Intramuscular Microvascular Flow Sensing for Flap Monitoring in a Porcine Model of Arterial and Venous Occlusion

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J Reconstr Microsurg 2023;39:231–237.

Abstract

Background Commercially available near infrared spectroscopy devices for continuous free flap tissue oxygenation (StO_2) monitoring can only be used on flaps with a cutaneous component. Additionally, differences in skin quality and pigmentation may alter StO_2 measurements. Here, we present a novel implantable heat convection probe that measures microvascular blood flow for peripheral monitoring of free flaps, and is not subject to the same issues that limit the clinical utility of near-infrared spectroscopy.

Methods The intratissue microvascular flow-sensing device includes a resistive heater, 4 thermistors, a small battery, and a Bluetooth chip, which allows connection to a smart device. Convection of applied heat is measured and mathematically transformed into a measurement of blood flow velocity. This was tested alongside Vioptix T.Ox in a porcine rectus abdominis myocutaneous flap model of arterial and venous occlusion. After flap elevation, the thermal device was deployed intramuscularly, and the cutaneous T.Ox device was applied. Acland clamps were alternately applied to the flap artery and veins to achieve 15 minutes periods of flap ischemia and congestion with a 15 minutes intervening recovery period. In total, five devices were tested in three flaps in three separate pigs over 16 vaso-occlusive events.

Results Flow measurements were responsive to both ischemia and congestion, and returned to baseline during recovery periods. Flow measurements corresponded closely with measured StO_2. Cross-correlation at zero lag showed agreement between

Keywords
- free flap
- perfusion monitoring
- microsurgery
- intramuscular

received
January 30, 2022
accepted
May 29, 2022
article published online
August 11, 2022

DOI https://doi.org/10.1055/s-0042-1755261.
ISSN 0743-684X.

these two sensing modalities. Two novel devices tested simultaneously on the same flap showed only minor variations in flow measurements.

Conclusion This novel probe is capable of detecting changes in tissue microcirculatory blood flow. This device performed well in a swine model of flap ischemia and congestion, and shows promise as a potentially useful clinical tool. Future studies will investigate performance in fasciocutaneous flaps and characterize longevity of the device over a period of several days.

After microsurgical anastomosis, free flaps are susceptible to malperfusion resultant from thrombosis, vasospasm, or compression of the vascular pedicle.[1–6] This can lead to partial or complete flap failure.[2] Previous studies have shown that flap failure typically occurs within the first 72 hours after free tissue transfer.[2–4,7] The likelihood of successful emergent surgical flap salvage after vascular occlusion is inversely related to the time before detection of malperfusion.[2,4,7] This makes rigorous flap monitoring an essential aspect of postoperative care.

Traditional flap monitoring strategies include intermittent physical examination and assessment of the pedicle with a cutaneous Doppler.[2] However, malperfusion may not always present with unequivocal changes in the physical or Doppler examination until late in its course.[8] Furthermore, these methods are unable to detect vascular compromise in buried flaps.[9] Implantable Doppler ultrasound devices have been in use for decades and can continuously assess the patency of the microvascular anastomoses. However, these devices have a high false-positive rate,[10] and can directly damage or deform the vascular pedicle during placement or removal.[11–13]

Near-infrared spectroscopy (NIRS) has been employed in the clinical setting for flap monitoring for several years. NIRS devices like the T.Ox (Vioptix Inc. Fremont, CA) have been widely adopted by many reconstructive surgeons and have several advantages.[11,14–16] They allow for continuous, remote monitoring of free flap tissue oxygenation (StO_2), and also detect malperfusion events with excellent sensitivity and specificity.[14] While NIRS has proven clinically useful, this technology does have limitations. Confounding factors like skin pigmentation and thickness, ambient light incursion, and instability at the skin–sensor interface can alter StO_2 measurements.[17,18] Additionally, commercially available NIRS sensors can only be used on flaps with a cutaneous paddle. There is also no defined threshold for StO_2 to indicate flap vascular compromise[16,19] and measurements can be skewed by systemic factors related to overall patient health.[19]

While the peripherally mounted (away from the pedicle) and continuous nature of the ViOptix T.Ox device is appealing from a safety and ease-of-use standpoint, the limitations discussed above have caused us to seek alternative means of continuous flap monitoring. Here, we present a novel probe for measuring microvascular blood flow as an alternative means for free flap monitoring. This intratissue implantable device is able to distinguish states of normal capillary circulation and stagnation based upon the tissue's convection of minute amounts of applied heat (heat convection). The proposed device is wireless, implantable, and is not subject to the same confounding variables as NIRS.

