Comparison of Laser Doppler Flowmeter and Radioactive Microspheres in Measuring Blood Flow in Pig Skin Flaps

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Laser Doppler flowmetry is a noninvasive technique commonly used to monitor skin perfusion after free-tissue transfer or replantation in reconstructive surgery. Several investigators have expressed concern about the reliability of the quantitative value provided by laser Doppler flowmeters (LDF) and the extent to which they reflect nutrient blood flow. This experiment was designed to compare quantitatively the skin blood flow in the pig measured by LDF and by 15-μm radioactive microspheres (RMs). It was observed that the skin blood flow rates measured by LDF and RMs in the normal skin and in acute random-pattern and arterialized skin flaps were highly correlated ($r = 0.93$, $P<.01$). However, the skin blood flow rates measured by LDF were consistently higher ($P\leq.05$) than the corresponding flow rates measured by RMs, and this discrepancy increased considerably at low skin blood flow rates (< 2 mL/min/100 g). We speculated that the LDF most likely measured both nutrient and arteriovenous shunt flow in the skin and that this arteriovenous shunt flow at least in part caused the discrepancy in skin blood flow rates measured by the LDF and RMs because the 15-μm RMs are known to measure nutrient blood flow only. The inherent variations and errors in LDF technique were also discussed.

INTRODUCTION

There is an increasing demand from reconstructive surgeons for a way to postoperatively monitor perfusion in flaps and replants for early detection of compromised skin circulation. As a result, a variety of instruments and techniques have been devised for detection of skin blood flow in flaps and replants by monitoring fluorescein dye penetration in skin tissue, transcutaneous oxygen tension, skin pH, and skin temperature and by photoplethysmography, surface ultrasound Doppler, implantable ultrasound Doppler, and laser Doppler flowmeter (LDF).[1,2] Among these techniques, LDF has stood out as a simple, noninvasive, continuous monitoring system that provides a quantitative measurement of skin blood flow. To date, LDF is used by many reconstructive surgeons for postoperative monitoring of skin perfusion in autogenous tissue transplants and replants.[2,3] Despite these advantages, however, there is still uncertainty as to the reliability and limitation of LDF in quantitative determination of skin blood flow.[1,4,5] Reports of skin blood flow detected by LDF in nonperfused skin in experimental skin flaps have heightened these concerns.[6–9] In addition, it is not clear whether LDF measures skin nutrient (tissue) blood flow or total skin blood flow (*i.e.*, nutrient and arteriovenous shunt flow).

The radioactive microsphere (15-μm diameter) technique, although invasive, has been widely accepted as a research laboratory technique for measurement of nutrient blood flow. Several laboratories have used RMs for measurement of skin flap nutrient blood flow in the pig.[10–12] RMs can be used in animal models to test the reliability of other clinical techniques for monitoring of nutrient (tissue) blood flow. Of particular interest to us was to use RMs to evaluate the LDF technique for quantification of nutrient (tissue) blood flow in normal skin and in different skin flap models in the pig.

MATERIALS AND METHODS

Animal Management

Six castrated Yorkshire pigs (12–14 kg) were used. The animal protocol was approved by The Hospital for Sick Children Animal Care Committee. Pigs were anesthetized with ketamine (25 mg/kg intramuscularly) and pentobarbital (20–25 mg/kg intravenously), intubated with an endotra-

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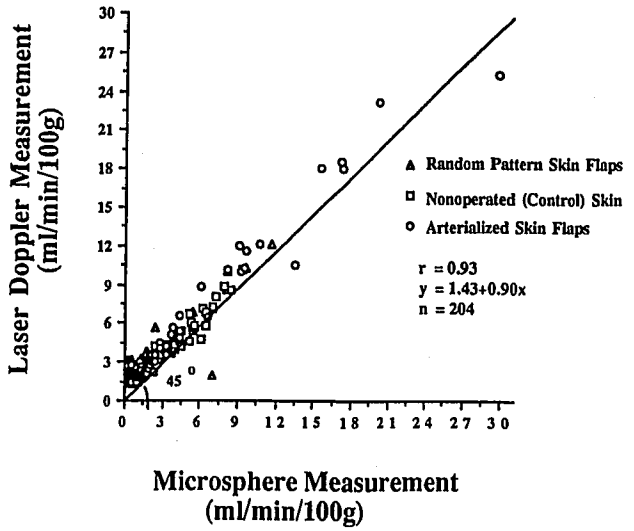

