BIOPHYSICAL MODEL OF A MUSCLE FATIGUE PROCESS INVOLVING Ca^{2+} RELEASE DYNAMICS UPON THE HIGH FREQUENCY ELECTRICAL STIMULATION

Piotr Kaczmarek
Poznań Univeristy of Technology, Insitute of Control and Information Engeenering, Piotrowo 3a, 60-395 Poznań, Poland

Keywords: Electrical stimulation, Muscle model, Calcium release, Muscle fatigue.

Abstract: The aim of this study is to create a model which enables to explain the muscle fibre contraction due to various stimulation programs. The model accounts for Ca^{2+} release dynamics both as a result of an action potential and of a stimulus shape, duration and frequency. It has been assumed that the stimulus can directly activate the voltage-dependent receptors (dihydropiridine receptors) responsible for a Ca^{2+} release. The stimulation programs consisted of standard stimulation trains made of low and middle frequency square pulses. High frequency modulating harmonic signals have been tested to investigate the fibre fatigue effect. It has been observed that fatigue effect factors depend on the selected stimulation program. The results reveal that the fatigue effect could be minimized by changing the shape and frequency of the stimulation waveform. Such the model could be useful for a preliminary selection and optimization of the stimulus shape and the stimulation trains, thus reducing the number of in vivo experiments.

1 INTRODUCTION

Electrical stimulation is a rehabilitation technique applied to increase muscles force, reduce spasticity, muscular atrophy and to decrease pain effects. It is also used to restitute a motion in handycaped subjects via Functional Electrostimulation (FES). In order to get an efficient FES system, the optimal stimulation programs have to be worked out. The former investigations revealed that muscle fatigue effect is greater as a result of electrical stimulation than as a result of a voluntary contraction (Kostyukov et al., 2000; Gissel, 2000). It has been reported that stimuli train frequency and a single pulse shape have the significant impact on the fatigue effect (Bennie et al., 2002). Therefore, the optimization of the stimulation programs is one of the most important aspects of the FES method. As far, the optimization has been limited to the identification of the optimal frequency of a stimulation pattern (Ding et al., 2003; Chou et al., 2005) or to a search for variable frequency pulse trains. (Mourselas and Granat, 1998).

The studies on the high frequency stimulation programs (>200Hz) as well as on the single pulse shapes as related to the muscle fatigue effect are missing.

The dynamics of Ca^{2+}ions transportation plays an important role in the muscle contraction process (Bottinelli and Reggiani, 2000; Benders et al., 1997; Delbono and Meissner, 1996). The change of the Ca^{2+}release rate is an important factor of the fatigue effect (Westerblad et al., 2000; Gissel, 2000). Therefore a majority of models reflecting potentiation and fatigue effects have been based on the Ca^{2+}dynamics. (Otazu et al., 2001; Ding et al., 2003; Riener and Quintern, 1997). In these models the impact of the stimulus shape as well as of the pulse width on the fatigue effect were not addressed. It is only assumed there that a single stimulus evokes an action potential (AP) in the muscle fibre, which activates a voltage-dependent dihydropiridine receptor (DHPR) resulting in Ca^{2+}release from the sarcoplasmic reticulum (SR). The amount and the release profile of the liberated Ca^{2+}ions are assumed to be constant, even though the physiological variability of the AP amplitude and shape (in the t-tubular system) is observed.

The in vivo experiments demonstrated that the stimulus amplitude and duration affect the calcium concentration ($[Ca^{2+}]$)(Delbono and Meissner, 1996;

Kaczmarek P. (2008).
BIOPHYSICAL MODEL OF A MUSCLE FATIGUE PROCESS INVOLVING Ca2+ RELEASE DYNAMICS UPON THE HIGH FREQUENCY ELECTRICAL STIMULATION.
In *Proceedings of the First International Conference on Bio-inspired Systems and Signal Processing*, pages 52-57
DOI: 10.5220/0001062300520057

Bakker et al., 1996; Benders et al., 1997). However, the direct influence of a neuro-muscular electrical stimulation (NMES) on the DHPR receptor behaviour was ignored in the models. Therefore the applicability of these models for testing stimulation trains composed of wider pulses is dubious and the trains frequency should be restricted to the maximal physiological frequency of the AP generation ($f_{stim} < 100$Hz).

