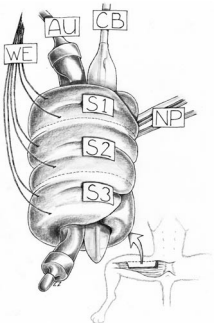
New Electrical Stimulation Techniques in Dynamic Myoplasty

Erik D.H. Zonnevylle



"An invasion of armies can be resisted,
but not an idea whose time has come"
-Victor Hugo-

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New Electrical Stimulation Techniques in Dynamic Myoplasty

Nieuwe Elektrische Stimulatie Technieken in Dynamische Spierplastieken

(met een samenvatting in het Nederlands)

Proefschrift

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to 35 mongrel dogs

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Contents

Chapter 1

General introduction

“Obstacles are those frightful things you see
when you take your eyes off your goal”
-Henry Ford-

Introduction

The wish for reconstruction of parts of the body is probably as old as their mutilation. For centuries documents have appeared reporting on sometimes brilliant efforts of creative minds to reconstruct what was lost, using surgery as a tool. Well-known and interesting to read are the reports concerning early reconstructions of the nose. The demand for surgical reconstruction was increased by the vast amount of casualties in the first world war. At the same time the need to share knowledge and experience brought together surgeons working in this evolving field of 'plastic surgery'. Today, plastic surgery is an independent medical specialty, in which reconstructive surgery plays a central role. The evolution of reconstructive surgery has had many accelerations, which were often preceded by the development of new surgical techniques. These new surgical techniques, almost always paved the way for an array of new applications. A good example is the development of the microsurgical anastomosis of small blood vessels. Rendering this technique made it possible to transplant tissue from less critical donor sites to more precarious recipient defects in a one-stage procedure. A multitude of flaps, consisting of all kinds of tissues, were developed to fill an even larger multitude of recipient defects.

The mean goal of those microsurgical procedures in the early sixties and seventies was to fill defects. After mastering the microsurgical technique, attention was directed to restoring form. Restoration of form is challenging and sometimes reaches artistic levels even with current surgical techniques. Today, besides the aesthetic side of reconstructive surgery, restoration of function plays an increasingly important role. However, in many instances its possibilities are limited due to the restriction of applicable key (surgical) techniques.

Therefore, this thesis reports on new techniques, which are believed to be meaningful in the development of reconstructive surgery dealing with functional muscular deficits.

Dynamic myoplasty

In reconstructive surgery, usually muscle flaps are used to cover large tissue defects. Often these muscle flaps (myoplasties) atrophy, because of denervation during transfer to the recipient site. However, these muscle flaps can be re-animated by re-innervation, which is made possible using neuroraphy techniques. This way myoplasties are created with an ability to contract. These are referred to as 'dynamic myoplasties' and they offer new surgical solutions to reconstructive surgery dealing with functional deficits.

Free vascularized and re-innervated muscle flap transfer has met with reasonable success [(Harii 1976 & Manktelow 1989)], but re-innervation of the flap, and thus motor control, is a fairly unpredictable process. The ability of the dynamic myoplasty to be animated to produce the desired performance often results in less than optimal function. Furthermore, a nerve providing the appropriate innervation signal is not always at hand. Therefore, it is often necessary to use an external electrical pulse generator, eliciting contractions in a dynamic myoplasty to perform activity at the recipient site.

Cardiomyoplasty and graciloplasty are dynamic myoplasties, which have reached clinical application. In cardiomyoplasty the entire latissimus dorsi muscle is wrapped around the myocardium and stimulated by an implantable pulse generator to contract rhythmically at a fairly high frequency (approaching every other heart beat) and in synchrony with the heart in order to assist its failing function.[(Carpentier 1985, Chachques 1987 & 1997)] In graciloplasty for urinary and fecal incontinence, the gracilis muscle is wrapped around the fecal or urinary outlet and stimulated to contract tetanically and continuously for periods of 2 to 4 hours to replace a failing native sphincter.[(Williams 1989, Baeten 1991 & Janknegt 1992)]

However, these clinical applications unveiled important problems for dynamic myoplasties; among the most significant and compromising are rapid muscle fatigue, muscle damage due to over-stimulation, ischemic lesions, abundant scar formation of the adjacent tissues and inadequate performance of tasks, among others, due to lack of refined stimulation control.[(Merell 1986, Williams 1991, Konsten 1993, Janknegt 1995, Grandjean 1996, Geerdes 1996, Chachques 1997, van Aalst 1998, Madoff 1999, Baeten 2000 &

Bardoel 2002) Outcomes in dynamic myoplasties therefore continue to be, to a large degree, unpredictable.

Muscle fiber types and training protocols

An approach used in dynamic myoplasty to avoid muscle failure due to fatigue is to train the muscle to enhance fatigue resistance.(Salmons 1969,1976, 1981 & 1994, Pette 1973 &1975, Koller 1994) Skeletal muscles are normally a mixture of various classes of muscle fibers with different qualities, ranging from slow and relative fatigue resistant (type 1) to fast and fatigue-prone (type 2b).(Buller 1960, Salmons 1969, Pette 1997, Scott 2001) The innervation and the function of the muscle determine the predominance of one fiber type over another. However, striated muscle is plastic in nature. When the stimulation signal changes, the ratio of the muscle fiber types changes accordingly. This change in signal can either be a physical exercise program, re-innervation of the muscle tissue by a different nerve or electrical stimulation.

In order to improve the endurance of dynamic myoplasties, muscle-training programs have been designed to transform the myoplasty using an electrical stimulation regimen. Most training regimens transform the muscle from a fatigue prone, fast twitch, glycolytic muscle with predominantly type II fibers into a non-fatiguing, slow twitch, oxidative muscle with predominantly type I fibers.(Salmons 1981)

The trade-off for producing fatigue resistance is a slower contracting muscle capable of generating less power than its innate character.(Salmons 1981 & 1994, Pette 1984, Chachques 1987, Koller 1994)

Sequential segmental neuromuscular stimulation

Fatigue reduction in dynamic myoplasty is mainly achieved by electrical stimulation training protocols at the cost of strength, responsiveness and the chance of ischemic lesions due to constant high pressures. In this thesis a different, more physiological approach was tested in an attempt to bypass most of these drawbacks.

Sustained contraction in skeletal muscle tissue is only possible for a brief moment, because locally increased pressure impairs perfusion. Therefore in physiological conditions skeletal muscle performs sustained work by recruiting different fibers at different times. In this way a constant force is maintained, while part of the muscle is working and part of the muscle is resting. This type of contraction enables the muscle to be perfused constantly: while the microcirculatory blood flow in the contracted part of the muscle is momentarily impaired, hyperemic flow in the resting part allows metabolites to be washed out and oxygenation to be increased.

Current electrical stimulation techniques apply a single source voltage, which causes all of the muscle fibers to contract simultaneously, impairing perfusion during contraction and leading to rapid fatiguing as explained above. In sequential segmental neuromuscular stimulation the skeletal muscle is partitioned into segments, which are alternately stimulated in a sequential order. This approach causes only one segment of the muscle to perform the necessary workload, thereby allowing the other non-stimulated segments to recover by reperfusion, as is the case in physiological contraction of the muscle and leads to a prolongation of the endurance of the muscle. Similar techniques have been shown to reduce muscle fatigue.(Petrofsky 1979, Pournezam 1988, Thoma 1991 & Lau 1995)

Closed-loop control

A different perspective to the same problem of current non-physiological electrical stimulation paradigms in dynamic myoplasties is their performance control.

Under normal circumstances, a given anatomical muscle can respond to moderate prolonged activity and brief intense activity. The exact possibility of response, generated by the muscle, depends on the distribution of myofiber types contained within the muscle and the pattern of activity in which they are engaged.(Saltin 1983 & Scott 2001) In normal physiology the performance of the muscle is tuned to the exact needs, saving performance potential and avoiding long sustained high internal pressures. However, current applications of dynamic

myoplasty do use tetanic stimulation, resulting in contraction of all myofibers simultaneously leading to irreversible muscle damage, when prolonged. Furthermore, this type of on/off control does not allow more intricate applications of dynamic myoplasty to be developed, in which fine tuned performance control is mandatory. Therefore, a method of electrically stimulating dynamic myoplasties was developed, more closely mimicking physiological muscle performance regulation.

Closed-loop performance feedback control creates the condition in which it is possible to precisely tune the amount of force generated by the dynamic myoplasties to the level necessary to carry out the required physiological function.[Quintern 1997 & Lemay 1997] Economizing peak exertion to the minimum has beneficial effects on muscle and surrounding tissues by reducing ischemic lesions, stricture and scar formation. Moreover, this reduction augments the endurance of the dynamic myoplasty and allows the development of dynamic myoplasty applications in which fine-tuned performance control is obligatory.

Closed-loop control depends upon the biofeedback, which is offered by implantable biosensors. This biofeedback can also be used to run algorithms, in programmable units of implantable stimulators, to process decisions concerning dynamic myoplasty performance. This offers refinement in complex tasks and creates possibilities for more demanding implementations of dynamic myoplasties.

Hypotheses

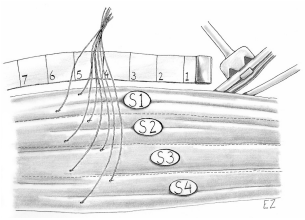
This thesis reports on research concerning improvement of electrical stimulation used in dynamic myoplasties. Hypotheses were formulated before experiments were conducted. Some expectations were met and some were not. The most important hypotheses are summarized below:

- Sequential stimulation is feasible and will result in a constant net performance, while different segments alternate during stimulation (supported, see chapter 2 & 3).
- Sequential stimulation will enhance blood perfusion of the stimulated muscle, when compared to conventional stimulation (supported, see chapter 2 & 3).
- Sequential stimulation will enhance fatigue resistance, when compared to conventional stimulation (supported, see chapter 2 & 3).
- Sequential stimulation will prevent the need to alter muscle fiber type, using electrical stimulation training regimens, in graciloplasty (disproved and rejected, see chapter 3)
- Closed-loop control is feasible in a sequentially stimulated dynamic myoplasty (supported, see chapter 4 & 5).
- Oscillation damping parameters are effective in closed-loop controlled sequentially stimulated dynamic myoplasties (supported, see chapter 4).
- Both amplitude and frequency modulation of the stimulation signal in closed-loop control are feasible in sequentially stimulated dynamic myoplasties (supported, see chapter 4).
- A combination of sequential stimulation, closed-loop control and function-controlling algorithms is feasible in dynamic myoplasty (supported, see chapter 5).
- A combination of sequential stimulation, closed-loop control and function-controlling algorithms improve the versatility of dynamic myoplasties (supported, see chapter 5).

- A combination of sequential stimulation, closed-loop control and function-controlling algorithms in a graciloplasty-model has the potential to function as a reasonable alternative to the native sphincter function (supported, see chapter 5).
- The results described in this thesis justify the effort of miniaturizing stimulating devices capable of combining sequential stimulation, closed-loop control and function-controlling algorithms, to implantable size (made plausible, see chapter 6).

Chapter 2

Sequential stimulation in an isometric setup



"Do not go where the path may lead, go instead where there is no path and leave a trail" -Ralph Waldo Emerson-

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Zonnevylle E, Somia N, Stremel R, Maldonado C, Werker P, Kon M and Barker J. Sequential segmental neuromuscular stimulation: an effective approach to enhance fatigue resistance. Plastic & Reconstructive Surgery 105: 667-673, 2000.

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Abstract

Electrical stimulation of skeletal muscle flaps is used clinically in applications that require contraction of muscle and force generation at its recipient site, for example, to assist a failing myocardium (cardiomyoplasty) or to reestablish urinary or fecal continence as a neo-sphincter (dynamic graciloplasty). A major problem in these applications (muscle fatigue) results from the non-physiological manner in which most of the fibers within the muscle are recruited in a single burst-like contraction. To circumvent this problem, current protocols call for the muscle to be put through a rigorous training regimen in order to transform it from a fatigue prone to a fatigue resistant state. This process takes several weeks during which, aside from becoming fatigue resistant, the muscle looses power and contraction speed.

In this study, we tested the feasibility of electrically stimulating a muscle flap in a more physiologic way. Namely, by stimulating different anatomical parts of the muscle sequentially rather than the entire muscle all at once. Sequential Segmental Neuromuscular Stimulation allows parts of the muscle to rest while other parts are contracting. In a paired designed acute dog study (n=7) we compared the effects of sequential stimulation to conventional stimulation on muscle fatigability and muscle blood perfusion in gracilis muscles: Sequential stimulation on one side and whole muscle stimulation on the other side. In sequential stimulation, electrodes were implanted in the muscles in such a way that four separate segments of each muscle could be stimulated separately. Then, each segment was stimulated in such a way that part of the muscle was always contracted while part was always resting. This type of stimulation permitted sequential yet continuous force generation. Muscles in both groups maintained an equal amount of continuous force. In sequential stimulation muscles separate segments were stimulated so that the duty cycle for any one segment was 25%, 50%, 75% or 100%, thus varying the amount of work/rest any one segment experiences at any one time. We found that with duty a cycle of 25, 50 and 75%, sequential stimulation produces significantly enhanced resistance to fatigue. In addition, we found that muscle perfusion was

significantly increased in these sequentially stimulated muscles compared to the controls, receiving whole muscle stimulation.

Therefore, it is concluded that sequential stimulation reduces muscle fatigue and enhances muscle blood flow during stimulation. These findings suggest that using sequential stimulation in clinical myoplasty procedures could obviate the need for prolonged training protocols and minimize muscle training associated problems.

Introduction

In reconstructive surgery, muscle flaps are most commonly used to cover large tissue defects. Recent advances in implantable electrical pulse generator technology has made it possible to electrically stimulate muscle flaps so that they perform work at their recipient site. This new use for muscle flaps has been given the name 'dynamic myoplasty'. In spite of the exciting potential for this new procedure, outcomes continue to be, to a large degree, unpredictable.

Examples of clinically applied dynamic myoplasty include cardiomyoplasty and graciloplasty. In cardiomyoplasty, the latissimus dorsi muscle flap is passed through the chest, wrapped around a failing heart and stimulated to contract repeatedly, without rest, in synchrony with the heart to assist in its pumping function.[Carpentier 1985 & Chachques 1997] In graciloplasty, the gracilis muscle flap is wrapped around the urinary or fecal outlet and is stimulated to contract constantly for up to 4 hours straight to replace the sphincter function.[Baeten 1991 & 2000, Williams 1991, Janknegt 1992]

In both of the above applications of dynamic myoplasty muscle fatigue of the transferred skeletal muscle poses a major problem. Sustained contraction in skeletal muscle tissue is only possible for a brief moment, caused by locally increased pressure, impairing perfusion. Therefore in physiologic conditions skeletal muscle performs sustained work by recruiting different fibers at different times. This way a constant force is maintained, while part of the muscle is working and part of the muscle is resting. This type of contraction enables the muscle to be perfused constantly: while the microcirculatory blood flow in the contracted part of the muscle is momentarily impaired, hyperemic flow in the resting part clears metabolites and replenish ATP, necessary for contraction.

The currently used electrical stimulation paradigms require the muscle to contract in a non-physiologic manor causing it to rapidly fatigue. Current methods cause all the fibers within the muscle flap to contract simultaneously and repeatedly without rest in synchrony with the heart (cardiomyoplasty) or constantly for up to 4 hours at a time (graciloplasty). This repeated, sometimes tetanic, muscle contraction causes a sustained increase in intra-muscular pressure and can lead to a decrease in muscle blood perfusion.

To overcome muscle fatigue in the currently applied stimulation protocols the muscle is put through a rigorous training regimen for prolonged periods before it is made to perform its definitive work of assisting a failing heart [(Koller 1994)] or restoring continence to a non-functioning sphincter. Most training regimens transform the muscle from a fatigue prone, fast twitch, glycolytic muscle with predominantly type II fibers into a non-fatiguing, slow twitch, oxidative muscle with predominantly type I fibers.[(Salmons 1981 & 1994)] This time consuming training regimen causes the muscle to loose power and reduces its contractile speed.

These drawbacks lead us to search for alternative methods of muscle stimulation. We performed a paired experiment using 7 dogs to evaluate the effects of a method we termed Sequential Segmental Neuromuscular Stimulation. This new method was compared to conventional whole muscle stimulation. The setup was designed to detect differences in fatigue resistance and muscle perfusion during stimulation.

Materials and Methods

In dogs both gracilis muscles were electrically stimulated, using four-channel sequential stimulation on one side and continuous, whole muscle stimulation on the other side. The distal ends of the muscles were fixed to force transducers and the muscles were stimulated to produce an equal and predetermined amount of force. The half time to fatigue and blood perfusion during stimulation was measured in all muscles.

Animal Care

Seven anesthetized dogs (15-20 kg; approx. 6 months of age) were used in this experiment. Prior to the experiment, animals were housed in separate cages at a controlled temperature (i.e., 22 °C) and with a 12-hour light/dark cycle. The animals were fed commercial dog diet and provided with water *ad libitum*. At the termination of the experiment, the dogs were euthanized with an overdose (10 ml, IV) of Beuthanisia (390 mg sodium pentobarbital and 50 mg sodium phenytoin per ml, Schering-Plough Animal Health Corp. Kenilworth, NJ).

The protocol for the use of dogs in this study was approved by the Institutional Animal Care and Use Committee (IACUC) and adhered to the NIH and APS "Guide for the care and use of laboratory animals". Studies were performed in the American Association of Laboratory Animal Care (AALAC) approved Research and Resource Center at the University of Louisville Health Sciences Center.

Muscle Stimulation and Fatigue/Blood Flow Measurement Equipment

Stimulation of the gracilis muscle was performed using an input/output device (CED1401^{plus}, Cambridge Electronic Devices, Cambridge, England) in combination with a personal computer (Gateway 2000, P5-133/16) and data acquisition software (Spike2, version 2, Cambridge Electronic Devices, Cambridge, England). Additional customized sequencer files and script files were developed especially for this purpose (see appendix). The output signals were led to a multiple channel custom-made switchbox designed to connect the stimulation channels with the appropriate electrodes. Four pairs of Teflon coated stainless steel wires (∅ 0.007 inch; Medwire®, Mount Vernon, NY) were used as leads for the sequential stimulation. Denuding the last 8 mm. of the wire created the electrode tips, while the other ends were connected to the switch box. Conventional "whole muscle" stimulation (controls) was performed using two single lead intra-muscular electrodes (SP-5577-30, Medtronic, Minneapolis, MN). Muscle contractile force generated by all muscles upon stimulation was measured using isometric force transducers (FT10C, Grass, Quincy, Mass.). Their signals were amplified (CED 1902, Cambridge Electronic Design, Cambridge, England) and recorded with the above mentioned input/output device. Gracilis flap blood perfusion was measured using a flow-probe placed on the flap's pedicle artery and a blood flow meter (1.5RB; T206, Transonic Systems Inc., Ithaca, NY). This signal was also amplified and recorded.

Muscle Preparation and Experimental Setup:

All animals were anesthetized (Pentothal 6-12 mg/kg, Abbott Laboratories, North Chicago, IL), intubated and ventilated (Halothane, 1.5%, oxygen 94.5%, nitrous oxygen 4%; Halocarbon, River Edge, NY) for surgery. The animals were positioned supine with their hind legs abducted. A medial, mid-thigh incision was made on both legs and the gracilis muscles were identified and lifted as single pedicled (i.e., one main vascular supply) muscle flaps. The canine gracilis muscle consists of a bulky posterior and a slimmer, almost parallel arranged, anterior part receiving the main pedicle. To simulate the long thin proportions of the human gracilis we tailored the muscle by resecting the posterior part using bipolar coagulation to divide the collateral vessels. The tailored anterior aspect of the muscle was preserved with a width of 35 mm. The proximal tendons (origins) were left intact, while the distal tendons (insertions) were cut and fixed to isometric force transducers. The bony pelvis to which the proximal gracilis muscles were left attached and the force transducers were secured to a metal scaffolding such that the muscle resting tension could be adjusted and fixed (see figure 1). In the gracilis flaps, receiving sequential stimulation, four pairs of electrodes were inserted, perpendicular to the long-axis, half way along its length, approximately 5 cm distal to where the main neuro-vascular pedicle entered the muscle. These paired electrodes divided the muscle into four parallel individually stimulatable segments. In control muscles (whole muscle stimulation) two electrodes (SP 5577-30, Medtronic) were inserted transversely from anterior to posterior, approximately 3 cm apart, into the contralateral gracilis muscle, also half way along its length and 4 and 7 cm distal from the neuro-vascular pedicle (see figure 2). All electrodes were connected to the switchbox, which itself was connected to the output of the CED1401^{plus}. The force transducers were connected to the signal amplifiers, which were connected to the input of the data acquisition device (CED1401^{plus}). After calibration, the blood flow probes (Transonic) were placed on the arteries of the neuro-vascular pedicles of both gracilis muscles. The probes were connected to the dual flow-measuring device, which was connected to the

CED1401^{plus} input/output device in order to record the arterial blood flow to both muscles (see figure 3).