Methods

Design of the Flow Probes

The implantable probes for measuring microvascular flow in muscle flaps are small and minimally invasive (width ~ 2 mm, thickness ~ 1 mm, ►**Fig. 1A–C**). A surface-mounted resistive heater and four thermistors (0.6 × 0.3 mm footprint) at different relative positions (0-, 0.75-, 1.5- and 8-mm relative to the heater) along the length of the probe act as the sensing components. During operation, local microcirculation partially dissipates the heat generated by the heating element through convection. Detection of temperature

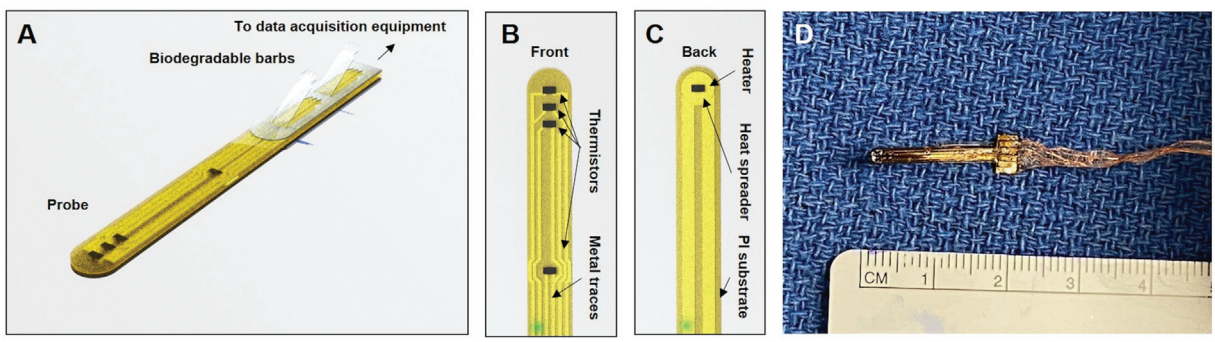

Fig. 1 (**A**) Schematic of the microvascular flow probe. (**B** and **C**) Schematics of the front side (**B**) and back side (**C**) of the flow probe, showing details of the sensing components. (**D**) A photograph of the microvascular flow probe.