The aim of this work is to analyse the influence of the stimulation parameters on the muscle contraction and fatigue effect. We present a novel model of a muscle fibre. The model is an extension of already known models, by introducing the direct interaction between the stimulus and the DHPR receptor activity as well as by incorporating the calcium release dynamics. These adds-on enable to study the muscle fatigue effect during various stimulation programs. In particular we analyse the influence of a train frequency and a single pulse-duration on the dynamics of calcium concentration and on the fatigue effect.

2 PHYSIOLOGICAL BACKGROUND

2.1 Excitation-Contraction Coupling

Depolarization of sarcolemma due to the physiological action potential (AP) or to stimulation, activates a sarcoplasmic reticulum (SR) Ca^{2+}release. The voltage signal is transformed into the Ca^{2+} release via a voltage-sensitive dihydropiridine receptor (DHPR), which activates some of the Ca^{2+}channels (ryanoidine receptor - RyR) in SR. This process is called Dihydripiridine-Induced Calcium Release (DICR). The amount of the activated RyRs is dependent on the stimulus intensity and the muscle fibre type. The number of RyR coupled with DHPR depends strongly on a fibre type, and is the largest for the slow fibres (Delbono and Meissner, 1996; Benders et al., 1997).

The uncoupled RyRs are activated as a result of the sarcoplasmic $[Ca^{2+}]$ increase. This effect, called Calcium-Induced Calcium Release (CICR), generates a positive feedback in the Ca^{2+}liberation process. Ca^{2+}ions are transported by a Ca^{2+}-ATPase pump from cytosol into SR. The pump efficiency is dependent on the $[Ca^{2+}]$ in the sarcoplasm. At the resting state the Ca-ATPase pump maintains the Ca^{2+}ions concentration about 10^4 higher in SR than in cytosol (Bottinelli and Reggiani, 2000).

Ca^{2+}diffuses in cytosol from the proximity of SR surface to the interior of the myofibrils, where a troponin (TN) is localized. TN is a part of a thin filament proteins. Whenever TN binds to Ca^{2+}, actin (the part of thin filaments) and myosin (the part of thick filaments) are able to interfere resulting in the myofibril contraction. In the sarcoplasmic space the Ca^{2+}can be buffered also by parvalbumin (PARV). The CaTN and CaPARV buffers decrease the concentration of free Ca^{2+}ions in cytosol.

2.2 Fatigue Effect

There is an experimental evidence that the muscles are subject to the faster fatigue under the electrical stimulation than during the voluntary contraction. Moreover, the stimulation of muscles having majority of the fast-type fibres induces stronger fatigue effect than with the slow-type muscles (Delbono and Meissner, 1996; Gissel, 2000).

The following reasons of the muscle fatigue are reported:

1. RyR receptor has an inactivating binding site for Ca^{2+}(Glukhovski et al., 1998) resulting in the inhibition of CICR during long-lasting stimulation as well as in response to APs.
2. The AP amplitude and shape changes in the t-tubular system under long-lasting AP (Wallinga et al., 1999; Bakker et al., 1996).
3. The Ca^{2+}liberation is inhibited due to the increase of Mg^{2+} concentration and decrease of [ATP] (Westerblad et al., 2000).
4. Calcium-phosphate precipitation in the SR (Westerblad et al., 2000)
5. Structural degeneration of the muscle fibres as a result of the eccentric, low frequency contraction (Westerblad et al., 2000).

In this paper only the two first factors will be discussed.

3 PROCESS MODEL

The proposed muscle fibre model is based on the model of Otazu et al. (Otazu et al., 2001), originally applied to study a potentiation and a catch-like effects in muscle fibres. It consisted of two blocks: the activation dynamics block (AD) and the contraction dynamics block (CD). The input to the AD subsystem is a potential of the sarcolemma activating the voltage-dependent DHPR receptors. In the original model it has been assumed that the muscle contraction is evoked only by APs. Each AP generates the

same membrane potential profile and thus the amplitude and dynamics of DCICR is kept constant during simulation.