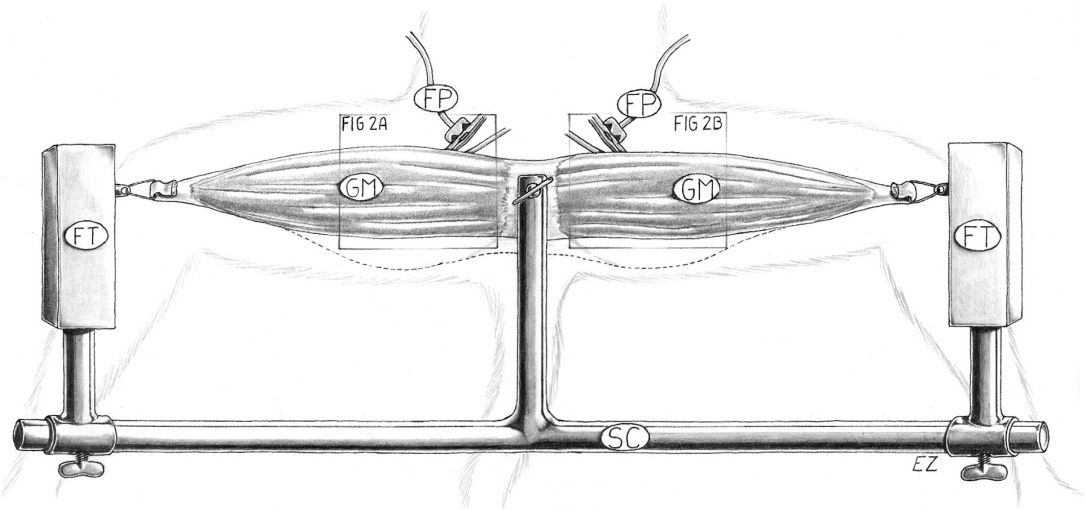


Figure 1: Simplified overview of the setup. Medial insertions of the gracilis muscles (GM) were left intact while the lateral insertions were attached to force transducers (FT). A metal scaffolding (SC) kept distances fixed. Flow-probes (FP) were attached to the single supplying arteries. Dashed lines outline the muscle parts, which were cut off during tailoring. Details concerning electrodes are depicted in Figures 2a and 2b.

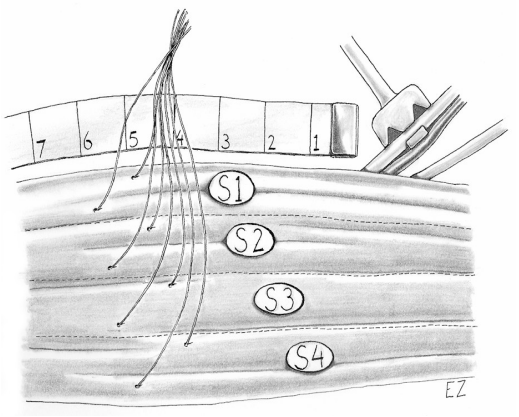


Figure 2a: Four pairs of Teflon coated stainless steel wires were inserted into the sequentially stimulated muscle around 5 cm. distal to the neuro-vascular pedicle. These were repositioned until only the intended isolated selection of the muscle (S1-4) contracted on electrical stimulation.

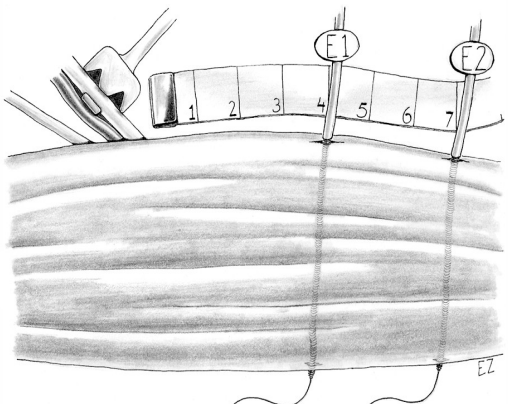


Figure 2b: Two single lead intra-muscular electrodes (E1-2) were inserted into the control muscle 4 and 7 cm distal from the neuro-vascular pedicle.

Pilot studies were conducted to determine the values for frequency and pulse-width, which would generate a complete tetanic contraction with the lowest possible stimulus intensity. This was found to occur at a frequency of 30 Hz and a pulse width of 500 μsec. The pulse shape consisted of a mono-phasic block. The force generated by the muscle was fixed and the stimulus intensity adjusted for every experiment. Generated force was chosen being of sufficient magnitude to fatigue the muscle, but not so large as to damage the muscle and prevent repeated tests. The four muscle segments were sequentially stimulated in 1.0 sec. steps.

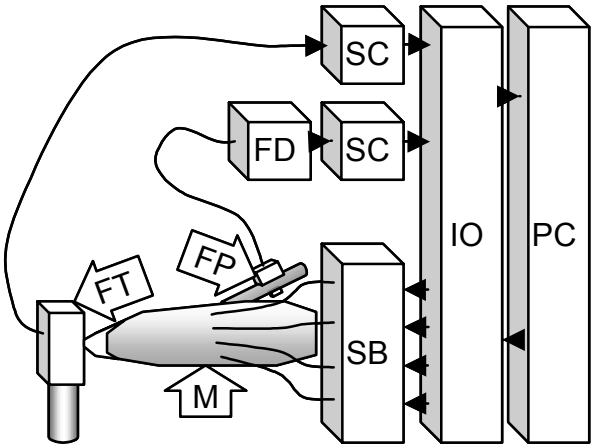


Figure 3: Schematic representation of the connections: The controlling personal computer (PC) was connected to the input/output (IO) device, which generated the electrical stimulation signal. This signal was led to the muscles (M) via the switchbox (SB) and the electrodes. The flow-probes (FP) were connected to the dual flow measurement device (FD), which delivered the arterial blood flow values via signal conditioners (SC) to the input/output device (IO). The force-transducers (FT) were also connected to the input/output device (IO) via signal conditioners (SC). All incoming signals were analogue-to-digitally converted and delivered to the controlling personal computer (PC).

Fatigue/Blood Flow Measurements

In both groups the distal ends of the muscles (sequential stimulation and whole muscle stimulation) were attached to force transducers and were stimulated at an intensity that generated an equal and predetermined starting force. Fatigue was determined by measuring the amount of time it took for a muscle to decrease the generated force to one-half of the starting force, i.e., half time to fatigue. Measurements were performed four times, alternating back and forth between each muscle, with 15-minutes resting periods between measurements.

The number of simultaneously stimulated muscle segments in the sequentially stimulated group determined the duty cycle of the segments and

also influenced the force generated. For example, the sequentially stimulated muscle was stimulated so that only one of the four segments was contracting at a time; this constituted a duty cycle of 25% and generated initially a constant force of 0.35 kiloponds. When two segments were stimulated simultaneously, a 50% duty cycle and 0.65 kiloponds of force were created. Three simultaneously stimulated segments gave a 75% duty cycle and produced a generated 1.0 kilopond of force. Finally, when all four segments of the sequentially stimulated muscle were simultaneously contracted a 100% duty cycle resulted, producing a 1.2 kiloponds force generation. The conventional (whole muscle) stimulation and the sequential stimulation were always compared at the same generated force. All measurements were terminated after 20 minutes, regardless the amount of occurred fatigue. The arterial resting flow and the mean of the flow, between 16-32 seconds after starting stimulation were measured for every muscle.

Statistics

To compare fatigue rates between sequential and conventional stimulation the ratio's of the half times to fatigue between both stimulation regimens were calculated for all four steps in the measurement protocol. Differences were also statistically analyzed using the paired t-test after checking the distribution of the values for normality using the Kolmogorov-Smirnov method. To compare perfusion in the muscles exposed to the two different stimulation regimens, the averaged gain of arterial blood-flow between 16 sec and 32 sec after start of stimulation was expressed as a percentage of resting flow. The percentages of both stimulation approaches were compared using the paired t-test for means after checking the distribution of the values for normality using the Kolmogorov-Smirnov method.

Results

The half times to fatigue (mean ± SEM) of sequential stimulation and whole muscle stimulated muscles are depicted in figure 4. During whole muscle stimulation, the half times to fatigue did not significantly vary for the various loads (51 ± 5 sec for the 0.35 kp load to 64 ± 13 sec for the 1.2 kp load). This was in contrast to sequential stimulation where a decreasing duty cycle markedly increased the half time to fatigue: 100%: 65 ± 6 sec.; 75%: 119 ± 7 sec; 50%: 262 ± 29 sec and 25% : 1,133 ± 33 sec. As the duty cycle decreased, the load on any individual muscle segment stayed relatively constant while the period of rest and recovery increased. When expressed as a ratio, sequential stimulation with a duty cycle of 25% performed 22.2 times better than conventional whole muscle stimulation. For 50% the ratio was 4.8; for 75% the ratio was 2.2 and for 100% there were no significant differences and thus the ratio was 1.0.

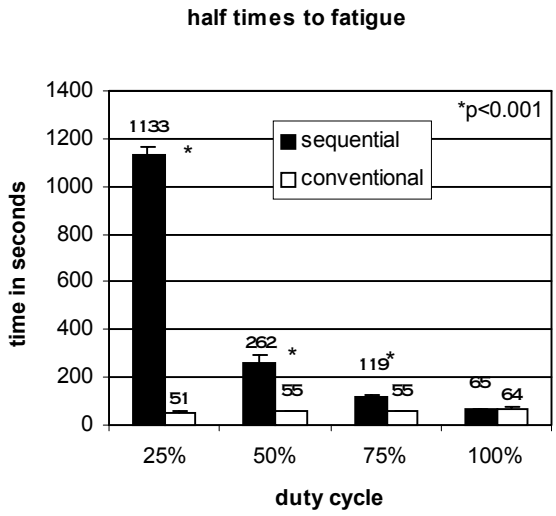


Figure 4: Half times to fatigue (mean and S.E.M. in seconds) of sequential and conventional stimulation for all four groups of duty cycles. Differences were statistically significant for 25%, 50% and 75%.

In figure 5 the averaged increase in blood-flow during stimulation is represented for the four measured duty cycles of sequential stimulation and their respective controls. At a duty cycle of 25% (and corresponding resting cycle of 75%) and a generated force of 0.35 kiloponds, blood-flow increased to an average of 257 ± 20% of the resting flow. For the duty cycle of 50% (and corresponding resting cycle of 50%), and 0.65 kiloponds of force, the averaged blood-flow increased to 302 ± 44%. For a 75% duty cycle (and corresponding resting cycle of 25%), and 0.90 kiloponds of force, blood flow increased to 272 ± 30%. Finally at a 100% duty cycle (and no resting cycle) and 1.2 kiloponds of force, blood flow increased to 145 ± 5%. The same forces generated with whole muscle stimulation showed minor increases in arterial blood-flow (134 ± 8% for the 0.35 kp. load to 133 ± 11% for the 1.2 kp. load).

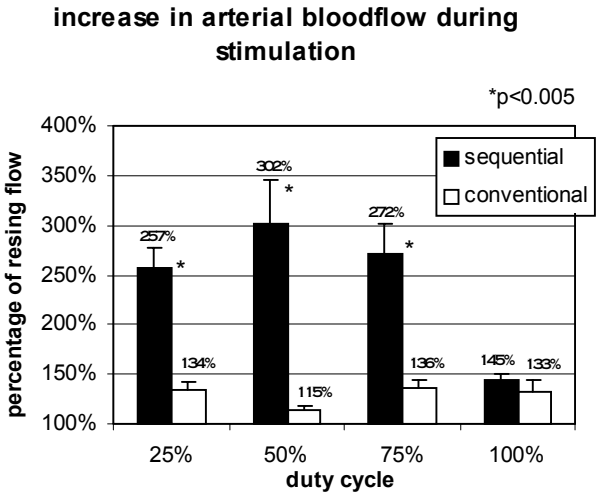


Figure 5: Increases in blood-flow (mean and S.E.M.) are expressed as percentages of the blood-flow in rest. Differences between sequential and conventional stimulation were statistically significant for 25%, 50% and 75%.

Discussion

In clinical cardiomyoplasty and graciloplasty procedures, latissimus dorsi and gracilis muscle fatigue is overcome by putting the muscles through a prolonged training regimen. This training transforms the normally fatigue prone, fast twitch, glycolytic, predominantly fiber type II skeletal muscle into a fatigue resistant, slow twitch, oxidative, predominantly fiber type I muscle.

While this rigorous training protocol transforms the muscle to fatigue resistant, it also causes the muscle to loose power and decreases its velocity of contraction. Other drawbacks associated with transforming skeletal muscle are the prolonged time required before the muscle can be used to assist the failing heart or replace sphincter function.

All or part of these drawbacks could contribute to the inconsistent outcomes reported in clinical cardiomyoplasty and graciloplasty.

Based on the above drawbacks we proposed a different approach to minimizing skeletal muscle fatigue, using a more physiologic approach. Rather than stimulating the entire muscle in one electrical burst and thus recruiting the same fibers simultaneously, we studied the feasibility of stimulating different muscle segments sequentially. The latter allows parts of the muscle to rest, while other parts work. To produce a precisely tuned amount of force, it was necessary to locate electrodes distant from the main neural pedicle, which is in contrast with current methods. Thus, small nerve branches were activated, rather than large sections of the muscle.

We compared both approaches in 7 pairs of dog gracilis muscles and found that sequential stimulation significantly reduced muscle fatigue compared to controls. In addition we found that smaller duty cycles correlate with greater fatigue reduction. Some care should be taken though, while interpreting the exact relation between the duty cycles and fatigue, since force generation was arbitrary selected and the order of measurements was not randomized because of small numbers (n=7).

These findings are in agreement with literature dealing with endurance enhancement in neuromuscular prosthesis research for gait, in which alternation between agonistic muscles proved to be beneficial.[Peckham 1970, Pournezam 1988] It is

also in agreement with observations described in the literature concerning the indirect or neural multi-channel stimulation, which was also developed to sequentially recruit separate parts of a muscle.[Petrofsky 1979, Baer 1990, Thoma 1991] Unfortunately, it was also reported that this type of peripheral nerve stimulation causes severe damage to the nerve and therefore never achieved widespread clinical application.

The significant improvement in muscle function observed in this acute model could certainly be attributed to the significant improvement in muscle blood flow during sequential stimulation as compared to whole muscle stimulation. Blood flow in the main pedicle artery, solely feeding the gracilis flap, was measured in a time window from 16 to 32 seconds in order to measure muscle perfusion during both stimulation regimens. This measuring time was pragmatically chosen, because we noted that after 16 seconds (four cycles of sequential stimulation) blood flow values had stabilized. Measurements were stopped after 32 seconds, because the subsequent rapid drop in force in the conventionally stimulated muscle caused increasing interference. This drop allowed partial hyperemic perfusion of the abandoned fatigued muscle fibers, causing the blood flow to slowly rise after the (arbitrary) measurement ending time of 32 seconds.

The work described in this report studied muscle fatigue and blood flow in sequential stimulation muscles prepared acutely. The question arises whether in long-term studies a sequentially stimulated muscle will experience transformation and how this will affect the long-term function.

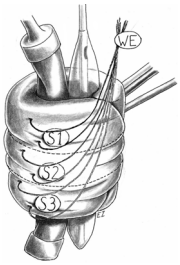
From the results of this experiment it can be stated that sequential stimulation provides a substantial gain in fatigue resistance over conventional stimulation, which is inversely progressive to the duty cycle. Thus, if a muscle flap used in a given myoplasty procedure is not required to contract maximally, as in graciloplasty for the treatment of urinary incontinence or fecal incontinence, sequential stimulation could improve endurance acutely. This could result in shorter training regimens and earlier full functioning of the myoplasty.

Conclusions

Sequential Segmental Neuromuscular Stimulation significantly enhances fatigue resistance in an inverse progressive ratio to the applied duty cycle. Reperfusion during sequential contraction is demonstrated and is assumed to contribute to the enhanced fatigue resistance.

Chapter 3

Sequential stimulation in a non-isometric setup



"The great tragedy of science:
the slaying of a beautiful hypothesis by
an ugly fact" -Thomas Huxley-

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Based upon the articles:

Perez Abadia G, Zonnevylle E, Somia N, Stremel R, Koenig S, Palacio M, , Werker P, Kon M, Maldonado C, Tobin R and Barker J. Dynamic graciloplasty: sequential segmental neuromuscular stimulation (SSNS) improves neo-sphincter performance. Surgical Forum 49: 669-671, 1998.

Zonnevylle E, Somia N, Perez Abadia G, Stremel R, Maldonado C, Werker P, Kon M and Barker J. Sequential segmental neuromuscular stimulation reduces fatigue and improves perfusion in dynamic graciloplasty. Annals of Plastic Surgery 45: 292-297, 2000.

and presented (in part) at:

the Annual Meeting of the Plastic Surgery Research Council 1998, Loma Linda, California, U.S.A.

the 84th Annual Surgical Forum of the American College of Surgeons 1998, Orlando, Florida, U.S.A.

Abstract

Dynamic graciloplasty is used as a treatment modality for total urinary incontinence caused by a paralyzed sphincter. A problem in this application is undesirable fatigue of the muscle caused by continuous electrical stimulation. Therefore, the neo-sphincter must be trained via a rigorous regimen in order to transform it from a fatigue prone to a fatigue resistant state. To avoid or shorten this training period, the application of sequential segmental neuromuscular stimulation was examined. This form of stimulation proved to be highly effective in acutely reducing fatigue caused by electrical stimulation.

The contractile function and perfusion of gracilis muscles employed as neo-sphincters were compared between conventional, single-channel, continuous stimulation and multi-channel, sequential stimulation in 8 dogs. The sequentially stimulated neo-sphincter proved to have a 2.9 times longer endurance (as measured by time to half-fatigue) and a better blood perfusion during stimulation and both differences were statistically significant. Clinically this will not outdate training of the muscle, however, sequential stimulation is likely to reduce the need for long and rigorous training protocols making dynamic graciloplasty more attractive as a method for treating urinary or fecal incontinence.

Introduction

Dynamic Graciloplasty is the technique in which the gracilis muscle flap is wrapped around the urinary or fecal outlets and used to function as a neo-sphincter. In currently used procedures, the gracilis muscle is incompletely raised and transposed with its distal end around the target outlet.[Williams 1989, Baeten 1991, Janknegt 1992, Williams 1993] Because of ischemia and poor function of this distal end, it was recently proposed to raise the gracilis muscle as an innervated free flap and use the most suitable part of the muscle for the wrap.[van Aalst 1996, 1998] Although this seems to be a major step forward in the field of custom made neo-sphincter construction, undesirable sphincter fatigue caused by the necessary electrical stimulation continues to be a major drawback.

In the currently applied procedures, fatigue is overcome by putting the muscle through a rigorous training regimen for prolonged periods. The muscle is transformed from a fatigue prone, fast twitch, glycolytic muscle with predominantly type II fibers into a fatigue resistant, slow twitch, oxidative muscle with predominantly type I fibers.[Salmons 1969, 1976, 1981 & 1994, Pette 1973 & 1997] The long training period required to transform the muscle and the subsequent prolonged period of incontinence, motivated searches for a better alternative to the currently used protocols for training skeletal muscle in dynamic graciloplasty.

In the previous chapter, the feasibility of sequential segmental neuromuscular stimulation was tested: electrically stimulating different segments of a muscle in an alternating fashion rather than the entire muscle all at once in order to increase acute endurance. In this chapter it is suggested that muscle fatigue during continuous electrical stimulation is mainly due to the fact that the same muscle fibers are being stimulated to contract without rest. Sequential / alternating fiber recruitment during stimulation provides interim rest of some segments, permitting temporary hyperemia of segments, while other segments are contracting. The periodic, temporary hyperemia allows metabolites to be washed out and oxygenation to be increased in the non-contractile muscle segments. This causes the endurance of the muscle to increase. Although the results of sequential stimulation were only known for acute isometric contraction, it was predicted that in the non-isometric case of the modified dynamic

graciloplasty the endurance could be prolonged using sequential stimulation. Theoretically, this could benefit patients by decreasing the period of electrical training of the neo-sphincter and therefore more rapidly provide continence post-operatively.

In this study, fatigue rates and perfusion of sequentially stimulated neo-sphincters were compared with conventional continuously stimulated neo-sphincters. Sequential segmental neuromuscular stimulation significantly decreased fatigue compared to the currently used method of continuous stimulation in dynamic graciloplasty.

Materials and Methods

In dogs both gracilis muscles were converted into neo-sphincters and electrically stimulated, using three-channel sequential stimulation on one side and conventional single channel continuous stimulation on the other side. The neo-sphincters were wrapped around balloon catheters attached to pressure-transducers and stimulated to produce an equal and predetermined amount of pressure. The time to decline to half the value of the initial pressure and the arterial blood flow were measured in all muscles.

Animal care

Eight mongrel dogs (15-20 kg; approx. 6 months of age) were used in this experiment. Prior to the experiment, animals were housed in separate cages at a controlled temperature (i.e., 22 °C) and with a 12-hour light/dark cycle. The animals were fed commercial dog diet and provided with water *ad libitum*. At the termination of the experiment, the dogs were euthanized with an overdose (10 ml, IV) of Beuthanisia (390 mg sodium pentobarbital and 50 mg sodium phenytoin per ml, Schering-Plough Animal Health Corp. Kenilworth, NJ). The protocol for the use of dogs in this study was approved by the Institutional Animal Care and Use Committee (IACUC) and adhered to the NIH and APS “Guide for the care and use of laboratory animals”. Studies were performed in the American Association of Laboratory Animal Care (AALAC) approved

Research and Resource Center at the University of Louisville Health Sciences Center.

