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Research

Skin Pigmentation Affects ViOptix T.Ox Performance in Variably Pigmented Preclinical Model of Flap Ischemia and Congestion

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Background: Free flap monitoring is more difficult in patients with dark skin because ischemia and congestion can be masked by pigmentation. For this reason, adjunct methods such as cutaneous near-infrared spectroscopy are of elevated importance in patients with highly pigmented skin. The purpose of this experiment is to determine if ViOpitx T.Ox performance is affected by cutaneous pigmentation.

Methods: Swine with naturally occurring areas of nonpigmented and pigmented skin were used. Pigmentation of each animal was assessed using spectrophotometry and histopathology. During normoxemia, tissue oxygenation (StO_2) measurements were taken of nonpigmented and pigmented skin using the T.Ox device. A bicolor pedicled rectus abdominis myocutaneous flap was raised, and T.Ox probe was adhered to adjacent areas of opposite coloration on the same flap. StO_2 was measured continuously during reversible episodes of flap ischemia and congestion (n = 4 swine, n = 6 flaps).

Results: There was not a significant difference between baseline StO_2 values of nonpigmented (49% ± 7.9%) and pigmented skin (47% ± 6.2%). The absolute change in StO_2 was significantly larger during both ischemia (6%) and congestion (16%) in nonpigmented skin compared with adjacent pigmented skin.

Conclusions: T.Ox detects flap ischemia and congestion in both highly pigmented and nonpigmented skin. However, surgeons need to be aware that StO_2 changes related to complete flap ischemia or congestion may be much more subtle than what is seen in nonpigmented skin. This study establishes a novel internally controlled porcine model that isolates the impact of skin pigmentation when assessing cutaneous devices measuring tissue oxygenation. *(Plast Reconstr Surg Glob Open 2024; 12:e5865; doi: 10.1097/GOX.0000000000005865; Published online 5 June 2024.)*

INTRODUCTION

Near-infrared spectroscopy (NIRS) is a useful modality for continuous monitoring of tissue oxygenation (StO_2) in cutaneous free flaps.[1–6] The T.Ox device (ViOptix, Inc., Fremont, Calif.) is a widely used NIRS-based monitor that has been shown to have excellent sensitivity (99.1%) and specificity (99.9%) for the detection of flap compromise.[2] Given that bedside visual clinical examination is substantially less reliable for the detection of tissue ischemia in patients with darker compared with lighter skin,[3,7,8] adjunct monitoring tools such as T.Ox are of particular importance in patients with higher levels of pigmentation.

Recent reports have raised concerns as to whether the T.Ox device performs equally well across a diverse range of skin tones.[9] Specifically, Gary et al[9] subjected volunteers to tourniquet-induced upper extremity ischemia while monitoring tissue oxygenation using the ViOptix T.Ox NIRS probe placed on fingertip. In all participants,

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NIRS T.Ox was able to successfully detect the acute onset of digital ischemia, but those with subjectively darker skin tones had a lower starting StO_2 and smaller change in StO_2. Although these results suggest T.Ox monitoring is impacted by melanin, this experimental design does not control for nonpigmentary differences that might contribute to interindividual variation and cannot necessarily be generalized to cutaneous flap monitoring where early tissue compromise generally reflects isolated arterial or venous occlusion.

In this article, we introduce a novel internally controlled approach for isolating the impact of pigmentation upon tissue oxygenation assessment devices in states of normal perfusion, ischemia, and venous congestion. This method leverages the natural phenomenon of variably pigmented swine with adjacent areas of pigmented and nonpigmented skin within the cutaneous paddle of a rectus abdominis myocutaneous flap. It is the aim of this work to (1) establish and characterize a preclinical internally controlled model that isolates the variable of skin pigmentation as it applies to flap monitoring, (2) evaluate if skin pigmentation affects the T.Ox reading in a state of normoxemia, and (3) determine if skin pigmentation impacts T.Ox measured tissue oxygenation in states of controlled flap ischemia and congestion (Fig. 1). We hypothesized that the baseline StO_2 reading would be lower in pigmented skin compared with adjacent nonpigmented skin and that changes resultant from flap ischemia and congestion would be of lesser magnitude in darkly pigmented skin.

Takeaways

Question: Is near-infrared spectroscopy performance affected by cutaneous pigmentation when monitoring during perfusion insults?

Findings: This study used a novel preclinical model of swine with naturally occurring adjacent areas of nonpigmented and pigmented skin. There was a significantly larger change in tissue oxygenation during both ischemia and congestion in nonpigmented compared with pigmented skin.

