Extracorporeal Free Flap Perfusion Using Extracorporeal Membrane Oxygenation Device

An Experimental Model

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Abstract: Extracorporeal perfusion of organs has a wide range of clinical applications like prolonged vital storage of organs, isolated applications of drugs, bridging time to transplant, and free composite tissue transfer without anastomosis, but there are a limited number of experimental models on this topic.

This study aimed to develop and evaluate a human extracorporeal free flap perfusion model using an extracorporeal membrane oxygenation device. Five patients undergoing esthetic abdominoplasty participated in this study. Deep inferior epigastric artery perforator flaps were obtained abdominoplasty flaps, which are normally medical waste, used in this model. Deep inferior epigastric artery perforator flaps were extracorporeally perfused with a mean of 6 days. The biochemical and pathological evaluations of the perfusions were discussed in the article.

Key Words: extracorporeal free flap perfusion, extracorporeal free flap perfusion experimental model, extracorporeal free flap transfer, extracorporeal free flap perfusion using ECMO

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Experimental normothermic perfusion of organs dates back to the pioneering study of Alexis Carrel as early as in 1930s.[1] There are a large number of studies in the literature regarding organ preservation and transplantation procedures that use extracorporeal perfusion (ECP).[2–4] Interestingly, this topic was not linked to plastic surgery studies for a long time, and the first successful report of free flap transfer through extracorporeal circulation without microvascular anastomosis was published by Wolff et al[5] in 2015.

Extracorporeal perfusion of organs has a wide range of clinical applications like prolonged vital storage of organs, isolated applications of drugs, bridging time to transplant, and free composite tissue transfer without anastomosis, but there are a limited number of experimental models on this topic.[4–12]

This study aimed to develop and evaluate a human extracorporeal free flap perfusion model using an extracorporeal membrane oxygenation (ECMO) device for further studies in this field. Using ECMO device, we tried to perfuse a deep inferior epigastric artery perforator (DIEP) flap, which was described by Kreidsrein et al[13] as an isolated perfused human skin flap model.

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MATERIALS AND METHOD

Between May 2016 and April 2018, 5 patients undergoing esthetic abdominoplasty participated in this study. Abdominoplasty flaps, which are normally medical waste, were used in this study.

All clinical procedures were performed according to the principles expressed in the Declaration of Helsinki. The study was approved by the Local Ethics Committee for Clinical Experiments at Medeniyet University (ethics committee approval, 2016/0221). Informed consent was obtained from all participants included in the study. Bıcakcılar kindly provided the ECMO device and oxygenators needed for this study. No other funding was received from any external source.

Surgical Technique

After routine abdominoplasty markings, standard abdominoplasty flaps were elevated by preserving 1 of the major paraumbilical perforators. A DIEP flap was prepared from the planned excision area based on the major perforator vessel. Zone 4 of the flaps was excised. The perforator was ligated and cut over the fascia, and no subfascial dissection was performed. After harvesting the DIEP flap, standard abdominoplasty was completed by another plastic surgeon in our team. The artery, concomitant veins, and 1 to 2 superficial veins were cannulated with appropriately sized peripheral venous catheters (16–20 gauge) and fixed with silk sutures 6-0. The flap was rinsed with heparinized Ringer's solution. Intra-arterial fluorescein was administered to the flap through the perforator artery, and the flap was observed under Wood's light. The excision was planned based on the nonperfused area. After the excision of the nonperfused areas, the flap was folded on itself, shaped into a tube, and sutured (Fig. 1). A drain was placed in the tube-shaped flap. To quantify edema formation, the flaps' weight was documented during the experiment. The flaps were kept in room temperature at intensive care unit of our department.