Materials

Stimulation was performed using an input/output device (CED1401^{plus}, Cambridge Electronic Design, Cambridge, England) in combination with a personal computer (Gateway 2000, P5-133/16) and data acquisition software (Spike2, version 2, Cambridge Electronic Design, Cambridge, England). Additional customized sequencer files and script files were developed especially for this purpose (see appendix). The three separate output signals were first led to optical linear stimulus isolators (A395's, World Precision Instruments, Sarasota, FL, USA) and thereafter to a multiple channel custom made switchbox. Three pairs of Teflon coated stainless steel wires (∅ 0.007 inch; Medwire®, Mount Vernon, NY) were used as leads for the sequential stimulation. Baring the last 8 mm. of the wire created the electrodes, while the other ends were connected to the switchbox. Two single electrode leads (SP-4300-35, Medtronic, Indianapolis, USA) were used for the control muscle. The pressure generated by the stimulated neo-sphincters was recorded by balloon dilatation catheters (BMX/8-3/5.8/120, Boston Scientific Corporation, Quincy, MA) connected to pressure-transducers (P23 ID, GultonStatham Inc., Ca, USA). Their signals were amplified using CED1902's (Cambridge Electronic Design, Cambridge, England) and recorded with the above mentioned input/output device. Arterial blood flow was measured using Doppler flow probes combined with a dual flow measurement device (T206, Transonic, Ithaca, New York, USA). The measurements were recorded using CED1902 pre-amplifiers and the CED 1401^{plus} input/output device.

Setup preparation

All animals were anesthetized (Pentothal 6-12 mg/kg, Abbott Laboratories, North Chicago, IL), intubated and ventilated (Halothane, 1.5%, oxygen 94.5%, nitrous oxide 4%; Halocarbon, River Edge, NY). They were positioned supine

with their hind legs abducted. Bilateral medial incisions were made through which both gracilis muscles were identified and lifted as single pedicled neuro-vascular flaps. The muscles were reduced to a width of 35 mm by removing the caudal aspect. At this point, two single leads were inserted into the conventionally stimulated muscle, four and seven cm. distal from the point of entry of the main pedicle (which includes the nerve at entry point). Starting from the proximal tendo-muscular transition, 12 cm. length was marked. These marked muscle-parts were cut from their origins and insertions. Each muscle was tensionless but tightly wrapped around a latex tube (∅ 8mm, wall thickness: 0.5 mm.) and a balloon catheter. Three pairs of Teflon coated leads were transversely inserted into the sequentially stimulated neo-sphincter, at an angle of 45 degrees to the direction of the fibers, 5 to 6 cm. distal from the main pedicle, dividing it into three parallel individually stimulatable units. Figure 1 depicts an overview of the setup, while figures 2a and 2b show the configuration of the electrodes after the wrap on the sequential and conventional side. The latex tubes were connected to a water column providing 40 cm. hydrostatic pressure. The balloon catheters were over-pressurized with 150 cm H_2O pressure in order to prevent collapse or deformation. The offset of 150 cm H_2O over-pressure was further corrected to zero by the data acquisition software. The balloons were connected to the pressure transducers. These were connected to the signal amplifiers (CED1902's), which in turn were connected to the input connectors of the data acquisition device (CED1401^{plus}). Flow probes were attached to the supplying arteries. These flow probes were then connected to the dual flow measurement device (T206), whose output channels were connected to signal amplifiers (CED1902's), which were connected to the input/output device (CED1401^{plus}). Figure 3 delineates an overview of the experimental setup, data acquisition and computer control for sequential segmental neuromuscular stimulation.

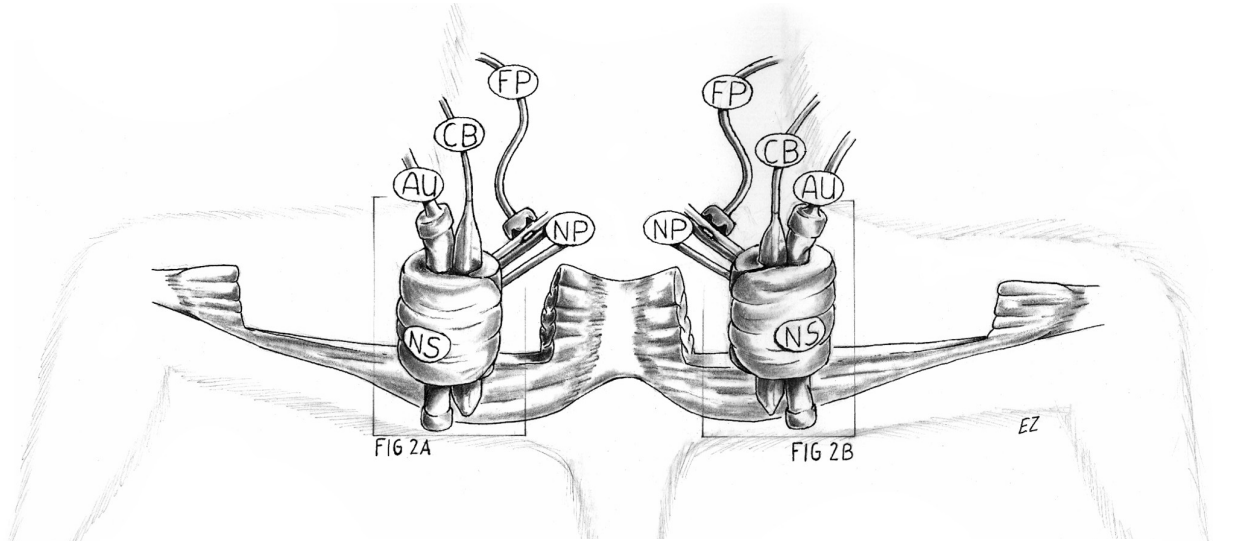


Figure 1: Surgical preparation. Gracilis muscles were turned into neo-sphincters (NS), wrapped around artificial urethrae (AU) and catheter balloons (CB). Flow probes (FP) were attached to the arteries of the single neurovascular pedicles (NP).

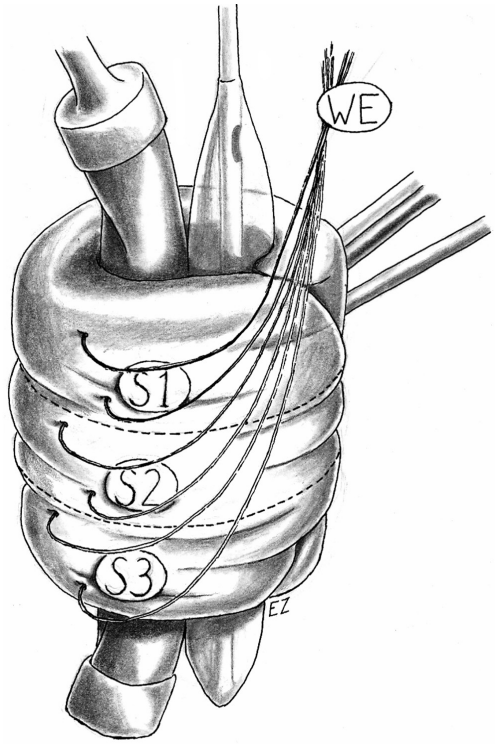


Figure 2a: Sequentially stimulated neo-sphincter. This neo-sphincter was sequentially stimulated using three segments (S1,S2,S3). Three pairs of wire-electrodes (WE) were transversely inserted into the muscle approximately 5 cm. distal to the pedicle. Electrodes were inserted in that way that each segment could be independently recruited and proportionally controlled modulating the amplitude of the signal.

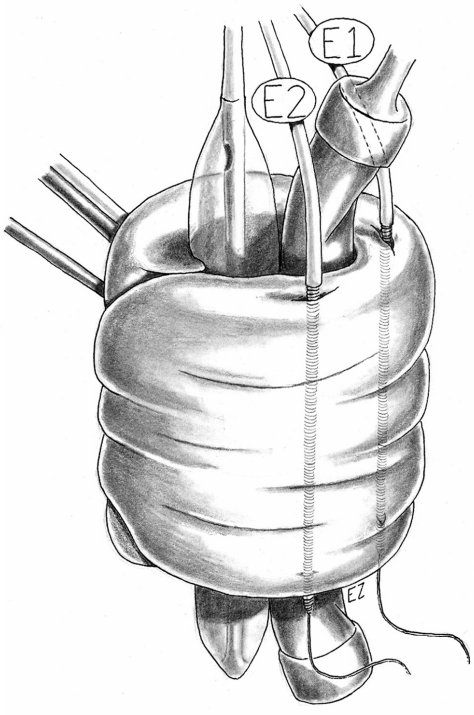


Figure 2b: Conventionally stimulated neo-sphincter. This neo-sphincter was conventionally stimulated acting as control. Two electrodes (E1,E2) were transversely inserted into the muscle 4 and 7 cm. distal to the pedicle. These positions ensured proportional control over the generated pressure, by modulating amplitude.

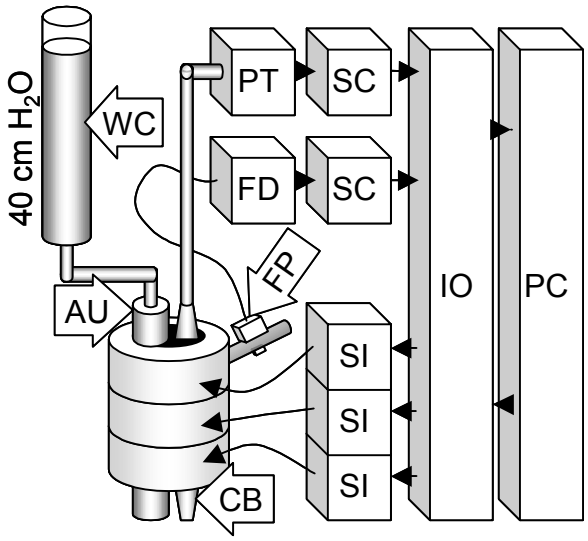


Figure 3: Diagrammatic representation of experimental preparation: water column, providing 40 cm static H_20 pressure (WC); artificial urethra, which collapses during stimulation (AU); catheter balloon (CB), transferring pressure to the pressure transducer (PT); flow probe (FP), acquiring blood flow with the flow measuring device (FD); signal conditioners (SC), pre-amplifying signals for the input/output device (IO); stimulus isolators, providing the stimulation signals (SI); personal computer (PC), controlling the stimulation via the input/output device and acquiring the measurements of generated pressure and arterial blood flow.

Stimulation parameters

Frequency and pulse-width were fixed values, determined in pilot studies, generating a complete tetanic contraction with the lowest possible stimulus amplitudes. The stimulus was optimized at a frequency of 30 Hz and a pulse-width of 500 microseconds. The pulse shape consisted of a mono-phasic block. The amplitudes were individually set for each of the three muscle segments of the sequentially stimulated neo-sphincter and also for the conventionally stimulated neo-sphincter to generate an initial pressure of 80 cm H_2O. During sequential stimulation each segment was stimulated for 1 second and rested for 2 seconds.

Measurements

Both neo-sphincters were stimulated to generate 80 cm H_2O pressure which collapsed the latex tubes and allowed the neo-sphincters to shorten during contraction. The elapsed time to decline to half the initial pressure (40 cm H_2O) due to fatigue was recorded. Arterial blood flow through the neo-sphincters was measured before stimulation. During stimulation, the blood flow was measured again for a period of 15 seconds, after an settling period of 15 seconds.

Analysis

Differences in endurance were quantified by comparing the elapsed times to fatigue between sequential stimulation and continuous stimulation. Statistical significance was demonstrated using the paired t-test for means, after checking the distribution of the values for normality using the Kolmogorov-Smirnov method.

The mean value of the arterial flow, measured between 15 and 30 seconds after stimulation started, was expressed as a percentage of the flow before stimulation. The differences between the relative changes in arterial blood flow of sequential and continuous stimulation were tested for statistical significance using a paired t-test for means after checking the distribution of the values for normality using the Kolmogorov-Smirnov method.

Results

During sequential stimulation, the averaged elapsed time to fatigue was 143 ± 17 seconds (mean ± sem). This is in contrast to conventional stimulation where the averaged elapsed time to fatigue was only 51 ± 6 seconds (see figure 4). Therefore, the ratio of the elapsed times to fatigue of the sequential and conventional stimulation was 2.9 ± 0.2. The difference is statistically significant ($p<0.05$).

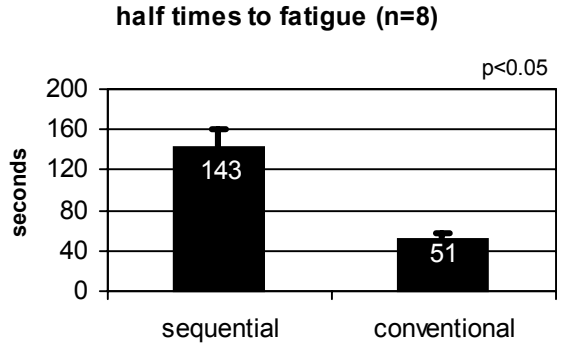


Figure 4: Averaged half times to fatigue (time for the stimulated neo-sphincter pressure to decrease from 80 to 40 cm H_20) for sequential and conventional stimulation. The difference between sequential and conventional stimulation is statistically significant.

In figure 5, the averaged percentage changes in arterial blood-flow during stimulation are represented for the sequential and conventional stimulation. The sequentially stimulated neo-sphincters showed an averaged blood-flow increase of 86 % above control. The same work achieved with conventional stimulation resulted in a *decrease* in arterial blood-flow below control of 37%. The difference is statistically significant ($p<0.05$).

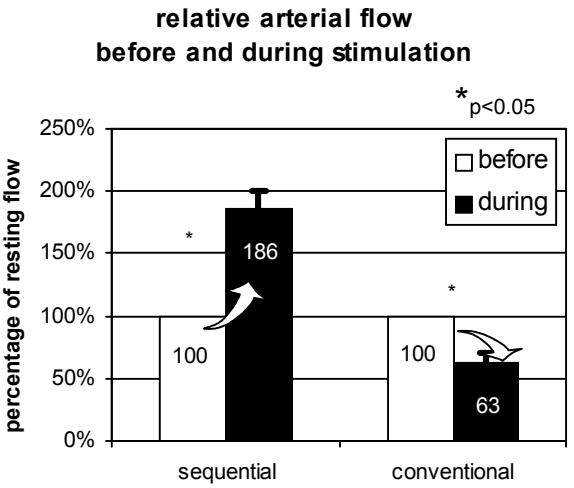


Figure 5: Relative changes in arterial blood flow during sequential and conventional stimulation compared to resting arterial flow. Resting blood flow was normalized as 100%. The difference between the flow changes during sequential and conventional stimulation is statistically significant.

Discussion

Dynamic graciloplasty is an exciting approach to the treatment of urine or fecal incontinence, but clinical trials have shown that the concept needs some refinement.[Konsten 1993, Baeten 1995, Janknegt 1995] A promising improvement is the innervated free flap approach developed by van Aalst et al. reported in 1996 and 1998. Although this approach might decrease scarring and improve effectiveness of the neo-sphincter in generating pressure, it still requires an eight-week training period to produce a fatigue resistant neo-sphincter at the

cost of strength and reaction speed. The importance of the latter is discussed in chapter 4 and 5 reporting the combination of sequential segmental neuromuscular stimulation with closed-loop control.

The previous chapter demonstrated the poor acute performance of dynamic myoplasties, when whole muscle recruiting electrical stimulation is applied. This stimulation continuously paces the same muscle fibers over and over again. This impedes local perfusion and results in metabolic acidosis, followed by a decrease in performance. Currently, rigorous training programs are used to alter the metabolic pathway of the involved skeletal muscle. The fast-twitch type II glycolytic fibers are replaced by slow-twitch type I oxidative fibers. In this way the muscle can better deal with the impeded perfusion, again, at the cost of strength and responsiveness. Furthermore, during the months of training following a graciloplasty procedure, the patient cannot enjoy the benefits of the procedure.

It is clear from the results that sequential stimulation of the neo-sphincter does acutely improve endurance substantially over continuous stimulation. The increase in arterial blood flow during sequential stimulation is in sharp contrast with the decrease measured in conventional stimulation. This supports the theoretical explanation of the prolonged endurance in sequential stimulation caused by temporary hyperemia of the intermittently resting muscle segments.

Because of practical reasons, the number of individually stimulated segments was chosen to be three in the experiment described in this chapter. This resulted in a duty cycle of 33%. Based on the results in the isometric gracilis preparation, described in chapter 2, it was anticipated that acute endurance could be prolonged 10-15 times, by extrapolating a duty cycle of 33% in the results graph of figure 4 in chapter 2. This proved not to be the case, since the difference was actually, only three times. Difference in experimental setup could be an explanation for this lower gain in endurance. The experiment above was deliberately chosen not to be isometric in order to resemble the actual procedure of dynamic graciloplasty. In the isometric preparation, sarcomeres in the muscle are kept in an ideal position to generate force, while the compressible artificial urethra allowed, at least for the inner part of the neo-sphincter, the more

unfavorable shortened position of the sarcomeres in the non-isometric preparation of this research. Furthermore, the vessel bed of the muscles in the isometric preparation was slightly stretched and kept stretched during stimulation, while the wrap around the artificial urethra and the balloon catheter caused hitching and torsion of the intra-muscular vessel bed, which was increased during stimulation. The latter caused higher resistance and subsequent lower perfusion. It is most likely that these differences at least partly caused the lower endurance in the experiment described in this chapter.

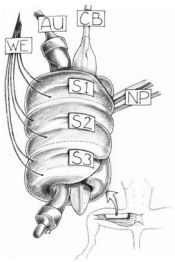
Conclusion

Sequential segmental neuromuscular stimulation does not outdate a training regimen in the clinical setting of dynamic graciloplasty. However, it acutely prolongs endurance and enhances perfusion. It is anticipated that it will substantially shorten the necessary training protocol, leading to an earlier state of acceptable continence for patients undergoing dynamic graciloplasty. Furthermore it is postulated that this form of stimulation makes it less necessary for the muscle to replace fast twitch, type II, glycolytic fibers by slow twitch, type I, oxidative fibers, preserving strength and responsiveness, which becomes important in the next two chapters.

Chapter 4

Closed-loop Control:

feasibility in a neo-sphincter model



"Minds are like parachutes; they work
best when open" -Lord Thomas Dewar-

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Based upon the articles:

Zonnevylle E, Perez Abadia G, Somia N, Stremel R, Maldonado C, Koenig S, Palacio M, Werker P, Kon M and Barker J. Feedback (closed-loop) control of a urinary graciloplasty neo-sphincter. Surgical Forum 49: 314-316, 1998.

Zonnevylle E, Somia N, Perez Abadia G, Stremel R, Maldonado C, Werker P, Kon M and Barker J. Three parameters optimizing closed-loop control in sequential segmental neuromuscular stimulation. Artificial Organs 23: 388-391, 1999.

Zonnevylle E, Perez Abadia G, Somia N, Kon M, Barker J, Koenig S, Ewert D and Stremel R. A technique for sequential segmental neuromuscular stimulation with closed-loop feedback control. Journal of Investigative Surgery 15(2): 91-99, 2002.

and presented (in part) at:

the 6th Vienna International Workshop on FES 1998, Vienna, Austria.

the 84th Annual Surgical Forum at the American College of Surgeons 1998, Orlando, Florida. U.S.A.

Abstract

In dynamic myoplasty, dysfunctional muscle is assisted or replaced with skeletal muscle from a donor site. Electrical stimulation is commonly used to train and animate the skeletal muscle to perform its new task. Owing to simultaneous, tetanic contractions of the entire myoplasty, muscles are deprived of perfusion and fatigue rapidly causing long-term problems such as excessive scarring and muscle ischemia. Sequential segmental neuromuscular stimulation contracts part of the muscle while other parts rest, thus significantly improving blood perfusion. Nevertheless, the muscle still fatigues. In this chapter, we report the feasibility of using closed-loop control to economize the contractions of the sequentially stimulated myoplasty. A simple stimulation algorithm was developed and tested on sequentially stimulated neo-sphincters designed from a canine gracilis muscle. Pressure generated in the lumen of the myoplasty neo-sphincters was used as feedback to regulate the stimulation signal via three control parameters, thereby optimizing the performance of the myoplasty. Additionally, we investigated and compared the efficiency of amplitude and frequency modulation techniques. Closed-loop control enabled the maintenance of target pressures within 10% deviation, using amplitude modulation and optimized control parameters. The large-scale stimulation/feedback setup was unsuitable for chronic experimentation, but can be used as a blueprint for a small-scale version to reveal the theoretical benefits of closed-loop control in chronic experimentation.

Introduction

Dynamic myoplasty is an evolving surgical procedure in which dysfunctional muscle tissue is assisted or replaced with skeletal muscle from a less critical donor site. Cardiomyoplasty and graciloplasty have been the most promising clinical applications of this technique to date. In cardiomyoplasty, the latissimus dorsi muscle is wrapped around the heart and electrically paced in synchrony with the cardiac rhythm, assisting the heart to contract more effectively.[Carpentier 1985, Chachques 1989 & 1997] In graciloplasty, the gracilis muscle is wrapped around either the urethra or anal canal and is continuously electrically stimulated to prevent incontinence.[Williams 1989, Baeten 1991& Janknegt 1992] Electrical stimulation has been most commonly accomplished by delivering a voltage pulse train to the latissimus and gracilis muscles.[Hallan 1990, Lucas 1991 & Grandjean 1996] More recently, biphasic pulse train input[Scheiner 1990] and differential electrode configurations have been employed. These techniques apply a single source voltage, which causes all of the muscle fibers to contract simultaneously. Continuous contraction of all of the muscle fibers is a non-physiological approach and consequently, the muscle fatigues quickly.

