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A Novel Single Compartment *In Vitro* model: Perflurocarbons for Electrophysiological studies of the Rat Urinary Bladder

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Abstract— **This study presents a novel single compartment model for *in vitro* electrophysiological studies of the rat urinary bladder. We tested the functionality and suitability of FC-770 (a Perflurocarbon) for *in vitro* recording of nerve activity arising from the bladder in a single compartment setup. We have also favorably tested stimulation of the bladder via the bladder nerves in FC-770. The organ viability was monitored by recording spontaneous contractions of the bladder for a certain time. We propose the use of FC-770 as a fluid for nerve recording/stimulation *in vitro* as well as for maintaining organ viability, over the commonly used two compartmental methods.**

I. Introduction

With an ever increasing focus on reduction of experimental animal use according to the principles of 3Rs-Replacement, Refinement and Reduction, *in vitro* experimentation has attracted interest in many fields of research. *In vitro* studies offer many advantages over *in vivo* studies [1], especially in whole-organ preparations for electrophysiological studies involving the stimulation of organ systems or the measurement of afferent nerve responses of the organs (e.g. urinary bladder) to various stimuli.

Although the concept of *in vitro* experimentation seems promising, the experimental setup is often complex. Electrophysiological studies on whole organ systems require an experimental setup capable of stimulation and/or recording from the nerves involved. It has to ensure i) the organ viability (since there is no blood supply) and ii) the electrical insulation of the measuring electrodes (to prevent the electrode being short-circuited). The literature frequently describes the use of two compartment models [1, 2, 3], for extracellular nerve stimulation and recording of the urinary bladder. The first compartment, normally filled with oxygenated Krebs solution, maintains the organ viability. The second compartment, filled with paraffin oil, acts as a recording chamber. The nerve attached to the organ (immersed in the viability chamber) is guided into the oil-filled recording chamber through a small gate and then sealed with silicon grease [1]. Guiding the nerve from the viability chamber to the recording chamber through the gate is a difficult task and can easily result in overstretching/ damage of the nerve (as experienced during our earlier experiments using this model). Suction electrodes are another type of two compartment setup, employed in electrophysiological recording from nerves [4, 5]. As the name suggests the nerve is sucked into a pipette like-structure and once placed, manipulation becomes difficult.

In this study we tested the functionality and suitability of a Perflurocarbon (PFC), FC-770 for *in vitro* recording of rat bladder afferent nerve activity in a single compartment setup. PFCs are organic compounds in which all hydrogen atoms have been replaced with fluorine. They are stable and inert compounds since their carbon-fluoride bonds do not interact with living tissue [6]. PFCs are characterized by their high capacity of dissolving respiratory gases at atmospheric pressure. In 1966, Clark and Gollan [6] showed that mice could breathe and survive in an oxygen-saturated PFC solution. Since then PFCs have found an ever increasing application in biomedical sciences. Use of PFCs for liquid ventilation in animals and humans has been reported [7,8]. PFCs have also been used for organ preservation. In 1988 Kuroda and Fujino [9] developed a cold storage two-layer method consisting of Euro-Collins's solution and PFCs for pancreas preservation. In a following study, [10] oxygen bubbled PFC was found to be a simpler and more effective preservation medium for simple cold storage of the pancreas. In other organs the success rate has shown to be organ and species dependent [11].

Considering the capability of PFCs in organ preservation and the high dielectric constant (k=1.9) [12], which represents the insulation properties of this fluid, we propose a novel, simplified , single compartment model for *in vitro* recording/stimulation of the rat bladder and associated nerves, which may greatly simplify neurophysiological/ electrophysiological studies of other isolated organs as well.

II. Materials and Methods

A. Perflurocarbons

3M™ Fluorinert™ Electronic Liquid FC-770, commonly known as FC-770 was used in the experiments.

B. Animals

A total of 9 animals (fig. 1) were used in this study. Approval for the animal experiments was obtained from the local Erasmus MC Animal Experiment Committee. All laboratory and experimental procedures were conducted in accordance with institutional guidelines.

