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Proof-of-Concept Studies for Marker-Based Ultrasound Doppler Analysis of Microvascular Anastomoses in a Modified Large Animal Model

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Abstract

Background—Despite attempts to solve the problem of flap monitoring, assessing the patency of vascular anastomoses postoperatively remains challenging. In addition, experimental data suggest that near-total vessel occlusion is necessary to produce significant changes in clinical appearance or monitoring devices. We sought to develop an ultrasound-based system that would provide definitive data on anastomotic function.

Methods—A system was developed consisting of a resorbable marker made from poly-lactic-co-glycolic acid (PLGA) implanted during the time of surgery coupled with ultrasound software to detect the anastomotic site and perform Doppler flow analysis. Surgical procedures consisting of microvascular free tissue transfer or femoral vessel cutdown were performed followed by marker placement, closure, and ultrasound monitoring. Transient vascular occlusion was produced via vessel-loop constriction. Permanent thrombosis was induced via an Arduino-controlled system applying current to the vessel intima.

Results—Four surgeries (one femoral vessel cutdown and three microvascular tissue transfer) were successfully performed in Yorkshire swine. The markers were readily visualized under ultrasound and provided a bounding area for Doppler analysis as well as orientation guidance. Transient spasm and partial occlusion were detected based on changes in Doppler data, while complete occlusion was evident as the total loss of color Doppler.

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Conflict of Interest

Under a licensing agreement between Sonavex Incorporated and the Johns Hopkins University, Drs. Coon, Prince, and Bector are entitled to royalties on an invention described in this article. Drs. Coon, Prince, and Bector are also co-founders/consultants to Sonavex. This arrangement has been reviewed and approved by the Johns Hopkins University in accordance with its conflict of interest policies.

Conclusion—In this preliminary report, we have conceptualized and developed a novel system that enables the real-time visualization of vascular pedicle flow at the bedside using Doppler ultrasound and a surgically implanted marker. In a large animal model, use of the system allowed identification of the anastomosis, flow analysis, and real-time detection of flow loss.

Keywords

free flap; microvascular reconstruction; flap monitoring; ultrasound; Doppler

Microvascular reconstruction represents a powerful tool for addressing complex reconstructive defects that often cannot be solved by other methods. Over the past 25 years, with the diffusion of knowledge and technology, free tissue transfer has become increasingly widespread and reliable. With the advent of new “free-style” perforator flaps and supermicrosurgery, patients can receive increasingly sophisticated reconstructive options that may decrease donor site morbidity and offer better functional and aesthetic outcomes.

A core aspect of free tissue transfer that has remained steady is the necessity of maintaining patent anastomoses postoperatively and, consequently, a need for interrogating the status of blood flow into the flap, as it is well established that time to anastomotic revision after occlusion directly correlates with the chances for salvage.¹ Many experimental solutions have been proposed to address this need, with some reaching clinical use.² Some attempts have been made to use color Doppler ultrasound as a noninvasive method of quantitatively assessing blood flow at the anastomosis itself. However, this approach has not seen clinical adoption because recipient vessel blood flow proximal to the anastomosis poses a significant false-positive risk and the difficulty of locating and analyzing the vessels under ultrasound precludes routine use for monitoring. We sought to assess the viability of a new approach to vascular monitoring using a resorbable echogenic marker placed adjacent to the vessels at the end of surgery to highlight their location for flow monitoring followed by Doppler blood flow analysis.

Methods

Marker Design

Based on a marker computer-aided design (CAD) template, marker prototypes were extruded from 50/50 poly-L-lactic-co-glycolic acid (PLGA) using a miniextrusion machine to produce echogenic, resorbable markers with an expected absorption time of 3 to 6 months. For the final two experiments, markers were sterilized using hydrogen peroxide plasma gas processing (STERRAD, Ethicon, West Somerville, NJ), a low temperature plasma sterilization system, so as not to deform or degrade the marker.³

System Setup

An Food and Drug Administration-approved Ultrasonix So-nixTOUCH (Analogic, Peabody, MA) ultrasound machine was used in research mode. A standard 5 to 14 MHz linear vascular probe was used for data acquisition. Using the Ulterius toolkit (Analogic), a network LAN connection was established and custom C++ (Microsoft Visual Studio, Redmond, WA) software using the ITK/VTK toolkits (Insight Segmentation and

Registration Toolkit and Visualization Toolkits; Kitware Inc, Clifton Park, New York) was used to acquire and analyze ultrasound data.