In order to improve endurance, muscle-training programs have been designed to transform the myoplasty from a fast twitch, fatigable muscle to a slow twitch, fatigue resistant muscle at a cost of reduced power.[Salmons 1969, 1976 1981 & 1994, Pette 1973 & 1975, 1984 & Chachques 1987] This training protocol generally takes up to 8 weeks, delaying the assistance from the myoplasty. Furthermore, in graciloplasty for urinary incontinence, continuous contraction of the myoplasty at the high pressures required maintaining continence during peak bladder pressure and (short) periods of extraneous pressure (e.g., coughing or lifting heavy objects) can cause muscle scarring, stricture, and ischemic lesions.[Williams 1993, Konsten 1993, Baeten 1995, Janknegt 1995, Geerdes 1996, van Aalst 1998 & Madoff 1999]

In chapters 2 and 3, sequential stimulation was introduced as an alternative methodology to single source electrical stimulation. In this technique the skeletal muscle is partitioned into segments, which are alternately stimulated in a sequential order. This approach causes only one segment of the muscle to perform the necessary workload, thereby allowing the other non-stimulated

segments to recover. This and similar techniques have been shown to reduce muscle fatigue[Petrofsky 1979, Pournezam 1988 & Thoma 1991]; however, the muscle is still susceptible to fatigue and eventually fails.

Ideally, a dynamic myoplasty should perform the required physiological function indefinitely. In this chapter, sequential segmental neuromuscular stimulation is combined with a new closed-loop pressure feedback control scheme. This created the condition in which it is possible to reduce the amount of work the myoplasty performs to the minimal level necessary to carry out the required physiological function for prolonged periods. As a model a neo-sphincter was chosen, created using a gracilis muscle as used in dynamic graciloplasty for treatment of incontinence.

Materials and Methods

In a dog experimental model (n=8), triple partitioned neo-sphincters were created around pressure measuring devices and sequentially stimulated. Via an input/output device, a computer acquired the generated pressures and provided the stimulation signals to the neo-sphincters. The measured pressures were compared to target pressures and deviations were corrected by adjusting the stimulation signals using a closed-loop pressure feedback control algorithm. Constants and parameters of this algorithm were evaluated for their ability to generate target pressures and minimize pressure fluctuation.

Surgical technique

After a pilot involving three dogs a final study design was composed in which both gracilis muscles of mongrel dogs (15-20 kg; approx. 6 months of age) were dissected from their surrounding tissue and left attached only by their primary blood/nerve supply. The muscles were then tailored into 3.5 cm X 12 cm size and were wrapped around artificial urethras and catheter balloons in the shape of neo-sphincters. Pressure in the artificial urethras was regulated using connected water columns, providing approximately 5 cm H_2O less pressure than the target pressure. The muscles were functionally divided into three separately

stimulatable units using three pairs of stainless steel wire electrodes connected to three isolated sources of electrical stimulation (see figure 1).

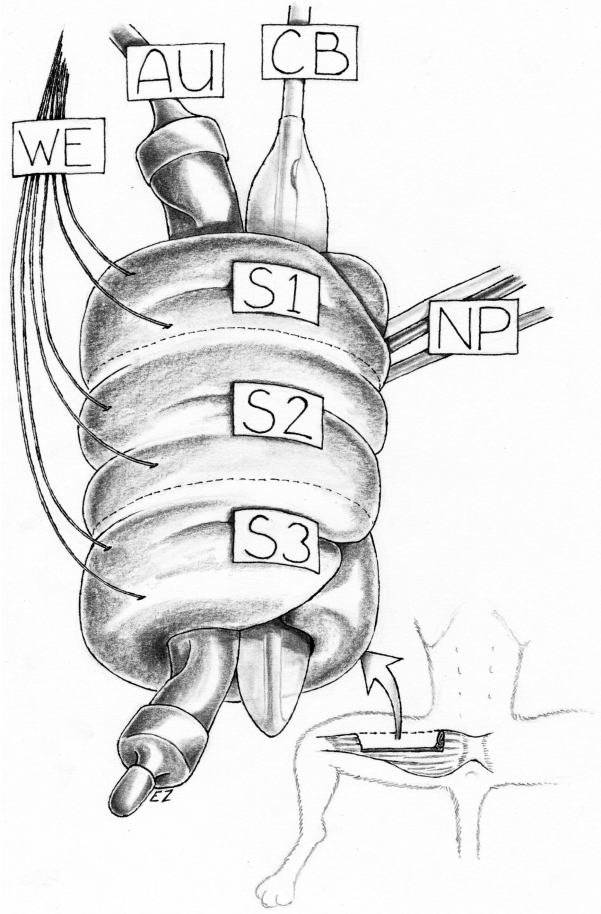


Figure 1. Surgical procedure: Neo-sphincters were constructed using canine gracilis muscles isolated on their main neurovascular pedicles (NP). Each neo-sphincter was divided into three individually stimulatable segments (S1, S2, S3, arbitrary borders by dashed lines), using three pairs of wire electrodes (WE). Neo-sphincters were wrapped around artificial urethras (AU) and pressure measuring catheter balloons (CB).

Sequential stimulation and pressure feedback control instrumentation

The stimulation setup consisted of a desktop computer (Gateway 2000 Pentium 200/80, Sioux Falls, SD), an input/output data acquisition (I/O) device (CED 1401^{plus}, Cambridge Electronic Design, Cambridge, England), three linear stimulus isolators (World Precision Instruments, Sarasota, FL), and Teflon-coated stainless steel wire stimulation leads (0.007 inch in diameter, Medwire®, Mount Vernon, NY). The desktop computer and I/O device, which generated digital-to-analog waveforms and/or analog-to-digital data files, were used to

produce three independent segmental stimulation voltage signals. These signals had user-selectable characteristics (i.e. amplitude, pulse width, etc.) that were programmed using custom written scripts (see appendix) in data acquisition software (Spike2, v2.21, Cambridge Electronic Design, Cambridge, England). The stimulation profiles that were generated were applied to the individual segments through isolation amplifiers via the stimulation leads. The isolation amplifiers eliminated potential conduction pathways between stimulated and non-stimulated leads, thereby preventing non-stimulated muscle segments from inadvertently contracting. Balloon dilatation catheters (BMX/8-3/5.8/120, Boston Scientific Corporation, Quincy, MA) were used to measure the pressures generated by artificial neo-sphincters, as described in the surgical technique section. The pressure in the balloon was measured using a pressure-transducer (P23 ID, GultonStatham Inc., CA, USA), which was amplified using a CED1902 signal conditioner (Cambridge Electronic Design, Cambridge, England). The measured pressure was converted from analog-to-digital format by the I/O device. Custom control software was used to adjust stimulation parameters using the difference between actual and target pressures (see figure 2).

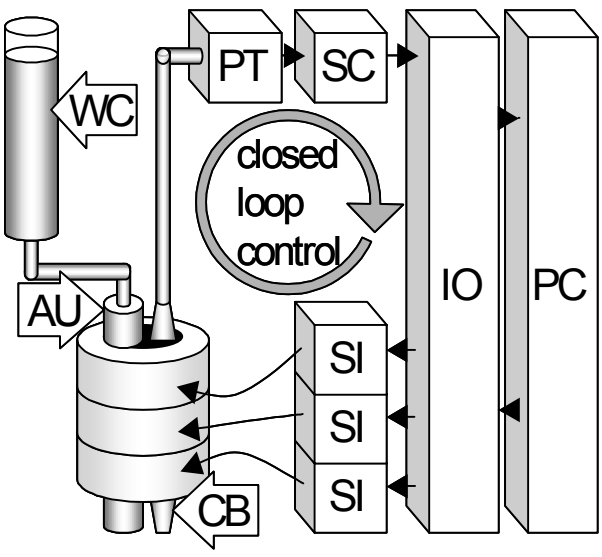


Figure 2. Segmental stimulation setup with closed-loop pressure feedback control: a personal computer (PC) ran a closed-loop control algorithm. Information was converted to stimulation signals with an input/output device (IO) and isolated via 3 linear stimulus isolators (SI). A catheter balloon (CB) transferred the actual pressure in the neo-sphincter to a pressure transducer (PT). A signal conditioner (SC) fed the actual pressure to the input/output device, which converted the signal and passed it to the personal computer as input for the closed-loop control algorithm. The neo-sphincter was also wrapped around an artificial urethra (AU), which collapsed during stimulation. The artificial urethra was connected to a water column (WC), providing static H_20 pressure lower than the target pressure.

Closed-loop control algorithm

Closed-loop control was accomplished using amplitude or frequency modulation. When amplitude modulation was selected, the output of the I/O device was a monophasic pulse train with variable amplitude, a pulse width of 500 μs, and pulse trains generated at 30 Hz. In the case of frequency modulation, the frequency was varied while the amplitude of each individual segment was fixed at 2.5 times its twitch threshold defined as the minimal value of constant current to produce a measurable twitch. A 'multiplication factor' was used to convert the pressure error to the stimulation parameter adjustment. The multiplication factor (K) was set at K = 2.0 for amplitude modulation and K = 0.5 for frequency modulation. When the difference between the actual pressure (P_A) and the target pressure (P_T) exceeded the 'correction threshold' the stimulation signal was adjusted at the rate of the 'correction frequency'. The stimulation signal was not adjusted during the interval after the start of stimulation of a new segment, which was defined as the 'transition time'. Segment stimulation periods were set to 1-second time intervals with individual segments being stimulated in a sequential order. The control algorithm equations for amplitude and frequency modulation (Equation 1) are shown below.

Equation 1

$$\Delta_{n+1} = K\left(P_T(n) - P_A(n)\right)$$

$$\textit{Correction Factor} = \sum_{n=1}^{\infty} \Delta_n$$

Study parameters and data acquisition

As described in the surgical technique section, an artificial neo-sphincter (graciloplasty) was created. The balloon pressure catheter was inflated to150 cm H_2O, in order to minimize its compliance. Subsequently, any pressure

generated by the neo-sphincter was measured as an increase in pressure over150 cm H_2O. First, optimal values of the muscle stimulation parameters were determined. This was performed by choosing a target pressure and determining the 'correction frequency', 'correction threshold', and 'transition time' that produced the shortest time to reach the target pressure, a mean measured pressure closest to the target pressure, and the smallest standard deviation of the mean measured pressure. These measurements were amplitude modulated and started at t=0 seconds with a minimal stimulation signal and a target pressure 30 cm H_2O higher than resting pressure (150+30=180 cm H_2O). After reaching and maintaining the target pressure, elapsed time was measured and recorded at a sampling frequency of 10 Hz. Thereafter, the mean and standard deviation of the actual pressure was measured and calculated over a 15-second time interval. Ideal settings for the 'correction frequency', 'correction threshold', and 'transition time' were experimentally determined using the ranges shown in table 1. The order of every 8 steps in these ranges was rotated over the 8 times the experiments were conducted, to avoid bias in the measurements caused by fatigue of the neo-sphincters.

Table 1. Parameter ranges used for determining ideal settings.

Parameter	Range	Increment
Correction Frequency	1 - 8 Hz	1 Hz
Correction Threshold	1 - 16 %	1,2,3,4,6,8,12,16%
Transition Time	0 - 0.7 seconds	0.1 seconds

Using the ideal settings for 'correction frequency', 'correction threshold', and 'transition time', determined earlier in the three pilot experiments, the above described measurements were performed on the contra-laterally created neo-sphincters, comparing amplitude modulation to frequency modulation for target pressures in the range of 10-80 cm H_2O (in steps of 10 cm H_2O) over resting pressure. The same rotation order was used in this part of the experiments.

Data analysis

The time to reach target pressure was calculated as the elapsed time between starting time and the time when target pressure was reached and maintained for three seconds (one full cycle of sequential stimulation) within a maximum allowed deviation of 20% of the target pressure. The shortest time (fastest response) was considered optimal corresponding to the ideal setting for each of the three control parameters. During the 15-second period after reaching the target pressure, 150 pressure samples were obtained. From these samples, the mean and standard deviation were calculated. The mean value most resembling the target pressure and the smallest standard deviation were considered optimal.

Statistical analysis, determining the overall (n=8) optimal values for the three muscle stimulation parameters, was performed using Friedman Repeated Measures Analysis of Variance on Ranks and All Pairwise Multiple Comparison Procedures (Dunnett's Method). Differences between amplitude and frequency modulation were analyzed using paired t-tests for means, after checking the distribution of the values for normality using the Kolmogorov-Smirnov method.

Results

Stimulation signals were adjusted to attain and maintain the set target pressures, indicating that pressure feedback was operational. In figure 3, the results of one measurement are depicted. The means of each individual segment are shown. This demonstrates that each segment, while acting individually, is undergoing its own path of corrections.

The value for the ‘correction frequency’ for the shortest time to reach the required target pressure was 8 Hz. The optimized value for the smallest standard deviation while maintaining target pressure was 4 Hz. A ‘correction frequency’ of 1 Hz. did not result in a controllable situation (see figure 4). The optimal value for the ‘correction threshold’ was 4% of the target pressure for both the shortest times to reach the required pressure and the smallest standard deviation (see figure 5). The optimal value for the ‘transition time’ was 0.3

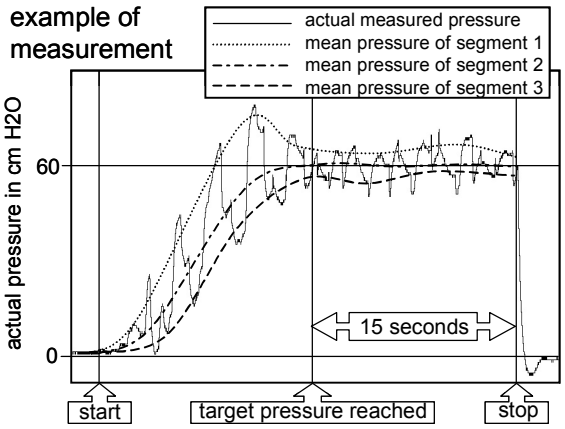


Figure 3. The stimulation signals were modulated in order to reach and maintain target pressure. In this example, the setup attained 60 cm H_2O using amplitude modulation (freq. corr.: 4 Hz, corr. thresh. 4%, transit time: 0.3 sec.). Segment 1 of the neo-sphincter overshot the target pressure before reaching it causing a correction of the stimulation signal, while segment 2 and 3 showed less variation. Thereafter the setup maintained the target pressure within a certain error for 15 seconds.

seconds for both the shortest time to reach the required pressure and the smallest standard deviation (see figure 6). All these findings were statistically significant ($p<0.05$). The means of the actual pressures were not statistically significant different and did not provide differentiation for optimizing settings of the tested stimulation parameters (see figures 4, 5 and 6).

Amplitude modulation more accurately maintained the range of target pressures than frequency modulation: The means of actual pressures more closely resembled target pressures and standard deviations were statistically significant smaller for all target pressures ($p<0.05$). However, using frequency modulation, target pressures were faster attained, when the gap between actual and target pressures was larger (see first graph in figure 7). Almost all differences were statistically significant ($p<0.05$) and are marked with an asterix in figure 7. The standard deviations resulting from the amplitude-modulated measurements were smaller than 10% of the target pressure (see figure 7). Thus, selecting the optimized values for the tested stimulation parameters in combination with amplitude modulation of the stimulation signal offers the ability to regulate the sequentially stimulated neo-sphincter pressure within 10% deviation of the target pressure in the range of 10-80 cm H_2O.

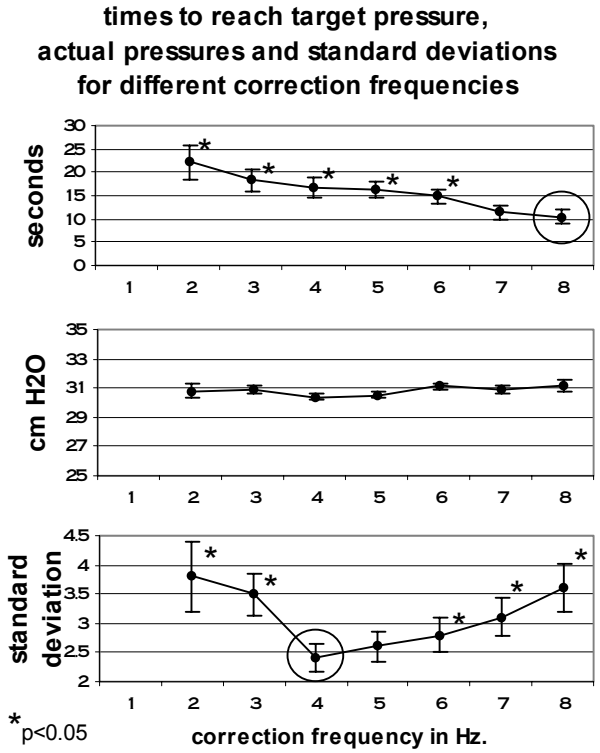


Figure 4. The 'correction frequencies' showed fastest time to reach target pressure at 8 Hz. and the optimized value for smallest standard deviation while maintaining target pressure was 4 Hz. No differentiation could be made concerning the mean of the actual pressures. A correction frequency of 1 Hz. did not result in a controlled situation.

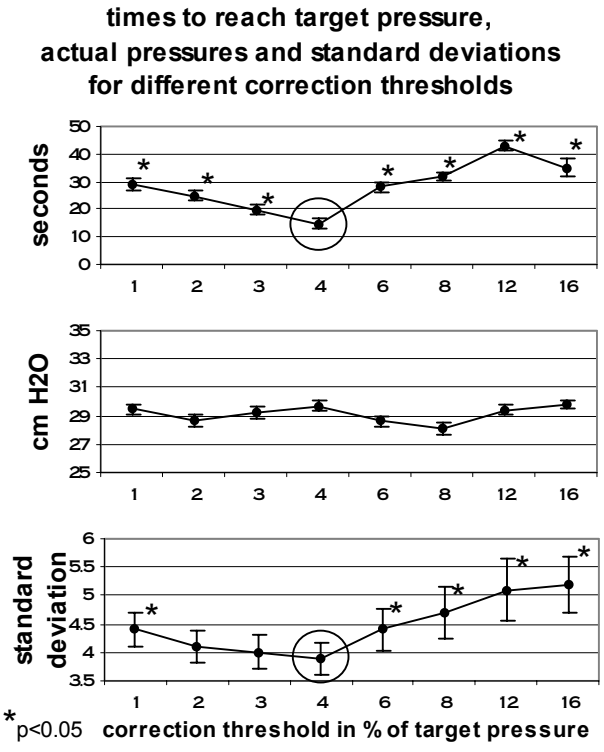


Figure 5. The optimal 'correction threshold' showed fastest time to reach target pressure and smallest standard deviation while allowing the actual pressure to deviate up to 4% of the target pressure before correction of the stimulation signal was executed. No differentiation could be made concerning the mean of the actual pressures.

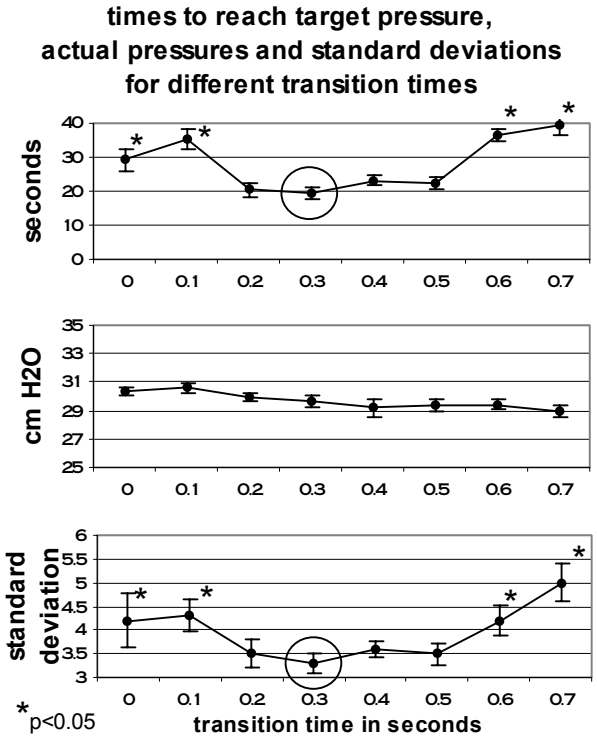


Figure 6. The optimal 'transition time' showed fastest time to reach target pressure and smallest standard deviation while maintaining a correction free period of 0.3 seconds after sequential stimulation switching to the next segment of the neo-sphincter. No differentiation could be made concerning the mean of the actual pressures.

