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Multispectral imaging of organ viability during uterine transplantation surgery in rabbits and sheep

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1.1 Uterine Transplantation Surgery

Absolute uterine factor infertility (AUFI) renders women unconditionally infertile due to the absence of the uterus (premenopausal hysterectomy or congenital reasons). UTx has been proposed as a potential solution in such cases. A large body of surgical research in animal transplant models, has resulted in the development of surgical techniques that offer the potential for pregnancy to women without a womb. An important predictor of the success of the transplant procedure is the degree to which perfusion is restored in the organ. An optical method of assessing the whole organ’s health is described in this paper within the context of an animal study.

1.2 Optical Imaging of Hemodynamics

Optical imaging of hemodynamics has been proposed as a treatment for permanent absolute uterine factor infertility (AUFI) in the case of the congenital absence or surgical removal of the uterus. Successful surgical attachment of the organ and its associated vasculature is essential for the organ’s reperfusion and long-term viability. Spectral imaging techniques have demonstrated the potential for the measurement of hemo-dynamics in medical applications. These involve the measurement of reflectance spectra by acquiring images of the tissue in different wavebands. Measures of tissue constituents at each pixel can then be extracted from these spectra through modeling of the light–tissue interaction. A multispectral imaging (MSI) laparoscope was used in sheep and rabbit UTx models to study short- and long-term changes in oxygen saturation following surgery. The whole organ was imaged in the donor and recipient animals in parallel with point measurements from a pulse oximeter. Imaging results confirmed the re-establishment of adequate perfusion in the transplanted organ after surgery. Coronal oxygenation trends measured with MSI are consistent with pulse oximeter readings, showing decreased \( \text{StO}_2 \) immediately after anastomosis of the blood vessels. Long-term results show recovery of \( \text{StO}_2 \) to preoperative levels. © The Authors. Published by SPIE under a Creative Commons Attribution 3.0 Unported License. Distribution or reproduction of this work in whole or in part requires full attribution of the original publication, including its DOI. [DOI: 10.1117/1.JBO.21.10.106006]

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1 Introduction

Recently published results by Brännström et al. have demonstrated the feasibility of uterine transplantation surgery (UTx) to offer the potential for pregnancy to women without a womb. An important predictor of the success of the transplant procedure is the degree to which perfusion is restored in the organ. An optical method of assessing the whole organ’s health is described in this paper within the context of an animal study.

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1 Introduction

Recently published results by Brännström et al.1 have demonstrated the feasibility of uterine transplantation surgery (UTx) to offer the potential for pregnancy to women without a womb. An important predictor of the success of the transplant procedure is the degree to which perfusion is restored in the organ. An optical method of assessing the whole organ’s health is described in this paper within the context of an animal study.

1.1 Uterine Transplantation Surgery

Absolute uterine factor infertility (AUFI) renders women unconditionally infertile due to the absence of the uterus (premenopausal hysterectomy or congenital reasons).2 UTx has been proposed as a potential solution in such cases. A large body of surgical research in animal transplant models,3 in addition to experience in fertility-sparing oncological surgery,4 has resulted in the development of surgical techniques that recently led to the first successful UTx in humans.5

Reperfusion of the organ, through connection of its major blood vessels to the recipient’s vasculature, is necessary to ensure long-term viability of the uterus. This is particularly important as the organ is deprived of oxygenated blood during periods of vascular clamping (“warm” ischaemia) or while preserved outside the body during surgery (“cold” ischaemia). Ischemia-reperfusion injury to the parenchyma and microcirculation may occur during these periods.6

In previous work by our group, a pulse oximeter probe was used to measure oxygen saturation and “perfusion index” (a relative measurement of blood volume) by attaching it directly to the organ.7 Although this method had the advantage of being readily available, it suffered from that fact that it only reflected arterial oxygenation and returned just single-point measurements.