Meaning: There is a decreased responsiveness of tissue oxygenation during occlusion in pigmented compared with nonpigmented tissue, and thus, smaller changes are potentially important in patients with dark skin.

METHODS

Swine Anesthesia

Animal research was performed with the approval of the Institutional Animal Care and Use Committee at Washington University School of Medicine. This was performed per the US Department of Agriculture Animal Welfare Regulations at an accredited facility. In this study, multipigmented swine with naturally occurring areas of nonpigmented and pigmented skin on the torso (n = 4 Hampshire-Yorkshire crossbred) were used. Anesthesia was induced intramuscularly with Telazol (4 mg/kg; Tzed, Dechra Veterinary Products), ketamine (2 mg/kg; ketamine hydrochloride injection, Dechra Veterinary Products), and xylazine (2 mg/kg; Rompun, Dechra Ventrinary Products) followed by maintenance with 1%–5% isoflurane (Isoflurane USP, Covetrus). At the end of the experiment, each animal was euthanized with a pentobarbital (Euthasol, Virbac).

Skin Characterization

The pigmentation of each animal was assessed using spectrophotometry and histopathology (n = 4 swine). In vivo spectrophotometry measurements were made using

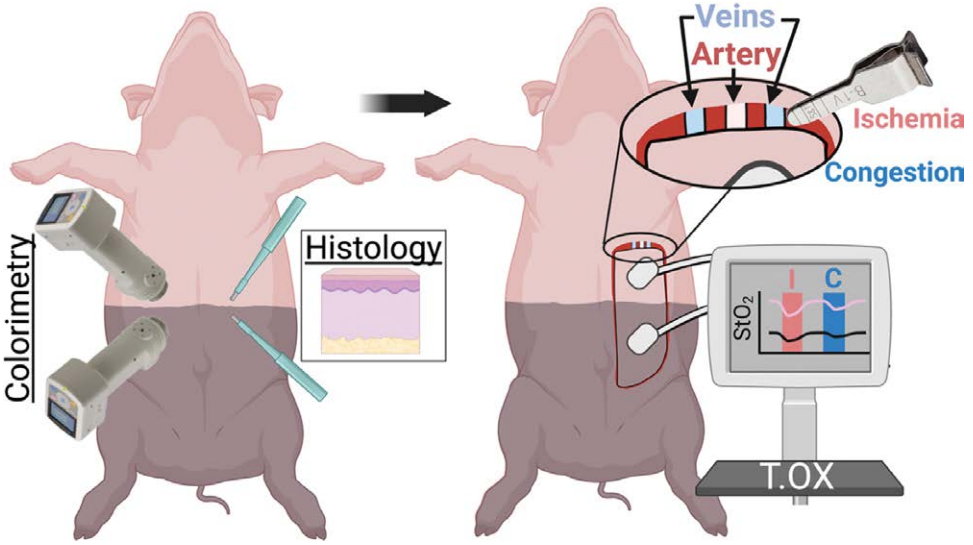

Fig. 1. The skin of multipigmented porcine will be characterized using colorimetry and histology before evaluating T.Ox flap monitoring performance in adjacent areas of nonpigmented and pigmented skin during arterial and venous occlusion.

a Konica Minolta CM 700d spectrophotometer (Ramsey, N.J.) to measure light reflectance from pigmented and nonpigmented sites. The CM 700d measured the perceived color in CIELAB color space (L*, a*, b*) of the skin[10] and then used the LAB coordinates to determine the individual typology angle {ITA = atan[(L – 50]/b) × 180/π}, which has been shown to be a surrogate for melanin content and is used widely as an objective assessment of skin pigmentation.[11]

Skin biopsies were obtained for histopathologic assessment. Biopsies were taken from nonpigmented and pigmented skin on the swine torso. Biopsies were placed in 10% phosphate-buffered formalin for at least 48 hours, embedded in paraffin, and sectioned (10 μm thick). Sections were stained with hematoxylin and eosin to assess the morphology of the skin and with Fontana-Masson to visualize melanin. Stain kits from Sigma-Aldrich (St. Louis, Mo.) were used for both stains (hematoxylin and eosin: HHS; Fontana-Masson: HT200).

Baseline Tissue Oxygenation Measurements

Although the animal was anesthetized and maintained at normoxemia, tissue oxygenation measurements were taken of nonpigmented and pigmented skin at baseline using the T.Ox device (ViOptix, Inc.; n = 1). A 6×6 grid of 1″×1″ squares was created on an area of nonpigmented skin, and three T.Ox measurements were taken at each grid location. A different T.Ox probe was used for each of the triplicate measurements. Spectrophotometry measurements using the Konica Minolta were also taken in triplicate at each grid location. This process was repeated on an area of pigmented skin.