The model proposed here accounts for the depolarization of the sarcolemma under direct influence of the stimulation pulses. Thereby it takes into account the fact that DICR profile and amplitude depend on the stimulus shape, amplitude and train frequency. Such a model let to study the muscle fibre behaviour under a high-frequency or a wide-pulse stimulation, when APs are not generated. Such the model could enable the preliminary optimization and selection of the stimuli and the stimulation trains reducing the number of *in vivo* experiments.

The model of a voltage activated channel reflects some properties of the DHPR receptor recorded *in vivo* during the stimulation with a high amplitude and the long lasting depolarization pulses (Delbono and Meissner, 1996; Bakker et al., 1996). The AD block produces the concentration of the TN bounded to the Ca^{2+}ions ([CaTN]).

3.1 Activation Dynamics

In this section the description of the myofibril model has been limited only to the aspects necessary for the analysis of stimulation effects. The full model with parameters values have been presented by Otazu *et al.* (Otazu et al., 2001).

The intracellular Ca^{2+}concentration is described by the stoichiometric equation:

$$\frac{d[Ca^{2+}]_{PROX}}{dt} = \gamma_{DICR} + \gamma_{CICR} + \gamma_{LEAK} - \gamma_{PUMP} - \frac{[Ca^{2+}]_{PROX} - [Ca^{2+}]_{DIST}}{\tau_{PROX}}, \quad (1)$$

where: γ_{DICR} is the rate of Ca^{2+}liberation process elicited by the voltage-dependent DHPR receptor (see section 3.2), γ_{CICR} is a rate of the Ca^{2+}release from SR through uncoupled-RyR, γ_{LEAK} denotes a constant Ca^{2+}efflux leakage, while γ_{PUMP} is a Ca-ATPase pump rate. $[Ca^{2+}]_{PROX}$ denotes a Ca^{2+}concentration nearby SR surface, while $[Ca^{2+}]_{DIST}$ is a Ca^{2+}concentration in the interior of the myofibrillar space, τ_{PROX} denotes a time constant of a diffusion process.

Previous results (Glukhovski et al., 1998) revealed that the RyR channel has two calcium binding sites: the first one for couple Ca^{2+} ions (activating site) and the second one for a single Ca^{2+} ion (inactivating site). The Ca^{2+} release rate is described by the probability of binding of two Ca^{2+}ions to the activation site (a) and the probability that the inactivation site is bound to a single Ca^{2+}molecule (i).

$$\gamma_{Ca} = f_{Ca}(1-i)a \quad (2)$$

$$\frac{da}{dt} = \alpha_a(1-a)[Ca^{2+}]^2 - \beta_a a \quad (3)$$

$$\frac{di}{dt} = \alpha_i(1-i)[Ca^{2+}] - \beta_i i \quad (4)$$

where f_{Ca} denotes the maximum rate of Ca^{2+} release through the uncoupled-RyR. The probability of binding of Ca^{2+} ion to the activation or inactivation site is represented by a coefficient α and depends on $[Ca^{2+}]$. A durability of the bond is characterized by β.

3.2 Voltage Activated Channel

It is difficult to evaluate unambiguously a relationship between the sarcolemma potential and the Ca^{2+} liberation rate (via the coupled RyRs) based on the recent experimental evidence, because the CICR effect is strictly dependent on the DICR effect. The interaction between the DICR and the CICR results in a complex dynamical system, therefore the decomposition of these two effects is difficult (Bakker et al., 1996; Delbono and Meissner, 1996). For the sake of simplicity, it is assumed that DICR release rate is proportional to the depolarization potential. Model of the RyR coupled with DHPR receptor reflects a voltage-dependent factor generating a slow decline in the Ca^{2+}release rate as an effect of the long-lasting depolarization (Delbono and Meissner, 1996). Moreover, the threshold depolarization potential (V_{th}), which reflects DHPR excitability, is taken into consideration (Delbono and Meissner, 1996; Bakker et al., 1996).

