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Non-radioactive coloured microsphere measurement of regional tissue blood flow for axial pattern flaps in rabbits

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SUMMARY. **We administered non-radioactive coloured microspheres (NRACM) to measure the regional tissue blood flow (RTBF) of eight axial pattern flaps and four kidneys in four rabbits using four repeated injections into each animal. As a control, we used radioactive microspheres (RAM) for six kidneys in three rabbits. There were no significant differences for RTBF values between NRACM and RAM, between the numbers of microspheres used (2×10^6 and 4×10^6), or between the number of injections. These results showed that NRACM is a useful and safe laboratory method.**

The measurement of the regional blood flow in soft tissues such as muscle, tendon, nerve and skin is important experimentally and clinically in order to evaluate viability. Several techniques, including using radioactive microspheres (Guba, 1980), an electromagnetic flowmeter (Awward *et al.*, 1983), an ultrasound doppler (Jones and Greenhalgh, 1983) and an isotope clearance technique (Young and Hopewell, 1983) have been reported. It has been generally accepted that the radioactive microsphere technique has been the "gold standard" method for measurement of experimental tissue blood flow since the report of Rudolph and Heymann (1967). An initial study on blood flow using various sized non-radioactive glass microspheres was performed by Prinzmetal and his colleagues (1948) to observe arteriovenous anastomoses in the visceral organs of rabbits and was encouraging. In this method, spheres were recovered and counted microscopically. Since their experiment, other authors have been using non-radioactive microspheres to measure the cochlear blood flow (Axelsson *et al.*, 1983; Angelborg *et al.*, 1987) or adrenal gland blood flow (Hamaji *et al.*, 1985) and myocardial blood flow (Shell *et al.*, 1985; Hale *et al.*, 1988). The injected glass microspheres were counted in thick histological sections. Serial sectioning and counting microspheres by microscope is more time consuming than determining the number of spheres by their radioactivity, so most investigators have used radioactive microspheres (RAM). However, the value of RAM is limited by energy overlap, high cost, radiation danger, difficulties in animal disposal and poor spatial resolution. Shell and his colleagues (1985) improved the material from which the non-radioactive microspheres were made, and also the techniques used for counting using a haemocytometer. Hale and his colleagues (1988) later performed a comparative experiment on the measurement of regional myocardial blood flow between NRACM and RAM, and demonstrated a good correlation between them. The aims of this study were: (1) to reconfirm the effectiveness of NRACM compared to RAM; (2) to determine the dose of NRACM in a rabbit experimental skin flap model for future research; and (3) to determine the feasibility of giving four repeated injections.

Materials and methods

In this study, 7 adult rabbits weighing 3 kg were used. Of these, 4 rabbits were used for measurement of NRACM and 3 were used for measurement of RAM as a control group.

NRACM 15 μm in diameter were obtained from E-Z Trac (Los Angeles, California). Microspheres of 5 different colours (red, green, orange, black and blue) were used in the 4 rabbits. Three different tissues were evaluated in each rabbit: (i) a 3.0×4.5 cm rabbit ear skin flap pedicled on the central nerve, artery and vein; (ii) an epigastric flap pedicled on the epigastric artery and vein; and (iii) the right kidney. In the control group of 3 rabbits, RAM 15 μm in size and labelled with ^{51}Cr were used and 3 pairs of kidneys were examined.

Surgical techniques (Fig. 1)

Each rabbit was anaesthetised with an intramuscular injection of ketamine (40 mg/kg), acepromazine (0.5 mg/kg) and atropine (0.001 mg). General anaesthesia was maintained by inhalation of oxygen, nitrous oxide and halothane (2%). The neck, ear and groin areas were shaved and cleaned with betadine solution. After performing tracheotomies, a tracheal tube was inserted and the rabbits were mechanically ventilated with room air; their general condition soon became stable with controlled respiration. A polyethylene catheter (internal diameter 0.2 mm, external diameter 0.5 mm, from Natume Co. Ltd, Japan) was filled with heparinised saline (15 i.u./ml) and inserted into the left ventricle from the left common carotid artery. From the change of arterial pressure gradients, it was confirmed that the top of the catheter was in the left