Complete Vascular Occlusion Model

A pedicled rectus abdominis myocutaneous flap was raised based on the superficial superior epigastric vein and deep superior epigastric artery (n = 6 flaps in four swine).[12] Each flap had areas of nonpigmented and pigmented skin, and a ViOptix T.Ox probe was adhered to immediately adjacent areas of opposite coloration (Fig. 2A). As previously described by our group, the flap malperfusion experiments consisted of 15-minute periods beginning with baseline establishment, followed by complete ischemia, flap recovery, venous congestion, and flap recovery (Fig. 2B).[6,13–15] Complete ischemia was induced by applying an Acland clamp to the deep superior epigastric artery. Complete congestion was created by applying Acland clamps to the deep and superficial superior epigastric veins. For flap recovery, the Acland clamp(s) were released to allow for re-establishment of a stable baseline reading. The experiment was then immediately repeated using the same flap. Although the experiment was attempted on bilateral flaps in each animal, two flaps were excluded due to sensor malfunction.

Reporting T.Ox Results

Because the raw StO_2 output of the T.Ox monitor is used clinically, we chose to report these data untransformed with changes calculated simply by the difference between baseline and nadir values. However, because we know that the T.Ox StO_2 value is a somewhat relative measure (with relative changes meaning more than the absolute value), it can be argued that these data might be better interpreted as a percent change from the baseline value. For this reason, relative changes were also

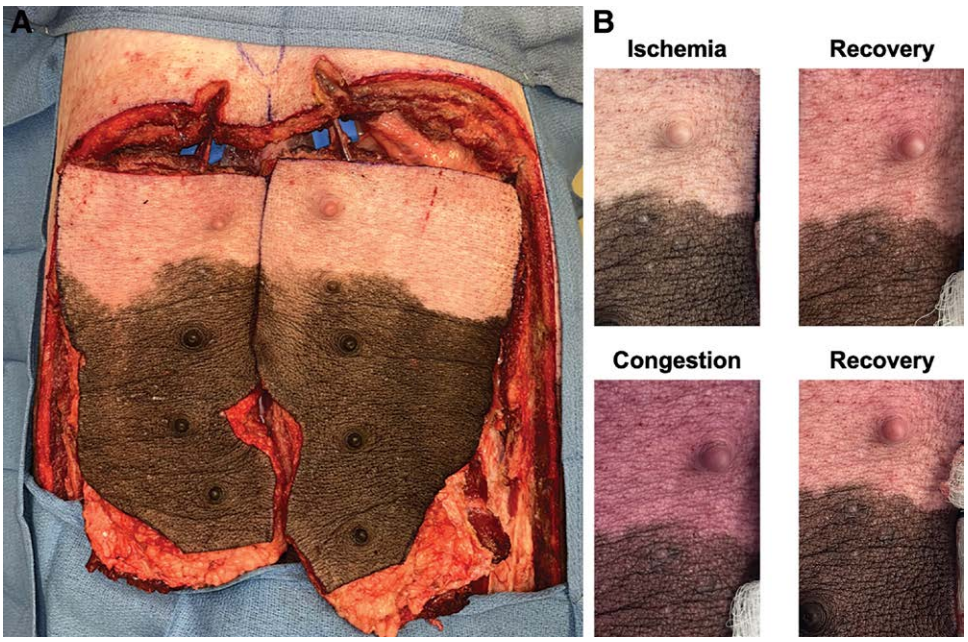

Fig. 2. Complete vascular occlusion model in multipigmented swine. A, Example of multipigmented porcine with areas of nonpigmented and pigmented skin on each rectus abdominis myocutaneous flap. B, During the complete vascular occlusion experiment, representative images of the multipigmented flap during each phase of testing.

calculated and reported in parallel to the untransformed data. Relative StO_2 values were calculated by dividing the StO_2 measurements by the initial StO_2 value at the start of the experiment. In the complete vascular occlusion model, the magnitude of StO_2 change was calculated during each episode of ischemia (n = 12) and congestion (n = 12) using the difference between StO_2 at baseline before vascular insult and the StO_2 nadir during vascular occlusion.