$$\gamma_{DICR} = g_{DHPR}(1-i_V)(V_m - E_{rest}) \quad (5)$$

$$\frac{di_V}{dt} = \alpha_V(1-i_V)(V_m - E_{rest}) - \beta_V i_V \quad (6)$$

where V_m is the sarcolemma potential, E_{rest} denotes a resting potential of the sarcolemma, g_{DHPR} denotes a proportional coefficient, i is related to the voltage-dependent DICR decline.

The parameters in eq. (5) and (6) were estimated based on *in vivo* results available for a soleus muscle (Delbono and Meissner, 1996), under the assumption that the refractory period of the DHPR is similar to a refractory period of sarcolemma (8ms). The value of g_{DHPR} was calculated assuming that AP (which amplitude reaches 20mV (Wallinga et al., 1999; Bakker et al., 1996)) generates the Ca^{2+}release according to Otazu et al. (Otazu et al., 2001). The V_{th} is calculated from the Voltage dependent of SR Ca^{2+}release results and the coefficients α_V and β_V were estimated by using least square method and digitalized results of the time dependence Ca^{2+}release. The obtained estimates are presented in tab. 1

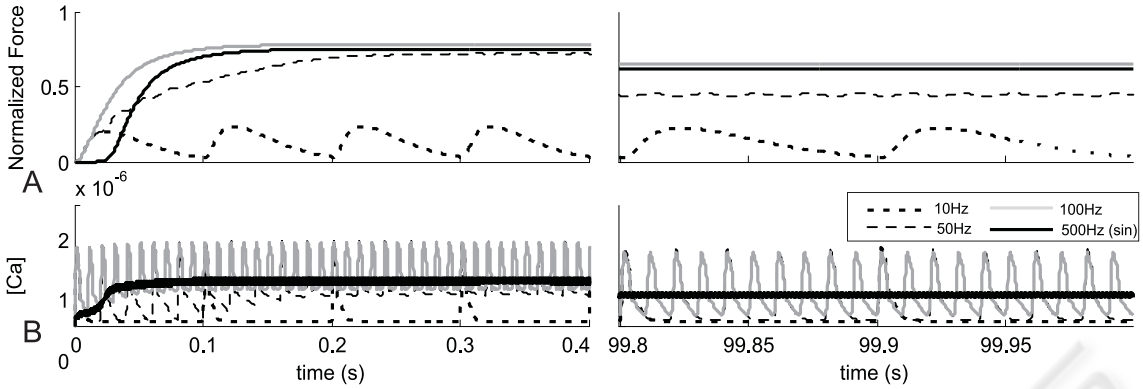

Figure 1: The contraction profiles (A) and $[Ca^{2+}]$ concentration (B) recorded at the beginning and at the end of 100s stimulation period for 10,50,100Hz trains of square-wave and modulated harmonically pulses (500Hz).

Table 1: Parameters of the voltage activated channel.

g_{DHPR} $M(mV\cdot s)^{-1}$	α_V $(mV\cdot s)^{-1}$	β_V s^{-1}	E_{rest} mV	V_{th} mV
$1.0e-3$	1.29	125	-80	-50

3.3 Contraction Dynamics

The input to the block modelling the contraction dynamics is a concentration of TN bound to Ca^{2+}([CaTN]) (Otazu et al., 2001). The contraction dynamics is described by a linear second-order element connected with two nonlinear elements: a threshold-type (connected to the input) and a Hill-type saturation (connected to the output). Such the behavioural model, accounts for the following physiological observations: the threshold level of the [CaTN] above which the contraction occurs, and the saturation of the [CaTN]-Force curve (Bottinelli and Reggiani, 2000).