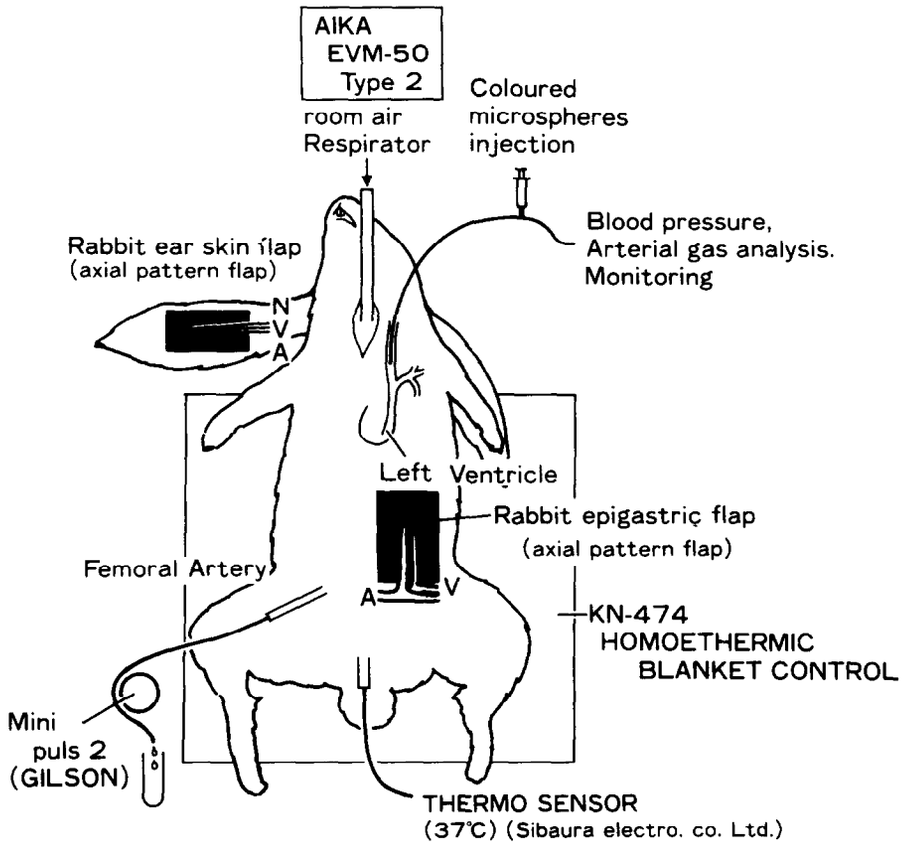

Fig. 1

Figure 1—Illustration of the operative procedure.

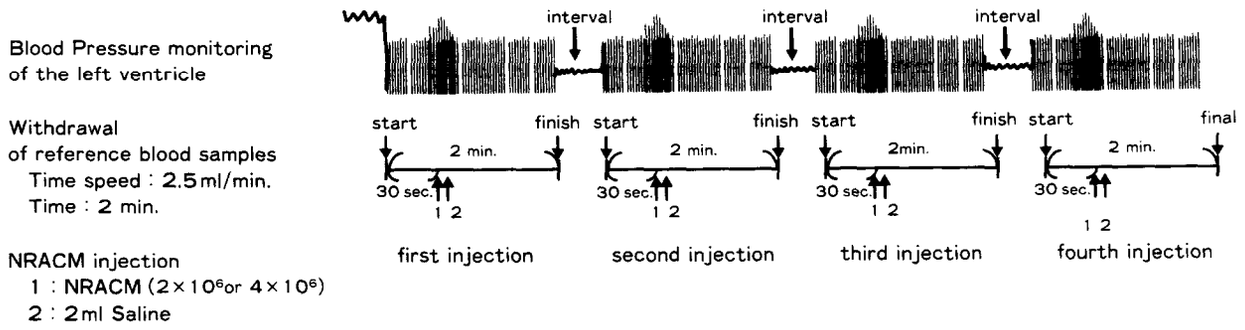

Fig. 2

Figure 2—The time chart of injections and the blood pressure of the left ventricle.

ventricle. Another catheter was inserted into the right femoral artery to take a reference blood sample. Then a 3.0 × 4.5 cm size axial pattern flap with central artery and veins on the right ear and a 4 × 6 cm size axial pattern flap on the left abdomen were elevated and sutured back into the same position. Rectal temperature was controlled at 37°C using a thermosensor (Sibaura Electro Co. Ltd, Japan) (Fig. 1).