Perfusion System

A pediatric ECMO device was used in this study. The device consisted of a centrifugal pump head (Revolution, Sorin, Milano, Italy) with tubing and a pediatric hallow fiber oxygenator (LILLIPUT II, Sorin, Milano, Italy) with a countercurrent heat exchanger. It was modified for the experiments, and a 150-mL cardiotomy reservoir with a filter (D901 LILLIPUT, SORIN) was added to the standard pediatric ECMO kit. The diameter of the tubing system was reduced to the appropriate size (16–20 G) (Fig. 2). The system was primed with heparinized (500 U/450 mL) fresh whole blood diluted with Ringer's lactate by 30% and hydroxyethyl starch by 10%. Fresh whole blood was obtained from the blood bank, which derived the resourced from volunteers at our hospital with the same blood type as the patient. Ceftriaxone (500 mg) was added to each unit of whole blood that was used in the experiment. After filling the 150 mL reservoir, the rest of the whole blood was kept sterile and cooled in appropriate conditions. Venous return was achieved by passive drainage and gravity into the reservoir of the system (Fig. 3).

All perfusions were initiated within 60 minutes after the harvesting of the flap. The blood pressure and flow rate before the flap were monitored continuously. Venous outflow was observed from the venous

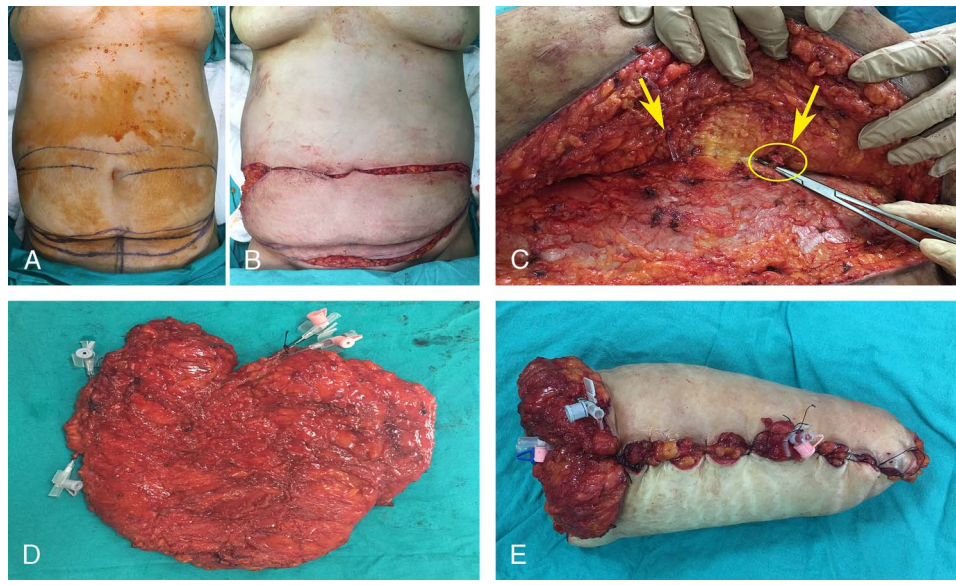

FIGURE 1. A, B, Preoperative and intraoperative pictures of the abdominoplasty excision area are shown. C, Paraumblical perforator is shown. D, Paraumbilical perforator vessels and superficial veins are cannulated. D, The flap was folded on itself, shaped into a tube, and sutured.

line of the flap; the flow rate and tension on the arterial line were set based on the venous outflow. The mean arterial flow rate was set to be 8 to 10 mL/min, which was adequate for a blood pressure of 90 to 100 mm/Hg on the arterial line. This flow rate was high normal values of deep inferior epigastric artery.[14] The temperature of the system was maintained at 34°C (93°F).

FIGURE 2. Flap is connected to the modified ECMO system.