1.2 Optical Imaging of Hemodynamics

Recently published results from our group have shown the feasibility of intraoperative visualization of oxygen saturation using a multispectral imaging (MSI) laparoscope.6 This technique, validated in a porcine abdominal procedure, was demonstrated to be capable of following changes in oxygenation across a wide range (30% to 100%), as well as being sensitive to the spatial heterogeneity of the tissue’s vasculature. Studies by other researchers have also demonstrated the effectiveness of this approach for assessing perfusion of the retina,2 brain,8 and kidneys.9 Spectral reflectance imaging has also been used to investigate cold and warm ischaemia-reperfusion injury on the kidneys.10
In this paper, we describe the use of our laparoscopic MSI system to assess changes in uterine oxygen saturation during transplant surgery on rabbit and sheep models. The system incorporates a standard surgical white light source and a liquid crystal tuneable filter (LCTF) to provide spectral selection. Results were obtained in parallel with pulse oximetry point measurements in short- and long-term survival studies. Detailed descriptions of the surgical method and results have been published separately.\textsuperscript{11,12} These measurements enable intraoperative quantitative measures of perfusion to be recorded along with spatial information, at different time points in addition to subjective observations. The results of this study help to identify typical trends in organ oxygen saturation that may occur during surgery and recovery, with reference to a baseline.

2 Materials and Methodology

All animal experiments described in this paper were conducted under UK Home Office licenses (70/7508 and 70/6927). Full details of the surgical procedures may be found in separate papers relating to the sheep\textsuperscript{11} and rabbit operations.\textsuperscript{12}

2.1 Multispectral Imaging Laparoscope

A laparoscopic MSI system developed by our group, and characterized and validated in a separate publication,\textsuperscript{8} has been used in the previous pilot studies of uterine transplantation procedures.\textsuperscript{13,14} The clinical imaging system comprises a 30-deg laparoscope (Karl Storz GmbH, Tuttingen, Germany), LCTF (Varispec, Cri, Inc.), and monochrome camera (DCU223M, Thorlabs Ltd., UK). A surgical xenon light source (xenon 300, Karl Storz GmbH, Tuttingen, Germany) was used for illumination. The laparoscope was secured to a flexible mounting arm and the whole system placed on a trolley that could be conveniently positioned at the bedside for measurements. A data cube of 13 images (500 to 620 nm) was acquired to generate reflected intensity spectra of the tissue at each spatial location. Exposure times of ∼200 ms/image were required, resulting in a total acquisition time of ∼3.2 s. A preprocessing image registration step using custom-written feature-tracking software\textsuperscript{8,16} was carried out to compensate for misalignments caused by breathing and peristalsis motion during this time. The spectral sensitivity of the optical system was corrected for using a white reflectance target (Spectralon, Labsphere, Inc.) before converting the data to absorbance spectra. Figure 1 shows the laparoscope in position during imaging of the rabbit uterus.

Relative concentrations of oxygenated (HbO\textsubscript{2}) and deoxygenated hemoglobin (Hb) were determined using linear least squares regression\textsuperscript{9} of the experimental data to the known pure component spectra.\textsuperscript{17} The sum of the concentrations (total hemoglobin; Hbt) was calculated, along with the concentration of Hb\textsubscript{O2} expressed as a percentage of Hbt (oxygen saturation; StO\textsubscript{2}). This approach is subject to the assumption that oxygen and deoxyhemoglobin are the only optical absorbers in the field-of-view, and that scattering and penetration depth are approximately constant across the spectrum. Pixel locations with a poor match ($r^2 < 0.9$) between experimental and model absorbance curves were discarded. All data processing was conducted offline using MATLAB (The Math Works, Inc.).

2.2 Intraoperative Imaging

Uterine oxygenation was measured during short-term (sheep) and long-term (rabbit) animal UTx viability studies. The sheep (~60 kg) study was conducted as an autotransplant under terminal anaesthesia, with the uterus removed, stored externally temporarily, and replaced into the same animal. New Zealand white rabbits (~4 kg) were used in a series of allotransplant procedures, where the uterus of a donor animal was transplanted to a recipient animal. The recipient subsequently recovered following surgery.