Power Calculations

To assess baseline tissue oxygenation in pigmented and nonpigmented skin, 88 measurements (44 for each coloration) would be necessary for 80% power detect a difference of three points assuming an SD equal to 5.[16] To compare the responsiveness of T.Ox in flap ischemia or congestion, 11 distinct arterial occlusion events would be necessary for 80% power to detect a difference in responsiveness equal to five points assuming an SD of 5 among the paired differences.[17]

Statistical Methods

Central tendency was reported as mean ± SD. ITA values of pigmented and nonpigmented skin were compared using an unpaired *t* test. For the baseline tissue oxygenation measurements, StO_2 values were compared between nonpigmented and pigmented skin using an unpaired *t* test. The change in StO_2 in pigmented and nonpigmented areas was compared using a paired *t* test. The level of significance was set at a *P* value of less than 0.05 in all statistical tests (GraphPad Prism).

RESULTS

Skin Characterization of Multipigmented Swine

Histological analysis of pigmented and nonpigmented areas of skin within each animal revealed melanin distribution patterns that mirror expected melanin distribution in pigmented and nonpigmented human skin.[11] In nonpigmented skin, melanin was absent, and in pigmented skin, melanin was concentrated within the basal layer of the epidermis (Fig. 3A). On areas of both nonpigmented and pigmented skin, ITA was measured in 36 locations three times in a swine (n = 108 total measurements per skin tone). ITA was 71.9±5.4 for nonpigmented and −7.6±10.5 for pigmented skin. This was a statistically significant difference ($P < 0.001$).

Spectrophotometric analysis of pigmented and nonpigmented skin across all swine demonstrated visually distinct reflectance curves from 400 to 700 nm (Fig. 3B), with pigmented skin exhibiting lower light reflectance than nonpigmented skin, particularly in red wavelengths (600–700 nm). Across all animals the ITA in the nonpigmented skin fell in the "intermediate" to "very lightly" pigmented ranges,[18] whereas, in the pigmented skin, ITA fell in the "dark" or "brown" pigmentation ranges. Animals 1 and 2 had visibly the darkest pigmented patches of skin which corresponded to the lowest ITA values, whereas animals 3 and 4 had less significant degrees of pigmentation that corresponded to more moderate ITA values. (**See figure, Supplemental Digital Content 1,** which displays the ITA for each area of pigmentation across all flaps, **http://links.lww.com/PRSGO/D256**.)

Pigmentation Effect on Baseline Tissue Oxygenation Measurements

Tissue oxygenation was measured 108 times each on areas of pigmented and nonpigmented skin. Baseline StO_2 values were 49% ± 7.9% for nonpigmented and 47% ± 6.2% for pigmented skin. This was not a statistically significant difference.

Pigmentation Effect in the Complete Vascular Occlusion Model

The effect of pigmentation on tissue oxygenation monitoring during vaso-occlusive events was tested in six flaps

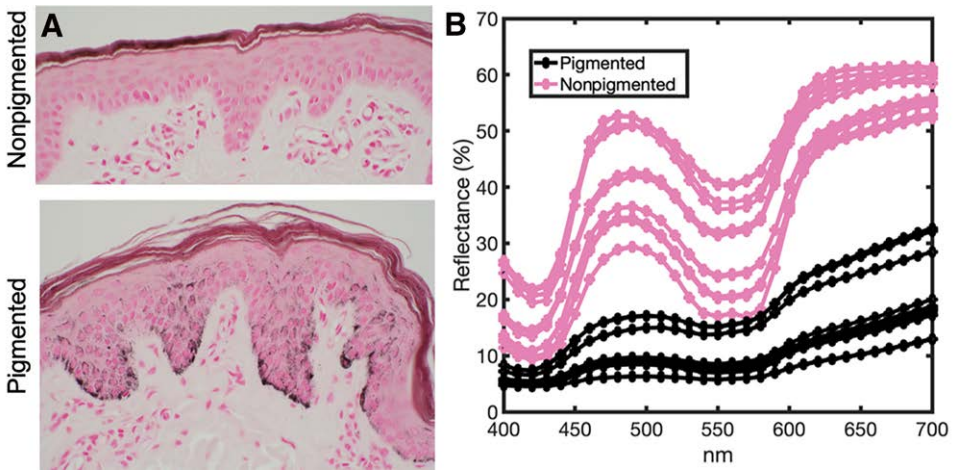

Fig. 3. Skin characterization of multipigmented swine. A, Representative images (400×) of Fontana-Masson staining of nonpigmented and pigmented areas of skin within the same swine. B, in vivo spectrophotometry measurements of nonpigmented and pigmented skin across all porcine.

in four separate animals. In representative images, visual assessment of the flap showed clear color changes associated with ischemia and congestion in nonpigmented skin, and these color changes were much less obvious in adjacent pigmented skin (Fig. 2B). StO_2 tracings showed precipitous changes occurring upon arterial clamping, arterial release, venous clamping, and venous release in both adjacent nonpigmented and pigmented flap areas (Fig. 4). The T.Ox signal quality was greater than 85 in all trials. During ischemia events, the absolute change in measured StO_2 was 6% larger in nonpigmented skin compared with adjacent pigmented skin. During congestion events, the absolute change in measured StO_2 was 16% larger in nonpigmented skin compared with adjacent pigmented skin. This was a statistically significant difference for both ischemia ($P = 0.005$) and congestion ($P = 0.0002$) (Table 1).