4 SIMULATIONS

4.1 Comparison of Two Modes of Stimulation

In our experiment the fatigue effect was studied during stimulation of the myofibril model lasting 100s. The standard stimulation with short stimuli (0.1ms) and frequency in the range of (2÷100)Hz was used. Each pulse was assumed to trigger an AP. Moreover, the persistent stimulation by (250÷1500)Hz sinusoidal trains was investigated. It was assumed that during transcutaneous NMES, the muscle fibre was depolarized by both positive and negative half-periods. The pulse polarity has a little influence on muscle activation as compared to the pulse amplitude. The intensity magnitude must be above the DHPR threshold (V_{th}) (Green and Laycock, 1990). The stimulation amplitude was selected in order to obtain myofibril contraction at the level observed with a traditional stimulation at the range (50Hz÷100)Hz. It has been assumed that the persistent stimulation inhibits the generation of APs (as in a TENS effect)(Bakker et al., 1996).

4.2 Evaluation of Fatigue Effect as Related to the Pulse-width

The influence of the depolarization on the fatigue effect was investigated in the following experiment. First the square stimulation pulses at the frequency 10, 30 and 50Hz with varying width in the range of 4÷20ms have been applied. Then, the modulation of the corresponding stimulation pulses with the harmonic 500Hz signal were applied with respect to 30Hz stimulation sequence. In both cases the generation of AP at the beginning of each stimulation period (30Hz) was enabled. The aim of this study was to determine whether the pulse-width or the pulse modulation can reduce the fatigue effect.

In our paper, the fatigue effect is characterized by two parameters: the relative force decrease (RFD) and the relative Ca^{2+}concentration decrease (RCD). These parameters are defined as:

$$RFD = \frac{F_{max}-F_{min}}{F_{max}}\cdot 100\% \quad (7)$$

$$RCD = \frac{[Ca^{2+}]_{max}-[Ca^{2+}]_{min}}{[Ca^{2+}]_{max}}\cdot 100\% \quad (8)$$

where F_{max} denote maximal force and $[Ca^{2+}]_{max}$ is a maxiumum calcium concentration, while F_{min} and

$[Ca^{2+}]_{min}$ are maximal a force and a calcium concentration, respectively at the end of stimulation experiment lasting 100s.

5 RESULTS AND CONCLUSIONS

5.1 Frequencial Effects

The fatigue effect under the traditional square-wave stimulation (1÷100)Hz is similar to the results of *in vivo* experiments (Westerblad et al., 2000; Chou et al., 2005). The relative force decrease (RFD) is greater for sub-tetanic (50Hz) contractions than for the fused tetani (100Hz) stimulation (fig. 1A and, 2A). However, this result does not reflect the change in Ca^{2+}concentration. The relative $[Ca^{2+}]$ decrease (RCD) is greater for the 100Hz than for the 50Hz stimulation (fig. 2B). The muscle stimulated with 100Hz pulses is more fatigue-resistant due to the non-linear relationship between the [CaTN] and the contraction force. The saturation of this function ensures that during fused contractions, the force changes are small even if the calcium concentration changes are significant (Westerblad et al., 2000). In the case of unfused contractions (1-30Hz) the rise of the stimulation frequency increases the fatigue effect (RFD) and RCD as well (fig. 2). However the RFD and the RCD values are lower in that case than during sub-tetani contractions (50Hz). In each case, the calcium concentration decrease is due to the inhibition of uncoupled-RyR (see eq. 4). The inhibition level depends on mean as well as on maximal calcium concentration. This can be observed in the frequency-RCD relation (fig. 2). Moreover such a significant force decrease in the case of sub-tetani contraction (50Hz) is due to the decay of the potentiation effect (Otazu et al., 2001). The results obtained with the harmonic high-frequency stimulation (HFS) reveal that the observed RFD is similar as for the 100Hz traditional stimulation (fig. 1A) and slightly depends on the pulse base-frequency (fig. 2A). However the RCD value is two times larger here than in the case of the traditional stimulation (fig. 2B). The calcium concentration decrease cannot be explained here as a result of uncoupled-RyR inhibition, because the maximal $[Ca^{2+}]$ level is significantly lower than during the traditional stimulation (fig. 1B), so the inhibition level must be lower as well. Therefore the main factor resulting in RCD increase must be the coupled RyRs habituation (eq. 6).

