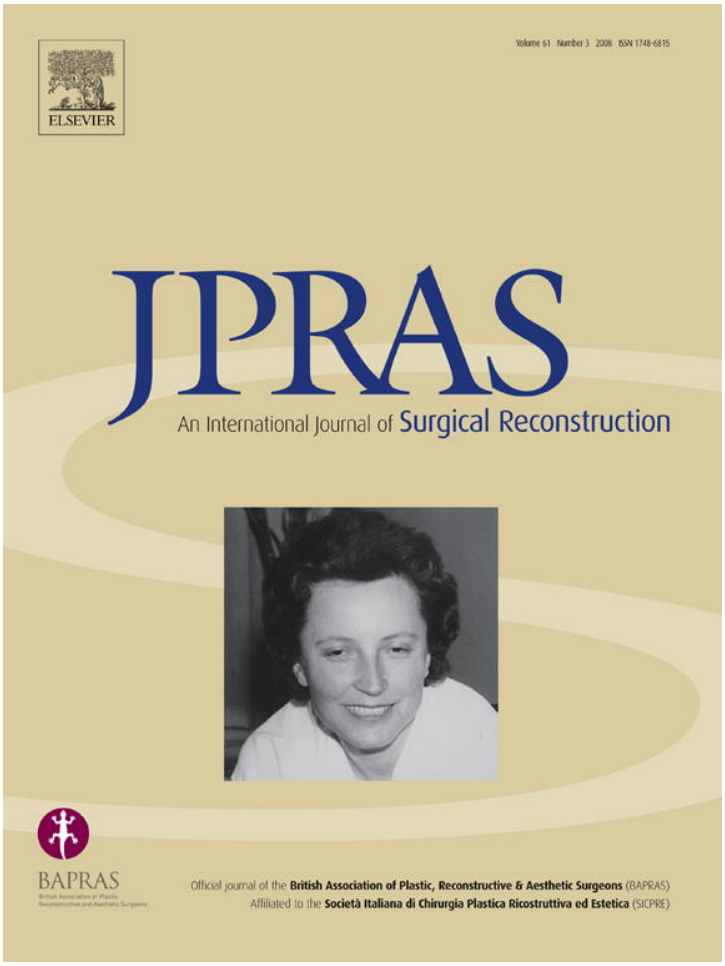

Journal of Plastic, Reconstructive & Aesthetic Surgery (2008) 61, 289–296

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JPRAS
An International Journal of Surgical Reconstruction
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# Evaluation of near infrared spectroscopy in monitoring postoperative regional tissue oxygen saturation for fibular flaps

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Received 22 January 2007; accepted 17 October 2007

**KEYWORDS**
Near infrared spectroscopy (NIRS);
Monitoring blood microcirculation;
Regional tissue oxygen saturation ($rSO_2$);
Autogenous vascular free fibular flaps

**Summary** The ability of near infrared spectroscopy (NIRS) to predict vascular compromise in vascular free flaps postoperatively has been assessed, and the extent of regional tissue oxygen saturation ($rSO_2$) after fibular flap transplantation was investigated quantitatively.

To validate the sensibility and precision of the technique, the following methods were used. (1) Forearm vessel obstructive tests were conducted in four healthy volunteers. (2) Measurement and analysis of bilateral $rSO_2$ at the mandibular body and ramus were performed in 40 healthy volunteers by NIRS in the morning and afternoon. (3) Measurement and analysis of $rSO_2$ in transplanted fibular flaps for 41 cases with mandibular reconstruction were performed by NIRS at postoperative days 1–6. The results were: NIRS had high sensibility and precision in monitoring $rSO_2$ of living tissues. No significant difference in the values of $rSO_2$ was found across different times or areas in the normal mandible. However, $rSO_2$ in the transplanted fibular flaps was reduced compared to the value on the control side. $rSO_2$ decreased gradually 4–12 hours postoperatively. After that period, $rSO_2$ increased gradually and approached the value of the control side at 20 h after the operation.

It can be concluded that NIRS is a reliable noninvasive method for monitoring blood circulation in transplanted tissues, particularly for buried flaps.


Autogenous vascular free fibular flap transplantation is an ideal choice for reconstruction of long-span mandibular defects. Three modalities of fibular flaps are used for mandibular reconstruction, including vascular fibular

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doi:10.1016/j.bjps.2007.10.047

bone-alone transplantation and osteocutaneous and osteomusculocutaneous fibular flap transplantation. In our department, the pure bony transplantation (i.e. fibular flap transfer) is commonly used. This kind of fibular flap is usually buried at a different depth within the normal soft tissues. However, it is very difficult to monitor blood flow for this kind of flap. Therefore, a skin island is applied during surgery to serve as the 'monitoring window' (Fig. 1) and is removed later. Near infrared spectroscopy (NIRS) may be a possible method for monitoring the viability of the buried fibular flap, in place of a skin island.