The transplant procedure involved removal of the uterus and its blood supply en bloc, in a microvascular patch, followed by flushing of the blood vessels with saline and a preservation solution (Custodiol HTK, Dr. Franz Köhler Chemie GmbH, Germany). The organ was stored on ice in a bath of the same preservation solution while the recipient was prepared. During implantation, the cornua and vagina were also attached to provide mechanical stability for the organ. Reconnection of the blood supply (anastomosis) was achieved either through an aorta–aorta/inferior vena cava (IVC)–IVC anastomosis (rabbit) or internal/external iliac anastomosis (sheep), as illustrated in Fig. 2.

Oxygenation measurements were made of the uterus in its native condition, during organ retrieval surgery in the donor, and immediately after implantation and reperfusion in the recipient. In the case of the allotransplant study, the recipient’s own uterus was also measured before a hysterectomy was performed. A pulse oximeter (Datex-Ohmeda 3600P; Datex-Ohmeda, Colorado) was used to measure oxygen saturation and perfusion index (PI) at the medial and lateral aspects of the right and left cornua (Fig. 2). These measurements were followed by MSI acquisitions of the entire uterus. Three MSI measurements were made at each site to reduce the risk of random error. Mean StO\textsubscript{2} was calculated in the regions-of-interest within the images that corresponded to the pulse oximeter measurement sites.

3 Results

The short-term (autotransplant) and long-term (allotransplant) MSI results are presented below and show reconstructed color images and StO\textsubscript{2} overlays. Imaging was completed in a total of seven rabbits and five sheep. Each transplant procedure is referred to by a UTx number (rabbits: 3 to 9; sheep: 1 to 5).
corresponding to the results described in our surgical papers.\textsuperscript{11,12} No imaging was performed in rabbit UTx 1 or 2.

3.1 Sheep Study (Autotransplantation)

Color and processed MSI data from the sheep transplants are shown in Fig. 3. On appearance alone, the uterus, in its native state, appears bright red/pink as it is well-perfused with oxygenated blood. The transplanted uterus (“graft”), while also red, is not as saturated as in the native state. The processed MSI data also support this, with the bright red and yellow shades indicating relatively high oxygenation in both cases. Areas that could not be adequately described by the absorbance model are seen as “gaps” in the processed images. This is particularly evident in

![Fig. 2 Schematic showing the harvested uterus, its major supplying blood vessels, and the points of anastomosis for the rabbit and sheep transplants. The numbered regions of interest indicate measurement points for oxygen saturation on the medial and lateral parts of the uterine cornua. The photograph on the right shows a rabbit uterus immediately prior to removal from the donor animal. The aorta and inferior vena cava appear below the organ following dissection of the microvascular patch.](image1)

![Fig. 3 Complete sheep imaging results showing the uterus in the donor, prior to harvesting, and the transplanted graft. UTx 1 and 4 were abandoned during implantation.](image2)
regions where specular highlights are present, such as those visible in Fig. 3.

The quantitative results are shown in Fig. 4. Transplant numbers 1 and 4 had to be abandoned due to intraoperative complications. The rest were completed as planned.

Baseline (donor) arterial oxygen saturation values recorded by the pulse oximeter are consistently high in all animals, varying from 88% to 100% (mean = 94%). Tissue oxygen saturation measured by the MSI device is lower, varying from 41% to 77% (mean = 66%). Intraorgan variability is visible in the mean values in the four ROIs of each case, whereas interorgan variability in StO2 is evident through comparison of the donor and recipient values across all procedures. In the completed procedures (UTx 2, 3, 5), lower oxygen saturation values were recorded in the transplanted graft by both methods, with the exception of the MSI results for UTx 3.