Surgical Procedure

Animal work was approved by the IACUC of both Johns Hopkins University and the University of Maryland. Previous studies had reported several surgical models in large animals. A porcine model based on a previously reported gracilis myocutaneous flap⁴ was modified to involve transfer of a fasciocutaneous flap from the groin based on the saphenous artery to the external carotid and internal jugular vessels. After all experiments had concluded, animals were euthanized with pentobarbital 100 mg/kg.

Thrombosis Induction

An electrolytic injury model was used to create thrombosis.⁵ A 6–0 suture needle soldered to the anode was placed in contact with the vessel lumen with the cathode wire placed in subcutaneous tissue. An Arduino microprocessor was used for current supply and measurement with a potentiometer-based current control. A current from 150 μ A to 1 mA was then applied for 10 minutes, followed by withdrawal of the needle and ultrasound monitoring as above. If thrombosis failed to form via electrolytic injury, it was further induced under ultrasound using a ferric chloride model.⁶ A 3-mm wide strip of filter was saturated with 50% (w/v) ferric chloride and rolled around the vessel of interest.

Results

Surgical Procedures and Normal Flow Measurement

A total of four procedures with marker placement (one femoral cutdown thrombosis test and three microvascular tissue transfers; see Fig. 2) were performed in Yorkshire swine. For the femoral cutdown, the femoral vessels were bluntly exposed before the femoral artery was transected and repaired with 7–0 polypropylene suture. For the free tissue transfers, a skin paddle ranging from 6 $\times$ 5 to 11 $\times$ 8 cm was centered over the palpable medial saphenous artery, including all subcutaneous and fascial tissue down to the thigh musculature (Fig. 1). The flap was then raised and the vessels dissected back to their takeoff at the femoral vessels before ligation. After flap transfer, 9–0 nylon was used for handsewn anastomosis of both the artery and vein. After satisfactory flow across the anastomosis, the marker was placed under the vessels and sutured into place using 4–0 Monocryl (Fig. 2). The flap was then inset with 4–0 nylon and ultrasound scanning was initiated.

Location of the pedicle vessels was straightforward due to the obvious presence of the marker signature on the ultrasound image in the region of interest. Due to the linear orientation provided by the marker channel, normal flow in both the arterial and venous pedicles could be visualized in the same axial view on color Doppler ultrasound. In the first of the three free flap procedures, a relatively small skin paddle was used and paradoxical flow back out of the artery was seen during diastole with little or no flow through the vein. Subsequent procedures used larger skin paddles with incorporation of a greater volume of fascia and subcutaneous tissue and this effect was no longer seen.

Transient Loss of Flow Detection

By wrapping a vessel loop around the vessel and tightening, flow could be interrupted and resumed repeatedly. Vessel occlusion was immediately observed as loss of Doppler signal on the screen. Several seconds after relaxation of the loop, flow returned and was detected.

Detection of Permanent Thrombosis

Generation of occlusive thrombosis via intimal vessel injury was successfully performed (Fig. 3). Forming clots were readily visible on B-mode ultrasound as echogenic material filling the vessel lumen. After thrombotic occlusion was complete, the loss of flow was readily apparent on color Doppler imaging (Fig. 4).

Discussion

Flap monitoring remains a subject of significant discussion within microsurgery, with few universally agreed upon principles except for the importance of clinical examination and early detection of thrombosis.² The latter principle, based on the concept that earlier detection is correlated with higher salvage rates, has been shown by several centers and has motivated much of the effort to develop better methods of vascular monitoring over the past 30 years.^{1,7-10} In addition to improved patient outcomes, the cost associated with such devices can be supported on the basis of cost effectiveness due to the tremendous expense incurred to the health care system by a total flap loss and the resulting lengthy hospital stay.^{11,12}

Although clinical examination remains the gold standard for flap monitoring, it is heavily reliant on the expertise of the bedside caregiver and changes may not become obvious until thrombosis has progressed significantly.^{9,10,13} Each technical solution developed to address these shortcomings has its own advantages and disadvantages. For example, devices such as the Cook Doppler suffer from a high false-positive rate¹⁴ and potential safety concerns,¹⁵ while the output of the ViOptix (Fremont, CA) can be difficult to interpret and requires near-total venous occlusion to produce a significant drop in tissue oxygenation.^{13,16} In a recent national survey each monitoring technology assessed (Cook Doppler, ViOptix Tissue Oxygenation Sensor, Laser Doppler, and Indocyanine Green Angiography) was used “frequently” by fewer than 25% of surgeons, suggesting significant room for improvement on existing devices.¹⁷