When the T.Ox StO_2 data were transformed to reflect relative change from a normalized baseline (Fig. 5), there was again a significantly larger decrease in relative StO_2 during ischemia ($P = 0.002$) and congestion ($P = 0.0015$) in nonpigmented compared with pigmented skin (Table 1). During congestion events, the change in relative StO_2 was more blunted in the flaps with a darker skin tone or lower ITA value compared with less dark-pigmented flaps (Fig. 6A). A similar trend was observed for ischemia events, although there was one outlier (flap 3) wherein the darker skin tone and had a larger change in StO_2 (Fig. 6B).

DISCUSSION

The clinical monitoring of cutaneous free flaps can be augmented by the use of continuous NIRS, which has been shown to yield increased rates of flap salvage. This

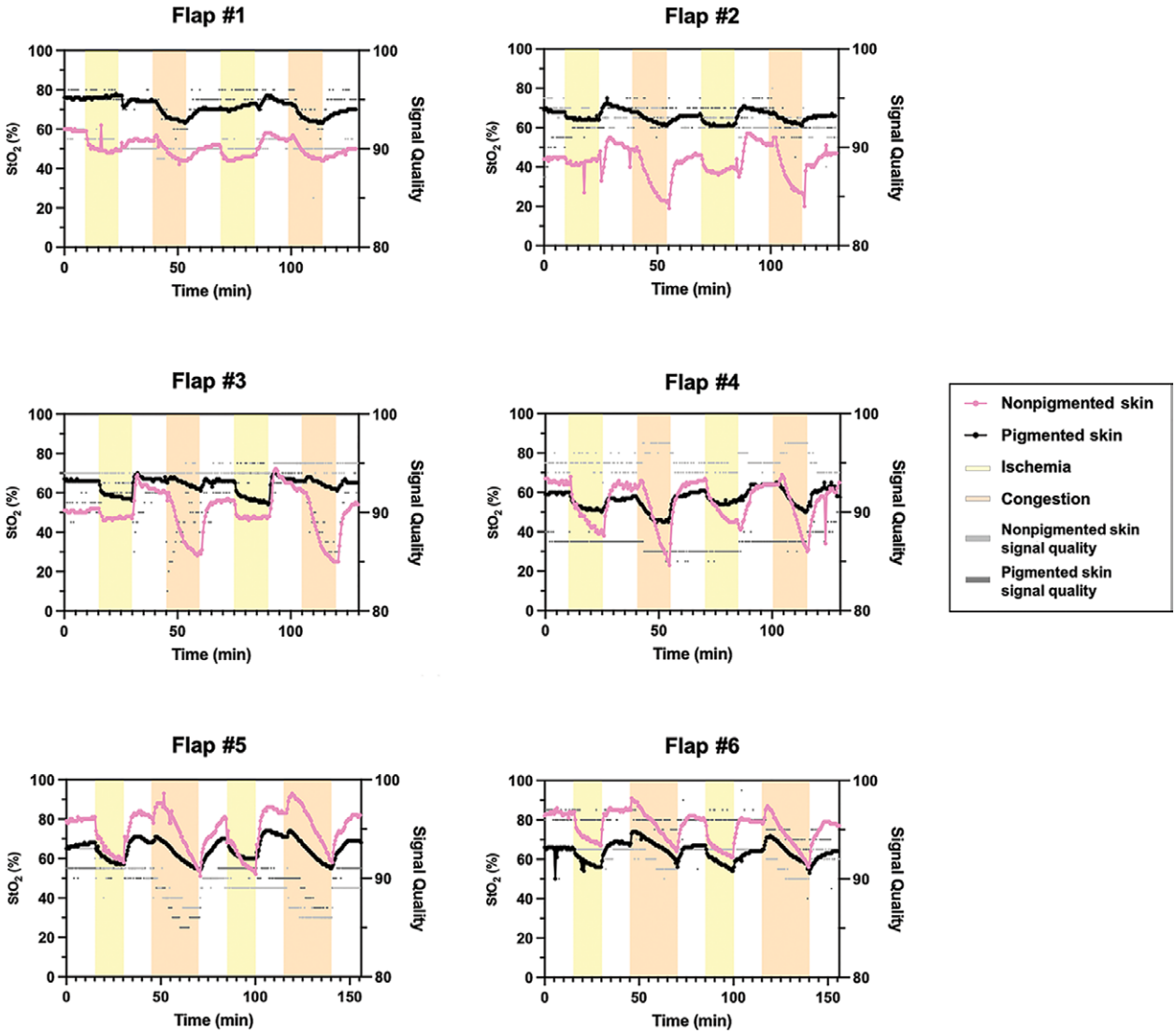

Fig. 4. Each graph shows simultaneous traces of tissue oxygenation (StO_2) recorded on adjacent areas of nonpigmented and pigmented skin on the same flap during the vascular occlusion model. After 15 minutes of stable baseline recordings, the cycle of 15-minute increments of ischemia, recovery, congestion, and recovery was repeated twice per flap.

adjunctive objective measurement of tissue oxygenation is particularly important in patients with dark skin because pigmentation compromises the clinical assessment of tissue perfusion.