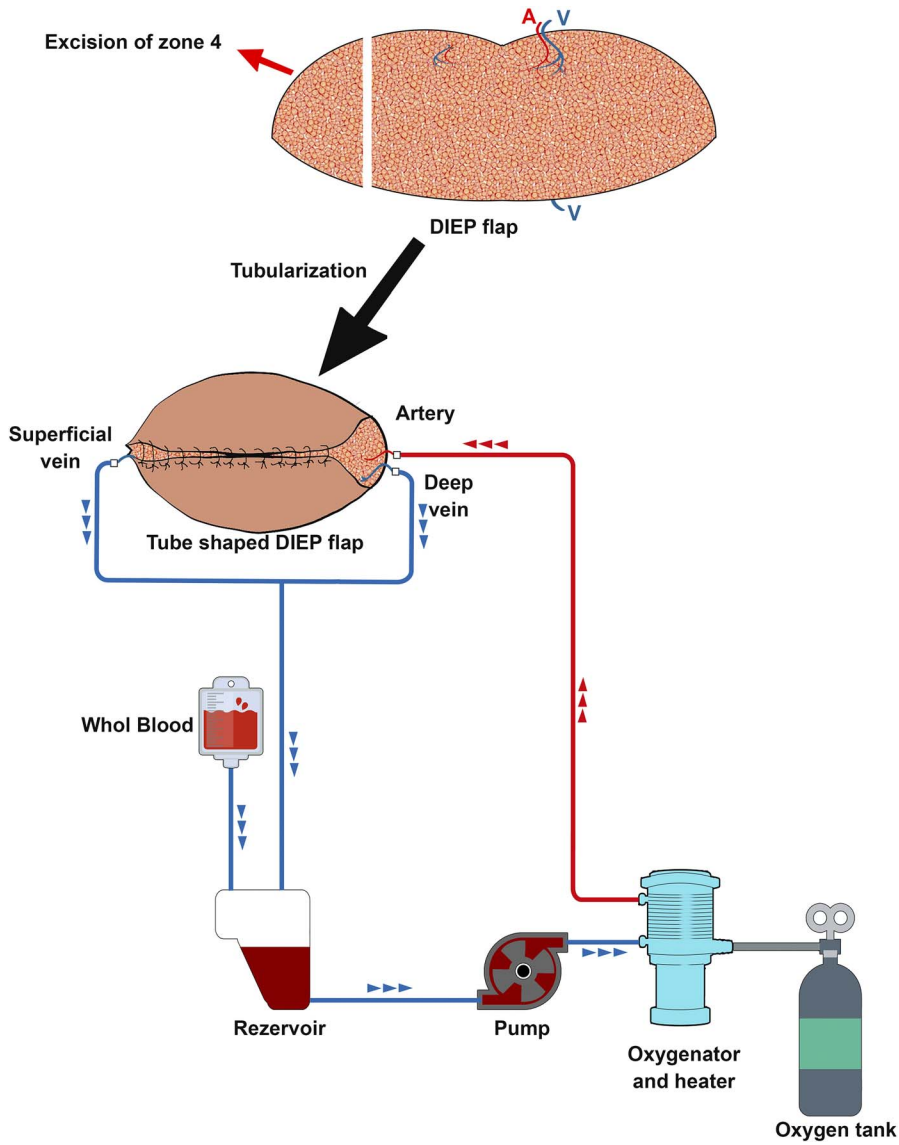

FIGURE 3. Schematic presentation of the modified ECMO system.

Assessment of Flap Perfusion

Flap color and capillary refill were observed clinically. Blood gas and laboratory analyses were performed for measurement of oxygen saturation (SO_2 %), pH value, oxygen partial pressure, carbon dioxide partial pressure, hemoglobin level, glucose, sodium bicarbonate buffer, potassium (K^+), and activated clotting time every hour in the first day and every 2 hours in the following days.

Correction of pH drops was achieved by adding sodium bicarbonate 8.4% into the reservoir. If the remaining blood was less than 30 mL, fresh whole blood was added to system. Pathological biopsies were taken daily from the central parts of the zones 1 and 3 of the flaps. The specimens were fixed in a 4% formalin solution, then embedded in paraffin, and stained with hematoxylin and eosin for histological analysis.

RESULTS

With initiation of ECP, a clear capillary refill was observed. Venous return started about 1 minute later. The dark color of the venous blood indicated lower oxygen levels (Fig. 4). As an indication of the maintained metabolism and tissue viability, mean arterial-venous oxygen partial pressure difference was 32% (99% vs 68%) till the fourth day (Fig. 5).