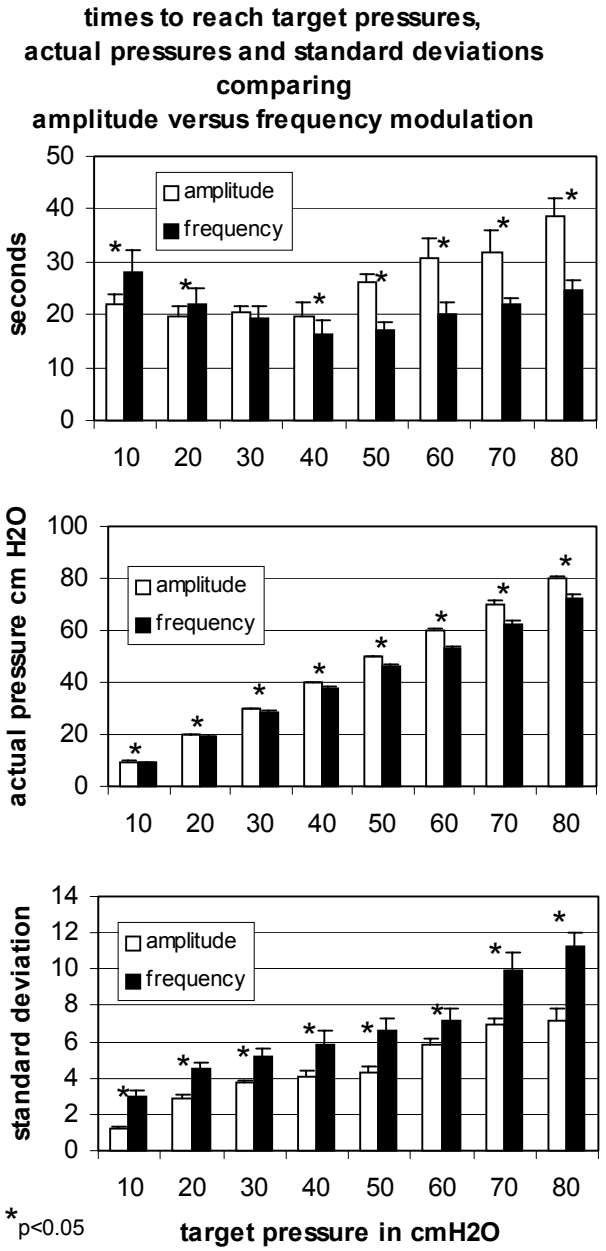


Figure 7. Target pressures were reached quicker using frequency modulation. However amplitude modulation was more accurate than frequency modulation in maintaining the range of target pressures: the standard deviations of the actual pressures were lower and the means more closely resembled target pressures.

Discussion

Physiological muscle fatigue is primarily due to depletion of local energy. This local energy is stored chemically in the muscle tissue as adenosine triphosphate (ATP). In the process of contraction, ATP is chemically downgraded into adenosine diphosphate (ADP). This breakdown of ATP releases the energy necessary for myofilament interaction and contraction. Metabolism of nutrients and the consumption of oxygen constantly regenerate ATP. The oxygen and nutrients are replenished by blood flowing through the muscle tissue. Therefore, a given amount of maintained muscle contraction demands a certain amount of metabolism that in turn requires a given amount of blood flow in the muscle tissue. When the demand exceeds the amount of replenishment (via the blood flow), ATP concentrations can only be maintained through non-oxygen requiring metabolism. The result is lactic acid production and muscle fatigue. Furthermore, contraction of muscle tissue increases tissue pressure, which is associated with vessel occlusion and reduced blood flow. Therefore, during electrical stimulation of the muscle, the force of the contraction can result in reduced regional blood flow, leading to fatigue.

Conventional stimulation techniques usually stimulate all muscle fibers simultaneously by applying a single stimulus that results in rapid muscle fatigue. An advance to the conventional technique is to stimulate segments of a muscle independently in a sequential fashion, such that one segment produces the required force for a short time interval, while the non-stimulated segments relax and recover by receiving adequate local reperfusion. The number of segments and stimulation time intervals can be optimized to further delay the onset of muscle fatigue, but cannot eliminate it entirely. Muscle fatigue is still caused by full contraction over prolonged periods, while maximum contractile force is not always necessary. A method of preventing long-term muscle fatigue would be to require the muscle to economically perform in proportion with the actual needs. Therefore, some form of performance regulating control needs to be established.

In this chapter, the concept of closed-loop control was used to regulate the performance of a sequentially stimulated myoplasty, proving its feasibility. However, closed-loop control has a tendency to oscillate. This is caused by the

constant cyclic repetition of evaluation of the current performance, followed by correction. Several parameters were introduced in the algorithm to damp oscillations and improve effectiveness.

Corrections have a latency before they are effective, which is system specific. If the corrections overtake their own application, caused by system latency, the closed-loop control starts to oscillate. Meanwhile, the performance directed with this closed-loop control has a tendency to diverge within a certain time, which needs correction. Therefore, the frequency of corrections was evaluated on efficiency and showed an optimum value at 4Hz, while maintaining target pressure. At lower frequencies, corrections were relatively late allowing greater deviations. At higher frequencies, adaptation of the neo-sphincter to corrected stimulation amplitudes was too slow to efficiently establish a new pressure before another round of evaluation resulted in another correction. This led to over-correction of the amplitudes and overshooting of the target pressure, which augmented oscillations. Using 8 Hz. as the 'correction frequency', target pressure was reached fastest, due to the rapid successive corrections. However, after reaching target pressure, larger oscillations occurred during maintenance of the target pressure, making 8 Hz. less favorable.

The control over performance output is not infinitely precise. Correcting for small deviations of performance output, while the corrected signal will not apply this intended correction promptly, will be repeated cycle by cycle until a threshold is passed and the performance output reacts with an overshoot of the intention. To damp these overshoots, a certain deviation must be allowed, while at the same time the threshold for corrections should be as low as the system allows, preventing gross deviations. The threshold for corrections showed an optimum in speed and efficiency at a value of 4% of the target pressure. With lower thresholds the algorithm also compensated for minimal differences between actual and target pressure. Being insufficient in this range under 4% of the target pressure, over-correction augmented oscillations. With higher thresholds the system was more indifferent to deviations, allowing unnecessary inefficiency.

The algorithm controlled all three segments of the sequentially stimulated neo-sphincter separately, each having its own amplitude and individual correction of this amplitude. When switching from one segment to the next, the muscle and thus the pressure feedback did show a delay. Therefore, it was possible that a correction in amplitude was executed on a just starting segment, based on pressure feedback information actually referring to the previously stimulated segment. This faulty information led to poor corrections in amplitude of the stimulation signal. Therefore, a transition time in which no corrections were fulfilled was introduced. An optimum was found around 0.3 seconds. Shorter transition times showed the above-described interference of different segments and their pressure feedback, resulting in higher standard deviations. Longer transition times also resulted in less efficiency, simply caused by the lack of corrections during stimulation.

Further refinements in accuracy can be achieved by further development of the controlling algorithms and by including additional parameters. Even greater accuracy can be expected from so called 'intelligent/self-learning' algorithms.

Amplitude modulation of the stimulation signal delivered more accurate performance regulation than frequency modulation, although, in our model, frequency modulation attained target pressure faster at start up, when larger differences between actual pressure and target pressure had to be bridged. These findings suggest that frequency modulation produces grosser neo-sphincter reaction. During experimentation it proved difficult to balance the size of change in the frequency and the resulting change in performance. On the other hand, modulation of the amplitude resulted in predictable reactions of the performance.

A major advantage of closed-loop control is that it is self-regulating under changing conditions. Short-term changes like pressure fluctuations in the abdomen (e.g. during lifting heavy objects) can be met while maintaining continence in graciloplasty. Adaptation to long-term changes is automatically executed. For instance, the stimulation signal can be turned up, while effectiveness of the electrodes decreases due to scar formation in the first months of implantation.

Ultimately, combining sequential stimulation and closed-loop control has the potential for fine-tuning the performance of a myoplasty so that it performs the precise function required, continuously and dynamically adapting according to need. In the case of dynamic graciloplasty to treat urinary or fecal incontinence this type of self-controlling stimulation would provide advantages over existing treatments. This acute experiment has laid the theoretical groundwork for further experiments that will develop and test the necessary devices and principles that will enable this concept to be applied in a chronic protocol.

Limitations

Some difficulties need to be overcome before the presented concept can be applied in chronic experiments. Among others, the methods used to gather or sense the feedback information must be improved. The currently used sensor setup is completely unsuitable for chronic experimentation, because of the size, immobility and constant need for re-calibration. However, sensor technique is rapidly evolving and the latest miniature pressure sensors are well suited to perform in downsized chronic closed-loop control setups. Other new sensors measuring stress, strain or tension could also be used in a whole new variety of applications of closed-loop controlled myoplasties, providing accurately regulated performance.

Another difficulty will be electrode design. Both sequential stimulation and closed-loop control require delicate and very precise placement of the electrode tips. The Teflon coated stainless steel wires with bared tips used in the current setup could be precisely placed after numerous attempts without inflicting too much damage to the muscle tissue. However, these electrodes will break during chronic experimentation, requiring more solid, probably helix-formed, versions. Insertion of more solid, thicker electrodes in muscle tissue inflicts much more damage and requires a perfect placement in one attempt. Therefore, more knowledge is required about electrode placement and design in closed-loop controlled sequentially stimulated dynamic myoplasty.

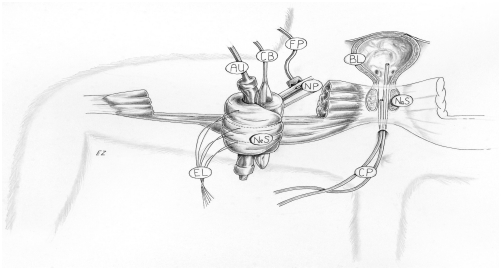
Conclusion

The concept of closed-loop control was explored to economize the performance of a sequentially stimulated dynamic myoplasty, in order to minimize muscle fatigue and long-term scar formation, when applied chronically. Based on our findings, this concept appears to be feasible and should be further developed toward application in a chronic setting. These findings contribute to the development of improved methods for applying dynamic myoplasty in the clinical setting.

Chapter 5

Sequential stimulation and closed-loop control:

application in a graciloplasty model



"Whenever you find yourself on the side of the majority, it's time to pause and reflect" -Mark Twain-

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Based upon the article:

Zonnevylle E, Perez Abadia G, Stremel R, Maldonado C, Kon M and Barker J. Seqential closed-loop electrical stimulation of a urinary neo-sphincter. (submitted).

Abstract

Muscle tissue transplantation applied to regain or dynamically assist contractile functions is known as “dynamic myoplasty”. Success rates of clinical applications are unpredictable, because of lack of endurance, ischemic lesions, abundant scar formation and inadequate performance of tasks due to lack of refined control. Electrical stimulation is used to control dynamic myoplasties and should be improved to reduce some of these drawbacks. Sequential segmental neuromuscular stimulation improves the endurance and closed-loop control offers refinement in rate of contraction of the muscle, while function-controlling stimulator algorithms present the possibility of performing more complex tasks.

An acute feasibility study was performed in anesthetized dogs combining these techniques. Electrically stimulated gracilis-based neo-sphincters were compared to native sphincters with regard to their ability to maintain continence. Measurements were made during fast bladder pressure changes, static high bladder pressure and slow filling of the bladder, mimicking among others posture changes, lifting heavy objects and diuresis.

In general, neo-sphincter and native sphincter performance showed no significant difference during these measurements. However, during high bladder pressures reaching 40 cm H_2O the neo-sphincters maintained positive pressure gradients, whereas most native sphincters relaxed. During slow filling of the bladder the neo-sphincters maintained a controlled positive pressure gradient for a prolonged time without any form of training. Furthermore, the accuracy of these maintained pressure gradients proved to be within the limits setup by the native sphincters. Refinements using more complicated self-learning function-controlling algorithms proved to be effective also and are briefly discussed.

In conclusion, a combination of sequential stimulation, closed-loop control and function-controlling algorithms proved feasible in this dynamic graciloplasty-model. Neo-sphincters were created, which would probably provide an acceptable performance, when the stimulation system could be implanted and further tested. Sizing this technique down to implantable proportions seems to be justified and will enable exploration of the possible benefits.

Introduction

In reconstructive surgery, dynamic myoplasty is a challenging but limited area of investigation with few clinical applications. Muscle tissue transplantation aiming to regain or dynamically assist contractile functions could theoretically fit a large number of purposes, but still remains futuristic for the most part. However, one example of such a clinical application is the dynamic graciloplasty. In graciloplasty, the gracilis muscle flap is wrapped around the urinary or fecal outlet and is stimulated to contract continuously to replace the native sphincter function.(Baeten 1991, Janknegt 1992, Williams 1991 & 1993) Nevertheless, success rates of clinical applications of dynamic myoplasties vary.(Grandjean 1996, Chachques 1997, Konsten 1993, Janknegt 1995, Geerdes 1996 & Baeten 2000) Some drawbacks hindering dynamic myoplasties are fatigue or lack of endurance of the muscle tissues, ischemic lesions, abundant scar formation of the adjacent tissues and inadequate performance of tasks due to lack of refined stimulation control.(Merell 1986, van Aalst 1998 & Bardoel 2002)

Most commonly a single source voltage pulse-train accomplishes stimulation of the entire dynamic myoplasty, resulting in non-physiologic complete, spasm-like muscle contraction and thus intra-muscular ischemia due to constant pressure on the microcirculation. As described in chapter 2 and 3, sequential stimulation provides an alternative methodology to single source electrical stimulation by partitioning the myoplasty into segments. These segments are alternately stimulated in a sequential order, allowing some segments to have temporary hyperemia, while other segments are contracting. This form of stimulation and similar techniques have shown to enhance intra-muscular perfusion and prolong endurance,(Peckham 1970, Pournezam 1988, Thoma 1991 & Lau 1995) meanwhile lessening the need for a training regimen, which builds endurance at the cost of responsiveness and power of the dynamic myoplasty by fiber transformation.(Salmons 1969, 1976, 1981 & 1994, Sréter 1973, Pette 1975 & 1984)

In chapter 4 the feasibility and effectiveness of closed-loop control is described. It offers the possibility for dynamic myoplasties to precisely tune the amount of force generated to the level necessary to carry out the required physiological function.(Quintern 1997 & Lemay 1997) Implantable sensors offer

biofeedback, which is used in closed-loop control. However, biofeedback can also provide input for computing devices running algorithms, which process decisions concerning dynamic myoplasty performance.

In this chapter the feasibility of a dynamic myoplasty combining sequential stimulation, closed-loop control and algorithm based function control is reported. While designing this study, complexity was kept to an absolute minimum and potential clinical application was not discarded. Therefore, dynamic graciloplasty to treat urine incontinence was selected. The basic design of this dynamic myoplasty was further elaborated with the above-described enhancements. In this acute study on dogs, gracilis based neo-sphincters were sequentially stimulated and the generated pressures were controlled in order to maintain positive pressure gradients with the bladder-pressure. Performance was compared to the native sphincters, while bladder-pressures were varied.

Materials and Methods

In dogs, both gracilis muscles were converted into neo-sphincters and electrically stimulated, using three-channel sequential stimulation with closed-loop pressure feedback control. A computer, combined with an input/output device, recorded pressures of neo-sphincters, native sphincters and the bladder. An algorithm calculated the actual pressure gradients between sphincters and the bladder, comparing it to a predetermined standard and provided modulated stimulation signals to the neo-sphincters. Performance of the neo-sphincters and the native sphincters was recorded and compared while slow and rapid bladder pressure changes were induced.

Animal care

Eight mongrel dogs (15-20 kg; approx. 6 months of age) were studied in this experiment. Prior to the experiment, animals were housed in separate cages at a controlled temperature (i.e., 22 °C) and with a 12-hour light/dark cycle. The animals were fed commercial dog diet and provided with water *ad libitum*. At the termination of the experiment, the dogs were euthanized with an overdose (10

ml, IV) of Beuthanisia (390 mg sodium pentobarbital and 50 mg sodium phenytoin per ml, Schering-Plough Animal Health Corp. Kenilworth, NJ). The protocol for the use of dogs in this study was approved by the Institutional Animal Care and Use Committee (IACUC) and adhered to the NIH and APS "Guide for the Care and Use of Laboratory Animals". Studies were performed in the American Association of Laboratory Animal Care (AALAC) approved Research and Resource Center at the University of Louisville Health Sciences Center.

Experimental Setup

Sixteen neo-sphincters were created in eight dogs using both gracilis muscles. These muscles were wrapped around compressible latex tubes containing fluid at a hydrostatic pressure equal to the bladder pressure and balloon dilatation catheters (BMX/8-3/5.8/120, Boston Scientific Corporation, Quincy, MA, USA; see figure 1).

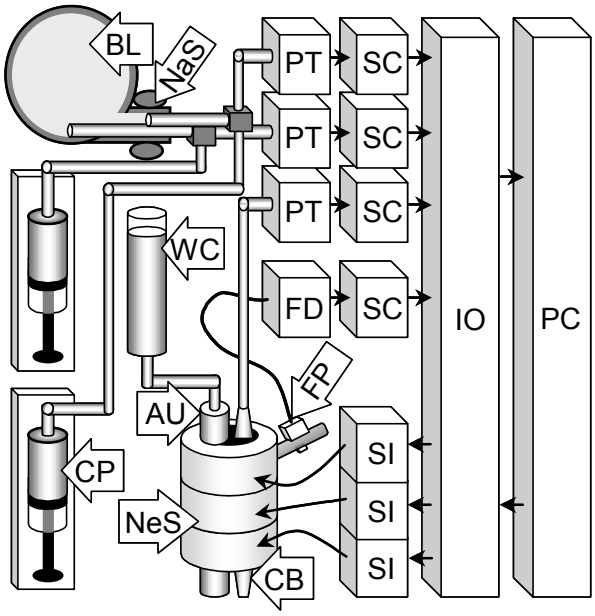


Figure 1: A personal computer (PC) ran an algorithm maintaining the pressure gradient between the neo-sphincter (NeS) and the bladder (BL) by stimulating the neo-sphincter and regulating its output using closed-loop pressure feedback control. Information was converted to modulated stimulation signals with an input/output device (IO) and isolated via 3 linear stimulus isolators (SI). A catheter balloon (CB) transferred the actual pressure in the neo-sphincter to a pressure transducer (PT). Two other pressure transducers were connected to the bladder and the native sphincter (NaS) combined with constant speed perfusors (CP). Three signal conditioners (SC) fed the actual pressures to the input/output device, which converted the signals and passed them to the personal computer as input for the algorithm. The neo-sphincter was also wrapped around an artificial urethra (AU), which collapsed during stimulation. The artificial urethra was connected to a water column (WC), providing static H_20 pressure equal to the bladder pressure. A flow probe (FP), combined with a flow-measuring device (FD) monitored blood perfusion of the neo-sphincter.

Stimulation of the neo-sphincters was performed using an input/output device (CED1401^{plus}, Cambridge Electronic Design, Cambridge, England) in combination with a personal computer (Gateway 2000, Pentium-200/80) and data acquisition software (Spike2, version 2.21, Cambridge Electronic Design, Cambridge, England). Additional customized sequencer files and script files were developed providing closed-loop control to the neo-sphincter (see appendix). Details about the closed-loop control are reported in chapter 4. In addition to the oscillation damping parameters described in chapter 4, an algorithm controlling the neo-sphincter bladder pressure gradient was incorporated in the present work. To create the stimulus, three separate output signals were first led to optical linear stimulus isolators (A395's, World Precision Instruments, Sarasota, FL, USA) and thereafter to a multiple channel custom made switchbox. A three-channel stimulation signal was provided to each neo-sphincter, using three pairs of Teflon coated stainless steel wires (∅ 0.007 inch; Medwire®, Mount Vernon, NY, USA) as leads. Baring the last 8 mm. of the wire created the electrodes, while the other ends were connected to the switchbox. Pressures generated by the stimulated neo-sphincters were recorded using the balloon dilatation catheters connected to pressure-transducers (P23 ID, GultonStatham Inc., CA, USA). Native sphincter pressures and bladder pressures were measured using urine catheters (8 french) combined with constant speed perfusors (model 600-900, Harvard Apparatus, Inc., Holliston, MA, USA) connected to pressure transducers (P23 ID, GultonStatham Inc., CA, USA). Doppler flow probes combined with a dual flow measurement device (T206, Transonic, Ithaca, NY, USA) measured arterial blood flow. Signals of all pressure transducers and flow probes were amplified by CED1902s (Cambridge Electronic Design, Cambridge, England) and recorded with the above mentioned input/output device.

Stimulation

The input/output device (CED 1401^{plus}, Cambridge Electronic Design, Cambridge, England) was used to generate a three-channel sequential stimulation signal (mono-phasic block-shaped pulse-trains; frequency 30 Hz;

pulse-width 500 μ-seconds; transition to next segment after 1.0 second). The customized sequencer and script files controlled the amplitudes of the stimulation signals for each individual segment.