Fig. 1. Relation of skin blood flow measured by LDF and 15-μm RMs in normal skin and acute random-pattern and arterialized skin flaps in the pig.

cheal tube (inner diameter = 5 mm), and mechanically ventilated with a mixture of nitrous oxide and oxygen (50/50) by a respirator (tidal volume ~ 15 mL/kg). A heating blanket was used to maintain constant body temperature, and the rectal temperature (37°–38° C) was monitored continuously. An intravenous line was established through an ear vein to infuse isotonic saline solution (2 mL/min) containing pentobarbital (10 mg/kg/hr) to maintain satisfactory anesthesia and mean arterial blood pressure, which was also monitored throughout the study.

Skin Flap Model

Random-pattern skin flaps. Two mirror-image 4 × 10-cm rectangles were marked on each flank of the pig. These rectangles were 4 cm from the dorsal midline of the pig and were 2 cm apart. A dorsally based 4 × 10-cm random-pattern flap was constructed on one of the rectangles on each flank of the pig. The remaining rectangle on each flank served as nonoperated intact control skin.

Arterialized skin flap. A 6 × 14-cm island buttock skin flap based on the circumflex iliac neurovascular bundle was constructed on both sides of the buttock in each pig. This skin flap model consisted of arterialized and random portions in the proximal and distal portions of the flap, respectively.

Determination of Skin Blood Flow

The intact skin and skin flaps were marked transversely into 2-cm–wide segments from the pedicle to the distal end for blood flow studies. Skin blood flow was measured by using the RMs and LDF technique 6 hours after the raising of skin flaps as detailed below.

Technique

A single-channel LDF equipped with the L-type skin surface probe (ALF 21, Transonic Systems Inc., Ithaca, N.Y.) was used. This system used a 2-mW infrared laser with a wavelength of 780 nm. The flow rate of the LDF was calibrated in mL/min/100 g tissue by the manufacturer. Skin blood flow was measured at each marked 2-cm segment along the intact control skin and skin flaps from the pedicle to the distal end. The lowest reading at which the LDF stabilized was taken as the final value. Three readings were taken at equidistance transversely across each 2-cm skin segment to obtain an average value for each segment. About 10 minutes after laser Doppler measurements were completed, RMs were injected to measure skin blood flow.

Technique

The principles and laboratory technique in using RMs for measurement of skin blood flow in the pig have been described in detail.[10]

Briefly stated, cobalt 57–labeled microspheres of 15.7 ± 0.5 μm in diameter (New England Nuclear, Boston, Mass.) were suspended in 8 mL of 0.9% saline containing 5% sucrose and 0.05% Tween 80. These RMs were subjected to 10 minutes of sonication and were vortexed vigorously for 1 minute before injection. RMs (~ 120,000/kg) were injected into the left ventricle over a period of 30 seconds through a carotid artery catheter. A withdrawal pump connected to a femoral artery catheter was turned on 10 seconds before and 30 seconds after injection of RMs. This reference blood sample was transferred to a counting vial. The intact skin and skin flaps were cut transversely into 2-cm segments. The radioactivities of these skin segments and the reference blood sample were determined with a gamma counter (1282 Compugamma CS, LKB Wallac, Finland). A microcomputer was programmed to calculate skin blood flow (mL/min/100 g) by using the formula reported previously.[10] The pig was killed with an overdose of pentobarbital (100 mg/kg) after this blood flow study.

RESULTS

With linear regression analysis it was observed that the skin blood flow rates measured by LDF in each 2-cm skin segment correlated highly with the corresponding skin blood flow rates measured by RMs in the nonoperated (control) skin ($r = 0.94$; n = 60, $P<.01$), random-pattern skin flaps ($r = 0.94$, n = 60, $P<.01$), and arterialized skin flaps ($r = 0.96$; n = 84, $P<.01$). The overall correlation coefficient (r) for all 2-cm skin segments was 0.93 (n = 204, $P<.01$; Fig. 1). For a total of 204 skin segments studied, in only 12 skin segments was the skin blood flow measured by the LDF similar to or lower than that measured by the RMs; these data points are located at or below the 45-degree line in Figure 1. The rest of the data points are located above the 45-degree line, indicating that the blood flow rates measured by LDF were consistently higher than the corresponding blood rates measured by RMs.