Injection method (Fig. 2)

A one-shot injection of NRACM was made 4 times in each animal into the left ventricle. A reference blood sample was drawn from the right femoral artery at the rate of 2.5 ml/min for 2 min. Thirty seconds after the start of drawing blood, a one-shot injection of NRACM immediately followed a one-shot injection of 2 ml saline solution made into the left ventricle. During injections, the blood pressure of the left ventricle was monitored, and was found to be unaffected by the injection (Fig. 3). After confirmation of stability of blood pressure in the left ventricle, the other different coloured microspheres were injected as soon as possible. The four injections were performed on each rabbit within 15 min. The details of colours and numbers of spheres are given in Table 1. Following the final injection of NRACM, each rabbit was killed with an intravenous injection of pentobarbitone and potassium chloride.

Counting techniques

After sacrifice, samples were collected and weighed immediately. A reference blood sample and NRACM

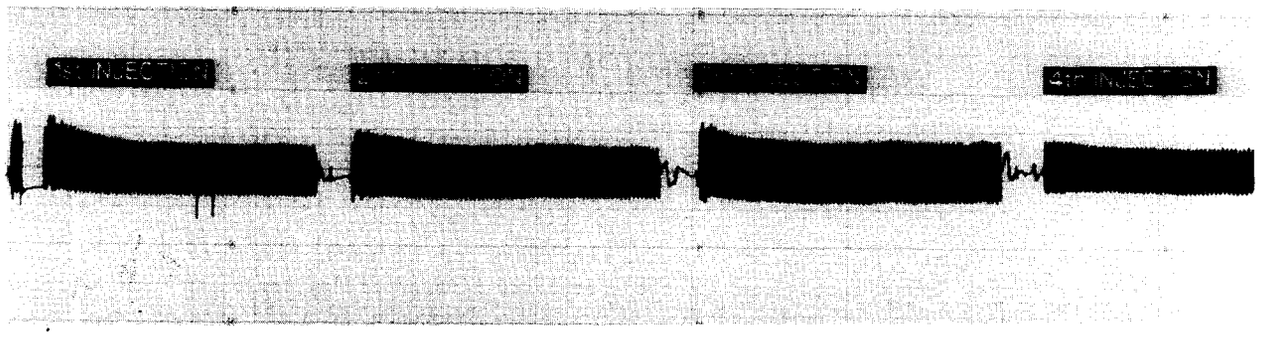

Fig. 3

Figure 3—Blood pressure of the left ventricle. Four repeated injections showed no measurable change in haemodynamics.

Table 1

Rabbit no.	*1st inj.*	*2nd inj.*	*3rd inj.*	*4th inj.*
1	Red*	Green**	Orange*	Black**
2	Blue*	Green**	Black**	Orange*
3	Red**	Orange**	Green*	Black**
4	Orange**	Black*	Green*	Blue**

* 2×10^6; ** 4×10^6.

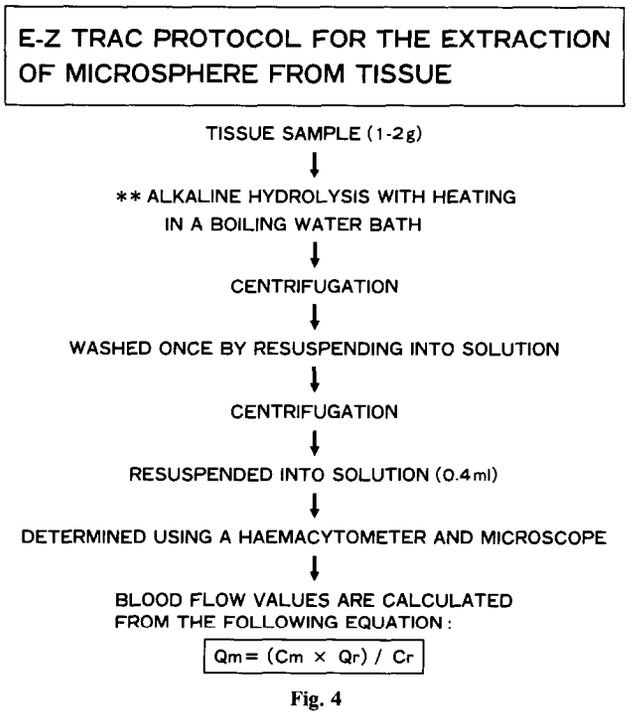

Fig. 4

Figure 4—A flow chart of counting techniques.