[3,7] Because the evidence mounts that the NIRS devices may perform differently in the presence of skin pigmentation,[9,19–21] it is critical to understand how this variable may impact function of the T.Ox device used for flap monitoring. In this study, we have established an internally controlled preclinical model to directly assess the impact of skin pigmentation on T.Ox performance while controlling for all other physiological parameters.

Table 1. The Magnitude of Change in ViOptix T.Ox StO_2 Measurements during Flap Ischemia and Congestion in Adjacent Nonpigmented and Pigmented Skin

	Absolute ΔStO_2	Relative ΔStO_2
Flap ischemia		
Nonpigmented skin	14% ± 7.1%	0.21 ± 0.08
Pigmented skin	7.9% ± 4.2%	0.12 ± 0.07
Flap congestion		
Nonpigmented skin	26% ± 8.8%	0.43 ± 0.19
Pigmented skin	10% ± 4.0%	0.15 ± 0.07

In these variably pigmented swine, melanin in the pigmented skin is distributed similar to pigmented human skin,[11] and there is a stark contrast between pigmented and nonpigmented skin in a single animal. At baseline, there was not a statistically significant difference in observed StO_2 between pigmented and nonpigmented skin. The T.Ox device clearly detected controlled ischemia and congestion in all tissues; however, the pigmented skin had smaller changes in StO_2 compared with immediately adjacent nonpigmented skin. This is true for the absolute units of change that we consider in clinical practice and also when changes were expressed as fractional deviation from baseline. Because T.Ox is usually interpreted as a