3.2 Rabbit Study (Allotransplant)

Macroscopically, the appearance of the rabbit uteri, shown in Fig. 5 immediately pre- and posttransplant, is similar to that of the sheep. The native organ in the donor and recipient (prehysectomy) appears bright and well-perfused, while appearing

![Fig. 4](image-url) Mean cornual StO2, plotted against sheep autotransplant number, for the four regions-of-interest in the donor uterus and transplanted graft. The error bars represent the standard deviation of repeated measures of the same organ. Pulse oximeter measurements (SpO2) for the same regions of the organ are plotted alongside the MSI results.

![Fig. 5](image-url) Complete rabbit MSI results showing images for the donor, the recipient’s native uterus and the transplanted graft. Pixels with r^2 < 0.9 are grayed out.
The long-term survivor (UTx 5) was imaged 90 days post-transplant during the surgical transfer of embryos to the transplant recipient’s uterus. Mean StO₂ in the organ recovered to 86% ± 1% from the immediate postoperative value of 47%, which is also higher than the original baseline reading of 80% ± 12% (medial aspect). The color and MSI images of the organ, shown in Fig. 7, indicate the changes that the organ has undergone in the intervening period. A significant number of postoperative adhesions have hidden the uterus’s distinctive shape, but it is clear that perfusion has been fully re-established.

4 Discussion

Donor uteri, in both rabbit and sheep procedures, were well-perfused and oxygenated as evident from the StO₂ images. Mean StO₂ values from the regions-of-interest varied from 62% to 91% (mean = 78%) in the rabbits, and from 41% to 77% (mean = 66%) in the sheep. Reperfusion of the organ was confirmed by detection of a pulsatile signal with the pulse oximeter and by the MSI images, which displayed a clear hemoglobin signal. Tissue oxygen saturation in the transplanted graft, as measured by the imager, was markedly decreased in all cases (with the exception of sheep UTx 3) compared to the native state. A paired t-test returns a significant difference between the StO₂ values pre- and post-UTx (p = 2.3 × 10⁻⁴) in the rabbit. This may be an effect related to the fact that even though oxygenated blood was flowing through the organ’s main vessels many microvessels within the tissue had still not dilated fully or been perfused with oxygenated blood. Thus, the more superficial tissue with lower oxygen content may have a comparatively stronger effect on the final StO₂ value recorded by the imager, which integrates the signal from both venous and arterial blood.

The MSI laparoscope uses the 500 to 620-nm range to estimate StO₂ due to the large difference in shape between the oxy- and deoxyhemoglobin absorption curves in this region. As mentioned in our previous work,⁶ this is under the assumption that each wavelength of detected light interrogates an equal volume of tissue. However, using values for whole blood,¹⁸ it can be seen that the effective attenuation coefficient (μₐeff = \sqrt{3μₐ(μₐ + μ₄')}), which influences penetration depth, varies by 48% across this range. This is similar to other similar MSI processing examples in the literature,¹⁹⁻²¹ and is a necessary balance among pathlength assumptions, sensitivity to StO₂ changes, quality of fit, and simplicity of computational implementation for imaging. It was found that the fitting range used here achieved better fit quality (r²) than a slightly narrower

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Fig. 6 Mean cornual oxygen saturation, plotted against transplant number, in the regions-of-interest in the donor animal, the recipient (native uterus), and the transplanted graft. Identification of the ROIs at embryo transfer (UTx 5) was not possible, due to adhesions (see Fig. 7), so mean StO₂ for the entire organ is shown instead. Error bars represent the standard deviation of repeated measurements on the same organ. Pulse oximeter measurements for the corresponding regions are included on the same plot.

Fig. 7 Long-term survival and embryo transfer. Rabbit test subject imaged 90 days after transplantation, during the embryo transfer procedure. The uterus, highlighted, has lost its distinctive shape due to adhesions. Background tissue has been manually masked out for clarity.
range (500 to 600 nm),\(^{19}\) whereas StO\(_2\) was unaffected. Reducing the range further resulted in ever decreasing \(r^2\) values, with 35% more pixels falling below the \(r^2\) threshold when the 520 to 580 nm range is used to perform the regression.