were extracted from the tissues basically in accordance with E-Z Trac protocol (Fig. 4). The tissue samples (1–2 g) were finely minced and underwent alkaline hydrolysis by heating in a boiling water bath. Then NRACM were extracted by centrifugation and numbers were determined by using a haemacytometer and microscope. Blood flow values were calculated from the equation: $Qm = (Cm \times Qr)/Cr$ where Qm is the regional blood flow per gram (ml/min/g), Cm is the microsphere count per gram of tissue, Qr is the withdrawal rate of the reference blood sample (ml/min), and Cr is the microsphere count in the reference blood sample.

In case of measurement using RAM in the other three rabbits, the procedure was the same as that of NRACM until sacrifice and both kidneys were counted by gamma counter.

Results

Except for the third injection in the No. 1 rabbit due to technical error, we were able to measure the blood flow of the flaps using the NRACM in all cases (Table 2). The flow values in axial pattern flaps in rabbit ears were $141.2 + 33.7$ ml/min/100 mg (2×10^6 NRACM) and $104.6 + 28.0$ ml/min/100 mg (4×10^6). In epigastric flaps, the values were $77.9 + 18.0$ ml/min 100 mg (2×10^6) and $38.2 + 11.9$ ml/min 100 mg (4×10^6). No significant differences were found in either of the blood flow values between 2×10^6 and 4×10^6 NRACM or between the numbers of injections (Fig. 5). Statistical analyses of NRACM and RAM values of the kidney

Table 2 Blood flow of axial pattern flap using NRACM

		Number of NRACM	
Axial pattern flap	*N*	*2×10^6*	*4×10^6*
Rabbit ear skin flap	4	141.2 ± 33.7	104 ± 28.0**
Epigastric flap	4	77.9 ± 18.0	38.2 ± 11.9**

Mean ± SED ml/min/100 mg.
** No significant difference.

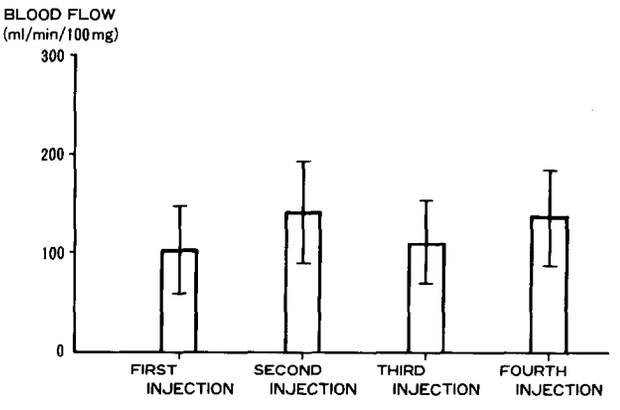

Fig. 5

Figure 5—Comparison between regional blood flow in rabbit ear skin flaps and repeated injections of NRACM. There are no significant differences between repeated injections.