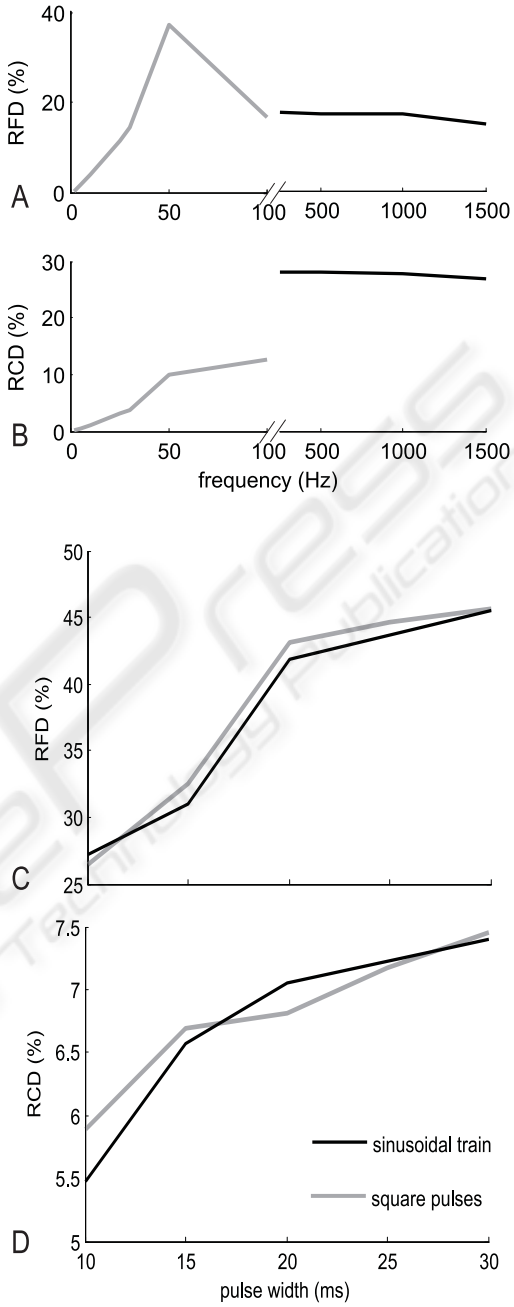

Figure 2: A relative force decrease (RFD) (A,C) and Ca^{2+}concentration decrease (RCD) (B,D) as a function of the stimulation frequency (A,B) and the pulse-width (C,D).

5.2 Pulse width Effect

The analysis of the pulse width influence on the muscle fatigue does not reveal any significant differences between the square pulses and the modulated sinusoidal stimulation (fig. 2B,C). However the sinus-modulated trains seem to be slightly better. Fatigue effect increases here as the pulse width grows, how-

ever for short pulses (10-15ms) it is significantly lower then for the traditional stimulation at 50Hz (fig. 2). In case of the modulated HFS, the RCD is over five times lower in comparison to the results of the harmonic persistent stimulation. This observation can be explained on the basis of the DICR model, because the modulated sinusoidal stimulation ensures the refractory period for the DHPR receptor.

5.3 Discussion

Presented myofibril model reflects effects of Ca^{2+} release from SR as a result of sarcolemma depolarization. It does not take into consideration the properties of the sarcolemma and other tissues which are stimulated during NMES. Thereby, the effect of direct influence of a transcutaneous stimulus on DHPR receptor can not be clearly established. It could be explained only on the basis of *in vivo* experiment results and on a muscle model reflecting myofibril properties, muscle fibres recruitation during stimulation and electrical properties of the skin and other tissues combined.

Modulated HFS trains seem to do better than the traditional stimulation programs, however the influence of such a stimulation on the fibre degeneration process should be investigated. Although the amplitude of repolarization pulses during HFS stimulation are 50% lower as compared to the short-pulses stimulation, the mean stimulation current is significantly higher (Bennie et al., 2002). In comparison with the wide-pulse stimulation the modulated HFS seems to be less painful due to the lower tissue impedance at a higher frequency. It should be mentioned that the presented model and results can be useful to evaluate stimulation programs under the hypothesis that the transcutaneus stimulation can trigger the DICR effect.