Since Jöbsis first used NIRS in monitoring cerebral and myocardial oxygen in 1977,[1] this technique has undergone great progress in its development and use. It has been used in various experimental and clinical settings to investigate tissue perfusion and oxygenation noninvasively, such as oxygen saturation in neonatal brain tissue[2] or in athletic muscles.[3] Its application in plastic and reconstructive surgery has only recently been reported.[4,5] However, there is no established technique for monitoring the oxygen saturation of buried flaps. Since 1995, a research team in Tsinghua University has performed serial investigations on NIRS and used it to assess changes in tissue oxygenation, haemoglobin oxygenation, and blood volume in a rhesus monkey with a prefabricated forearm flap.[6] Based on the steady-state spatially resolved spectroscopy (SRS) algorithm developed by Farrell et al.,[7] our team developed a NIRS oximeter (TSNIR-3).[8] This enables the monitoring and evaluation of the regional tissue oxygen saturation ($rSO_2$) and tissue viability following flap transfer. The current study evaluated the application of TSNIR-3 in monitoring microcirculation of fibular flaps after mandibular reconstruction with three specific goals: (1) to test the sensibility and precision of the NIRS oximeter; (2) to measure and analyse the $rSO_2$ of the normal mandible; and (3) to determine the normal measurements of regional tissue oxygen saturation ($rSO_2$) after fibular flap transplantation.

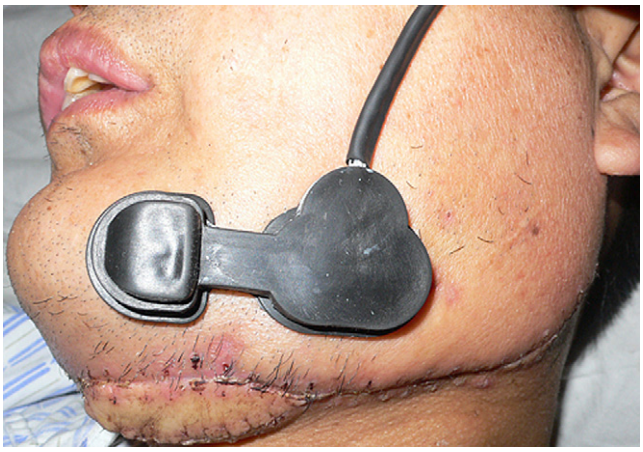

**Figure 1** The NIRS monitoring detector and the skin island of the fibular flap which serves as the 'monitoring window' for the time being.

## Materials and methods

### Equipment

The TSNIR-3 was designed by the Department of Biomedical Engineering, School of Medicine, Tsinghua University, based on the SRS algorithm. The sensor of the NIRS oximeter consisted of a two-wavelength near infrared light source, with emitting wavelengths of 760 nm and 850 nm, and three near infrared PIN (position indicator) detectors. According to our previous serial research,[2,6,9–11] if we put the light source and the detector at a certain distance on the human tissue surface, the emitted light can be detected. The average penetration depth of near infrared light is generally half the distance between the light source and the detector. For example, if the distance is 30 mm, the average penetration depth is about 15 mm. If the distance is 40 mm, the average penetration depth is about 20 mm. The distances between the light source and the three detectors in TSNIR-3 were 20 mm, 30 mm, and 40 mm, respectively. In order to gain the $rSO_2$ in the depth of mandible or transferred fibular bone under the soft tissue, two detectors (30 mm and 40 mm) of the NIRS oximeter were selected to be used in the following investigations, controlled by the software. The probe was sterilised with 75% alcohol before every monitoring, then the probe was lightly (no pressure) put on the skin surface overlying the monitoring area during the monitoring stage (Fig. 1).

### Sensibility and precision test of TSNIR-3

In order to test the sensibility and precision of the TSNIR-3 and to prepare it for the clinical application of monitoring $rSO_2$ in the transplanted fibular flaps, four healthy adult volunteers were recruited to participate in this study. Two kinds of different forearm vascular occlusion tests were performed in every case as follows:

*Measurement of normal $rSO_2$ in human forearm*. The blood pressure of every volunteer was measured, and the values of the systolic and diastolic pressure were recorded. The TSNIR-3 detector was placed on the radial skin of the forearm, and the researchers waited until the volunteer was calm. Then, the $rSO_2$ was measured and used as the normal control value.

*Forearm venous occlusion test*. The sleeve-pressing method was used to perform the forearm venous occlusion experiment. The pressure was increased over the diastolic pressure with the aid of the blood pressure measuring system and maintained for 2 min. The pressure was then released to the normal state. The $rSO_2$ of the forearm was measured using the TSNIR-3 at the time of the occlusion, immediately and 2 min after venous occlusion, and immediately and 2 min after the release of the occlusion.