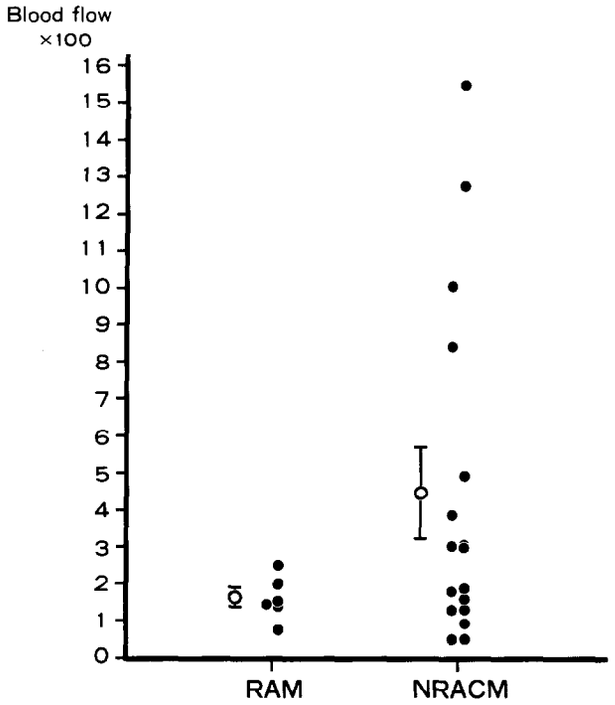

Fig. 6

Figure 6—Comparison of renal blood flow using RAM and NRACM (blood flow ml/min/100 mg): RAM (n = 6); NRACM (n = 16). There are no significant differences between RAMs and NRACMs; however, the counting data of NRACM showed more scatter than that of RAM.

blood flow showed no significant differences between them (Fig. 6).

Discussion

Pang and his colleagues (1984) stated that an ideal technique for determination of blood flow for laboratory research should have the following characteristics: (1) It should measure separately and simultaneously the quantitative capillary blood flow to the skin, muscle, and bone; (2) It should provide consecutive and highly reproducible determinations of capillary blood flow; (3) It should be inexpensive and easy to perform; and (4) It should be noninvasive. Several techniques have been reported for laboratory research. It has been generally accepted that the RAM technique is the best method for measurement of experimental tissue blood flow. The disadvantages of this technique are that it requires special equipment, disposal of animals is inconvenient, dealing with radiation can be dangerous, and its cost is high. To solve these problems, NRACM provide an effective alternative method. This method has already been evaluated in a comparative experiment on the measurement of myocardial blood flow, and a good correlation with RAM results was demonstrated (Hale *et al.*, 1988). Since then, the spotlight has focused on NRACM as a new method for measurement.

Our results indicated no significant difference between NRACM and RAM values of kidney blood flow. Our data using NRACM, however, showed more scatter than that of RAM, perhaps as a result of differences in counting technique. Perhaps, as suggested by others (Hale *et al.*, 1988), future developments in automated sample processing and microsphere counting will resolve this problem.

It is generally accepted that the blood flow in skin flaps in the immediate postoperative stages is unstable (Daniel and Kerrigan, 1979; Awward *et al.*, 1983; Hendel *et al.*, 1983a, b). Hence, to reconfirm the correlation of regional blood flow between RAM and NRACM, we believe that the skin flap just after surgery was not adequate and a tissue which has the property of autoregulation for circulation, such as brain, heart and kidney, should be selected for evaluation in future.

We have shown that there was no significant difference in results between the numbers of NRACM injected (2×10^6 or 4×10^6), and between the number of injections. One injection of 2×10^6 NRACM is sufficient for the measurement of tissue blood flow in our rabbit model, and multiple injections were possible without any measurable change in haemodynamics with 4 repeated injections within the first 30 min. Buckberg and his colleagues (1971) also reported that the three repeated RAM injections (over 300000 microspheres) can be given to rats with no measurable change in haemodynamics.

The importance of being able to repeat microsphere injection is that it is possible to compare flow in different situations, studying for example regional blood flow pre- and post-operatively (Aalto and Slatis, 1984), the influence of exercise (Simkin *et al.*, 1990) or flow status pre- and post-injection of some drugs (Hale *et al.*, 1988). Hence, our experiment results show that it is possible to observe the influence of three various loads between four injections.

Further advantages of NRACM measurement are that there is no need for special equipment or complicated manipulations of animals and it is a non-radioactive and safe system. Furthermore, it is lower in cost than RAM (about 1/5 cost of ^{51}Cr RAM used in this experimental method), multiple injections can be carried out in the same animal, and it is stable, which enables measurement of flow values later in freeze-stored tissues. In addition, the distribution or flow pattern of blood flow can be seen by direct histological observation and can be correlated with blood flow. The disadvantages of NRACM are that considerable time is required for the recovery of microspheres (average time 4 h) and counting data showed slightly more scatter than RAM.

We conclude that the NRACM technique is a very safe and useful method for the measurement of blood flow in flaps.

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