text{opt}}$, then (23) and the normalized form of (9). The material parameters $\alpha$, $\beta_1$ and $\kappa_{\text{AM}}$ which describe muscle contraction were fitted to two experiments: (i) the active isometric stress development at optimal muscle length between 0 and 5 min after activation (see Fig. 8(a)), and (ii) the isotonic quick-release velocity data performed at 10 min after activation for different afterload $P_{\text{AL}}$ (the index AL stands for afterload), taken from the literature (Dillon et al., 1981), see Fig. 9(a). The values are $\alpha = 26.7$ kPa, $\beta_1 = 0.5$ min$^{-1}$ and $\kappa_{\text{AM}} =$ 204 kPa. It was required that the filament sliding $\Delta\pi_{\text{fs}}$ does not exceed the maximal elastic elongation of the cross-bridges $\pi_{\text{e, opt}}$ during isometric contraction when fitting the mechanical material parameters, see Fig. 8(b). For muscle extension, the load-bearing capacity of an active CU, say $P_{\text{LBC}}$, was set to $P_{\text{LBC}} = 118$ kPa, which is based on data from the literature (Dillon et al., 1981), and the material parameters $\beta_2$ and $\kappa_{\text{AM}}$ were fitted to isometric stress development at optimal muscle length exposed to length-controlled quick-stretches of $\Delta\lambda = 0.025\lambda_{\text{opt}}$ at 1.5 min after activation (Dillon et al., 1981), see Fig. 9(b). The values are $\beta_2 = 0.125$ min$^{-1}$ and $\kappa_{\text{AM}} = 61.1$ kPa. With the fitted parameters, we could predict additional muscle-extension simulations at times 0.75 and 3 min, see also Fig. 9(b). Subsequently, the force–velocity curve could be extended to simulate velocity for both muscle shortening and extension at different
afterloads, see Fig. 9(a). Unfortunately no experiments of muscle extension velocity conducted on pig carotid media was found in the literature to compare the predictions. However, sudden muscle extension velocity performed on rat portal vein (Hellstrand and Arner, 1980) shows a similar behavior as in our simulation.

The material parameters $\mu_p$, $C_1$, and $C_2$ describing the passive (parallel) element were fitted to the passive length-tension experiments conducted in the present work, see Fig. 4, i.e., $\mu_p = 0.84$ kPa, $C_1 = 3.15$ kPa and $C_2 = 0.035$.

The proposed model managed to predict the length-tension experiment, see Fig. 4, with the fitted model parameters, as

---

**Table 1**

<table>
<thead>
<tr>
<th>Chemical model</th>
<th>$\eta$ (min$^{-1}$)</th>
<th>$h$ (-)</th>
<th>ED$_{50}$ (mM)</th>
<th>$k_{MLC}$ (min$^{-1}$)</th>
<th>$k_1$ (min$^{-1}$)</th>
<th>$k_4$ (min$^{-1}$)</th>
<th>$k_7$ (min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21.55</td>
<td>4</td>
<td>0.37 (Rembold, 1990)</td>
<td>9.76</td>
<td>4</td>
<td>0.05</td>
<td>0.002</td>
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</tbody>
</table>

<table>
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<tr>
<th>Mechanical model—active</th>
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</thead>
<tbody>
<tr>
<td>$\mu_a$ (MPa)</td>
</tr>
<tr>
<td>5.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Filament overlap function</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_0$ (kPa)</td>
</tr>
<tr>
<td>44.5</td>
</tr>
</tbody>
</table>

---
summarized in Table 1. The model simulations of the complete active isometric stress development for different isometric muscle lengths (see Fig. 5) are in very good agreement with the average of our experimental results.

3.4. Validation of model parameter \( \mu_a \)

The definition of the active mechanical parameters, as summarized in Table 1, are partly based on experimental observation, but the estimated values can be difficult to validate. The parameter \( \mu_a = \frac{L_{CU} E_{eb} N_{CU}}{2 \delta} \), e.g., can be investigated. The stiffness \( E_{eb} \) of a single cross-bridge has been estimated on rabbit skeletal actomyosin to \( E_{eb} = 1.75 \times 10^{-3} \text{N m}^{-1} \) (Offer and Ranatunga, 2010), and the spacing \( \delta \) between the actomyosin binding sites has been reported as \( \delta = 36 \times 10^{-9} \text{ m} \) (Steffen et al., 2001). The length \( L_{CU} \) of a CU is in the same range as a smooth muscle myosin filament approximated to \( L_{CU} = 2.2 \times 10^{-6} \text{ m} \) (Hai and Murphy, 1988b). Together with the fitted values of \( \mu_a \), the density of CUs in smooth muscle tissue can be estimated to \( N_{CU} = 0.5581 \times 10^9 \text{ number of contractile units per mm}^2 \). Observations through electron microscopy have indicated a myosin filament density of \( 10^7 \text{ filaments per mm}^2 \), which is \( 10^6 \text{ filaments per mm}^2 \) (Gillis et al., 1988), a good agreement with our estimate. Note that there can be a significant level of error on the measurement of the cross-bridge stiffness \( E_{eb} \) due to the compliance of the thin and thick filaments and the fraction of attached cross-bridges at the moment of measurements (cf. Offer and Ranatunga, 2010).