Surgical preparation

All animals were anesthetized (Pentothal 6-12 mg/kg, Abbott Laboratories, North Chicago, IL, USA), intubated and ventilated (Halothane, 1.5%, oxygen 94.5%, nitrous oxide 4%; Halocarbon, River Edge, NY, USA). Animals were placed in the supine position with their hind legs abducted. Bilateral medial incisions were made through which both gracilis muscles were identified and lifted as single pedicled neuro-vascular flaps. The muscles were reduced to a 35 mm width by removing the caudal aspect. Starting from the proximal musculo-tendinious junction, 12 cm. length was marked. These marked muscle-parts were cut from their origins and insertions. Each muscle was tensionless but closely wrapped around a latex tube (∅ 8mm, wall thickness: 0.5 mm.) and a balloon catheter. Three pairs of Teflon coated electrodes were transversely inserted into the neo-sphincters, at an angle of 45 degrees to the direction of the fibers, 5 to 6 cm. distal from the main pedicle, dividing them into three parallel individually stimulatable units (see also figure 2). The latex tubes were connected to a water column providing the hydrostatic pressure equivalent of the bladder pressure. The balloon catheter was inflated to150 cm H_2O, in order to minimize its compliance. Subsequently, any pressure generated by the neo-sphincter was measured as an increase in pressure over 150 cm H_2O. The tips of urine catheters were positioned in the urethra at the level of the native sphincter and in the bladder. The connected constant speed perfusors provided saline at 1 ml.min.$^{-1}$. Flow probes were connected to the arteries of the neuro-vascular pedicles.

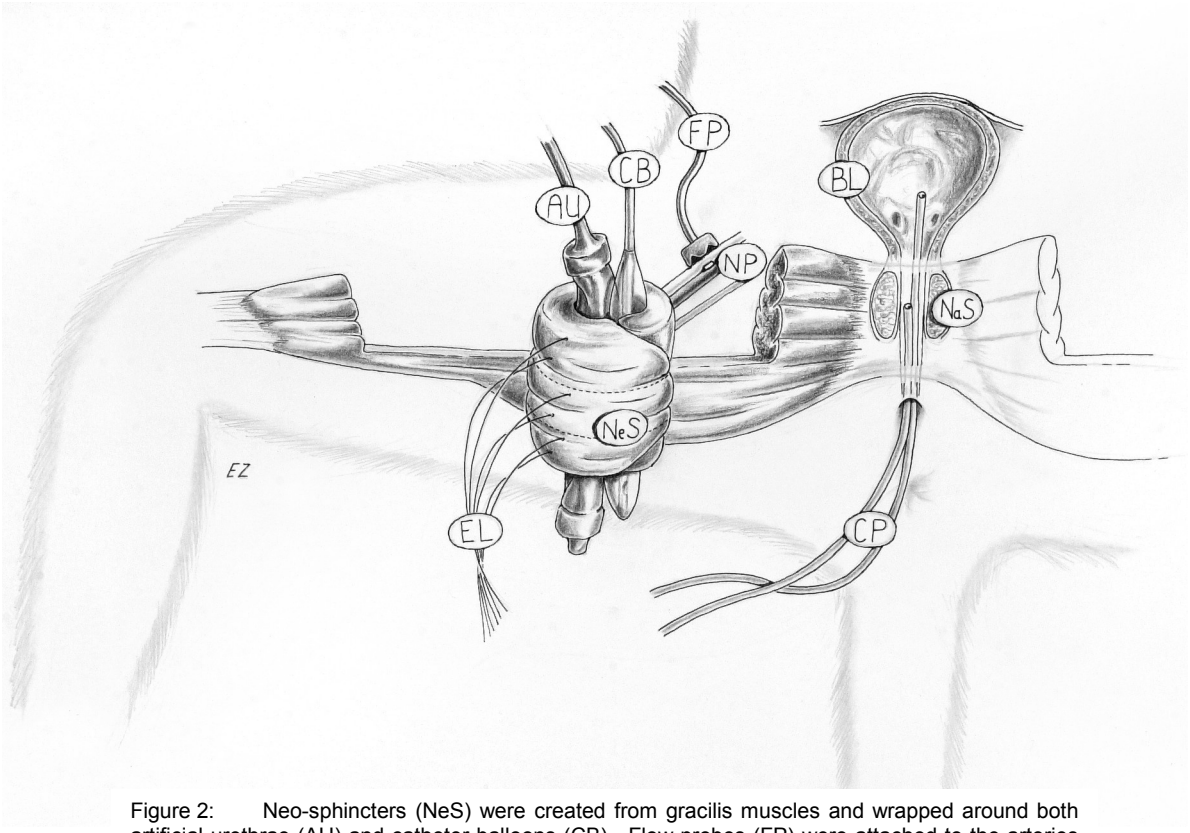


Figure 2: Neo-sphincters (NeS) were created from gracilis muscles and wrapped around both artificial urethrae (AU) and catheter balloons (CB). Flow probes (FP) were attached to the arteries of the single neurovascular pedicles (NP). Catheter probes (CP) were positioned in the bladder (BL) and in the urethra at the level of the native sphincter (NaS).

Measurements

In each dog two neo-sphincters were created and two different sets of measurements were performed in which the adjustments of the neo-sphincters and the native sphincters were assessed. One set contained measurements during rapid bladder pressure changes ('rapid-adjustment measurements') and the other set contained measurements during slow bladder pressure changes ('slow-adjustment measurements'). During all measurements the neo-sphincters were set to produce a 5 cm H_2O positive pressure gradient with the actual bladder pressure using closed-loop control with a tolerance of 2 cm H_2O.

Prior to the rapid adjustment measurements the bladder was filled with 150 ml of saline providing a bladder pressure of approximately 2-5 cm H_2O. Four measurement-blocks were performed, monitoring neo-sphincter and native sphincter pressures during different target pressures of the bladder. While controlling the actual bladder pressure on a computer-screen the abdominal pressure (and thus the bladder pressure) was manually raised within 5 seconds to a target pressure of 20, 30, 40 and 50 cm H_2O for a 60-second block. Thereafter manual pressure was released and bladder pressure dropped back to its baseline within 5 seconds. Pressures of the neo-sphincter, native sphincter and bladder were sampled at a frequency of 10 Hz. during these measurement-blocks. Measurements were spaced in time for at least 6 minutes and target pressures were rotated during subsequent experiments, using the Latin square method, to avoid bias caused by fatigue of the neo-sphincter.

Prior to the slow-adjustment measurement-block the bladder was emptied. During measurement the bladder was filled with saline at a rate of 4 $ml.min.^{-1}$ and pressures of the neo-sphincter, native sphincter, bladder and arterial flow to the neo-sphincter were sampled at a frequency of 10 Hz. until the neo-sphincter or the native sphincter failed to maintain continence due to respectively fatigue or reflex relaxation.

Data analysis

For the rapid-adjustment measurements, the performance of the neo-sphincter and native sphincter were compared during the change of bladder pressure and during the four 60-second blocks of maintained high levels of bladder pressure.

During the 5-second periods of bladder pressure changes, the rate of change (in cm $H_2O.sec.^{-1}$) varied and could be positive (at pressure raise) and negative (at pressure fall) ranging from roughly +6 down to -6 cm H_2O.sec.-1. The actual pressure gradients between the bladder and both the neo-sphincter and native sphincter were captured at representative data points. These points were determined using filtering techniques in which the derivative of the sphincter and bladder pressure had to have a linear relation for at least 1 second. The data-

points were plotted against the rate of bladder pressure change ($\delta P_b/\delta t$; see also figure 3). Cubic regression lines were calculated for both the neo-sphincter and native sphincter adjustment performance (SPSS 9.0, SPSS Inc, Chicago, IL, USA).

During the four 60-second blocks of maintained high bladder pressure, the means and the standard deviation of the pressure gradients between the bladder and both the neo-sphincters and native sphincters were calculated and compared using paired t-tests for each level of bladder pressure (see also figure 3). Also during the periods of continence, while performing the slow adjustment measurements, the means and the standard deviation of the pressure gradients between the bladder and both the neo-sphincters and native sphincters were calculated and compared using paired t-tests.

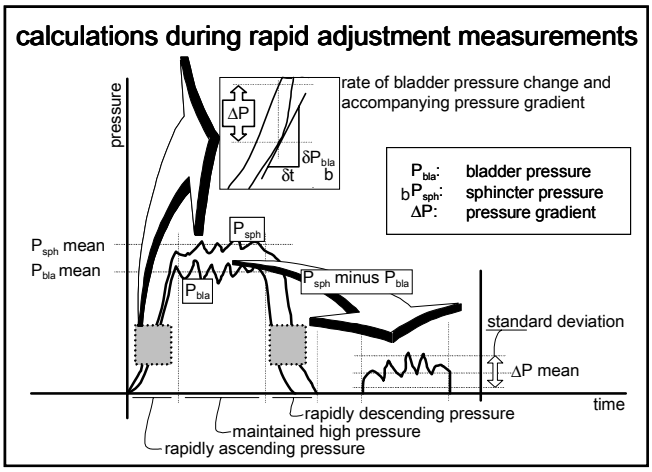


Figure 3: Throughout rapid-adjustment measurements data pairs of the first derivative of the bladder pressure ($\delta P_{bla}/\delta t$) and the pressure gradient of the sphincters (ΔP) were collected during rapid ascending and descending bladder pressures. Furthermore, during maintained high bladder pressure the mean of the sphincters and bladder pressures (P_{sph}mean, P_{bla}mean) as well as the mean and standard deviation of the pressure gradients between sphincters and bladder were calculated from 600 samples collected in 60 seconds.

Results

During the rapid-adjustment measurements, 5-second periods of bladder pressure change both preceded and followed the 60 second-blocks of maintained high bladder pressure. From the recorded data it was possible to assess 158 relevant data-points linking the rate of bladder pressure change and

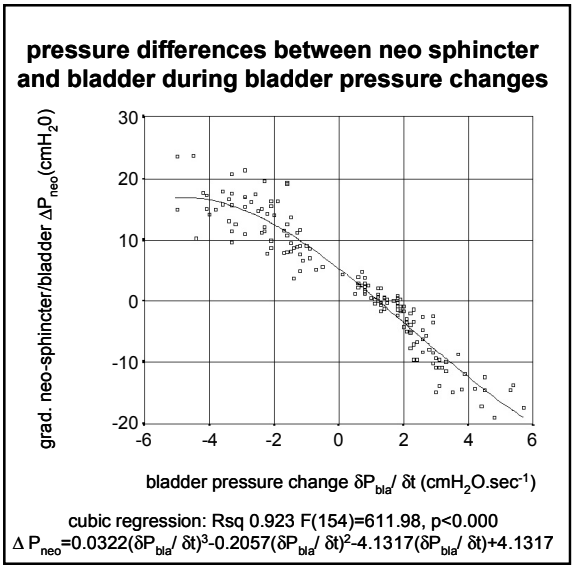


Figure 4a: Plot of the data pairs of the neo-sphincter/bladder pressure gradients (ΔP_{neo}) and the first derivative of the bladder pressure ($\delta P_{bla}/\delta t$). A best fitting cubic regression line was calculated and is drawn in the plot. The controlling algorithm could not provide positive pressure gradients, while bladder pressures rose faster than 1 cm $H_2O.sec^{-1}$.

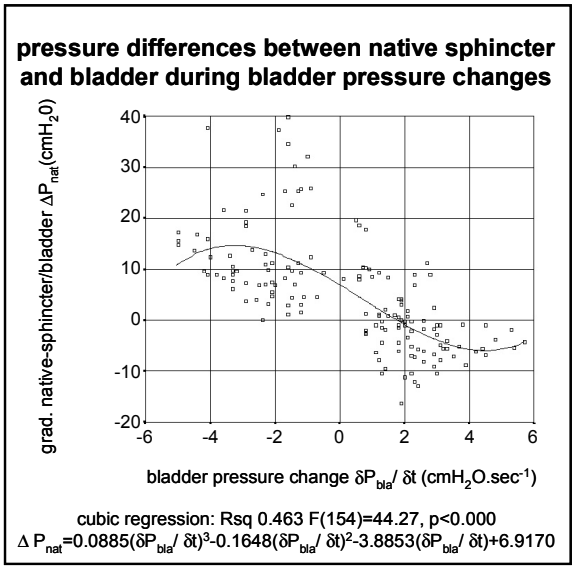


Figure 4b: Plot of the data pairs of the native sphincter/bladder pressure gradients (ΔP_{nat}) and the first derivative of the bladder pressure ($\delta P_{bla}/\delta t$). A best fitting cubic regression line was calculated and is drawn in the plot. In general the native sphincters lost positive pressure gradients, while bladder pressures rose faster than 2 cm $H_2O.sec^{-1}$.

pressure gradients between the bladder pressure and the neo-sphincter as well as the native sphincter pressures. Plots for both the neo-sphincter and the native sphincter performance are depicted in figure 4a and 4b. Cubic regression lines show that especially the neo-sphincters performed predictably ($F(154)=611.98$) and were able to provide a positive neo-sphincter/bladder pressure gradient up to a changing bladder pressure of 1 cm $H_2O.sec.^{-1}$. More rapidly increasing bladder pressures resulted in negative pressure gradients and occlusion of the artificial urethras was (sometimes) lost. The native sphincters showed more or less the same plot but proved less predictable ($F(154)=44.27$) and positive pressure gradients were lost at bladder pressure changes higher than 2 cm $H_2O.sec.^{-1}$.

Performance of the neo-sphincters compared to the native sphincters during the 60-second blocks of high bladder pressures are depicted in figure 5a-c. During all tested bladder pressures the neo-sphincters were capable of maintaining a positive pressure gradient (Fig 5a). The native sphincter generally 'failed' at pressures above 40 cm H_2O, resulting in emptying of the bladder. No statistical differences were found between performance of the neo-sphincters and the native sphincters, except at bladder pressures of 50 cm H_2O in favor of the neo-sphincter (see also figure 5b). The quality of constantly maintaining a pressure gradient between the sphincters and the bladder is determined by calculating the standard deviation of all pressure gradient samples per measurement block as depicted in figure 5c. No statistical differences were found between the quality of the neo-sphincter and the native sphincter.

During the slow adjustment measurements, the performance of the neo-sphincters and the native sphincters were compared while maintaining a pressure gradient with a filling bladder. Figure 6 depicts a typical plot of these measurements combined with averaged outcome values. After almost 49 minutes (mean and SEM: 48.8 +/-7.2) one of both the neo- or native sphincters could not maintain the necessary pressure gradient. At that point, the bladder pressure had reached an averaged pressure of 16 cm H_2O (mean and SEM: 15.9 +/-2.6). In all but two cases the neo-sphincter could not maintain the pressure gradient due to fatigue.

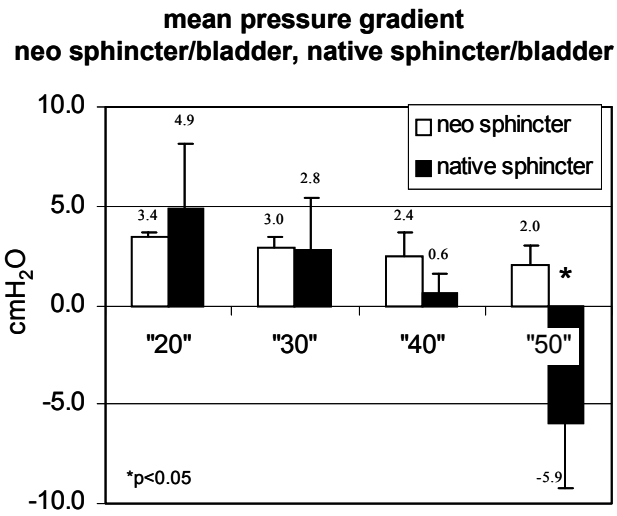


Figure 5a: The means of the pressure gradients performed by both neo-sphincters and native sphincters were equal for bladder pressures ranging from 20 to 40 cm H_2O pressure. However, at 50 cm H_2O bladder pressure a statistically significant difference was found in favor of the neo-sphincter.

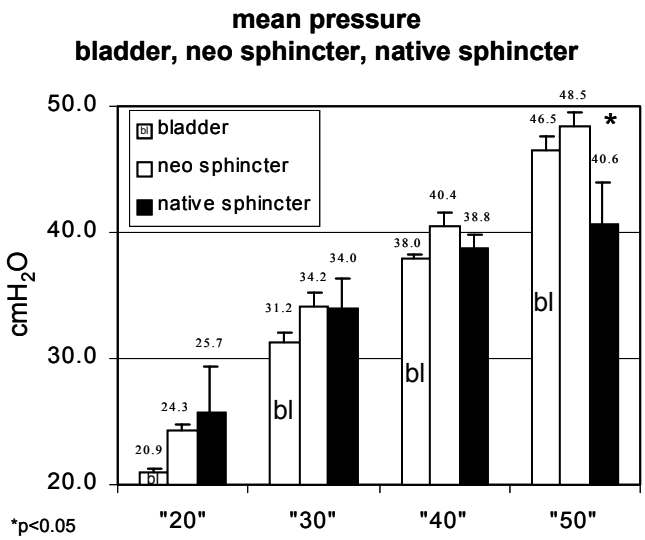


Figure 5b: The means of the neo-sphincter and native sphincter pressures were equal in reaction on high bladder pressures. However, above 40 cm H_2O bladder pressure a statistically significant difference was found in favor of the neo-sphincter.

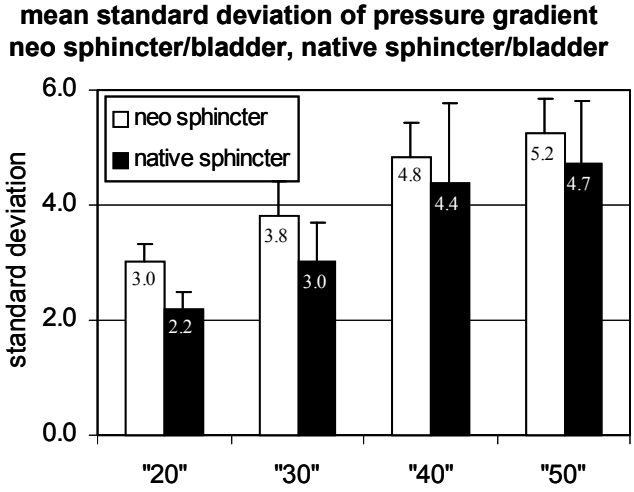


Figure 5c: The mean standard deviations of the pressure gradients indicate their quality of evenness. Performance of both neo-sphincters and native sphincters showed no statistically significant differences for all tested bladder pressures.

Reaching this point at least one segment of the sequentially stimulated neo-sphincter needed a higher stimulation amplitude than the maximum of 5 mAmp. to generate the requested pressure. In the other two cases however, the native sphincter reflexively relaxed and bladder pressure dropped before neo-sphincter fatigue occurred. The relative blood-flow increase of the neo-sphincters during stimulation was more than twofold (mean and SEM: 229% +/-30%). The mean pressure gradient between bladder and both sphincters during the 8 experiments was 3.1 cm H_2O with a SEM of +/-0.2 for the neo-sphincters and 10.1 +/-2.6 cm H_2O for the native sphincters.

The mean of the standard deviations, reflecting the accuracy of the mean pressure gradients, was 2.1 with a SEM of +/-0.2 for the neo-sphincters and 3.7 +/-0.3 for the native sphincters. Both differences were statistically significant (see figure 7).

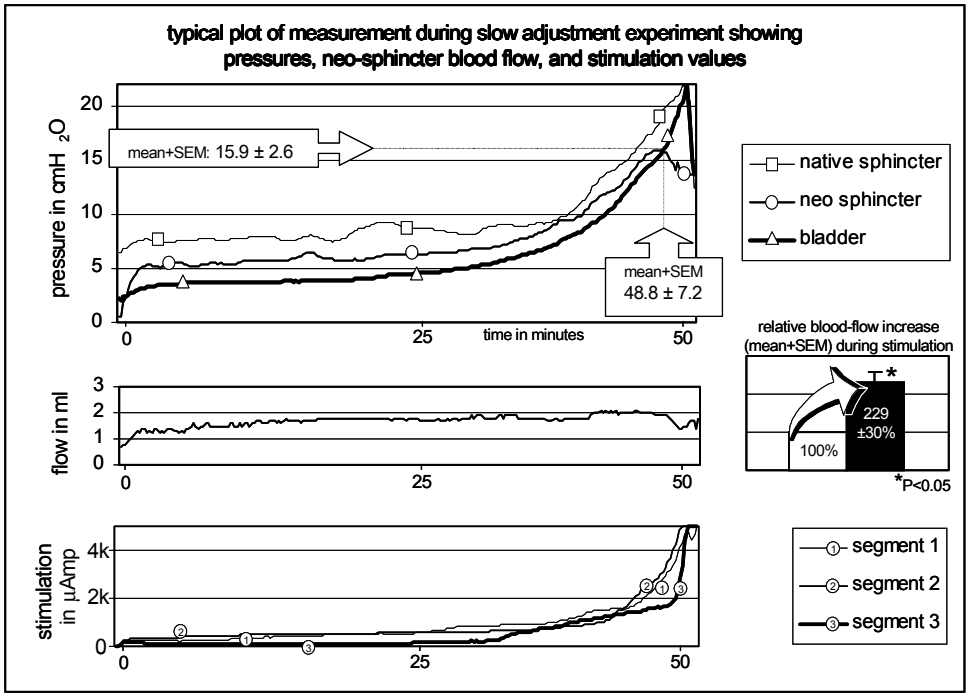


Figure 6: During the slow-adjustment measurements the bladder was constantly filled and pressure elevated, while the neo- and native sphincters maintained a pressure gradient. The perfusion of the neo-sphincters increased significantly during stimulation. In this typical plot the mean and standard error values are depicted.