There was a mean value of 40 mL/h leakage of perfusate from the row surface of the flaps throughout the experiment process. We had to replace 1.3 units of whole blood daily to manage the blood loss. We used a mean of 7.6 units of whole blood for every flap.

Extracorporeal perfusion was maintained for a mean time of 6 days (range, 4–7 days). No clinically apparent flap infection was observed. No complete flap necrosis or thrombosis was observed. After the fourth day of perfusion, venous outflow started to decrease, and superficial epidermal loss in the zone 1 area was observed in 4 flaps (correlated with blood gas analysis). In 1 flap, the experiment was terminated on the fourth day because of the thrombosis of the oxygenator. The mean total weight increase of the flaps was 38% during the experiment.

Histologically regular tissue architecture and intact epithelium were observed in all flaps that survived till the fourth day. The specimens on the fifth day showed dermoepidermal separation. On the sixth

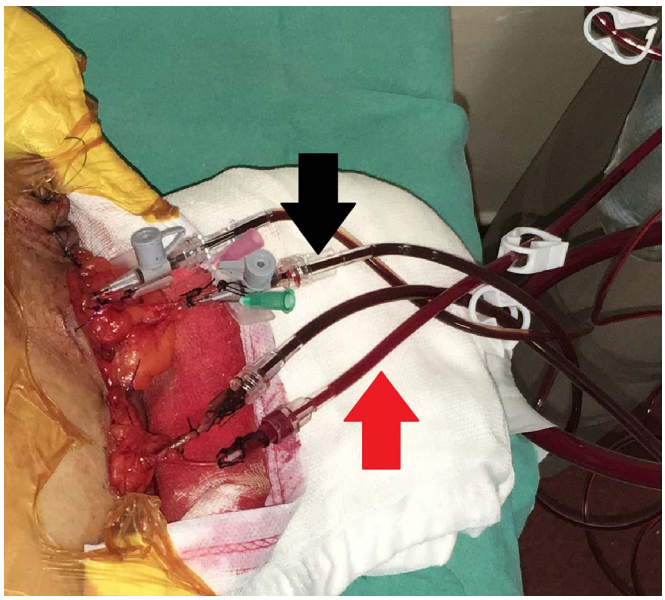

FIGURE 4. The color difference between the arterial (red arrow) and venous (black arrow) blood within the modified ECMO system.

day, separation was more evident, and the seventh day specimens showed apparent epitheliolysis (Fig. 6).

The pH levels stayed within normal values till day 4, but after the fourth day, they showed a sharp decrease (Fig. 7). Blood gas analysis showed initial K+ elevation but stayed stable throughout the experiment. Hemoglobin levels were low but stable owing to hemodilution. Glucose levels were above normal values throughout the experiment. The lactate levels showed a sharp increase on the first day of the perfusion and stayed stable but were elevated till the fourth day (Fig. 8).

DISCUSSION

In recent years, normothermic ex vivo perfusion of organs before transplantation was translated from an experimental laboratory technique into clinical practice with promising results.[4] Normothermic extracorporeal perfusion was demonstrated to be a feasible and well-tolerated method of organ preservation.[15] Although there are a large number of studies on ECP of organs in transplantation surgery, there are only a limited number of studies in composite tissue preservation and transplantation procedures in the field of plastic surgery.[5,7,12,16–19]

Extracorporeal perfusion of tissues has a wide range of clinical applications like prolonged vital storage of organs, isolated applications of drugs, gene and stem cell therapies, bridging time to transplant, saving time for free flap rescue, and free composite tissue transfer without anastomosis.[5–8,10,20] There are only a few experimental models related to this topic.[5,9,10,12,13] The aim of this study was to develop a human extracorporeal free flap perfusion model using ECMO device for further studies in this field.