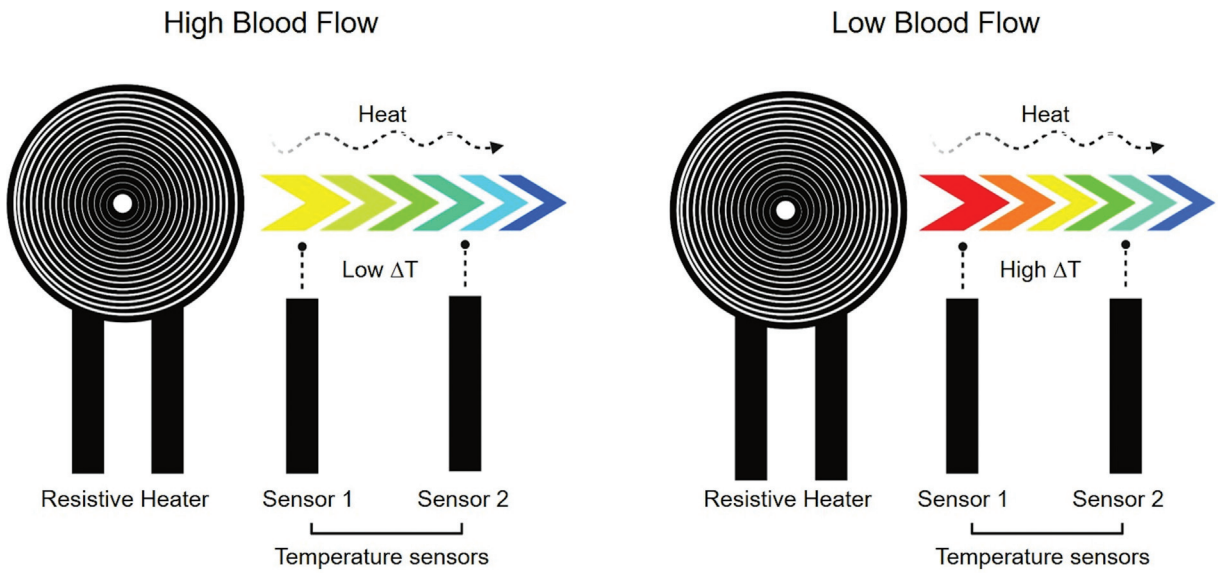

Fig. 2 A visual model of the microvascular flow probes. Microcirculatory flow velocity is calculated from tissue heat convection. A small amount of heat is applied to the tissue by the resistive heater. The heat is convected away from the resistive heater and is detected by temperature sensors. Heat convection is rapid in the presence of high microcirculatory blood flow and slow when microcirculatory blood flow is low. Created with BioRender.com.

changes around the heater by measuring the resistance of the thermistors yields a quantitative measure of flow velocity around the probe (►**Fig. 2**). Thin biocompatible polyimide (PI, 75 µm) serves as the substrate; ultrathin, inert Au/Pt films (50/300 nm) deposited on both sides of the PI and patterned by laser ablation serve as conductive wires connecting to the sensing components. A laser-patterned circular gold film (diameter 1.5 mm, thickness 25 µm) around the heater helps to spread the heat uniformly. Top and bottom encapsulations (PI, 75 µm) prevent biofluid penetration. Optional biodegradable barbs (cellulose acetate, 0.25 mm) cladded on the flow probe allow auto stabilization within the muscle tissue during use, followed by easy probe removal after barb degradation. Connecting the implantable probe to a voltage source and a multichannel Ohmmeter, or a Bluetooth low-energy wireless system implemented with equivalent functions[20] enables real-time data acquisition. The conceptual underpinnings, technical specifications, and bench top validation of this technology have been previously described by our group.[21]

Porcine Model

Animal research was performed after obtaining approval of the Institutional Animal Care and Use Committee at BLINDED School of Medicine. This was performed according to U.S. Department of Agriculture Animal Welfare Regulations at an accredited facility. Three live pigs were utilized for this study. Anesthesia was induced with Telazol, ketamine, and xylazine followed by maintenance with inhaled isoflurane. After completion of all experimentation, the animals were euthanized with pentobarbital.

Pedicled rectus abdominis myocutaneous flaps based upon the deep superior epigastric artery and veins were raised with the superficial superior epigastric vein. This technique (which we have described previously[22,23]) was modified from the methods described by Bodin et al.[24] Visualization of bright red bleeding from flap periphery confirmed flap viability.

Experimental Design

Deployment of the intramuscular microvascular flow device was achieved via stab incision with a 15-blade perpendicular to the axis of the muscle fibers on the underside of the rectus abdominis muscle (Supplemental Video,; available online only). Pedicle vessels were carefully avoided. The microvascular flow probe was then inserted into the muscle incision (►**Fig. 3A**). The flap was then returned to its anatomic position, while the device was connected to an iPad running the measurement software via Bluetooth (►**Fig. 3B**). A T.Ox

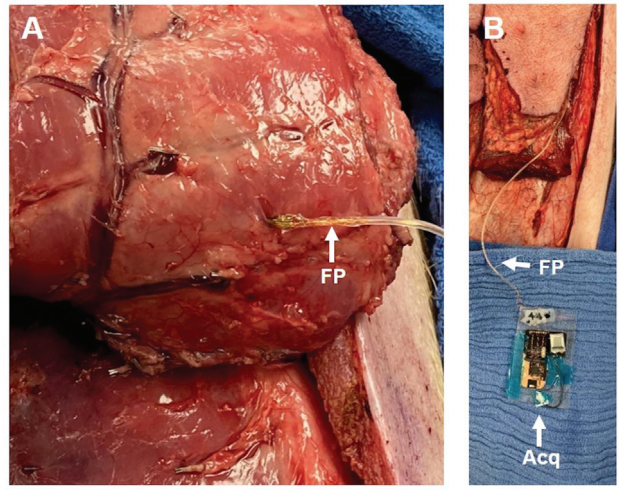

Fig. 3 (**A**) Microvascular flow probe (**FP**) implanted within the rectus abdominis. (**B**) Data acquisition equipment (acq) connected to the probe. A Bluetooth chip allows connection to a smart device (not shown).

probe was attached to the central portion of the skin paddle. The myocutaneous flap was monitored continuously for the duration of the experiment.

