

# Post-graduate Teaching in Microsurgery Using a Combination of Alternative Non-animal and In Vivo Methods

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## Summary

Over recent years alternative, non-animal methods have been developed for use in microsurgical training courses, for the training of suture techniques, dexterity and hand-eye coordination. Nevertheless, it is still hard to imagine that alternative methods will be able to replace in vivo teaching completely, because properties of biological tissue are different from those of latex or other artificial materials. Also the dynamics of biological processes, such as a pulsating bloodstream, are difficult to simulate in alternative models. However, the number of animals used in microsurgical training courses can be limited by combining non-animal methods with in vivo training. Moreover, individual techniques can be improved greatly by using non-animal methods. In a five-day introductory course in microsurgical and experimental techniques at the Biomedical Laboratory, University of Southern Denmark, we have limited the average number of animals to one rat per day per participant, by using alternative non-animal methods extensively on the first course day. Nearly all participants were able to produce a successful end-to-end anastomosis of the femoral artery during the second or third day of the course. The alternative methods used included a training device for microsurgical anastomosis and the MD PVC-Rat, an artificial rat with life-size latex abdominal vessels.

## Introduction

The application of microsurgical techniques is rapidly gaining importance in experimental research. The development of transgenic mice has increased the need for microsurgical techniques in experimental procedures, as has the appearance of small implantable radio transmitters for the measurement of physiological parameters (telemetry). Microsurgical techniques are also used for the placement of indwelling catheters or probes, and for experimental organ transplantations. Microsurgical training requires considerable time and resources, including the use of laboratory animals. However, the use of

laboratory animals, being a sensitive topic, should be kept to a minimum, including when used in microsurgical training. Moreover, EU legislation requires that the use of animals in experimental procedures is avoided when alternative methods are available.

Alternative methods in surgical training include suture exercises on a piece of latex glove, with the aim of improving manual dexterity and hand-eye coordination (Crosby *et al.*, 1995; Fanua *et al.*, 2001). A special training device (Figure 1), to refine the latex glove method, has been introduced (Van Dongen *et al.*, 1996). One of the first reports on alternatives to animal models was made on the application of the foliage leaf in microsurgical training (Kaufman *et al.*, 1984). More recently, the MD PVC-Rat (Figure 2), an artificial rat with life-size latex abdominal vessels, was developed for training of microsurgical techniques (Remie,

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Figure 1: The anastomosis device consists of two PVC rings with a piece of latex glove clamped in between the two parts.

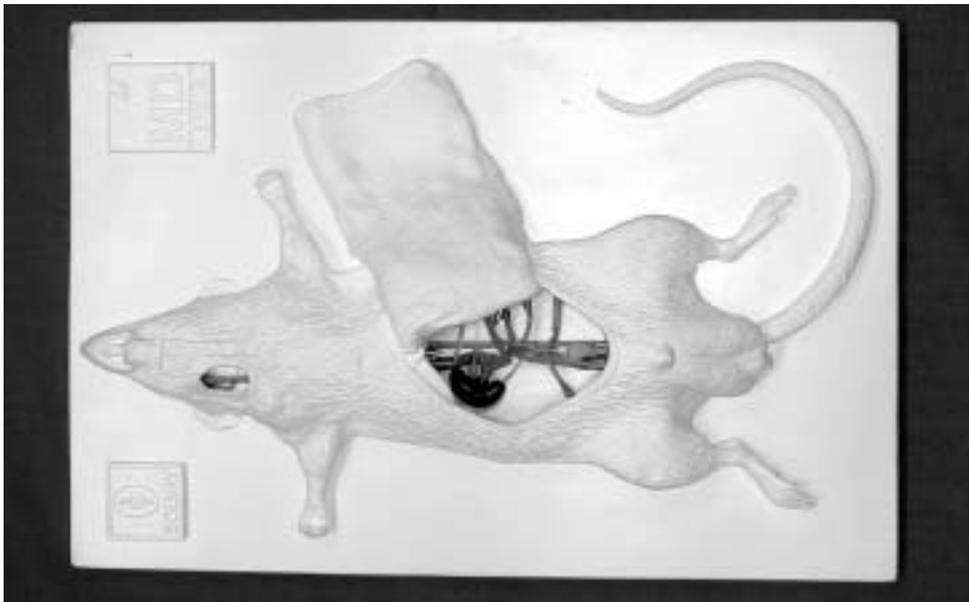


Figure 2: The MD PVC-Rat with life-size latex abdominal vessels.

2001). More unusual methods of training hand-eye coordination under a microscope are the removal of printed letters from a newspaper with a fine hypodermic needle and the removal of a fibre from a gauze pad, followed by its repositioning (*Miko et al., 2001*). Future training of microsurgical techniques may include the use of virtual reality simulators (*Erel et al., 2001*).

### **Microsurgical training at the Biomedical Laboratory:**

Microsurgical training has been performed at the Biomedical Laboratory, University of Southern Denmark, since the end of the 1970s. Presently, Odense University Hospital is the specialist centre for hand surgery in Denmark, and microsurgical training on rats has been an essential step in gaining this position. Before the first reattachment of severed fingers was performed on a patient in 1977, a larger number of rats was used for microsurgical training (*Pless and Barfred, 1978*). Training consisted of a short basic course, followed by an individual apprentice programme which included the performance of end-to-end and end-to-side anastomosis, with a free flap as the final test piece. Techniques were practised on anaesthetised rats until a satisfactory result was achieved. From 1982 to 1995, 146 medical doctors participated in the programme (*Salomon et al., 1996*). Today, microsurgical training is limited to a three-day introductory course, arranged by the National Health Board, and a five-day speciality course for plastic and hand surgeons. During the last seven years, an additional training course for biomedical researchers has been organised. This course in experimental microsurgery lasts 5 days, and is aimed at investigators using microsurgical techniques in experimental procedures, such as catheterization of vessels and ducts, implantation of telemetry transmitters, and experimental organ transplantation in rats and mice. The number of participants is limited to 12 per course and the number of instructors is four, guaranteeing almost individual attention to each participant to secure high quality training.