An isolated perfused human skin flap was first described by Kreidsrein et al[13] in 1990. In their study, they used a transverse paraumbilical skin flap based on perforator vessels from the deep inferior epigastric system, using the tissue discarded after abdominal dermolipectomy. In their model, the flaps were perfused with oxygenated Krebs-Henseleit buffer containing albumin for 4 hours, and they showed that the flaps were metabolically active throughout the experimental procedure. The isolated perfused human skin flap model was modified and used in our study. The flaps were folded and shaped into tubes on themselves. The aim of tubing was to minimize the raw surface area for both reducing the insensible fluid loss and prevention of infection in the long term. We also used whole blood for perfusing the flaps,

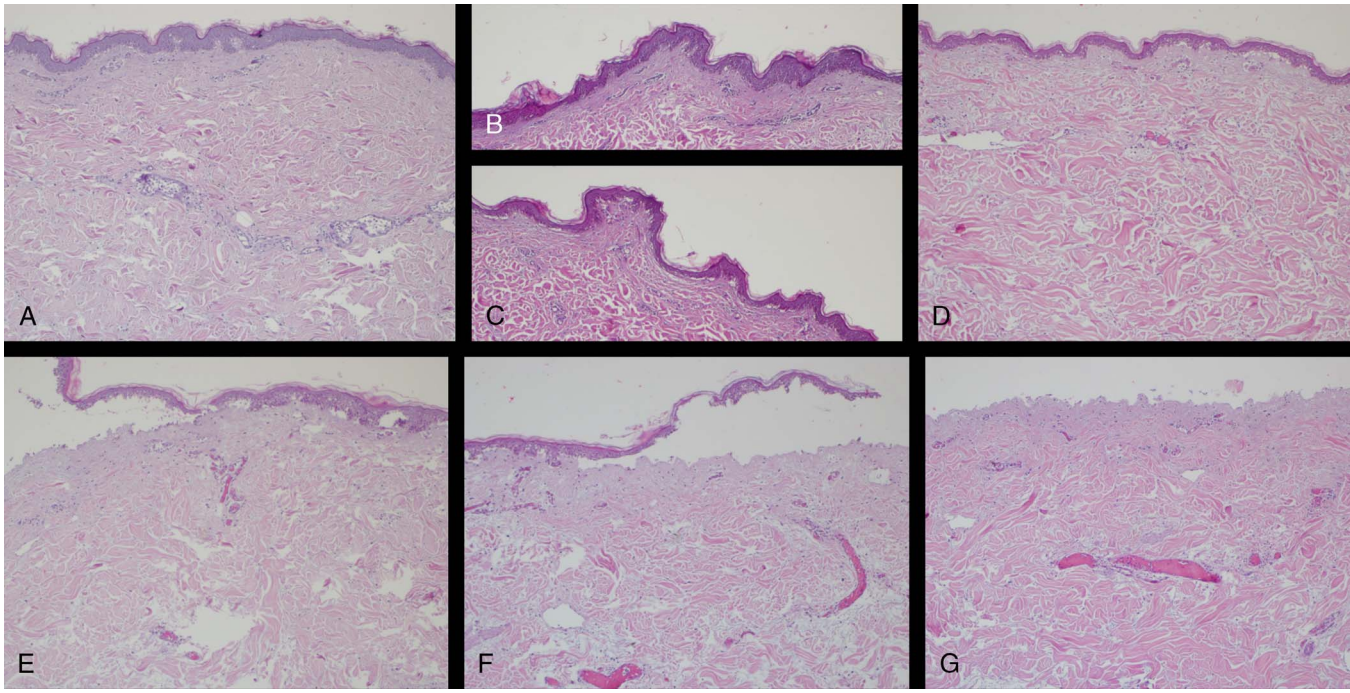

FIGURE 5. A–D, First, second, third, and fourth day pictures of the pathological examination show the intact skin epithelium. E, Fifth day pictures show dermoepidermal separation. F, Dermoepidermal separation is more severe on sixth day specimen. G, Seventh day specimen shows total epidermal loss.