3.5. Muscle shortening at different times and for different length changes

Through the proposed model the effects of the filament overlap, filament sliding and phosphorylated/denphosphorylated cross-bridges can be studied. In this section, the contractile behaviors for two different scenarios are studied: length-controlled muscle shortening at different times after activation, and for different muscle length changes.

3.5.1. Muscle shortening at different times after activation

The simulation of the isometric contraction was performed through the same intracellular calcium transient as used for the parameter fitting in Fig. 6(a), and at \( \lambda = \lambda_{opt} = 1.5 \). The active stress behavior for a length-controlled sudden muscle change of \( \Delta \lambda = -0.005 \) was studied at two different times, 1 min and 4 min, see Fig. 10(a). These times were chosen due to the (high)

3.5.2. Effects of filament overlap due to significant muscle shortening

Also here the simulation of the isometric contraction was performed through the same calcium transient as used for the parameter fitting in Fig. 6(a), but at \( \lambda = 2.0 \). The active stress behavior was simulated for a length-controlled muscle change of \( \Delta \lambda = -0.5 \) at 4 min after activation, see Fig. 10(b). The steady-state value of the active isometric stress simulation reached a higher value after the quick-release than before the release. The muscle was initially activated at a stretch \( \lambda = 2 \), which is larger than the optimal stretch resulting in a low filament overlap and in a shortening to the optimal length, where maximal filament overlap was obtained. The model simulation indicated that the rise of active stress behavior after the quick-release can be explained through structural changes in the CU and not by changes in \([\text{Ca}^{2+}]_i\). A similar active stress behavior has been seen in experiments performed on pig carotid artery (Rembold and Murphy, 1990) and shown through measurements of \([\text{Ca}^{2+}]_i\) that the rise in active steady-state stress was not due to any changes in calcium. Due to the significant length change, we assumed that the attached cross-bridges \((n_{AMP}, n_{AM})\) switched to their unattached state \((n_{AMP}, n_{AM})\) instantly after the quick-release.

4. Discussion

The proposed coupled model is an extended version of a model documented in Murtada et al. (2010a). It is not only able to predict the behavior of isometric contraction but also isotonic quick-releases, sudden extensions and length-tension relationships. The model is also able to simulate muscle relaxation, which, to the authors’ knowledge, has not been possible with any of the previous muscle models documented in the literature. The chemical part of the mechanochemical model is characterized by the calcium-phosphorylation kinetics, which couples intracellular

![Fig. 10](image-url)
calcium $[\text{Ca}^{2+}]$, to myosin phosphorylation, and finally to load-carrying cross-bridges. The mechanical part considers the filament overlap and a realistic cross-bridge evolution law which is based on the filament sliding behavior. The material parameters in the mechanochemical model are fitted to experimental data generated in the present work and to data documented in the literature.

To obtain well defined mechanical data we performed new length-tension experiments. The experiments were conducted with medial layers, extracted from pig common carotid arteries. Isometric contraction was initiated with 80 mM KCl in 2.5 mM CaCl$_2$ PSS. However, the experimental data of pig common carotid tissues taken from Rembold and Murphy (1988) were performed in 1.6 mM CaCl$_2$ PSS, and activated at 109 mM KCl. Due to the difference in activation and extracellular calcium, the intracellular calcium and myosin phosphorylation may be different for the two experiments. Both experiments, however, used high K$^+$ depolarization in a high concentration of CaCl$_2$ (compare with Kamm et al., 1989, Fig. 3 therein), thus the $[\text{Ca}^{2+}]$ transients in both experiments were considered to be within the same range.

There are not that much data available on smooth muscle depolymerization obtained from carotid arteries, where both [Ca$^{2+}$]$_j$ and myosin phosphorylation are measured. The same intracellular calcium transient [Ca$^{2+}$]$_j$ was used for all isometric contraction simulations, but there are some indications that the [Ca$^{2+}$]$_j$ may be dependent on the muscle length (Rembold and Murphy, 1990), which would effect the myosin phosphorylation and the active contractile force. A muscle length dependence of the myosin phosphorylation has been identified on skinned pig carotid arteries (Hai, 1991), and this needs to be considered. A length-dependent modulation of the chemical rate constants would contribute to a more realistic coupled model in which the muscle length would influence both the mechanical and the chemical parameters. However, to do so, a detailed study of myosin phosphorylation at different lengths would be necessary.