In our clinical experience, attempts to use color Doppler alone for anastomosis interrogation on suspicion of a problem had failed for a variety of reasons, including (1) difficulty finding pedicles that were not immediately subcutaneous; (2) challenges in reliably distinguishing patent anastomotic flow from residual proximal recipient vessel flow; (3) problems obtaining clean cross-sectional views of the vessels for flow analysis due to pedicle tortuosity; and (4) differentiating inability to locate flow as being thrombosis versus simply not finding the vessels in the scanned area. The fourth issue was the most critical—given the six degrees of freedom allowed by ultrasound, an error in probe position of as little as a few millimeters can cause the scan to completely skip the vessel area, particularly with anastomoses that are more than 1 to 2 cm from the skin after inset. We therefore sought to

address each of these problems by developing a system that included a mechanism to guide the operator to the correct location for scanning and confirm that vessel flow ought to be visible.

For a bioresorbable marker, we chose to use a system involving poly-lactic-co-glycolic acid (PLGA). PLGA is a copolymer consisting of both glycolic acid and lactic acid.¹⁸ It degrades by hydrolysis into its respective monomers, which are well tolerated, and the expected degradation time can be tuned based on the PLA:PGA ratio and molecular weight.¹⁹ Due to its excellent biocompatibility and several decades of clinical experience, PLGA is one of the most widely used bioresorbable polymers, commonly employed in both medical device and controlled drug release applications.^{18,20} Sterilization of these polymers without adverse effects can be challenging due to their intolerance of high temperatures (i. e., > 50°C). Based on the prior literature, we chose to use plasma sterilization, which performed well.²¹

Based on the past literature, it was believed that inclusion of the vein, artery, and overlying adipofascial tissue in the porcine flap model we used would be adequate to provide sufficient end capillary circulation. However, in prior reports, there was no method of directly visualizing flow. We found that without the inclusion of a larger volume of soft tissue, there was paradoxical rebound flow back out of the artery in diastole. The small amount of flow making it through to the vein was probably enough to maintain tissue viability. In a survival model, effects such as chokepoint dilation and loss of sympathetic tone may alter these flow dynamics over time. Similar effects may occur clinically but go unrecognized in flaps which feature high-flow recipient vessels, a large caliber pedicle, and relatively small amount of flap tissue (e.g., radial forearm flaps for head and neck reconstruction). This interesting finding highlights the potential of direct observation of anastomotic flow to provide new insights into unanswered questions in microvascular surgery, such as the time course of progression from initial thrombosis to full occlusion.

Significant work remains to be done beyond this early feasibility assessment. Head-to-head comparisons against other flap monitoring technologies and survival studies demonstrating that marker resorption properties are as expected are important steps. We recognize that the current system would only be usable by a practiced physician or ultrasonographer; additional work will also focus on ultrasound software and algorithms to simplify the process of navigating the surgical site and interpreting Doppler data received by the ultrasound.

Finally, no existing monitoring method reliably detects partial thrombosis (e.g., animal models suggest nearly 100% venous occlusion is necessary to desaturate the Vioptix device¹⁶). In contrast, Doppler ultrasound has been used for decades to successfully detect partial occlusion and thrombosis in applications such as carotid stenosis and deep venous thrombosis.²² Quantitative analysis of Doppler data over serial monitoring time points offers the potential for detection of decreases in bulk volumetric flow, which could for the first time allow operative re-exploration before complete thrombosis formation, potentially simplifying anastomotic revision and/or improving salvage outcomes. Clinically relevant large animal studies will be necessary to determine the feasibility of this aim and corresponding value in improving outcomes that it would confer.

Conclusion

In this proof-of-concept work, we have demonstrated a hybrid system consisting of a resorbable ultrasound marker implanted at the surgical site and Doppler ultrasound with custom analysis software permits location and flow analysis in a large animal model of microvascular reconstructive surgery, including real-time detection of flow loss. Future efforts will focus on demonstrating the biocompatibility of the marker and developing software to simplify the scanning process so that it can be used by bedside care providers.

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Fig. 1. Saphenous artery-based porcine free tissue transfer including flap marking (left) and flap inset.

Fig. 2.
Microvascular anastomosis of vessels followed by marker placement and flap inset.

Fig. 3.
Arduino microprocessor-controlled electrolytic injury model of thrombosis induction.

Fig. 4.

(Left) In vivo visualization of arterial (red) and venous (blue) flow in healthy vessels. Marker edges are indicated by solid line. A, artery lumen; V, vein lumen. (Right) Visual confirmation of loss of blood flow after transient flow interruption.