Model of Complete Vascular Occlusion

Stable baseline measurements were obtained for 15 minutes before an Acland clamp was applied to the deep superior epigastric artery, achieving complete ischemia. Ischemia was maintained for 15 minutes. The clamps were then released and the flap was allowed to recover for 15 minutes. Acland clamps were then applied to the deep and superficial superior epigastric veins, causing venous congestion. Clamps again remained in place for 15 minutes before the clamps were released and venous drainage restored. Flaps were again allowed to recover for 15 minutes. The experimental cycle was then repeated for each flap. Experiments were repeated in their entirety on 3 separate days with new pigs and devices to demonstrate reproducibility.

Flow Sensing: Calculation

Thermal power from the heater spreads into the surrounding environment through conduction mechanisms that include the effects of flowing biofluids, primarily blood. The following analytical formula describes ΔT, the temperature increase compared with the base temperature, as a function of thermal and geometric parameters:

$$\Delta T = \frac{qR/k}{1 + 0.76s\sqrt{uR/\alpha}} F\left(\frac{r}{R}\right)$$

where q and R are the power density and radius of the heater, k and α are the effective thermal conductivity and dissipation of the medium (water), r is the distance away from the heater, s is the fluid content in the medium, u is the flow velocity of the fluid, and F is a monotonic decreasing function. For the configuration of the flow probes described here, $F = 1.024$, 0.661, 0.283, and 0.071 for the four thermistors, respectively. Previous reports indicate $s \sim 4\% \pm 1\%$ for the porcine muscle.[25] Converting the decrease of ΔT induced by the flow yields quantitative values for the flow velocity u.

Statistical Methods

Normalized cross-correlation at zero lag was used to describe the correspondence between the flow velocity and T.Ox sensors being compared.

Results

In total, five devices were tested in three flaps in three separate pigs over 16 unique vaso-occlusive events. In the first animal, a flap was raised and two microvascular flow devices were tested simultaneously on the same flap alongside T.Ox. Another flow device was tested in a second series of vaso-occlusive events on the same animal. In the second and third animals, single devices were tested alongside T.Ox. The experiments were successfully performed without complication, with expected changes in StO_2 and flap coloration observed in response to, ischemia, congestion, and recovery (►**Fig. 4A–C**). Continuous monitoring was accomplished through each cycle of experiments. No connection loss was observed with either T.Ox or our flow devices. Flow measurements were responsive to both ischemia and congestion, and returned to baseline during recovery periods. A representative graph of microvascular blood flow and StO_2 during experiment 1 is shown in ►**Fig. 5**. Cross-correlation at zero lag showed agreement between these two sensing modalities, ranging from 0.739 to 0.933. Two novel devices tested simultaneously on the