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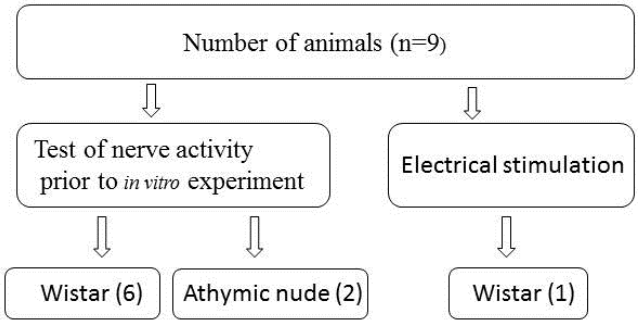

Figure 1 : Number of animals used for *in vitro* experiments

C. Surgical procedures and experimental protocol

C.1 In vivo experiments

For the *in vivo* measurements, the rats were anaesthetized with urethane (1 g/kg). Bladder filling (0.11 ml/min) and measurement of pressure was performed by inserting a 23G needle at the top of the bladder, the other end of the needle was connected to a pressure transducer and an infusion pump. The postganglionic bladder nerves, presumably branches of the pelvic nerve, were mounted on a bipolar platinum-iridium electrode.

The bladders were repeatedly filled with saline (0.9% NaCl) and AA (Acetic acid 0.5%) until they looked relatively full under the microscope. The afferent nerve activity was recorded and amplified by a DISA 15C01 EMG amplifier (gain: 100-200,000) and band-pass filtered with a Krohn-Hite 3944 filter (Bessel, 4th order, 200-2000 Hz). Pressure and nerve activity were sampled at 25Hz and 25 kHz respectively and a 30 second period of bladder pressure and corresponding nerve activity was stored using a custom written Labview® program. Recorded signals were processed with a custom written MATLAB® program.

C.2 In vitro experiments

After the *in vivo* measurements the rats were euthanized by injecting an overdose of KCl in the heart. The bladder along with prostate, urethra, attached nerves and surrounding tissue was taken out and placed in a pre-warmed (37 C) Krebs solution. After a careful removal of the seminal vesicles and unwanted surrounding tissue, the bladder along with the bladder nerves and urethra were transferred to a preheated (~30 C) FC-770 chamber. Bladder filling was performed in the same way as described in preceding section. After filling the bladder with saline, a mechanical stimulus was applied by pushing a cotton swab on the bladder for a period of ~ 4-5 seconds until there was a discernible increase in the nerve activity. The bladder pressure and nerve activity were stored, as described in the preceding section.

C.3 Three types of measurements were done:

1) Nerve activity measurements

In vivo

A test of nerve activity was conducted *in vivo* prior to the *in vitro* experiments (procedure described in the following section). The animals used were:

- 6 Wistar rats (mean weight 431 ± 37g)
- 2 Athymic nude rats (mean weight 368 ± 7g)

A selection criterion was based on the *in vivo* measurements: The rats in which nerve activity was recorded successfully *in vivo* in at least 25% of the measurements, were selected for further *in vitro* experiments.

In vitro

Nerve activity was represented as number of peaks crossing a certain threshold (baseline). The 30 seconds measurements were divided into windows of 5 seconds. A nerve activity measurement was considered successful only when the number of peaks in nerve activity were maximum in the region corresponding to the maximum of the pressure.

2) Electrical stimulation

1 Wistar rat (weight 427g) was used only for electrical stimulation *in vitro*. No nerve activity measurements were done on this rat. A test of electrical breakdown of FC-770 was conducted. The voltage was increased in steps of 1 volt (pulse width 400 μs and stimulation frequency 10 Hz), until an electrical breakdown (which could be seen as bubbling near the electrode-nerve contact) was observed under the microscope. The pelvic nerve was electrically stimulated with rectangular pulses with amplitude increasing in steps of 1 volt, keeping the frequency of stimulation constant, up to a maximum of 7 volt (determined through the electrical breakdown test) or until a bladder contraction was observed.

3) Test for organ viability

To test if FC-770 did maintain organ viability *in vitro* we recorded the pressure during spontaneous contractions of the bladder, upon filling with saline. The spontaneous contractions were also observed under the microscope.

III. RESULTS

1) Nerve activity measurements

In 4 out of the 6 Wistar rats nerve activity was measured successfully *in vivo*. None of the two Athymic nude rats showed any nerve activity *in vivo*.

In 1 of the 4 wistar rats, nerve activity was recorded *in vivo* after replacing the paraffin oil with FC-770 (Figure 2). The effect of FC-770 on the nerves was tested by comparing the amplitude of the nerve activity recorded in FC-770 with the nerve activity recorded in paraffin oil. No significant difference was found.