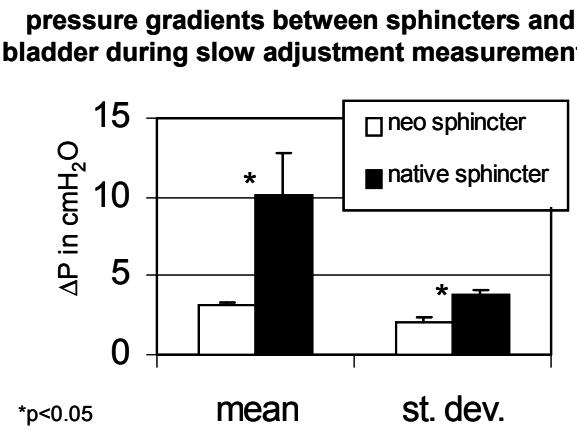


Figure 7: Overall mean pressure gradients and the standard deviations were statistically significantly different between neo-sphincters and native sphincters during the slow adjustment measurements.

Discussion

Dynamic myoplasties add animated function to reconstructive surgery. The number of potential applications could be substantial if the control over the myoplasty was less difficult. After transposition or transplantation the muscle tissue is lacking a pattern of impulse activity appropriate to its new role. Therefore, the inappropriate input needs to be superimposed by electrical neuromuscular stimulation.

In this chapter several innovations in dynamic myoplasty control were combined. As described in chapter 2 and 3, sequential stimulation has been shown to improve the endurance by allowing reperfusion of the resting segments during muscle performance. The extra reperfusion was at least twofold when compared to the conventionally stimulated control muscles. In the present experiment the perfusion of the neo-sphincters increased to a mean of 229% of the resting perfusion. However, no conventionally stimulated control muscles were incorporated in the present study design, and therefore the effectiveness of the sequential stimulation in this setup remains speculative. Furthermore, in this setup a 33% duty cycle was used. This implied that only one-third of the neo-sphincter could be used to generate the pressure needed at any time. Pilot-studies showed that one third of the neo-sphincter-design could generate a pressure higher than 200 cm H_2O, which allowed a large enough range of

pressures necessary for the experimental designs. However, in other dynamic myoplasty applications requiring high performance output, division of the muscle into weaker separate segments, necessary for sequential stimulation, could make the muscle incapable of reaching maximal performance peaks necessary to meet the requirements of that specific function. For these applications sequential stimulation does not suffice.

Closed-loop control made it possible to precisely tune the neo-sphincter pressure to the required values by constantly repeated corrections of the stimulation amplitude. The required neo-sphincter pressure values were calculated by the function-controlling algorithm, which monitored the pressure gradient between neo-sphincter and bladder.

A major advantage of closed-loop control is its self-regulation under changing conditions. However, this continuous correction towards a goal value also creates the possibility of over-correction, thus leading to oscillations. Therefore, in the present experiment, the same oscillation-damping parameters were incorporated as described in chapter 4. The stability and quality of the closed-loop control can be assessed by calculation of the standard deviation of the produced neo-sphincter pressures. No statistical differences were found between the calculated standard deviation of the neo-sphincters and the native sphincters during pressure gradient maintenance in the rapid-adjustment measurements. However, during the slow adjustment measurements the neo-sphincters performed better by having statistically lower standard deviations. These two findings imply that the principle of closed-loop control can meet an acceptable standard for clinical application. However, limited durability of biosensors, displacement of- and fibrotic changes around biosensors are just a few of the current drawbacks, making closed-loop control vulnerable to failure in chronic applications.

Maintaining a pressure gradient while bladder pressure changed rapidly showed that the neo-sphincter failed when bladder pressure changed faster than 1 cm $H_2O.sec.^{-1}$ (see also figure 4a). This is probably due to the fact that the algorithm calculated corrections of the stimulation signals in a linear fashion.

Exponential corrections however could provide a more accurate reaction at the cost of a higher risk of oscillations (see equation 1).

Equation 1

Linear: $$S_{(n+1)} = S_n + \kappa_1 (P_g - P_a)$$

Exponential: $$S_{(n+1)} = \begin{cases} S_n + (e^{\kappa_2 |Pg-Pa|} - 1) \wedge Pg - Pa \geq 0 \\ S_n - (e^{\kappa_2 |Pg-Pa|} - 1) \wedge Pg - Pa < 0 \end{cases}$$

Equation 1: $S_{(n+1)}$: next stimulation signal; S_n: actual stimulation signal; κ_1 & κ_2: correction factors; P_g: goal pressure; P_a: actual pressure

In the experiments the rapid-adjustment measurements mimicked the situation of sudden abdominal/bladder pressure rises and falls due to posture changes, lifting objects, etc. The slow-adjustment measurements mimicked diuresis. The function-controlling algorithm proved to be capable of responding accurately to these bladder pressure changes by constantly regaining and maintaining a positive pressure gradient. The value of approximately 49 minutes of 'untrained neo-sphincter continence' is encouraging, but also relative, since this value depends largely on the rate of bladder pressure rise and thus diuresis rates. Furthermore, distribution of abdominal and bladder pressure, adding to the native sphincter pressure as in normal physiology, was not considered.

The use of sensing programmable stimulators adds possibilities to implement among other things self-learning processes. For instance, in the above-described setup it often happened that one of the three neo-sphincter segments fatigued faster than the other two, leading to termination of the measurement. Therefore, pilot experiments were conducted after termination of the planned measurements, repeating the slow adjustment-measurements with adapted algorithms. These algorithms changed the fixed 33% duty cycle per neo-sphincter segment to a variable duty cycle, depending on the relative fatigue of each segment, so that a more fatigued segment performed a shorter duty cycle. Relative individual segmental fatigue was assessed comparing the necessary

actual stimulation amplitudes for the three segments (see equation 2). Using these algorithms, the already fatigued neo-sphincters performed another complete cycle of slow-adjustment measurements assuring a positive pressure gradient with the re-filling bladder for another 30 to 60 minutes.

Equation 2

$$DC_x = 100 * S_x^{-1} * (S_1^{-1} + S_2^{-1} + S_3^{-1})^{-1} \wedge \{x \in 1,2,3\}$$

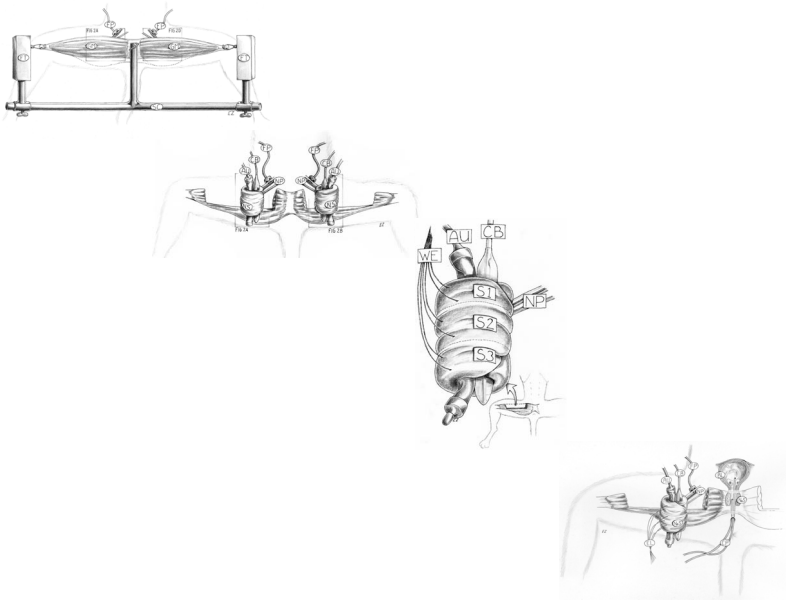
Equation 2: DC_x: duty cycle of segment x in %; S_x: actual stimulation signal of segment x (simplified from algorithms actually used)

Conclusion

A combination of sequential stimulation, closed-loop control and function-controlling algorithms is feasible in dynamic graciloplasty. This combination mimics the physiological function of the native sphincter in a more natural way than the currently applied tetanic and continuous stimulation in dynamic graciloplasty. Therefore, it is postulated that these stimulation enhancements will lead to better functioning dynamic graciloplasties. However, many problems need to be solved before an implantable device is capable of delivering this form of stimulation. For instance, the simple but delicate electrodes used in this study and obligatory for proportional control over the muscle tissue need further refinements to become useful for chronic implantation. Current rapid evolution of implantable sensors, circuitry and powerful but small batteries[Jarvis 2001, Loeb 2001, Bijak 2001 & Ferrarin 2001] will provide the opportunity to the future use of enhanced electrical stimulation.

Chapter 6

General discussion



"A scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die and a new generation grows up that is familiar with it"
-Max Planck-

Erik Zonnevylle, MD
Richard Stremel, PhD

Based (in part) upon the article:

Stremel R, Zonnevylle E. Re-animation of muscle flaps for improved function in dynamic myoplasty. Microsurgery 21: 281-286, 2001.

Introduction

The re-animation of tissue flaps is an important advance in the field of reconstructive surgery. While tissue flaps are used to repair large tissue defects and restore form (such as in breast reconstruction), contractile flaps (dynamic myoplasties) restore function, offering exciting new surgical solutions to clinical reconstructive problems.

Dynamic myoplasty requires significant surgical skills including microsurgical neuroraphy and anastomosis of arteries and veins. These techniques are critical when transplanting tissue flaps from one body location to another. While free vascularized muscle flap transfer has met with reasonable success [Harii 1976 & Manktelow 1989], re-innervation of the flap, and thus motor control, is a slow process. During this re-innervation process, the nonfunctioning transferred muscle atrophies and the ability of the muscle flap to be animated to produce the desired performance often results in less than optimal function. But also in the case of a pedicled graft leaving the nerve intact, the dynamic myoplasty is lacking a pattern of impulse activity appropriate to its new role. Therefore, it is often necessary to use external electrical pulse generators eliciting appropriate muscular contractions and in the case of free flaps preventing muscle atrophy, during a re-innervation phase.

Most dynamic myoplasty applications currently require that the transferred muscle flap must perform work that is different from that to which it was accustomed. Examples include cardiomyoplasty, in which the entire latissimus dorsi muscle (a muscle whose normal function is adduction and rotation of the humerus) is wrapped around the myocardium. It is then stimulated by an implantable pulse generator to contract rhythmically at a fairly high frequency (approaching every other heart beat) and in synchrony with the heart.[Carpentier 1985, Chachques 1987 & 1997] Another example is graciloplasty for urinary and fecal incontinence. The gracilis muscle (a muscle whose normal function is adduction of the thigh and flexion of the knee) is wrapped around the fecal or urinary outlet and stimulated to contract tetanically and continuously for periods of 2 to 4 hours.[Williams 1989, Baeten 1991 & Janknegt 1992] These new functional requirements pose important problems for these muscles; among the most significant and

compromising of these are rapid muscle fatigue, muscle damage due to over-stimulation, and ischemic lesions due to constant high pressures.[Williams 1991, Konsten 1993, Janknegt 1995, Geerdes 1996 & Madoff 1999)]

An approach used in dynamic myoplasty to avoid muscle failure due to fatigue is to train the muscle to enhance fatigue resistance.[Salmons 1976 & 1981 & Koller 1994)] Currently used training protocols require an 8-week period of stimulation at increasing frequency until the muscle is incompletely converted to a fatigue-resistant fiber type. Skeletal or striated muscles are normally a mixture of fatigue-resistant, slow-twitch, oxidative (type 1) and fatigue-prone, fast-twitch, glycolytic (type 2b) muscle fibers. Type 2a fibers have fast twitch, but fatigue resistant, oxidative qualities and are considered to be an intermediate fiber type.[Jarvis 1996, Mayne 1996, Pette 1997, Sutherland 1998 & Scott 2001)] The innervation and the function of the muscle determine the predominance of one fiber type over another, and all fiber types in a given motor unit are the same. However, striated muscle is plastic in nature, and the training regimen in cardiomyoplasty and graciloplasty transforms the muscle to predominantly type 1 fibers. The trade-off for producing fatigue resistance is a slower contracting muscle capable of generating less power than its original character.[Salmons 1981 & 1994, Pette 1992)]

While preliminary outcomes in these clinical applications of dynamic myoplasty appear to be producing promising results, in general, the results are variable. The clinical trials of both cardiomyoplasty and graciloplasty are promising but have met with difficulties.[Williams 1991, Chachques 1997 & Madoff 1999)] Many factors probably contribute to these difficulties. One major reason for the variable outcomes relates to the way the dynamic myoplasty is controlled using electrical stimulation. Under normal circumstances, a given anatomical muscle can respond and adapt to prolonged sustained activity, or brief intense activity. The response generated by the muscle depends on the types of motor units (and therefore myofiber types) contained within the muscle and the pattern of activity in which they are engaged.[Saltin 1983, Scott 2001)] Current applications of dynamic myoplasty use tetanic stimulation and contraction of all myofibers simultaneously. This tetanic type contraction occurs rarely in normal conditions and leads to irreversible muscle damage, when prolonged. Furthermore, this

type of on/off control does not allow more intricate applications of dynamic myoplasty to be developed, in which fine tuned performance control is mandatory. Therefore, a method of electrically stimulating striated muscle flaps was developed, mimicking physiological muscle contraction more closely. The ultimate purpose was the production of effective contraction within dynamic myoplasties without an undue wait for training, without compromising force generation, without ischemia during prolonged contractions and with precise control of the generated performance. To some extent these purposes were met in this thesis.

Sequential stimulation

The conventional stimulation approach in cardiomyoplasty and graciloplasty involves stimulation of the nerve where it enters the muscle. This approach produces neuromuscular stimulation through excitation of the motor nerves. Excitation of the motor nerve leads to excitation of all the motor units associated with this motor nerve. A consequence of this conventional stimulation is simultaneous excitation and contraction of the entire muscle flap. Therefore, a muscle flap must be divided into separately excitable segments to develop a stimulation approach that permits segmental recovery from contraction, during contraction of other segments. A dynamic myoplasty muscle is divided into multiple individually excitable segments by placing pairs of stimulating electrodes (anodes and cathodes) in strategic locations. If these segments are induced to contract in a sequential fashion, one segment rests while adjacent segments generate the necessary force required from the dynamic myoplasty. We termed this method of stimulation, sequential segmental neuromuscular stimulation. In the development of sequential segmental neuromuscular stimulation, our hypothesis was that this modulus of stimulation would mimic true physiological contractions of muscle more closely and thus enhances fatigue resistance of dynamic myoplasties.

Application of sequential stimulation

Our investigations of sequential stimulation as a method to reduce fatigue were focused on stimulation of the canine gracilis muscle. In anesthetized dogs, the gracilis muscles were lifted as uni-pedicled muscle flaps. To mimic human proportions, the muscles were tailored to a width of 3.5 cm by removing the posterior aspects of each muscle. The experimentation setup consisted of a simple fixation of the pelvis while each gracilis muscle was attached to an isometric force transducer (see figure 1 in chapter 2). Electrode placement was critical since it was important to establish four segments of the gracilis capable of generating equal force in each segment (see figure 2 in chapter 2). The stimulus intensity was adjusted to generate a pre-determined force that was the same for both sequential and conventional stimulation during any single comparison.

Since sequential stimulation offers the ability to use variable portions of the entire gracilis muscle, the amount of time spent in contraction versus relaxation was considered important to muscle force generation and fatigue resistance. Thus, a duty cycle was determined for each segment based on the number of segments contracting simultaneously. For instance, if only one of four segments were contracting at a time, then the duty cycle was 25% and the resting cycle was 75% because the muscle was working 25% of the time and resting 75% of the stimulation period. If all four segments were contracting simultaneously, the duty cycle was 100% and the contraction was equivalent to that generated by conventional stimulation.

During these experiments, the time required for the generated force to decrease by one half (i.e., the half-time to fatigue) was determined, together with the arterial blood flow. The results were quite striking and unequivocal. Sequential segmental neuromuscular stimulation significantly enhanced fatigue resistance in an inverse progressive fashion with respect to the duty cycle. During the contractions, the muscle blood flow increased more than 100% when compared to conventional stimulation for duty cycles of 25, 50 and 75 percent.

The success of these experiments suggested that the application of this stimulus paradigm to the functioning of the graciloplasty neo-sphincter could

provide fatigue resistance due to improved perfusion and avoid prolonged training, which implies loss of strength and responsiveness. This would permit earlier functional use of the neo-sphincter and preservation of reactivity, which is important for the possibility to acutely adjust performance (see section on closed-loop control). Therefore, the contractile function and perfusion of gracilis neo-sphincters were studied using both sequential and conventional stimulation. In anesthetized dogs, both gracilis muscles were converted into neo-sphincters (see figure 1 and 2 in chapter 3) and electrically stimulated. The stimulation approach consisted of three-channel sequential stimulation on one side (a duty cycle of 33%) and conventional stimulation on the other. Both neo-sphincters were stimulated at an intensity that generated 80 cm H_2O pressure. To ensure that the neo-sphincters could shorten and reduce their lumen, latex tubes connected to a 40 cm H_2O hydrostatic pressure column were also inserted in the neo-sphincter lumen alongside rigid balloon pressure catheters. The time necessary for the neo-sphincter to fatigue to one half of the initial pressure was determined. The results of this neo-sphincter application showed that sequential stimulation was significantly superior to conventional stimulation. Blood flow in the sequentially stimulated neo-sphincter actually increased by 86% while perfusion decreased by 37% in the conventionally stimulated neo-sphincters. Despite these results, sequential stimulation enhanced the endurance of the neo-sphincter for only a few more minutes. When used in clinical applications an untrained sequentially stimulated neo-sphincter would result in short continence times, which would not be acceptable.

Discussion on sequential stimulation

A universal complication of dynamic myoplasties is muscle fatigue. Muscles require maintenance of adenosine triphosphate (ATP) within the myofibers to generate force through the contractile interaction of actin and myosin proteins. In the absence of adequate oxygen delivery, muscle dependence on aerobic mechanisms of metabolism for ATP generation will become limited and dependence on anaerobic metabolic mechanisms will prevail. A consequence

of this shift to anaerobic metabolism is a reduction in ATP production and an increase in lactic acid production. While the etiology of muscle fatigue is complex and can result from many contributing factors, the combination of decreased ATP availability and decreased intracellular pH from lactic acidosis certainly contributes to the failure of muscle to produce contractile force.[Fitts 1996]

In dynamic myoplasty, muscle tissue fatigues primarily for two reasons: it is inadequately perfused and/or it is stimulated to perform in a way that is incompatible with its basic muscle fiber type. Currently used stimulation devices produce prolonged tetanic skeletal muscle contractions. As muscle tissue contracts, the individual myofibers within the muscle place significant pressure on the blood vessels within the muscle. Due to these high transmural pressures, a tetanic contraction has the effect of occluding blood flow through the muscle during the contraction. This reduction of perfusion due to internal muscle pressure contributes to the failure to deliver adequate oxygen and nutrients to the contracting muscle, leading to increasing lactic acid and decreasing ATP, which precipitates fatigue of the muscle and failure to generate force despite continued stimulation. This can be partially circumvented by transforming the muscle into a predominantly oxidative type 1 fiber containing muscle, using chronic stimulation and muscle training. The oxidative pathway replenishes the ATP, while the type 1 fibers deplete the available resources to a lesser extent. However, this process costs strength and responsiveness. Availability of resources on the other hand can be increased somewhat by augmenting perfusion of the performing muscle tissue as is clearly the case in sequential stimulation. The lesser performance of the non-isometric sequentially stimulated myoplasty, reported in chapter 3, when compared to the isometric sequentially stimulated myoplasty of chapter 2, is at least partly due to the kinking of the intra-muscular blood-vessels and allowed shortening of the muscle during stimulation in the non-isometric setup. This induced increased resistance of the intra-muscular vessels, leading to less effective perfusion, and relative decreased fatigue resistance in the non-isometric setup, when compared to its isometric counterpart.

Peckham et al. and Petrofsky described various experimental and clinical applications of fatigue resistance improving types of stimulus paradigm.[Peckham 1970 &1976, Petrofsky 1979] It has been demonstrated in humans that sequential alternation of stimulus between agonistic muscle groups enhances fatigue resistance.[Peckham 1970 & Pournezam 1988] Peckham et al. used a random electrode placement in cat skeletal muscle to stimulate contraction in three segments.[Peckham 1976] This random approach was likely to lead to overlap of stimulus area and precipitate muscle fatigue. Almost all studies utilizing multiple stimulation channels have also employed a common electrical ground, causing spread of the stimulation pulse. In the studies presented in this thesis, multiple differential channels with stimulus isolators were employed. Furthermore, discrete electrode placement in more distal parts of the muscle permitted exclusion of recruitment of the main motor nerve branches supplying the muscle. In this way, it was possible to recruit local intra-muscular nerves, which produced far less overlap of recruited segments. Petrofsky used a rotary electrode array to stimulate the peripheral nerve directly to sequentially recruit motor units and reduce fatigue. Unfortunately, peripheral nerve stimulation is associated with nerve injury and has not achieved broad clinical application. More recently, Lau et al. compared the effects of stimulating rabbit triceps at two to three different sites on fatigue resistance.[Lau 1995] Sequential stimulation proved to be superior to whole muscle and alternating segment stimulation in producing fatigue resistance. These observations underscore the results of the studies of chapter 2 and 3.