*Forearm total vascular occlusion test*. With the same method as mentioned above, the pressure was increased over the systolic pressure to ensure the artery and venous blood flow of the forearm was impeded completely, and this complete occlusion was maintained for 2 min. The pressure was then released and allowed to recover to the normal state. The $rSO_2$ of the forearm was measured using the TSNIR-3 at the time of occlusion, immediately

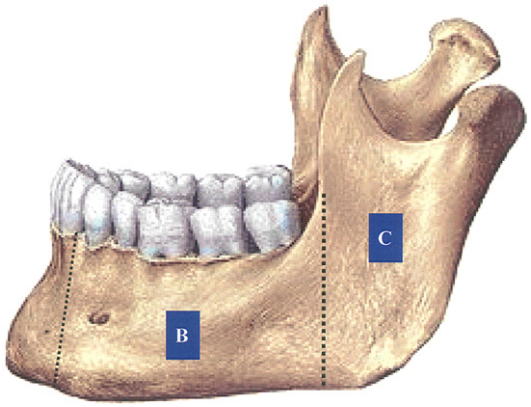


**Figure 2** Measuring position of the normal mandible (B, mandibular body; C, mandibular ramus).

and 2 min after total vascular occlusion, and immediately and 2 min after the release of the occlusion.

### Measurement and analysis of $rSO_2$ in the normal mandible

Forty healthy adult volunteers (20 males and 20 females), who ranged in age from 21 to 70 years with a mean age of 33 years, participated in this part of the study. The $rSO_2$values in the bilateral mandibular body and ramus (Fig. 2B, C area) were measured and analysed using the TSNIR-3 in the morning (9:00 am) and afternoon (4:00 pm). *t*-Tests and ANOVAs were used to compare the values of $rSO_2$ at different times and different positions with the aid of statistics software SPSS 10.0.

### Investigation of $rSO_2$ in the transplanted fibular flaps

Forty-one patients undergoing autogenous mandibular reconstruction by vascular fibular flap transplantation were randomly included in this part of the study. Some cases used a skin flap because of soft tissue defects; a small number of complicated cases used a skin island for monitoring the blood flow postoperatively. The patients' ages ranged from 14 to 73 years, with a mean of 42 years. The ratio of males to females was 3:1. In all 41 cases, the $rSO_2$ of the free fibular flaps was measured and analysed using TSNIR-3 on postoperative days 1—6. The measurements were performed once every 4 h within the first 24 h postoperation, and twice per day in the following 5 days. The remaining healthy mandibles were used as controls.

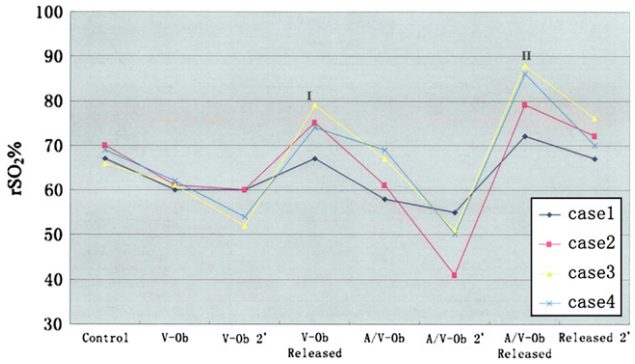


**Figure 3** Results of sensibility and precision test using the TSNIR-3 (V, vein; Ob, obstruction; A, artery).

The paired *t*-test was used to compare the $rSO_2$ values between the transplanted fibular flaps and the remaining healthy mandible.

## Results

### Sensibility and precision of the TSNIR-3

The measurements from the forearm vascular occlusion test in the four volunteers are shown in Table 1. Based on these data, the $rSO_2$ curve is shown in Fig. 3. In the venous occlusion test, the $rSO_2$ in the forearm decreased significantly because the venous outflow was obstructed suddenly. After 2 min, it decreased to a relatively stable level, and the range of this decrease was 7—15%. When the occlusion was released, the $rSO_2$ value rebounded rapidly and became a little higher than the normal value (Fig. 3: peak I). In the total vascular occlusion test, the $rSO_2$ of the forearm decreased much more rapidly than that found with the venous occlusion. The range of this decrease was 12—24%. After 2 min, it was at a relatively stable level. When the occlusion was released, the $rSO_2$ value recovered to normal or slightly higher than normal (Fig. 3: peak II) and remained there for 2 min. It then approached normal gradually. The results of the ANOVA examining $rSO_2$ across different time stages in each group indicated a significant difference ($P < 0.05$) within each group.