In the other 3 rats nerve activity was recorded *in vitro*. In 2 of those rats 4 successful measurements of nerve activity were done (fig 3). The third rat failed to show any nerve activity *in vitro*.

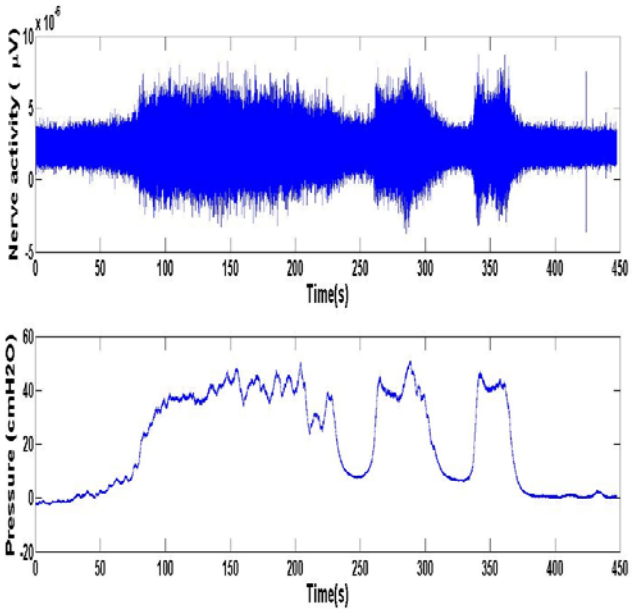

Figure 2: *In vivo* nerve activity measurement in FC-770

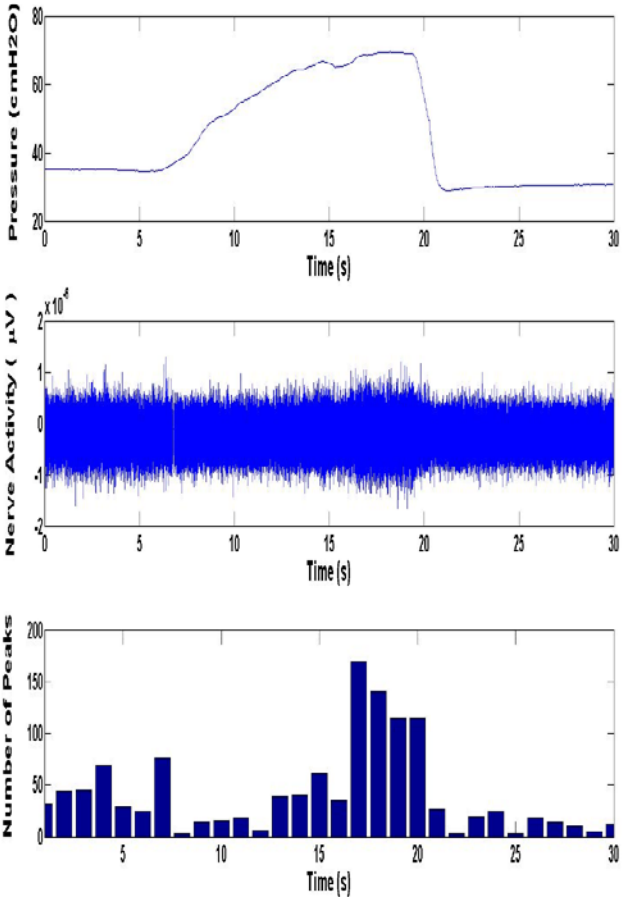

Figure 3: *In vitro* nerve activity measurements on application of a mechanical stimulus to the bladder

2) Electrical stimulation

The electrical breakdown voltage of FC-770s was found to be ~ 7 volts, which was considered the maximum stimulation voltage. In one Wistar rat bladder high amplitude contractions were evoked upon electrical stimulation (fig. 4). The bladder was stimulated until a peak in the pressure was observed and then stimulation was switched off, which resulted in an immediate decline in pressure. Subsequently it was stimulated again.