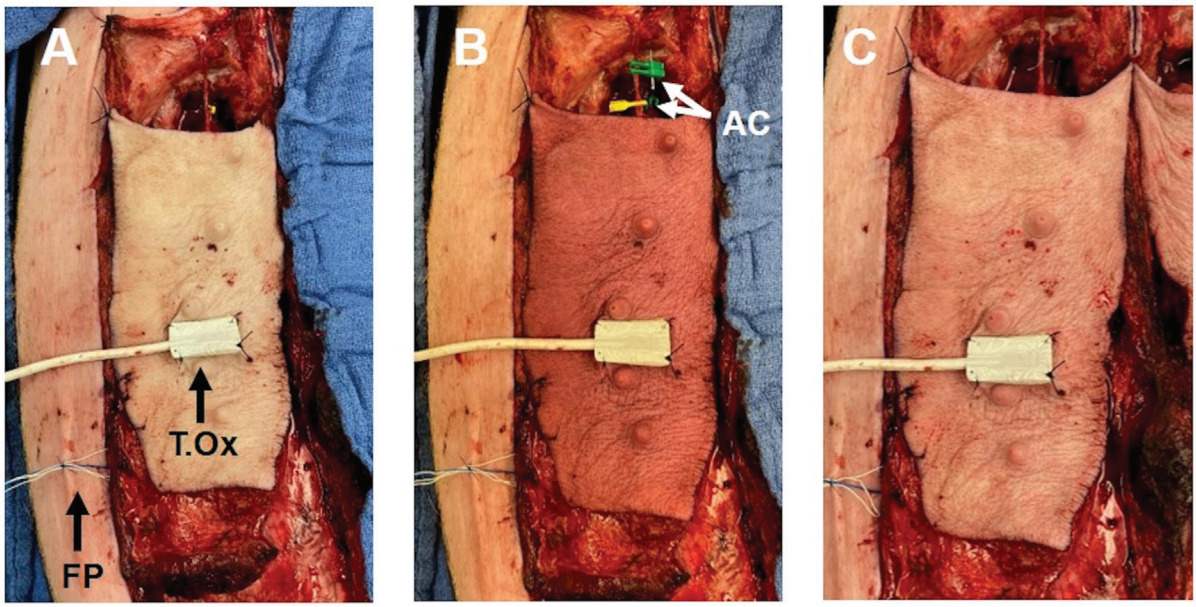

Fig. 4 Rectus abdominis myocutaneous flap during (**A**) ischemia, (**B**) congestion, and (**C**) recovery. Complete vascular occlusion was achieved by applying Acland clamps to the vascular pedicle (AC). T.Ox probes were attached to the skin paddle. Flow probes (**FP**) were inserted into the rectus abdominis muscle.

same flap showed only minor variations in flow measurements, with an interdevice cross-correlation of 0.953.

Discussion

Current wireless monitoring devices include the Vioptix T.Ox device, which is a popular flap monitoring device that employs NIRS technology. The device has many advantages, allowing for continuous and remote monitoring of flap viability. However, variations in skin thickness and pigmentation result in considerable interpatient variability with respect to acceptable measured StO_2. Furthermore, external factors such as ambient light, fluid incursion, and cutaneous interface instability can lead to signal alteration or loss and false positive alarms.[17,18] Additionally, these devices require a skin component for measurement.[19] Several alternative devices have been proposed, such as fully implantable ultrasound Doppler systems[26,27] and Doppler-equipped venous couplers.[28] These offer improvements on the design of the commonly-used Cook Doppler, which requires an external wire and offers no opportunities for remote monitoring.[8] However, all of these devices require central deployment directly upon the vascular pedicle, risking damage or deformation of the pedicle during placement and removal.[11–13] In this chapter, we introduce a novel device capable of detecting changes in tissue heat convection that are reflective of quantitative microcirculatory blood flow velocity.

Our novel microvascular flow probes detect changes in tissue thermal properties, which are reflective of quantitative microcirculatory (capillary level, nondirectional) blood flow velocity. When blood is circulating around the implantable intramuscular probe, convection of applied heat is rapid (high heat convection). When flow is slow or absent, heat is not convected away from the heating element (low heat convection). Completely wireless and water resistant, these devices are not at risk of signal change or loss due to tension on an external wire, and are tolerant of moisture at the flap-device interface. Furthermore, these devices are implantable and are not reliant on a skin paddle for measurement, and thus are completely unaffected by variable tissue coloration or ambient lighting. While measurement of flap temperature has been utilized in the past without much success,[29,30] it should be clarified that this device does not aim to infer perfusion from a measurement of the flap's temperature. Instead, the aim is to track changes in the tissue's thermal properties. Furthermore, while the application of heat to living tissue could theoretically pose a safety hazard, this is not a problem as the maximum change in local tissue temperature observed with application of this minute amount of heat was 4°C. Additionally, the device is easily removable with a gentle tug, which obviates any concern for medium or long-term foreign body reaction/toxicity.