### Measurement and analysis of $rSO_2$ levels in the normal mandible

The $rSO_2$ values in different mandibular positions were measured in 34 healthy volunteers at 9 am and 35 cases

**Table 1** Results of the sensibility and precision test of the TSNIR-3 ($rSO_2$%)

| Case | Control | Venous occlusion test | | | Total vascular occlusion test | | | |
|---|---|---|---|---|---|---|---|---|
| | | Immediately | Post 2 min | Released | Immediately | Post 2 min | Released | Released 2 min |
| 1 | 67 | 60 | 60 | 67 | 58 | 55 | 72 | 67 |
| 2 | 70 | 61 | 60 | 75 | 61 | 41 | 79 | 72 |
| 3 | 66 | 61 | 52 | 79 | 67 | 51 | 88 | 76 |
| 4 | 69 | 62 | 54 | 74 | 69 | 50 | 86 | 70 |

**Table 2** The results of $rSO_2$ in the normal mandible ($M \pm SD$,%)

| Time | Cases | Mandibular body | | Mandibular ramus | |
|---|---|---|---|---|---|
| | | Left side | Right side | Left side | Right side |
| 9am | 34 | $70.32 \pm 3.67$ | $68.38 \pm 3.24$ | $70.35 \pm 3.50$ | $69.62 \pm 3.04$ |
| 4pm | 35 | $69.74 \pm 3.14$ | $69.37 \pm 3.72$ | $70.49 \pm 3.83$ | $69.51 \pm 4.21$ |

at 4 pm, respectively, and at both 9 am and 4 pm in 29 cases. The results are shown in Table 2. The $rSO_2$ levels were not significantly different between the morning and afternoon ($P > 0.05$). There was no significant difference in the $rSO_2$ levels in the mandibular body or ramus or between the different sides ($P > 0.05$).

## Measurement and analysis of $rSO_2$ in fibular flap transplantation

Out of the 41 cases with fibular flap transplantation, one transplantation failed to survive after the operation and that patient's data are not included in the present results. The measurement and analysis of the results for the fibular flaps are shown in Table 3, and Fig. 4 shows the $rSO_2$ curve. From the data and the curve, the changes in $rSO_2$ levels in free fibular flaps were identified. There was a significant difference in $rSO_2$ levels between the transferred fibular bone and the control side of the mandible ($P$ value in Table 4). The $rSO_2$ levels in the fibular flaps were lower than those on the control side, but these differences were not large. The range of this decrease was 1.33—4.25%, indicating that the blood perfusion in the fibular flaps was altered but within the normal range. Fig. 4 shows the levels of $rSO_2$ in the fibular flaps in the postoperative 144 h (6 days). The $rSO_2$ levels of the control mandible were stable in approximately 70% of the cases. However, in the transferred fibular flaps, the $rSO_2$ levels decreased immediately after the operation and approached their lowest values 4—12 h after the operation. The change in $rSO_2$ levels ($\Delta rSO_2$%) was 4.2% at the lowest point. The $rSO_2$ values then increased gradually and approached control values 20 h after the operation. We did not find any cases of false positive results in the monitor and in the skin island.

## Measurement and analysis of $rSO_2$ values in the failed case with fibular flap transplantation

The failed case was a female patient aged 38 years, who suffered from mandibular gingival malignant melanoma. The fibula with skin flap was used for the mandible and surrounding soft tissue reconstruction. Venous crisis was found by NIRS monitoring and the skin flap 10 h after the operation. Anastomosis of the vein was immediately performed again, and the skin flap was taken out. Nonetheless, it suffered from intraoral infections on postoperative day 7. The flap underwent necrosis beginning from the distal end of the transferred bone. The flap was monitored by TSNIR-3, and the results are shown in Table 4 and Fig. 5, which was consistent with the changing trend of $rSO_2$ in the fibular flap at the 4th hour after the first operation. During postoperative hours 6—8, the skin island of the fibular flaps showed hyperaemia, followed by extravasation.

**Table 3** The results of $rSO_2$ in 40 cases of alive fibular flap transplantation ($rSO_2$%), ($M \pm SD$,%)

| Time (Post-op h) | No. of cases | Transferred fibular bone ($rSO_2$%) | Control group ($rSO_2$%) | $\Delta rSO_2$% | $P$ value |
|---|---|---|---|---|---|
| 0 | 40 | $71.20 \pm 4.16$ | $74.15 \pm 4.09$ | $2.95 \pm 4.02$ | 0.00 |
| 4 | 40 | $70.68 \pm 3.63$ | $74.93 \pm 4.74$ | $4.25 \pm 4.69$ | 0.00 |
| 8 | 40 | $69.43 \pm 4.49$ | $73.58 \pm 4.45$ | $4.15 \pm 3.96$ | 0.00 |
| 12 | 40 | $69.80 \pm 4.27$ | $73.98 \pm 3.99$ | $4.18 \pm 4.75$ | 0.00 |
| 16 | 38 | $71.24 \pm 4.98$ | $74.29 \pm 4.00$ | $3.05 \pm 4.31$ | 0.00 |
| 20 | 33 | $73.03 \pm 5.34$ | $75.33 \pm 3.26$ | $2.30 \pm 4.95$ | 0.012 |
| 24 | 35 | $70.83 \pm 5.32$ | $74.77 \pm 4.58$ | $3.94 \pm 5.98$ | 0.00 |
| 36 | 27 | $73.48 \pm 5.25$ | $74.81 \pm 5.53$ | $1.33 \pm 4.29$ | 0.118 |
| 48 | 40 | $71.85 \pm 5.27$ | $75.23 \pm 4.41$ | $3.38 \pm 4.64$ | 0.00 |
| 60 | 31 | $72.71 \pm 5.37$ | $74.81 \pm 4.09$ | $2.10 \pm 5.90$ | 0.057 |
| 72 | 37 | $72.19 \pm 5.57$ | $74.97 \pm 4.26$ | $2.78 \pm 5.57$ | 0.004 |
| 84 | 34 | $72.06 \pm 5.56$ | $74.50 \pm 3.74$ | $2.44 \pm 5.06$ | 0.008 |
| 96 | 22 | $71.55 \pm 5.48$ | $74.82 \pm 3.70$ | $3.27 \pm 5.82$ | 0.015 |
| 108 | 29 | $71.79 \pm 4.68$ | $74.76 \pm 3.92$ | $2.97 \pm 5.12$ | 0.004 |
| 120 | 16 | $75.63 \pm 2.87$ | $77.38 \pm 3.63$ | $1.75 \pm 3.80$ | 0.089 |
| 132 | 24 | $72.38 \pm 5.52$ | $74.21 \pm 4.24$ | $1.83 \pm 5.25$ | 0.10 |
| 144 | 17 | $72.53 \pm 4.85$ | $74.76 \pm 4.29$ | $2.24 \pm 3.25$ | 0.012 |
| Total | 543 | $71.66 \pm 5.01$ | $74.68 \pm 4.21$ | $3.02 \pm 4.87$ | 0.012 |