The skin blood flows measured by LDF and RMs in 2-cm skin segments at the same distance from the base were also compared by using the paired t test. The skin blood flow rates measured by LDF in skin segments 2, 4, 6, 8, and 10 cm from the base were consistently higher ($P \leq .05$) than the corresponding skin blood flow rates measured by RMs in the nonoperated (control) skin and in random-pattern skin flaps (Table I). Similarly, the skin blood flow rates measured by

TABLE I.
Comparison of Skin Blood Flow Measured by Radioactive Microspheres and Laser Doppler Flowmeter in Random-Pattern Skin Flaps and Nonoperated Control Skin. Values are mean ± SEM; n = 12.

Distance From Pedicle (cm)	Skin Blood Flow (mL/min/100 g)			
	Random-Pattern Skin Flap		Nonoperated Skin	
	RMs	LDF	RMs	LDF
10	0.13±0.04	1.74±0.10*	3.23±0.65	3.58±0.53
8	0.25±0.12	1.96±0.13*	3.30±0.55	3.62±0.49*
6	0.60±0.10	2.16±0.13*	3.01±0.53	3.79±0.56*
4	2.00±0.40	3.46±0.44*	3.27±0.48	3.98±0.39*
2	4.88±0.94	5.57±0.90*	5.46±0.80	6.09±0.78*

*Significant difference ($P \leq .05$) in mean skin blood flow measured by RMs and LDF in 2-cm skin segments at the same distance from the base (by paired *t* test).

TABLE II.
Comparison of Skin Blood Flow Measured by Radioactive Microspheres and Laser Doppler Flowmeter in Arterialized Skin Flaps. Values are mean ± SEM; n = 12.

Distance From Pedicle (cm)	Skin Blood Flow (mL/min/100 g) in Arterialized Skin Flaps	
	RMs	LDF
14	0.41±0.13	1.98±0.46*
12	0.76±0.16	2.03±0.05*
10	1.99±0.47	2.98±0.71*
8	2.23±1.04	2.93±0.38*
6	3.89±1.39	4.91±1.40*
4	6.04±1.86	7.43±2.01*
2	8.21±2.36	9.11±1.86*

*Significant difference ($P \leq .05$) in mean skin blood flow measured by RMs and LDF in 2-cm skin segments at the same distance from the base (by paired *t* test).

LDF in skin segments 2, 4, 6, 8, 10, 12, and 14 cm from the base were consistently higher ($P \leq .05$) than the corresponding blood flow rates measured by RMs in the arterialized skin flaps (Table II). This observation confirmed the observation in Figure 1 in that the skin blood flow rates measured by LDF were higher than the corresponding flow rates measured by RMs in the pig skin.

Both LDF and RMs detected a relatively uniform skin blood flow between 2 and 10 cm from the base in the nonoperated (control) skin (Table I) and a gradual decrease in skin blood flow from the pedicle toward the distal and (*i.e.*, gradient blood flow) in the random-pattern (Table I) and arterialized skin flaps (Table II). On average, the skin blood flow rates measured by LDF were 16% ± 3% higher than those measured by RMs in the normal skin. The average discrepancy between LDF and RMs in the measurement of skin blood flow was 22% ± 4% in arterialized skin flaps, where the skin blood flows measured by RMs were higher than 2 mL/min/100 g (Table II). However, the discrepancy in skin blood flow rates measured by LDF and RMs increased considerably in the random-pattern and arterial skin flaps, where the skin blood flow rates measured by RMs were at or below 2 mL/min/100 g (Tables I and II).