We hypothesized that our microvascular flow devices could accurately detect flap ischemia and congestion. To test this hypothesis, we deployed our devices in a rectus abdominis myocutaneous flap model of complete arterial and venous occlusion alongside skin-mounted T.Ox devices. This allowed us to compare the performance of our devices to a device that is accepted to yield measurements reflective of tissue perfusion in ideal circumstances. As expected, we observed immediate drops in StO_2 in response to arterial and venous occlusion. During occlusive events, the novel thermal microvascular flow probes detected precipitous declines in microcirculatory flow velocity. Both blood flow velocity and StO_2 increased after release of the Acland clamps and reperfusion. In all experiments, blood flow velocity approached zero during pedicle occlusion, and closely correlated with StO_2 through all phases of occlusion and recovery. Cross-correlation at zero lag showed high agreement between these two sensing modalities (0.739–0.933). Some variations were noted between the StO_2 and flow velocity tracings. These inconsistencies may be explained by the differences in the tissues being assessed by the novel device (muscle) and T.Ox (skin), or by the simple fact that one measurement reflects blood flow, and the other represents oxygenation. Despite these differences, we were able to show that this device produces a data stream that is visually and intuitively equivalent to the familiar T.Ox curves.

To assess interdevice concordance and reproducibility of results, we tested two microvascular flow devices simultaneously in the same flap, under the same conditions. These devices showed high agreement, with a cross-correlation at zero lag of 0.953 (►**Fig. 5**). Small measurement variations can be accounted for by their different positions within the flap. While the myocutaneous flap was isolated on its vascular pedicle and elevation included removing all collateral vessels to the tissue, variations in perfusion within the flap can be expected due to intraflap hemodynamics and small variations in local tissue characteristics.

This study was limited in several ways. First, we used an Acland clamp to achieve complete vascular occlusion in our flap model. While this allowed us to achieve immediate and complete vascular occlusion, flap failure by vascular thrombosis in the clinical setting may be more complex[31] and blood flow may not instantly go from "on" to "off." The

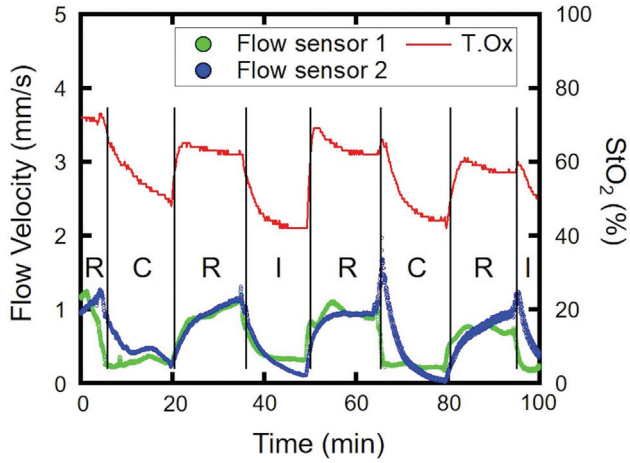

Fig. 5 A representative graph of microvascular blood flow velocity and StO_2 during periods of ischemia (I), congestion (C), and recovery (R) is shown. Two microvascular flow devices were tested simultaneously (flow sensor 1 & 2) and showed high agreement (cross-correlation at zero lag: 0.953).

absence of microvascular anastomosis in this model was helpful in avoiding undesired and irreversible pedicle thrombosis, but could theoretically impact the flow characteristics of the tissue. Additionally, these experiments were completed in a nonsurvival model under general anesthesia, preventing us from assessing the ability of our technology to assess flap perfusion in a breathing, moving animal over period of days. Additional testing is necessary to test the durability of these devices, and asses their ongoing performance over a period of several days. Further, while we have claimed that this device should be insensitive to tissue pigmentation and ambient light, this hypothesis was not tested in this study. Finally, we could not assess the clinical sensitivity or specificity of our devices in this highly controlled large animal model. However, this preclinical work is an important first step toward evaluating the efficacy of our technology in the clinical setting.

This novel implantable intramuscular probe is capable of detecting changes in tissue microcirculatory blood flow by measuring changes in convection of applied heat. This device performed well as a continuous flap monitor in a swine model of flap ischemia and congestion, and shows promise as a potentially useful clinical tool. Because flow measurements are not affected by variable lighting and the light absorbing properties of tissue, this monitoring strategy has the potential to provide more consistent information than NIRS. Our study demonstrated satisfactory function within muscle tissue. Future studies will investigate performance in fasciocutaneous flaps (which may have lower flow rates) and characterize longevity of the device over a period of several days. Ultimately, trials in the clinical environment are mandatory to assess real-world advantages and disadvantages of this new technology compared with existing options.

Conflict of Interest
None declared.

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