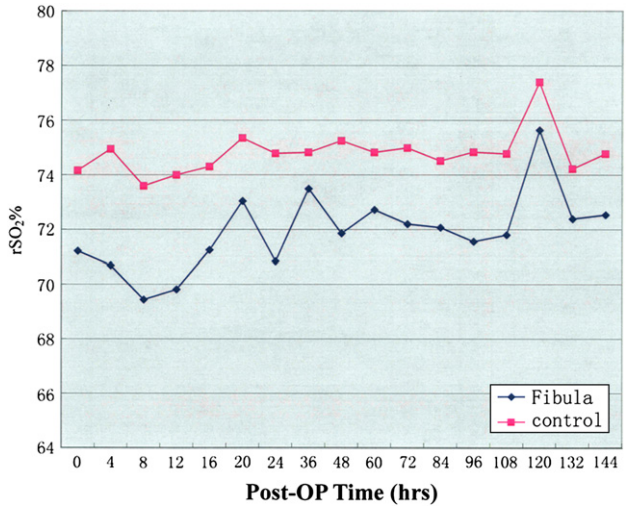


**Figure 4** Results of $rSO_2$ ($rSO_2$%) in 40 cases of alive fibular flap transplantation for 6 days postoperatively.

The skin colour changed gradually from fresh red to dark red. The $rSO_2$ value was changed from point A to point B (Fig. 5). At 10 h postoperation, the venous anastomosis was performed again. During the first 6 h after the operation, the $rSO_2$ value was under 60%. At the 10th hour after the operation, $rSO_2$ levels slowly recovered to normal values (Fig. 5, point C). When necrosis of the flap occurred after the second operation, the $rSO_2$ value of the transferred fibular flap decreased to less than 60%. The $rSO_2$ value in the necrotic fibular flap was lower than that in the control mandible (Fig. 6).

**Table 4** Measured results of $rSO_2$ in the failed case ($rSO_2$%)

| Time (Post-op h) | Transferred fibular bone ($rSO_2$%) | Control group ($rSO_2$%) | $\Delta rSO_2$% |
|---|---|---|---|
| 0 | 55 | 63 | 8 |
| 4 | 66 | 68 | 2 |
| 8 | 64 | 66 | 2 |
| 10 | 70 | 68 | −2 |
| 0(anastomosis again) | 48 | 61 | 13 |
| 4 | 49 | 63 | 14 |
| 8 | 52 | 63 | 9 |
| 12 | 61 | 63 | 2 |
| 16 | 63 | 70 | 7 |
| 20 | 60 | 61 | 1 |
| 24 | 65 | 68 | 3 |
| 36 | 59 | 64 | 5 |
| 48 | 58 | 73 | 15 |
| 60 | 60 | 63 | 3 |
| 72 | 61 | 68 | 7 |
| 84 | 61 | 64 | 3 |
| 96 | 61 | 64 | 3 |
| 108 | 62 | 72 | 10 |
| 120 | 52 | 56 | 4 |