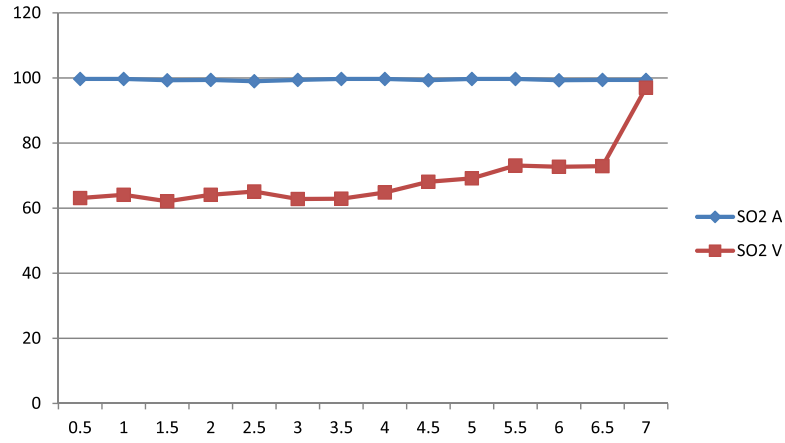

FIGURE 6. Oxygen saturation differences of the arterial and venous blood samples are shown.

and the venous blood was recruited back into the system. The flaps were connected and perfused with the modified ECMO system.

There are numerous advantages of using a DIEP flap in this model. The DIEP flap, which we frequently used in the past in microvascular breast reconstruction, is a medical waste in abdominoplasty operations. In such operations, it is easily accessible without any additional morbidity to the patient. The relatively large diameter of the vessels of this flap makes cannulation easy in comparison with animal models. Clinical observation of flap perfusion is also relatively easier through the abdominal skin. One of the other advantages of using a human extracorporeal free flap perfusion model is easy access to whole blood from voluntary individuals, and this blood is used for perfusion of the flaps. The whole blood that is used for perfusion in animal models is limited in quantity and usually obtained from sacrificed animals.[10,20]

Extracorporeal membrane oxygenation is a system for temporary support of heart and lung function by partial cardiopulmonary bypass for up to 3 to 4 weeks (up to 75% of cardiac output).[21] It is used for patients who have reversible cardiopulmonary failure from pulmonary, cardiac, or other diseases. Commercially available adult ECMO systems are generally used in composite tissue perfusion studies.[7,22] The prime volume of these systems is high, and it is hard to adapt them into the relatively small vessels of flaps. We used a pediatric ECMO circuit in our model. The pediatric ECMO circuit had a low (90 mL) prime volume, and it was suitable for long-term usage.[21] This pediatric ECMO circuit had a centrifugal pump (Revolution, SORIN) and a hollow fiber oxygenator (LILLIPUT II, SORIN) with a 150-mL filtered cardiotomy reservoir (D901 LILLIPUT, SORIN). There is no reservoir in the standard ECMO circuit, and it is connected directly to the common vessels of the patient. If the system is used in free flap perfusion without a reservoir, it is not possible to monitor and replace blood loss. With continuous blood loss from the row surface of the flap, air bubbles enter the circuit, and it is no longer possible to perfuse the flap. This problem is solved with a filtered blood reservoir connected to the system. The cost of the system was US $2400 for single experiment.

In our experiment, 5 flaps were extracorporeally perfused with a mean time of 6 days. On the fourth day of the perfusion, the venous outflow started to decrease, and on the sixth day, it completely stopped. The perfusion of the flaps continued till the cessation of venous outflow.

In the blood gas analysis, we observed an oxygen saturation difference in the arterial and venous blood samples till the fourth day, which was correlated with the pathological observations. Both oxygen saturation difference and histological examinations showed the intact metabolic activity and viability of the flaps at least for 4 days, but because the flaps were not reimplanted onto the body, it was not possible to prove the exact viability of the flaps. The flaps were evaluated histologically for the viability, and the absence of evaluation of cellular changes on molecular level is a limitation of this study.