The latch-state model by Hai and Murphy (1988a) used in the present work only considers thick filament-regulated (myosin phosphorylation/dephosphorylation) contraction. However, Hai and Kim (2005) extended the latch-state model by including thin-filament-based regulatory mechanisms, which could also be implemented in a straightforward way in the present model; that would, however, increase the number of rate constants.

We have introduced a novel approach to consider the filament overlap through a parabolic function which is fitted to experimental data. It is a straightforward approach but the parameters in the overlap function do not have a physical meaning. The myosin and actin filaments in the smooth muscle have varying length, and by implementing the statistical distribution of the filament lengths, a structural homogeneous function of the filament overlap function could be obtained which could replace the current parabolic overlap function. It has also been observed that the smooth muscle contractile units have a statistical orientation distribution (Walsmsley and Murphy, 1987), which has shown to have an effect on the optimal muscle length besides the filament overlap (Murtada et al., 2010b). The myosin filaments are considered to be stable and they are assumed that they do not depolymerize. However, some studies indicate that myosin filaments are unstable and that they may change length and or series/parallel arrangement as a function of muscle length and stimulation conditions (Ford et al., 1994; Smolensky et al., 2005), which would suggest that smooth muscle may have other length-dependent properties, other than the filament overlap.

The evolution law of the filament sliding $P_{i}^s$ is described through the novel Eq. (19), which is an extended version of Hill's equation and it is able to predict a realistic behavior of muscle shortening and extension. The original Hill's equation describes the force–velocity relationship for muscle shortening but since smooth muscle is often subjected to extension it is important that the filament sliding relationship also manages a realistic force–velocity relationship for muscle extension (cf. Hanks and Stephens, 1981). In the original work of the mechanical model (Murtada et al., 2010a), the filament sliding behavior was described through a linear function $(P_{i}^s = \eta (P_2 - P_1))$ and was not able to predict the correct hyperbolic force–velocity relationship.

The present filament sliding law has one additional material parameter ($P_{i}^r$) which is related to the maximal stress carried by active smooth muscle. In Dillon et al. (1981), $P_{i}^r$ was reported to be around 1.6 times the maximal active isometric stress but other works have reported a higher value (Singer et al., 1986).

Isotonic experiments are an efficient approach to find vital information about the contractile mechanisms in smooth muscles. From an isotonic experiment the muscle length behavior for a certain afterload can be studied. Information such as shortening velocity at a certain time and elastic recoil can be extracted from the muscle length behavior.

The elastic recoil is related to the elastic stretch of the cross-bridges. When smooth muscle is isometrically contracted at optimal muscle length, the attached cross-bridges are stretched to the optimal elastic elongation. The cross-bridges have a different elastic elongation depending on the isotonic stretch. Here it should also be reminded that the passive (parallel) element is also stretched at this moment. When the muscle is quick-released in an isotonic manner with zero afterload, the muscle tissue will recoil elastically to a shorter length due to the passive element and the elastic cross-bridges. If the attached cross-bridges would stay attached during the complete elastic recoil, then the elastic recoil could be explained in two parts: the first part is the recoil of the elastic elongation of the cross-bridges during that both the elastic cross-bridges and the passive element want to shorten to their reference length; the second part of the elastic recoil is the stretching of the attached cross-bridges due to the passive element. During this part the passive element wants to shorten while the attached cross-bridges would get elongated again. This suggests that the total measured elastic recoil would be larger than the elastic elongation of the cross-bridges obtained during isometric contraction.

The actin filaments in the smooth muscle CU are attached to dense bodies which act as binding sites for actin filaments, and similar to $Z$-discs found in skeletal muscle. The actin filaments have polarity and are anchored to the dense bodies with a certain S1 region) of the myosin motor is pulling, hence in which direction the filament sliding does occur. A consequence to the polarity-dependent sliding is that a myosin motor would not be able to continue to work in a similar manner once it has slide passed a dense body. Considering that myosin heads from the same myosin filament located on different sides of a dense body do not work against each other, the efficient filament overlap could be estimated as the overlap between the myosin and the actin filaments located at one side of the dense body.

The outcome from the mechanical model is sensitive to the fraction of phosphorylated and dephosphorylated attached cross-bridges which is obtained from the chemical model. Thus, the chosen chemical model is of high importance and will influence the mechanical contraction. This is an important factor when studying different types of smooth muscles (e.g., artery wall, urinary bladder, airway smooth muscle, etc.). It is important to emphasize that the proposed coupled framework can be applied to both phasic and tonic smooth muscles; hence the same form of equations (but with a different set of parameters) could be used to model different smooth muscles. In order to have a realistic chemical model describing the coupling between $[\text{Ca}^{2+}]$ and the...
fraction of attached cross-bridges, experimental data are extremely valuable. Additional measurements of $[\text{Ca}^{2+}]$ through fura-2 and more extensive myosin phosphorylation data would provide a better basis for developing realistic and accurate chemical models, which together with a realistic mechanical model constitutes a robust framework for better understanding the active behavior of smooth muscle tissue.

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References