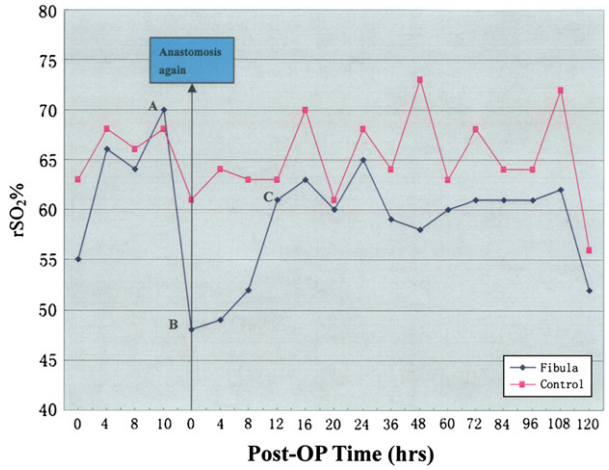


**Figure 5** Measured results of the failure case ($rSO_2$%).

## Discussion

During the last 7 years, over 1500 operations for cases with autogenous vascular free flaps were performed in our department by two microsurgery teams. In those cases, half of the free flaps were fibular flaps. Most of them were monitored postoperatively using a monitoring window (i.e. 'skin island'). Because the mandible defect did not typically include soft tissue, the skin island is not necessary for the reconstruction and is normally removed after the monitoring stage (e.g. postoperative day 7). Some microsurgeons consider the skin island as an ideal monitoring method for the deep buried flaps. However, it is invasive for patients and, thus, a noninvasive, low-cost, portable bedside monitoring method would be highly useful. With the development of microsurgical techniques over the last decade, the success rate of free flaps has greatly improved. Postoperative blood flow monitoring after free tissue transplantation has progressed considerably, especially for monitoring the success of microvascular anastomosis and analysing the perfusion of the transferred tissue.[12]

However, for the buried flaps, there is no ideal method as yet. According to Jones,[13] the ideal monitor should be

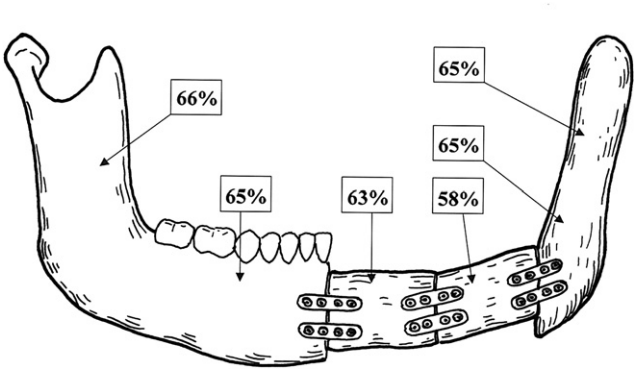


**Figure 6** Six points of necrotic fibular bone were measured in the failure case at the 8th postoperative day. The $rSO_2$ in the distal part of the necrotic fibular flap was lower than the controlled mandibular bone.

noninvasive, reliable, objectively repeatable, promptly reactive to blood flow changes, appropriate for continuous monitoring in all kinds of free tissue transfer, usable for the unskilled, and economically available. Currently, most microsurgeons rely on clinical experience to assess these changes, though techniques such as ultrasound,[14] hydrogen clearance technique[15] and laser Doppler[16] have been used. These methods are limited, however, in measuring changes close to the surface, and some methods are complex and expensive for clinical application. NIRS is a relatively new, noninvasive technique that allows continuous and immediate monitoring of changes in oxygen saturation within a flap at a variety of tissue depths.

Early in development, NIRS was used in reconstructive surgical experiments by Irwin[17] and Hayden.[18] Hayden et al. monitored tissue oxygen saturation and quantities of deoxygenated haemoglobin and oxygenated haemoglobin when flaps underwent venous or arterial occlusions with the aid of NIRS. The oxygen saturation (percentage of oxygenated haemoglobin) was calculated as the difference between two light intensities (860—750 nm) at two time periods: preoperative oxygen saturation (80%) and during arterial occlusion (0%) with NIRS. Following this work, clinical applications of NIRS were reported by plastic and reconstructive surgeons. Scheufler et al.[4] monitored cutaneous microcirculation with NIRS in breast skin flaps after inferior pedicle reduction mammaplasty. In addition, they investigated the pedicled transverse rectus abdominis musculocutaneous (TRAM) flap oxygenation and perfusion by NIRS and colour-coded duplex sonography.[5] Prior to the present study, however, there was no report on the monitoring of oxygen saturation in buried flaps using NIRS. Hence, the current work began investigating the use of NIRS in monitoring free flaps in 1999.

Based on the modified Lambert-Beer law, some ranges (e.g. ranges in wavelength from 700 to 900 nm) of the near infrared spectrum can easily penetrate into human tissues.[19] Oxygenated haemoglobin ($HbO_2$) and deoxygenated haemoglobin (Hb) are the main absorbers in human tissues, and their absorption spectra are significantly different in the near infrared band.[20] NIRS is, thus, used in detecting different tissue oxygenation levels. Furthermore, some NIRS oximeters are designed to calculate the concentration changes of $HbO_2$ and Hb and can be used in monitoring the changes in the free transferred flaps in plastic surgery.[6,21] However, these are not useful in continuously assessing tissue oxygenation, and it is difficult to apply them to the monitoring of free flaps postoperatively.