The pulse oximeter also showed a statistically significant decrease in cornual oxygen saturation post-UTx (rabbit: \(p = 5.5 \times 10^{-4}\); sheep: \(p = 5.8 \times 10^{-5}\)), although the absolute values still remained higher than the MSI results. This is expected as the device uses arterial pulsations to measure the degree of optical absorption, and is thus principally reflective of arterial oxygen saturation. There was no significant difference in StO\(_2\) or SpO\(_2\) between the donor and recipient (native uterus) in the rabbit procedures (\(p = 0.73\) and \(p = 0.83\), respectively). There was a significant difference in PI between donor and graft in the rabbit procedures (\(p = 0.02\)), but not in the sheep procedures (\(p = 0.32\)).

Although both MSI and pulse oximetry detected a similar trend in the detection of a drop in oxygenation after transplantation, there was no statistically significant correlation between the absolute values of StO\(_2\) and SpO\(_2\). The main reason for this is most likely due to the significant differences between the two modalities in how the signal is acquired. MSI uses diffuse reflectance, integrating the signal from all blood (arterial and venous) within the top 2 to 3 mm of the tissue. The pulse oximeter, on the other hand, operates in transmission mode, measuring the signal from arterial blood in the uterine cornua between the jaws of the device.

In the long-term surviving rabbit (UTx 5), StO\(_2\) was measured at values close to that of baseline levels, indicating recovery of the microvasculature and confirming full reperfusion of the organ. The presence of adhesions meant that pulse oximetry was not possible.

5 Conclusions

MSI has been used for the first time to visualize organ oxygen saturation during UTx in rabbit and sheep models. The technique enabled noncontact quantitative assessment of the changes in uterine StO\(_2\) and blood volume. Pseudocolor maps allowed visualization of the spatial distribution of oxygenated blood, whereas regions-of-interest could also be selected for numerical comparison of StO\(_2\) at various stages in the transplant process.

MSI allows monitoring of organ perfusion during transplant surgery by quantitatively imaging tissue oxygen saturation. Although a number of commercial point probes exist (e.g., O2C; LEA Medizintechnik GmbH, Germany; Intra; Ox; ViOptix, Inc.), there is currently no established method to perform StO\(_2\) imaging clinically, and the results presented here show its potential for use in future human studies. The device was capable of indicating restoration of perfusion following anastomosis of the organ’s vasculature, as well as long-term recovery postoperatively. Neither the sheep nor the rabbit procedures described in this paper demonstrated any postoperative complications that could be directly linked to insufficient StO\(_2\). However, the consistently high StO\(_2\) values in the donor and recipient animals prior to surgery served as a useful baseline from which to track longitudinal changes. Future preclinical organ imaging studies, with controlled levels of perfusion, will help to quantify the ability of intraoperative StO\(_2\) as a predictor of tissue viability.

The main technical improvements needed to make this technique more suitable for clinical use involve reduction of the number of wavebands required for the StO\(_2\) calculation to increase acquisition speed, and development of more computationally efficient, parallel-processing methods. To this end, our group and collaborators have been investigating data reduction techniques,\(^{22}\) use of a fast filter wheel MSI camera,\(^{23}\) and implementation of machine learning algorithms.\(^{24}\) The imaging hardware is compatible with existing sterilizable medical instruments such as laparoscopes and rigid cystoscopes, meaning that incorporation of MSI into human UTx studies is feasible. It is hoped that StO\(_2\) measurements intraoperatively and later, transvaginally, may help to quantify the health of the organ, track its recovery after surgery, and possibly indicate the onset of any rejection episode. This type of imaging is of particular importance as some of the reported human UTx failures have been strongly linked with incomplete reperfusion of the uterus.\(^{25,26}\)

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