### **Course syllabus**

The course in experimental microsurgery starts with training of hand-eye coordination and suture techniques under a microscope (Leica Microsystems, Denmark) on the first day. In this exercise suture material such as Ethilon 8-0 monofilament (Johnson & Johnson, Denmark) is used. Smaller suture material should be avoided at this stage to avoid difficulties in handling. Sutures are practised on the anastomosis device, a PVC ring system in which a latex glove is clamped (*Van Dongen et al., 1996*), and on the MD PVC-Rat, a model with life-size latex abdominal vessels (*Remie, 2001*). As few instruments as possible, only a needle holder, a micro-forceps and a pair of micro-scissors, are applied during this stage. On the anastomosis device, several patterns can be made, varying from single sutures in various angles to tube formation, followed by end-to-end and end-to-side anastomosis (*Crosby, 1995*). In the MD PVC-Rat end-to-end and end-to-side anastomosis can be made in relation to anatomical topography, and course participants can get used to working in cavities.

Hereafter, catheterization of the jugular vein and end-to-end anastomosis of the femoral artery and vein are practised in the rat, using 10/0 suture material and a standard set of laboratory microsurgical instruments (S&T, Switzerland). Additional techniques, such as fistulation of gut segments (*Kloots et al., 1995*), catheterization of ducts (bile, pancreatic or thoracic duct), arterial bypass formation, arterio-venous shunts (*Van Dongen et al., 1990; Acland, 1977*) and the formation of a free flap (*Acland, 1977*) can be practised as well.

The techniques are practised on anaesthetized rats, and several anaesthetic protocols are possible. A balanced anaesthesia, which can be supplemented if necessary, is vital to reduce anaesthetic losses. Fentanyl-fluanisone in combination with midazolam has been the anaesthetic of choice, but alternatives, such as ketamine in combination with xylazine or fentanyl in combination with droperidol and midazolam are also possible (*Flecknell, 1996; Kohn et al., 1997*). With these anaesthetic

procedures, in combination with placement of the anaesthetised rat on a heating pad and injection of 5-10 ml saline subcutaneously as perioperative fluid therapy, it is possible to maintain anaesthesia for 4-5 hours without cardiovascular depression.

The importance of sterile operation techniques are taught by the study of Popp and Brennan (*Popp & Brennan, 1981*).

**Number of rats used**

Table 1 presents the number of rats used during 1998-2003. On average 1.03 rats per day per participant were used, which is just over 5 rats per participant for the entire course. This low number was achieved because no animals were used on the first day, anaesthetic deaths were minimized and techniques were practised in low risk order. We found that techniques can be practised satisfactorily on the anastomosis device and MD PVC -Rat, since nearly all participants are able to produce a well-functioning end-to-end anastomosis of the femoral artery in an anaesthetised rat on the second or third day of the course. On days 2 and 4 more than one technique is practised in one rat. Catheterisation of the jugular vein and end-to-end anastomosis of the femoral artery, on day 2, and end-to-end anastomosis of the femoral vein and end-to-end anastomosis of the abdominal aorta, on day 4, can be performed in one rat without a large risk of lethal accidents. Since the end-to-end anastomosis of the abdominal

aorta is more risky than the end-to-end anastomosis of the femoral vein, this technique should be practised last, even though it seems more logical to practise arterial anastomosis before venous anastomosis because they are easier to perform. Apart from lethal accidents during end-to-end anastomosis of the abdominal aorta, the most common cause of losing rats is anaesthetic death. Often course participants work very concentratedly on the anastomosis, forgetting to keep an eye on the level of anaesthesia in the rat. Therefore, it is very important to produce a stable, long-lasting anaesthesia, and to train course participants in early recognition of cardiovascular depression in rats, if a further reduction of the number of rats is desirable.

**Discussion**

It is possible to train microsurgical techniques without an excessive use of animals. The use of animals is not necessary for training of hand-eye coordination and suturing techniques. This can be done efficiently on the anastomosis device and the MD PVC-Rat. The performance of course participants is greatly improved by using alternative non-animal methods for microsurgical training, and nearly all course participants are able to produce a successful end-to-end anastomosis of the femoral artery during the second or third day of the course. Anaesthetic death is the most common cause of animal loss, and course participants need to be trained

Table 1: Number of rats used at the introductory course in microsurgical and experimental techniques at the Biomedical Laboratory during 1998-2003

<b>Year</b>	<b>Number of rats</b>	<b>Number of participants</b>	<b>Number of rats per day per participant</b>
1998	58	8	1.45
1999	37	8	0.93
2000	56	12	0.93
2001	51	11	0.93
2002	62	12	1.03
2003	45	9	1.00
<b>1998-2003</b>	<b>309</b>	<b>60</b>	<b>1.03</b>

in recognizing early signs of cardiovascular depression. However, the MD PVC -Rat makes course participants more confident, thereby creating a surplus of capacity so that attention can be paid to the anaesthetic status of the rat.

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