The principle of the steady-state spatially resolved spectroscopy (SRS) enabled the development of a new kind of NIRS oximeter that can detect regional tissue oxygen saturation ($rSO_2$) and provide useful information about tissue perfusion at any moment postoperatively. Based on this principle, we designed the TSNIR-3 and used it to monitor the blood circulation of free fibular flaps. It includes a main body (Fig. 7), one set of software and a probe (Fig. 8). The buried flap being detected is under the overlying tissues such as the epidermis and subcutaneous tissues, whose optical characteristics were described in detail in our previous research.[2,6,10] Huang et al.[2] introduced application of monitoring cerebral oxygenation for healthy neonates and neonates with hypoxic-ischaemic

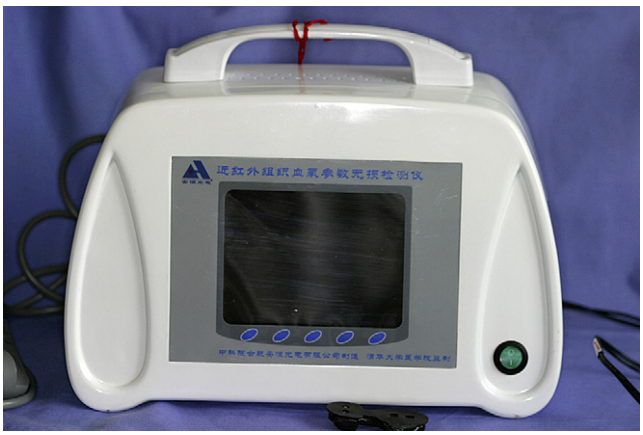

**Figure 7** Outline of the new product TSAH-100 NIRS.

encephalopathy using TSNIR-3. Furthermore, Teng et al.[10] monitored cerebral oxygen saturation in adults during cardiopulmonary bypass using the same device. They proved that NIRS could penetrate all human tissue, including bony tissue, not only from the basic principles but also the clinical tests. According to their basic research, the distances between the light source and the two detectors being used should be properly chosen according to the thickness of the overlying tissues, thereby ensuring that the $rSO_2$ of the buried fibular flap (including fibular bone, marrows and some soft tissues, e.g. muscular sleeves), but not of the surrounding autochthonous tissues, were measured definitely. Because the thickness of the overlying soft tissue in the mandibular area in most Chinese people is 15—20 mm normally, the distance was chosen as 30 mm and 40 mm for Chinese adults in the current study. Besides $rScO_2$, the concentration changes of $Hb(\Delta C_{Hb})$ and $HbO_2$

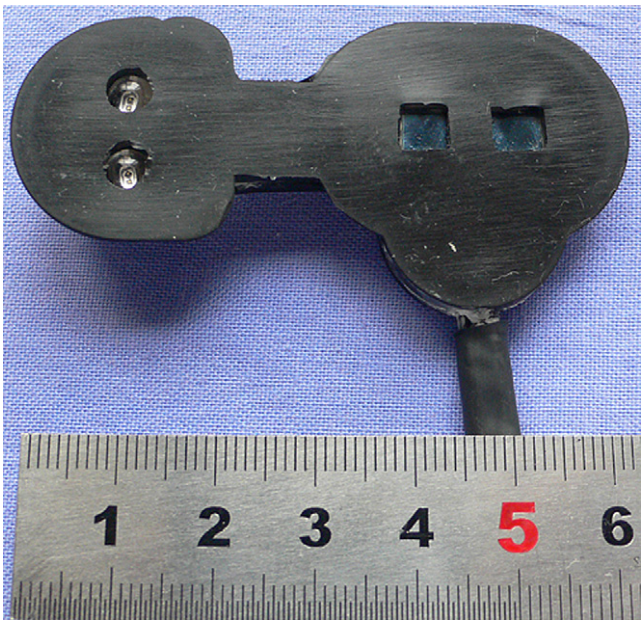


**Figure 8** The new probe of the TSAH-100 NIRS with two different distances (30 mm and 40 mm). Left: light source; Right: detectors.

($\Delta C_{HbO2}$) compared with their original values are also noninvasively monitored by our oximeter simultaneously, according to our animal experimental research.[6] The values of $\Delta C_{Hb}$ and $\Delta C_{HbO2}$ should be very useful for determination of arterial or venous crisis. The current study focused on assessing its ability to monitor the absolute value of tissue $rSO_2$ in real time.

The difference of skin colour during detection is mainly the difference between the amounts of melanin in the epidermis. More melanin only results in more attenuation of the near infrared light by the tissue. Thus, if we increase the incident light intensity appropriately, the tissue oxygenation parameters of Negroid patients can also be monitored using NIRS. In our NIRS oximeter, the intensity of the incident light can be adjusted automatically and self-adaptively according to the tissue being detected.