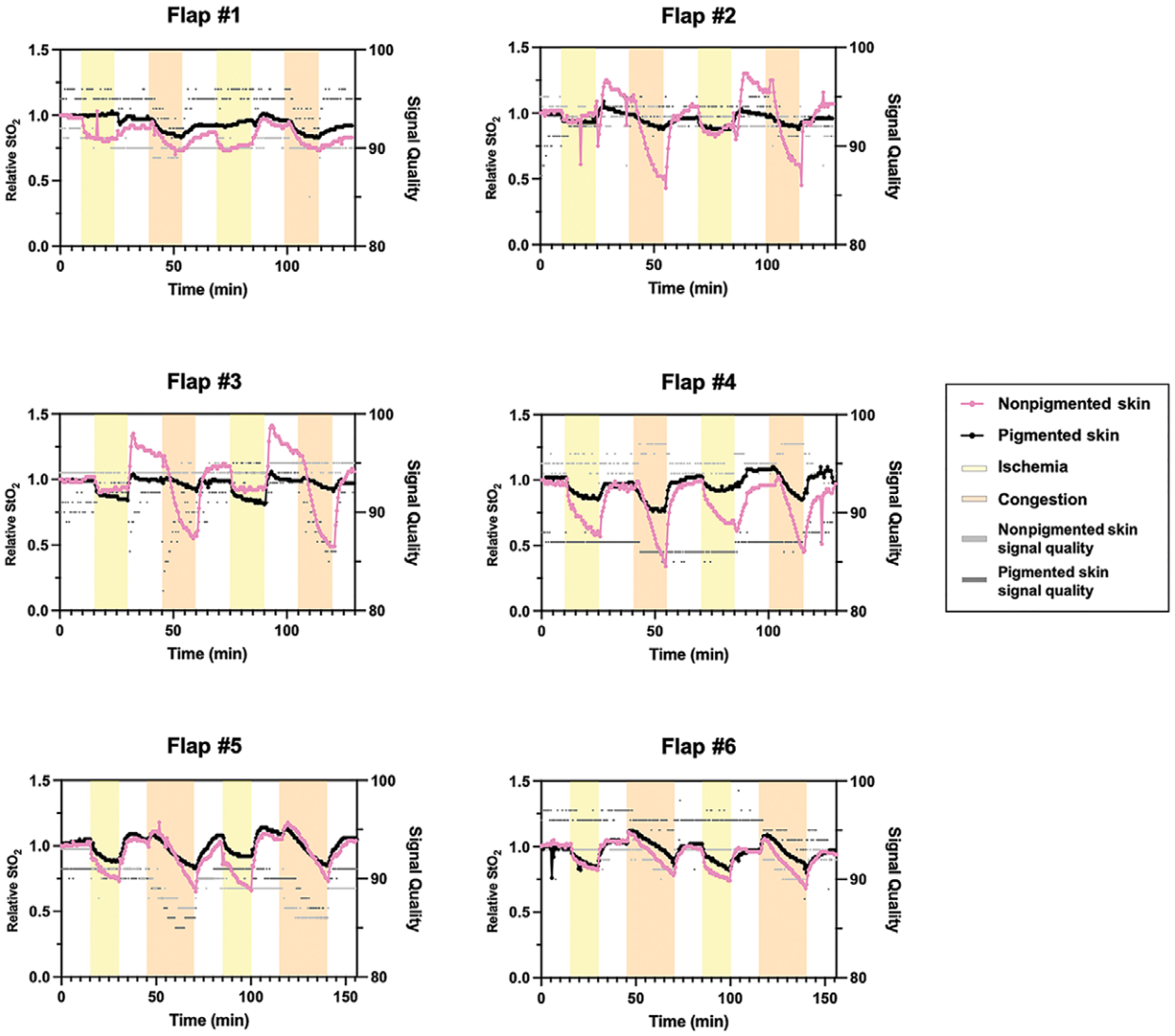

Fig. 5. Each graph shows the relative StO_2 recorded simultaneously on adjacent areas of nonpigmented and pigmented skin on the same flap during the vascular occlusion model.

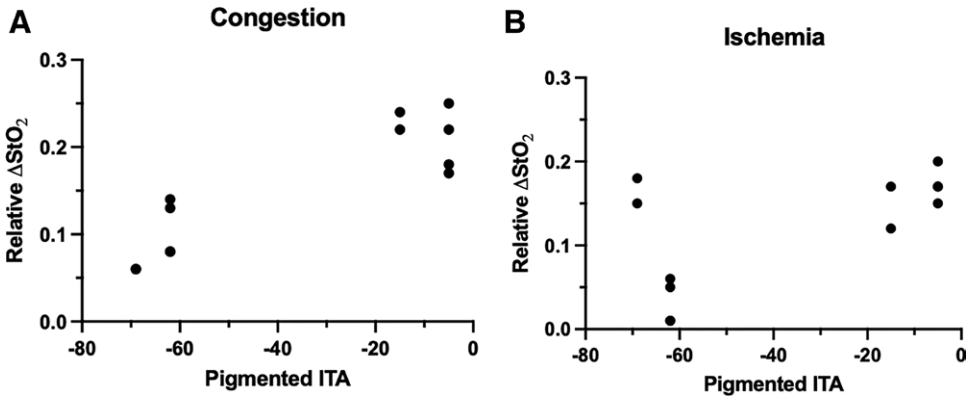

Fig. 6. Pigmentation effect on relative StO_2. During congestion (A) and ischemia (B) events, swine with a lower ITA value of their pigmented skin had smaller changes in relative StO_2 compared with swine with less dark-pigmented skin.

relative measure of tissue oxygenation, we believe that the latter dataset is more mathematically rigorous. That said, regardless of the analytical method one prefers, the conclusion is the same.

Although Gary et al[9] found decreased baseline StO_2 (–8.6%) in pigmented fingertips compared with nonpigmented fingertips, our study found no significant StO_2 difference in adjacent areas of nonpigmented and pigmented tissue during normoxemia despite being powered to detect a difference as small as 3%. Contrastingly, both studies found that the T.Ox device was less dynamic in the presence of darker skin tones.[9] With regard to the contrasting findings during normoxemia, we would argue that the current experiment using adjacent areas of contrasting skin in the same organism is better suited to make this assessment than any design using separate individuals, in which factors such as skin thickness, comorbidities, and/or variable perfusion could confound the comparison.