ACKNOWLEDGEMENTS

The work was partially supported by the Polish Ministry of Education and Science, project no. 1445/T11/2004/27

REFERENCES

Bakker, A. J., Head, S. I., and Stephenson, D. G. (1996). Measurement of membrane potential and myoplasmic [ca2+] in developing rat myotubes at rest and in response to stimulation. *Cell Calcium*, 19(5):409 – 418.

Benders, A. A., Oosterhof, A., Wevers, R. A., and Veerkamp, J. H. (1997). Excitation-contraction coupling of cultured human skeletal muscle cells and the relation between basal cytosolic ca2+ and excitability. *Cell Calcium*, 21(1):81 – 91.

Bennie, S. D., Petrofsky, J. S., Nisperos, J., Tsurudome, M., and Laymon, M. (2002). Toward the optimal waveform for electrical stimulation of human muscle. *Eur J Appl Physiol*, 88(1-2):13 – 19.

Bottinelli, R. and Reggiani, C. (2000). Human skeletal muscle fibres: molecular and functional diversity. *Progress in Biophysics & Molecular Biology*, 73:195 – 262.

Chou, L.-W., Ding, J., Wexler, A. S., and Binder-Macleod, S. A. (2005). Predicting optimal electrical stimulation for repetitive human muscle activation. *J Electromyography Kinesiology*, 15:300–309.

Delbono, O. and Meissner, G. (1996). Sarcoplasmic reticulum ca2+ release in rat slow- and fast-twitch muscles. *J. Membr. Biol.*, 151(2):123 – 130.

Ding, J., Wexler, A. S., and Binder-Macleod, S. A. (2003). A mathematical model for fatigue minimization during functional electrical stimulation. *Journal of Electromyography and Kinesiology*, 13:575–588.

Gissel, H. (2000). Ca2+ accumulation and cell damage in skeletal muscle during low frequency stimulation. *Eur J Appl Physiol*, 83(2-3):175 – 180.

Glukhovski, A., Adam, D., Amitzur, G., and Sideman, S. (1998). Mechanism of ca2+ release from the sarcoplasmic reticulum: a computer model. *Ann Biomed Eng*, 26:213–229.

Green, R. and Laycock, J. (1990). Objective methods for evaluation interferential therapy in the treatment of incontinence. *IEEE Trans Biomed Eng*, 37(6):615–623.

Kostyukov, A. I., Hellstrom, F., Korchak, O. E., Radovanovic, S., Ljubisavljevic, M., Windhorst, U., and Johansson, H. (2000). Fatigue effects in the cat gastrocnemius during frequency-modulated efferent stimulation. *Neuroscience*, 97(4):789 – 799.

Mourselas, N. and Granat, M. H. (1998). Evaluation of patterned stimulation for use in surface electrical stimulation systems. *Medical Engineering and Physics*, 20:319–324.

Otazu, G. H., Futami, R., and Hoshimiya, N. (2001). A muscle activation model of variable stimulation frequency response and stimulation history, based on positive feedback in calcium dynamics. *Biol Cybern*, 84(3):193 – 206.

Riener, R. and Quintern, J. (1997). A physiologically based model of muscle activation verified by electrical stimulation. *Biochemistry and Bioenergetics*, 43:257–264.

Wallinga, W., Meijer, S. L., Alberink, M. J., Vliek, M., Wienk, E. D., and Ypey, D. L. (1999). Modelling action potentials and membrane currents of mammalian skeletal muscle fibres in coherence with potassium concentration changes in the t-tubular system. *Eur Biophys J*, 28(4):317 – 329.

Westerblad, H., Bruton, J. D., Allen, D. G., and Lannergren, J. (2000). Functional significance of ca2+ in long-lasting fatigue of skeletal muscle. *Eur J Appl Physiol*, 83(2-3):166 – 174.