In order to ensure the sensibility and precision of the detector and its capability to monitor buried microcirculation in fibular flaps after mandibular reconstruction, the present study used the forearm obstructive test. The results suggest that TSNIR-3 is a highly sensitive and reliable method to monitor the $rSO_2$ levels of normal human tissue, especially when the blood flow is impeded in free flaps. The $rSO_2$ levels proved to be quite stable and promptly reactive to blood flow changes. Thus, these results indicated that the TSNIR-3 could be used to perform the other parts of the study.

In order to discover whether TSNIR-3 could measure the $rSO_2$ of the mandible and if it can distinguish the changes of blood flow in the transferred tissue, two control groups were designed in this study. One group consists of the healthy mandibles and the other group is the remaining healthy mandibles in the operated group. At first, the $rSO_2$ levels were compared in different positions of the normal mandible and at different times of day. The results indicate that the $rSO_2$ levels distribute equally in the healthy mandible of adults and do not vary significantly with the time of day or positions of the normal mandible. Furthermore, in the operated group, the mean values of $rSO_2$ were significantly different between the transferred side and the control side. The results indicate that the $rSO_2$ levels of the fibular flap decreased gradually (4—16 h) after the operation. This decrease is likely due to the time required to establish new microcirculation in the buried transferred tissue. Also in this study, vascular crisis occurred in one patient during this stage. Based on our clinical experience, over two-thirds of vascular crises occurs in the first 24 h after the operation, suggesting that monitoring the changes in microcirculation in the transferred tissue during this stage is very important. Therefore, early reliable detection of adverse circulatory changes within a transferred flap during this stage and early re-exploration are vital to minimise free flap failure. Comparison of these two groups showed that the changes of $rSO_2$ in the transferred fibular flap can be monitored by TSNIR-3; however, the value of $rSO_2$ also showed some differences between the healthy mandible and the remaining healthy mandible in the operated group (Tables 2 and 3). In the operated group it was a little higher than in the normal mandible; the floating value is limited to 5%. We considered that the reason may be the local blood flow increased after operation, not only in the operated side but also in the remaining healthy mandible. Because there were limited normal samples and only one failed case in this study, we cannot get the threshold values for vascular failures. The values of $rSO_2$ in the healthy mandible are only considered reference values for the human mandible, but not a standard normal value. The values of $rSO_2$ in the remaining healthy mandible and transferred fibular flap were measured at the same time, and they were significantly different, thus it is suggested that we need to increase the normal mandibular samples for the measurement of the normal $rSO_2$ value, and we also need some more failed cases for the measurement of the threshold values for vascular failures in further research. Accordingly, we recommend using the values of $rSO_2$ in the remaining healthy mandible as a control group in the current measurement. Although the failed case provided only a limited reference, the measurement of TSNIR-3 was very helpful in determining the venous crisis. As Fig. 5 showed that the value of $rSO_2$ on the operated side suddenly increased over the control side at 10 h after the operation (Fig. 5, point A), it reminded us of the possibility of venous crisis. The colour changes of the skin flap in this case also supported the above result; furthermore, because the microthrombus had been formed in the microcapillary of the fibular flap, the $rSO_2$ in the flap showed a significant decrease after the second anastomosis (Table 4, Fig. 5), then it slowly increased, consistent with clinical observation. However, because we had to take out the skin flap due to the microthrombus in the microcirculation of the skin flap in the second operation, the mucosa was directly closed. After 5 days, an intraoral wound infection was found in the transferred side; at the beginning the fibular bone had some bleeding, gradually the whole bone stopped bleeding completely, and the measurement of the $rSO_2$ showed synchronal changes. It showed below 60% in the control side may be due to the intraoral infection expanding.

As mentioned above, results suggest that NIRS provides a sensitive and reliable postoperative monitor of tissue viability following transfer of free flaps, particularly for buried flaps, because it can harvest the absolute value of the deep tissue $rSO_2$ in real time. However, there is only one failed case in the present study, and this is not enough to achieve a standard for evaluation of vascular crisis. Based on our series of studies, the new production TSAH-100 NIRS (Figs. 7 and 8) has been designed, and was used in our clinical monitoring of the free flap after transplantation. We hope that new data could achieve a relatively reliable diagnosis standard for vascular crisis after free flap transplantation and this device may be widely used in plastic surgery in the future.

In conclusion, the present research was a pilot study on the application of near infrared spectroscopy in monitoring free flaps. The principle of the steady-state spatially resolved spectroscopy enabled the development of a new kind of NIRS, the oximeter-TSNIR-3. The forearm vessel obstructive test and measurement of the normal mandible validated its sensibility and precision. Furthermore, it was used in monitoring free fibular flaps and determined some regular rules for changes in $rSO_2$ levels in the buried free flaps. TSNIR-3 is a portable bedside monitor that can show real-time changes in the regional oxygen saturation within a flap at a variety of tissue depths. As it is very

important to monitor the oxygenation profiles of the buried transferred tissue accurately and rapidly in the first 24 h after surgery, TSNIR-3 provides an ideal noninvasive method for the monitoring of free fibular flaps.

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