Although the prior literature on T.Ox and skin pigmentation is quite limited,[3,9] there has been much more work on how pigmentation impacts pulse oximetry, which is another widely used application of NIRS. Recent work has found that pulse oximeters are more likely to provide falsely normoxemic StO_2 in hypoxemic patients who self-identify as Black.[19] Because a racial bias in oximetry has potential to disproportionately harm patients with dark skin, the Food & Drug Administration has called for investigation in this area.[22,23] However, progress has been hampered by the absence of a preclinical model of tissue hypoxemia which isolates the variable of pigmentation. The establishment and characterization of this model is perhaps the most important implication of this study, and we believe that variably pigmented swine are well suited for the ongoing investigation into how skin color may lead to differential NIRS performance in a wide variety of clinical applications.

The two major implications of this study are, first, that pigmentation does not bias the T.Ox reading in any specific direction during normoxemia. As such, the observation of a low baseline reading should not be attributed to skin pigmentation, and patients with dark skin pigmentation should not be expected to generate lower T.Ox values. Second, the T.Ox measurements associated with acute arterial and venous compromise will be smaller in patients with pigmented skin, compared with those with less pigmentation. The size of this effect which blunts the observed change was six absolute units or 11% of the baseline signal for arterial compromise, and 16 absolute units or 28% of the baseline for venous compromise. The finding that changes in T.Ox are more subtle in patients with dark skin means that it may be easier to miss early signs of flap failure. This could result in a delayed return to the operating room and an increased risk of flap failure. When treating patients with dark skin, it may be beneficial to modify alarm settings on the ViOptix machine or modify nursing call criteria, such that smaller changes generate an alert.

Strengths of this study include use of variably pigmented swine to create bicolor flaps that truly isolate the impact of pigmentation on tissue oxygenation assessment. This critical design feature represents an improvement upon prior studies that used subjects of different skin tones and, thus, can be confounded by other types of interindividual variation. Furthermore, the use of a flap model with separate control of arterial inflow and venous outflow is an improvement upon previous models that used a limb tourniquet, which imperfectly approximates both arterial and venous thrombosis. Finally, this study used four animals, each with slightly different and rigorously characterized pigmentation. The consistency of the findings across some degree of pigmentary variation increases the applicability of our findings to the diverse range of human pigmentation.

A potential confounder of this study is that the pigmented tissue is in the distal aspect of each flap. If this was an important confounder of StO_2 measurements, one would expect to see a decreased baseline StO_2 in the distal, pigmented area; however, this was not found. Additionally, if the proximal/distal assessment affected the degree of responsiveness during ischemia and congestion, one would expect the distal tip to be more sensitive to perfusion insults. This is in the opposite direction of our observations, and therefore we do not think this issue is important. Furthermore, the two T.Ox sensors

were placed close to each other, on opposite sides of the pigment demarcation line (rather than at opposite extremes of the flap) to minimize confounding by any proximal to distal perfusion gradient. Ideally, we would have animals with pigment in the opposite position; unfortunately, this is not a marking pattern that is regularly available. Additional limitations of this study include that, despite histology showing melanin deposition and skin architecture that was similar to human tissue, no animal model can be assumed to perfectly replicate human physiology. Additionally, although our study included detailed characterization of the variable coloration seen in four animals, data were analyzed by grouping the measurements into dichotomous pigmented and nonpigmented groups.

Future work will include investigation of a "dose–response" curve that would describe how increasing degrees of pigmentation (across the whole human skin color spectrum) impact T.Ox function. Finally, we will aim to apply this experimental model for the study of pulse oximeters and other light-based tissue assessment devices and develop computational methods to account for the impact of skin pigmentation.

CONCLUSIONS

This study documents the establishment of a novel, internally controlled porcine model that isolates the impact of skin pigmentation upon T.Ox responsiveness to flap compromise. Although there was no baseline bias in T.Ox readings, decreased responsiveness to both arterial and venous occlusion was found in pigmented compared with nonpigmented tissue. Surgeons need to calibrate their responsiveness to T.Ox changes based on skin pigmentation and recognize that, in patients with dark skin, smaller changes are potentially important.

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DISCLOSURES

Dr. Pet has a patent "Novel Wireless Probes for Tissue Perfusion Monitoring" pending. Dr. Shmuylovich has patent PCT/US2022/021048, "Hemodilution detector" pending and is cofounder of Armor Medical Inc. Dr. Westman has received research funding from 3M. Dr. Pet has received research funding from Checkpoint Inc, 3M, and Kent Imaging, Inc. and is also a scientific consultant for KLISBio. The other authors have no financial interest to declare. Funding for this study was received from the Division of Plastic Surgery and the Division of Dermatology at Washington University School of Medicine.

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