DISCUSSION

In the present experiment, we compared quantitatively the skin blood flow rates measured by LDF and RMs in normal skin and in acute random-pattern and arterialized skin flaps in the pig. Three important observations were made from this experiment: *1.* there was a high correlation ($P < .01$) in skin blood flow rates measured by RMs and LDF (Fig. 1); *2.* the skin blood flow rates measured by LDF were consistently higher than the corresponding rates measured by RMs; and *3.* the discrepancy between the measurement of skin blood flow by LDF and RMs was quite consistent in normal skin samples (16% ± 3%) and in skin flaps (22% ± 4%) at flow rates higher than 2 mL/min/100 g, but the discrepancy was considerably higher (>160%) when the flow rate was below 2 mL/min/100 g as measured by RMs (Tables I and II).

Several possibilities may explain the discrepancies between LDF and RMs in the measurement of skin blood flow: *1.* RMs consistently underestimated the skin nutrient blood flow; *2.* LDF measured nutrient and arteriovenous shunt flow in the pig skin; or *3.* LDF consistently overestimated the skin blood flow due to error in calibration or mechanical problems.

It is unlikely that RMs could have consistently underestimated the skin nutrient blood flow. Theoretically, all 15-μm RMs should have been trapped in the small arterioles (<15 μm) immediately before the capillary beds; thus, RMs, were more likely to slightly overestimate rather than underestimate skin nutrient blood flow. Furthermore, although our previous observations[10] indicated that the random error in the RMs technique could increase considerably at low skin blood flow rates (<1 mL/min/100 g), this random error could not account for the considerably large discrepancy between LDF and RMs in the measurement of skin blood flow at low flow rates (<2 mL/min/100 g) in the present experiment.

There is also the possibility that high LDF readings could be attributed to improper calibration of baseline or damage in the fiberoptic cable of the probe.[11] If calibration error were responsible for the high LDF readings, one would expect that the discrepancy between LDF and RMs in the measurement of skin blood flow should be relatively consistent. However, the discrepancy between LDF and RMs in the measurement of skin blood flow varied considerably at different ranges of flow rates (Tables I and II).

We[12] and other investigators[13–14] have observed previously that the arteriovenous shunt flow in the normal skin and acute random-pattern skin flaps on the flanks of the pig was more than 60% of the total blood flow. Therefore, it is likely that LDF measured nutrient and some arteriovenous shunt flow in the present experiment, which would explain why skin blood flow rates measured by LDF were consistently

higher in the normal skin (16% ± 3%) and acute skin flaps (22% ± 4%) compared to the corresponding flow rates measured by RMs and why these discrepancies were quite consistent at flow rates higher than 2 mL/min/100 g (Tables I and II). By the same token, it could be speculated by extrapolation that the arteriovenous shunt flow in the distal portion of the skin flap was considerably higher and thus resulted in a considerably larger discrepancy in the measurement of skin blood flow by LDF and RMs in this area. Reinisch[15] has reported that distal ischemia in acute random-pattern skin flaps in the pig was the result of a considerably high arteriovenous shunt flow in this area; this observation supports the aforementioned speculation. However, we[12] and other investigators[14] observed little or no blood flow in the distal portion of random-pattern skin flaps between 6 and 10 cm from the pedicle and also failed in confirm high arteriovenous shunt flow rate at the distal portion of these flaps. Alternatively, it could be speculated that the LDF used in our experiment was not sensitive enough for detection of changes in flow rates below 2mL/min/100 g. This speculation is supported by the observation that there was little or no change in skin blood flow rates measured by LDF in the distal portion of the random-pattern skin flaps (6 to 10 cm from the pedicle) and arterialized skin flaps (10 to 14 cm from the pedicle; Tables I and II).

There are different models of LDF made by different manufacturers, and obviously there are differences in sensitivities and calibration among these models. We can also expect variation in calibration even within the same model. Therefore, it is not proper to draw specific conclusion concerning the application and accuracy of LDF for monitoring of skin blood flow on the basis of observations obtained from one LDF used in the present experiment. However, the discrepancies in measurement of skin blood flow between LDF and RMs observed from the present experiment should draw the attention of LDF users to the potential inherent errors in using LDF. Specifically, the LDF most likely detects both nutrient and arteriovenous shunt flow in the skin; the sensitivity of LDF may vary with flow rates, and LDF reliability at low flow rates should be cautioned. Furthermore, since most LDFs are calibrated with normal tissues, the quantitative values provided by LDF are most likely subject to variability depending on the pathologic condition of the skin tissue, especially at low flow rates.

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