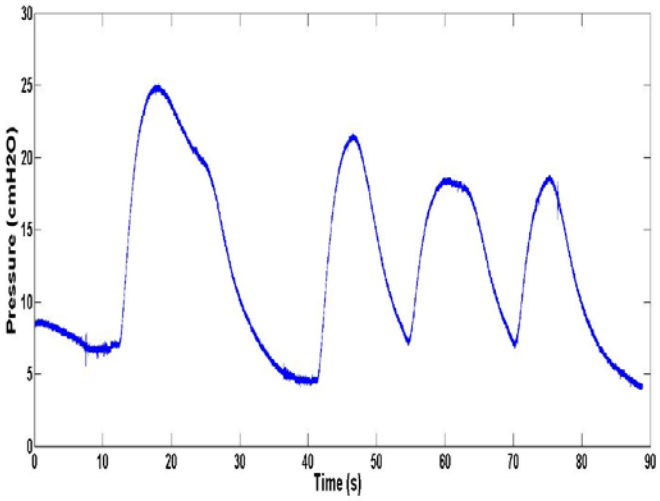

Figure 4 : *In vitro* electrical stimulation of the bladder

3) Test of organ viability

Bladder viability was tested by continuous filling of the bladder with saline. In almost all the *in vitro* preparations spontaneous bladder contractions were observed for ~2 hours after sacrificing the rats(fig. 5).

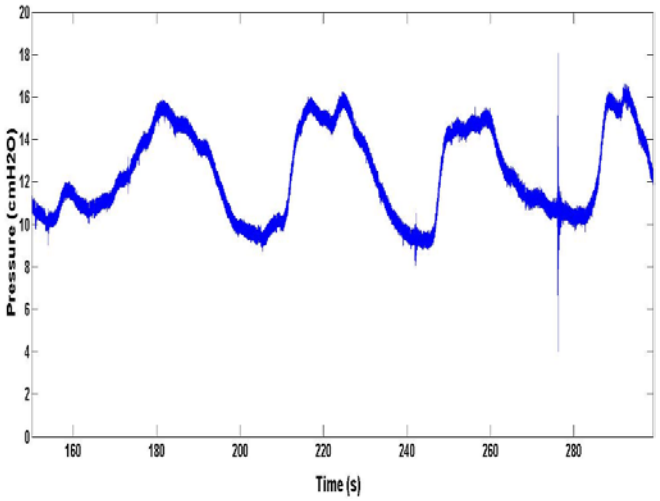

Figure 5: Spontaneous contractions of the bladder on saline instillations *in vitro*.

IV. Discussion

The aim of this study was to test the functionality and suitability of FC-770 for *in vitro* recording of nerve activity as well as electrical stimulation of the rat urinary bladder via

the bladder nerves. Our preliminary results indicate that FC-770 enables a simplified, one compartment substitute to the prevalent two compartment models for *in vitro* nerve recording and stimulation. Considering the simplicity and ease of manipulation, it lessens the chance of damage to organs and nerves, hence enhancing the number of successful experiments. The evidence supporting this conclusion can be summarized as follows:

- We were able to record 4 episodes of afferent nerve response in 3 animals upon mechanical stimulation of the bladder *in vitro*. The mechanical stimulation was necessary to cause a greater distension of the bladder, in addition to the distension caused by the filling fluid and probably caused the activation of mechanosensitive afferent fibers.
- To verify the effect of FC-770 on the amplitude of nerve activity, in one *in vivo* preparation, the paraffin oil was removed and nerve activity was recorded in the FC-770. There was no significant change in the amplitude of the signals recorded in paraffin oil and FC-770. It can be inferred from the nerve recordings that FC-770 provides the necessary electric insulation of the electrodes, which is the reason that up to now two compartmental models were used.
- Apart from recording from the nerves, electrophysiological studies sometime require stimulation of the organ via the associated nerves. In one rat the electrical stimulation evoked bladder contractions that corresponded well with the stimulation. The breakdown voltage of FC-770 (~7 volts AC, 15 Hz) is high enough for most neurophysiological stimulation experiments.
- Due to the high oxygen carrying capacity (approx. 25 times more than blood), FC-770 provides good organ viability, which was confirmed by the spontaneous contractions of the bladder throughout the experiments.

Additionally, the FC-770 is immiscible with aqueous and hydrophobic solutions, which facilitates the removal of blood or any other unwanted liquid from the chamber. As blood has a lower density than FC-770 it will float on the surface, and can be easily removed with the help of a pipette.

Although the results appear to be promising, certain shortcomings must be considered. Since FC-770 has a high density [12], the bladder floats on the surface of the fluid. This requires fixation of bladder to the bottom of the chamber [10]. The recorded nerve activity appears to be consistent with bladder pressure rises, however more experiments have to be done to precisely measure the effect of FC-770 on the nerve functionality. Histological investigations have to be considered in order to test the effect of FC-770 on nerve and bladder wall.

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