Closed-loop control

In the previous paragraphs a relationship is demonstrated between rate of exertion, duty cycle and rate of perfusion in a dynamic myoplasty. Furthermore, depletion of ATP, which is counteracted by perfusion and progressed by exertion, limits, among others, the performance and thus the applicability of dynamic myoplasties. With sequential stimulation already enhancing perfusion, it is reasonable to economize exertion to further maximize the possibilities of

dynamic myoplasties. Therefore a condition is created in which it is possible to reduce the amount of work the myoplasty performs to the minimal level necessary to carry out the required physiological function for prolonged periods. Furthermore, reducing peak exertion to the minimum must have beneficial effects on surrounding tissues by reducing ischemic lesions, stricture and scar formation.

Closed-loop control is a powerful cybernetic tool in muscle stimulation[Lemay 1997, Quintern 1997 & Ferrarin 2001] and offers the possibility of continuously controlling the performance of dynamic myoplasties by cyclically comparing the actual performance to a target performance. Differences between these two are used to compute corrections, which are applied in the stimulation signal and evaluated in the next cycle.

Biosensors feed the closed-loop control tool with the actual performance. These sensors can offer all kinds of biofeedback. This biofeedback can also provide input for computing devices running algorithms, which process all sorts of decisions concerning dynamic myoplasty performance. This 'artificial intelligence' (ranging from simple algorithms to sophisticated self-learning control systems) offers refinement in complex tasks, opening up possibilities for more demanding implementations of dynamic myoplasties.

Application of closed-loop control

A simple closed-loop control stimulation algorithm was developed and tested on sequentially stimulated neo-sphincters designed from canine gracilis muscles (see chapter 4). Pressure, generated in the lumen of the neo-sphincters, was used as feedback to regulate the stimulation signal. The typical property of closed-loop control to oscillate around the target performance was countered using three oscillation-damping parameters. Effectiveness of these parameters was determined by calculating the standard deviation of the actual measured pressures for ranges of parameter values. Each oscillation-damping parameter showed a significant effect on the standard deviations; the lowest standard deviations corresponded with the optimal value settings for each parameter.

Additionally, the efficiency of amplitude and frequency modulation techniques were investigated and compared using a similar procedure. Amplitude modulation systematically showed lower standard deviations, but frequency modulation allowed faster bridging of large differences between actual and target pressures. Closed-loop control enabled the maintenance of a variety of target pressures within 10% deviation, using optimized oscillation-damping parameters and amplitude modulation.

Versatile dynamic myoplasties can be designed, when the blood perfusion enhancement of sequential stimulation and the performance regulation of closed-loop control are combined with function-controlling algorithms executing more complex tasks. In chapter 5 a graciloplasty-model (for urinary incontinence) is described using the sequentially stimulated closed-loop controlled neo-sphincter of chapter 4. In this model the function-controlling algorithm was of a low complexity: a pressure gradient between the neo-sphincter and the bladder was maintained, while the bladder pressure was rapidly and slowly changed, mimicking respectively change of posture, lifting heavy objects, etc. and diuresis filling the bladder. Performance of the neo-sphincter was compared to the native sphincter. The graciloplasty-model proved to be satisfyingly functional: rapid bladder pressure changes were effectively compensated with neo-sphincter pressure corrections and without any form of training the neo-sphincter was capable of maintaining continence during pressure increase in the bladder due to diuresis for prolonged periods. Overall the graciloplasty-model functioned as a reasonable alternative for the native sphincter.

Discussion on closed-loop control

In this thesis, closed-loop control is advocated as an efficient cybernetic tool to gain proportional control over the performance output of a dynamic myoplasty. In order to apply proportional control, it is essential to reliably and reproducibly control small increments and decrements in the performance output of the muscle. Learning by trial and error we found that the anode and cathode

electrodes had to be placed distally enough, not to evoke larger common trunks of the intra-muscularly diverging innervating motor nerve, and proximally enough to stimulate small branches of this motor nerve before they finished at the motor end-plates. A 45 degrees angle between the axis of the electrical field, built up between the anode and cathode on one hand, and the direction of the muscle fibers and intra-muscular motor nerve branches on the other, allowed for a workable, pseudo-linear ratio between the amount of recruited motor-units and signal strength. This important finding made it possible to proportionally control the output performance with the signal strength in a reliable and reproducible manner. This proportional control allowed closed-loop control to precisely direct the performance output of the neo-sphincter.

Closed-loop control is unstable by its nature. This lack of stability is caused by the constant cyclic repetition of evaluation of the current performance, followed by correction. The application of corrections has a latency, which is system specific. If the corrections overtake their own application, caused by system latency, the closed-loop control starts to oscillate. So, the more cycles per time unit the evaluation is executed beyond this point, the more unstable the system becomes. On the other hand however, the performance directed with this closed-loop control has a tendency to diverge within a certain time and needs correction, before these diversions become larger than prudent. Therefore, an ideal frequency of corrections exists, in which corrections are made fast enough to prevent large diversions in performance, but not too fast to cause oscillations. The ‘correction frequency’, described in chapter 4, showed this optimum and proved to have a reproducible value. Furthermore it is important to realize that the control over performance output is not infinitely precise. Therefore it is not reasonable to correct for small deviations of performance output, when the corrected signal will not apply this intended correction in the first place. Adversely, these corrections of the stimulation signal will be repeated cycle by cycle until a threshold is passed and the performance output reacts with an overshoot of the intention. This over-steering of the closed-loop control will also result in oscillations. Therefore, a certain deviation must be allowed to prevent this adverse effect. On the other hand, the

threshold for corrections should be as low as the system allows, preventing gross deviations. In the experiments described in chapter 4, the anticipated optimized 'correction threshold' was found. Concerning the sequential stimulation, it was also important to consider the latency of application of the stimulation signal corrections. It is important to consider the segments of the sequentially stimulated dynamic myoplasty as independent units, which have their individual steering parameters (see also figure 3 in chapter 4), but with a common target. An evaluation of the performance output is meaningless, when the sequential stimulation has just switched to a next segment: in this case, due to latency, the performance output by the previous segment is still existent. An evaluation during this latency will lead to inappropriate corrections in the stimulus signal of the starting segment, which has its own independent setting of stimulation signal. This causes faults in the stimulation signal and thus deviations at the start of a segment. Therefore, it is necessary to introduce a short period in which no evaluations and corrections are executed after switching to the next segment in sequentially closed-loop controlled stimulation. However, when the 'transition time' is chosen too long, the period of absence of corrections will lead to unnecessary deviations of the target performance. Again, a trade-off between system latency and early corrections of the stimulus signal leads to an optimized value, which was described in chapter 4. It could be of interest to investigate if the evaluation of the performance during the start of a next segment can be of use during the next start of the previous segment. This implies a phase shifting between sequential stimulation and closed-loop control, determined by the latency of the system.

In the first paragraph of this section the importance of proportional control over performance is described. Amplitude modulation of the stimulation signal provided a more precisely proportioned performance output, when compared to frequency modulation. The modulation of the signal was computed using a simple linear algorithm in which the correction of the amplitude (and frequency in the case of frequency modulation) was determined by multiplying the performance deviation with an empirically fixed correction factor. However, the use of this linear algorithm to compute the amplitude corrections from the

performance deviations did not simultaneously allow executing fine-tuning on one hand and fast bridging of large performance changes on the other, using the same fixed correction factor. Therefore it could be of interest to use an exponential design for this algorithm. Another possibility to deal with the apparent opposite interests of fine-tuning and the capability of quickly bridging large changes in performance, could be the combination of amplitude and frequency modulation: the latter proved to be capable of bridging large performance changes faster, while amplitude modulation can add fine-tuning.

A combination of sequential segmental neuromuscular stimulation, closed-loop control and function-controlling algorithms provides versatility to dynamic myoplasties. In chapter 5 a successful application is described in which a neo-sphincter maintains a pressure gradient with changing bladder pressures. Based on this model it proved possible to upgrade the function-controlling algorithm to a more sophisticated version. The upgraded version could learn the relative state of fatigue of each segment of the triple partitioned neo-sphincter at that moment, by simply comparing the amplitude changes of each segment. With this information the duty cycle of each segment was adjusted, taking into account their individual state of fatigue relative to the other segments. This improved the endurance of the neo-sphincter, which was otherwise limited by the fastest fatiguing segment. This underscores that the above-described combination adds some versatility to the dynamic myoplasty.

Using the present knowledge, but disregarding the lack of availability of versatile programmable implantable stimulators, the ideal neo-sphincter design should partition the muscle into two sections. The first more cranially located section of the neo-sphincter should contain two sequentially stimulated segments. By training, these two segments should be transformed into units containing mainly slow twitch fibers. The other more distally located section should contain three non-transformed fast twitch sequentially stimulated segments. Both sections should have their own independently functioning closed-loop control systems. The slow twitch partition should be used to maintain a mean pressure gradient with the bladder pressure, based upon the integrated pressure gradient information of probably a minute. The fast twitch

partition however, should be used to safeguard a pressure gradient during acute moments of bladder pressure raise, which temporarily exceeds the slow twitch neo-sphincter pressure. Therefore, its functioning should be based upon the actual pressure gradient between the bladder and the slow twitch partition of the neo-sphincter. In this concept, the slow twitch partition functions like an involuntary smooth muscle sphincter, while the fast twitch partition is better compared to a striated voluntary sphincter.

In fact, replacement of any sphincter in the body (e.g., anal, dynamic myoplasty for stomal continence etc.) could benefit from similar stimulation approaches. However, the experiments described in this thesis were accomplished via a computer/data acquisition system with the size of a fridge, limiting the experiments to an acute design. The technology for the miniaturization of this more sophisticated stimulation system exists and is slowly becoming available.[Jarvis 2001] Moreover, feedback of all sorts of data from miniature implantable sensors is nowadays feasible with modern electronics. However, the use of leads/electrodes in chronic designs remains vulnerable due to the chance of displacement and breakage. A revolutionary design, dealing with this problem, is the BION^{tm} system, which disregards all leads, by introducing radio-controlled stimulation (BION1^{tm}) and sensing (BION2^{tm}) units.[Loeb 2001] Altogether, the results presented in this thesis justify the effort of miniaturizing the current computer/data acquisition system, so it can be tested in chronic experimental designs.

Conclusion

Sequential segmental neuromuscular stimulation improves the (re)perfusion of dynamic myoplasties during performance of their tasks. This improved perfusion enhances fatigue resistance in an inverse progressive fashion with respect to the duty cycle.

These effects are more pronounced in an isometric setup with stretched out and fixed muscle tissue than in a non-isometric setup, which allows shortening of the muscle tissue.

Closed-loop control is a sufficient cybernetic tool to regulate the performance generated by sequentially stimulated dynamic myoplasties. The use of optimized damping parameters, can successfully reduce the inherent problem of oscillations in closed-loop control, whereas amplitude modulation of the stimulation signal provides better proportional control over the performance of dynamic myoplasties, then frequency modulation.

A combination of sequential stimulation, closed-loop control and a function-controlling algorithm offers more versatile dynamic myoplasties, broadening the abilities of reconstructive surgeons in the repair of functional muscle defects.

All results justify the development of a comparable, but implantable system, which can be used in chronic studies to further elucidate the benefits of sophisticated electrical stimulation in dynamic myoplasty.

"The important thing is not to stop questioning" -Albert Einstein-

Summary

Summary

It has become common practice in reconstructive surgery to transpose or transplant a variety of autologous tissues to fill defects at a recipient site. Using muscle tissue, it becomes possible to dynamically assist or replace an impaired or lost function. For these procedures the term 'dynamic myoplasty' is generally used. In dynamic myoplasty, control of timing and rate of contraction of the transposed or transplanted muscle tissue is substantial, but presently not fully within reach. Currently used electrical stimulation protocols, which stimulate innervating nerves or the muscle tissue itself, supply gross on-off spasm-like contractions of the whole dynamic myoplasty muscle. This leads to rapid fatigue, which can be partly overcome using training protocols converting the dynamic myoplasty into a more fatigue resistant state at the cost of strength and responsiveness. Furthermore ischemic lesions, fibrotic changes and abundant scarring due to constant high tissue pressures are widespread reported in these procedures.

This thesis reports on efforts to gain more control over dynamic myoplasties, in order to overcome some of the drawbacks of currently used electrical stimulation on one hand, and to give rise to more refined applications of dynamic myoplasties on the other.

Conventional stimulation techniques recruit all or most of the muscle fibers simultaneously and continuously. Therefore, sequential segmental neuromuscular stimulation was introduced, in which only segments of the transferred muscle tissue are stimulated in a sequential fashion, providing continuous work, but with alternating partitions. This way, the temporary resting segments are reperfused during functioning of the dynamic myoplasty. The muscles studied were the canine gracilis and all experiments were acute studies in anesthetized animals. Comparison of sequential and conventional stimulation revealed the predicted increase in muscle fatigue resistance and muscle blood flow. These effects were more pronounced in the isometric setup with stretched out and fixed gracilis muscles reported in chapter 2, than in a non-isometric setup with gracilis based neo-sphincters, which were allowed to shorten during stimulation, as described in chapter 3. The shortening of the muscle tissue and

kinking of the intra-muscular blood vessels within the neo-sphincters could be a reason for the less distinct increase of perfusion and subsequent fatigue resistance during non-isometric contractions.

Apart from improving perfusion, reducing the load of the dynamic myoplasty will improve its endurance as well. Furthermore, it is likely that reducing the load will decrease ischemia, leading to less fibrotic changes and subsequent scarring of the involved tissues. To economize the load of the dynamic myoplasty to exact (and changing) needs, control over the contraction rate of the muscle tissue needs to be precise. Closed-loop control proved to be a good cybernetic tool to regulate the pressures generated by gracilis based sequentially stimulated neo-sphincters in an acute dog study as was elucidated in chapter 4. The inherent problem in closed-loop control of oscillations was successfully reduced to less than 10% of the target-pressures by using optimized correction frequencies, correction thresholds and transition times. Modulation of the stimulation amplitudes proved to be superior over modulation of the stimulation frequency in order to tune the performance of the neo-sphincters.

Having increased fatigue resistance and being able to precisely control the rate of performance, a dynamic myoplasty should be able to perform more complex tasks. Therefore, an acute dog study was designed in which gracilis based neo-sphincters were made to maintain a pressure gradient with the bladder during fast and slowly changing bladder pressures, using a combination of sequential stimulation, closed-loop control and a function-controlling algorithm. Results, set out in chapter 5, showed that the neo-sphincter was able to maintain the desired pressure gradient with the bladder during fast and slowly changing bladder pressures. Accuracy showed no significant difference when compared to the native sphincter, which served as control. Addition of a simplified self-learning component in the algorithm, adapting the duty cycle of individual segments to their relative state of fatigue, proved feasible and meaningful in this study design.

Overall this thesis reports improvement in fatigue resistance and control over a dynamic myoplasty using sequential stimulation, closed-loop control and function-controlling algorithms in acute studies. A comparable implantable

system, can be used in chronic studies. Availability of this implantable system should be able to confirm the lesser need for muscle fiber type transformation, using training regimens, and also reveal other benefits as prevention of ischemic lesions, fibrotic changes and abundant scar formation. All these improvements should offer more versatile dynamic myoplasties, broadening the abilities of reconstructive surgeons in the repair of functional defects.

Samenvatting in het Nederlands

Samenvatting

In de reconstructieve chirurgie is het steeds meer gebruikelijk om verschillende eigen weefselsoorten te transponeren of te transplanteren teneinde wonddefecten te sluiten. Door spierweefsel te gebruiken is het mogelijk om verzwakte of verloren functies dynamisch te ondersteunen dan wel te vervangen. Voor deze ingrepen wordt veelal de term 'dynamische spierplastiek' gebruikt. Bij het gebruik van dynamische spierplastieken is regulatie van het tijdstip en de mate van contractie van het getransponeerde dan wel getransplanteerde spierweefsel belangrijk, maar op dit moment nog niet geheel haalbaar. De huidige elektrische stimulatie protocollen, welke de innerverende zenuwen dan wel het spierweefsel zelf stimuleren, veroorzaken grove alles-of-niets, spastisch-achtige contracties van de gehele dynamische spierplastiek, hetgeen leidt tot snelle vermoeidheid van de spierplastiek. Deze vermoeidheid kan deels worden tegengegaan door het toepassen van trainingsprotocollen, welke dynamische spierplastieken converteren naar een minder snel vermoeibare status, echter ten koste van kracht en reactiviteit. Daarnaast komt bij deze manier van stimuleren ischemische laesies, fibrotisering en overmatige littekenvorming van de betrokken weefsels voor, veroorzaakt door de continu hoge druk.

Dit proefschrift beschrijft een manier om meer controle te krijgen over dynamische spierplastieken; enerzijds door nadelen van de huidig gebruikte elektrische stimulatie te voorkomen en anderzijds door de mogelijkheid te creëren voor meer verfijnde toepassingen van dynamische spierplastieken.

Conventionele stimulatie technieken rekruteren alle of de meeste spiervezels gelijktijdig en continu. Derhalve werd sequentiële segmentale zenuw-spier stimulatie geïntroduceerd, hetgeen alleen segmenten van het verplaatste spierweefsel stimuleert op een sequentiële manier, waarbij wel continu spierarbeid wordt verricht, maar met steeds afwisselende spiergedeeltes. Bij deze methode worden de tijdelijk rustende segmenten gereperfundeerd terwijl de dynamische spierplastiek toch continu functioneert. Voor de experimenten beschreven in dit proefschrift werden de gracilis spieren van honden gebruikt. Alle experimenten waren acute studies en vonden plaats onder algehele

narcose. Bij het vergelijken van sequentiële met conventionele stimulatie liet de sequentiële stimulatie inderdaad de voorspelde afname in spier vermoeidheid en de toename van perfusie van de spieren zien. Deze effecten waren meer uitgesproken in de isometrische opstelling met de opgespannen en gefixeerde gracilis spieren beschreven in hoofdstuk 2, dan in de niet-isometrische opstelling met van gracilis spieren nieuwgevormde sluitspieren, welke gedurende de stimulatie mochten verkorten, zoals gerapporteerd in hoofdstuk 3. Het verkorten van het spierweefsel en het kinken van de bloedvaten in het spierweefsel van de nieuwgevormde sluitspieren zou een verklaring kunnen zijn voor de minder uitgesproken toename in perfusie en vermoeidheidsresistentie gedurende non-isometrische contracties.

Naast het verbeteren van de perfusie, middels sequentiële stimulatie, zal het verkleinen van de belasting van de dynamische spierplastiek het uithoudingsvermogen van deze verder verbeteren. Bovendien is het aannemelijk dat bij een verkleining van de belasting de kans op ischemie afneemt, hetgeen leidt tot minder fibrotische veranderingen en daaruit volgende littekenvorming in en rond de betrokken weefsels. Om de belasting van de dynamische spierplastiek zo economisch mogelijk af te stemmen op de (veranderende) behoefte, is een precieze regulatie van de mate van contractie noodzakelijk. Terugkoppeling gestuurde controle bleek een geschikte besturingsmethode om de druk te reguleren welke werd gegenereerd door van gracilis spieren gemaakte sluitspieren, zoals beschreven in hoofdstuk 4. Door gebruik te maken van geoptimaliseerde correctie frequenties, correctie drempels en overgangspauzes werd het inherente probleem van oscillaties in terugkoppeling gestuurde controle succesvol gereduceerd tot minder dan 10% van de doeldruk. Modulatie van de stimulatie amplitudes bewees een nauwkeuriger methode te zijn dan modulatie van de stimulatie frequenties bij het reguleren van de gegenereerde drukken in de nieuwgevormde sluitspieren.

Een verbetering van de vermoeidheidsresistentie en de mogelijkheid om de mate van contractie nauwkeurig te reguleren maken het mogelijk om meer complexe taken uit te laten voeren door een dynamische spierplastiek. Derhalve werd een experiment ontwikkeld, waarin van gracilis spieren gemaakte

sluitspieren een drukgradiënt met de blaas onderhielden, gedurende snelle en langzame blaasdruk veranderingen, waarbij gebruik werd gemaakt van een combinatie van sequentiële stimulatie, terugkoppeling gestuurde controle en functie-regulerende algoritmen. Resultaten, gerapporteerd in hoofdstuk 5, toonden dat de nieuwgevormde sluitspieren in staat bleken de gewenste drukgradiënt met de blaas te onderhouden gedurende zowel snelle als ook geleidelijke veranderingen van de blaasdruk. De nauwkeurigheid van de nieuwgevormde sluitspieren was vergelijkbaar met de originele sluitspieren, welke als controle fungeerden. Toevoeging van een versimpelde zelflerende component in het besturingsalgoritme, welke de mate van inzet van individuele segmenten aanpast aan de mate van hun relatieve vermoeidheid, bleek mogelijk en zinvol in deze experimentele opzet.

Resumerend, dit proefschrift rapporteert verbetering in vermoeidheidsresistentie en prestatieregulatie in dynamische spierplastieken door gebruik te maken van sequentiële stimulatie, terugkoppeling gestuurde controle en functie-regulerende algoritmen in acute experimenten. Een vergelijkbaar implanteerbaar systeem zou kunnen worden toegepast in chronische experimenten. Beschikbaarheid van zo'n implanteerbaar systeem zou de verminderde noodzakelijkheid voor spiervezeltype transformatie middels training moeten kunnen aantonen, naast andere verbeteringen zoals het voorkómen van ischemische laesies, fibrotische veranderingen en overmatige littekenformatie. Al deze verbeteringen zullen de veelzijdigheid van dynamische plastieken vergroten, teneinde reconstructief chirurgen meer mogelijkheden te bieden bij het herstellen van functionele defecten.

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