It is hard to evaluate the results of the blood gas analysis in our experiment, because we continuously replaced the blood loss with fresh blood and intervened to the pH drops by addition of sodium bicarbonate to the system. However, the blood gas analysis may show the elevation or decrease trends of the parameters.

The pH levels stayed within normal values till day 4, but after the fourth day, they showed a sharp decrease. The K+ levels were elevated at the beginning of the experiment, but they stayed stable within normal

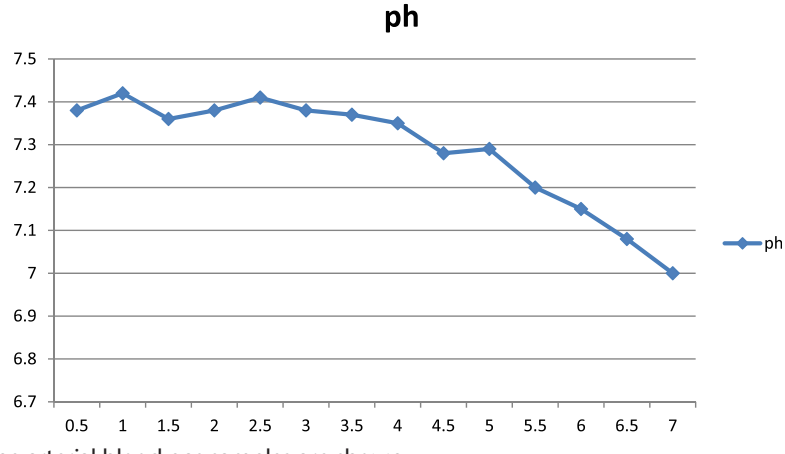

FIGURE 7. Ph changes of the arterial blood gas samples are shown.

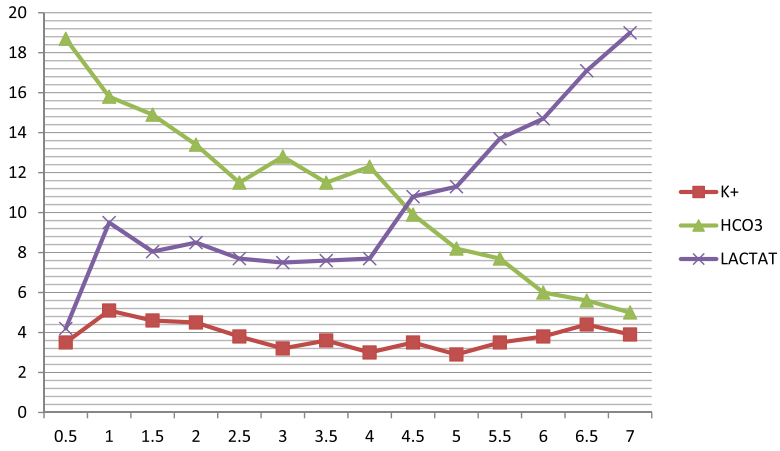

FIGURE 8. The change of the K^+, lactate, and sodium bicarbonate buffer levels are shown.

values throughout the experiment. Along with the normal pH levels, normal K+ levels showed the quality of the perfusion for at least 4 days.

Lactate is the normal end point of the anaerobic breakdown of glucose in tissues. Elevated lactate levels may show inadequate perfusion, with a concurrent shift toward increased anaerobic metabolism. Lactate is cleared from the liver and may accumulate in case of decreased clearance.[23] In our experiment, the lactate levels showed a sharp increase on the first day of the perfusion and stayed stable but were elevated till the fourth day. After the fourth day, they were also elevated sharply.

The elevated lactate levels in our experiment may be the result of the absence of a clearance mechanism like the liver connected to the perfusion system. Elevated lactate levels may also show the inadequate perfusion of the flap. However, as we mentioned earlier, the pH and K levels were within normal values till the fourth day in our experiment, which shows the quality of the perfusion.

Lactate is an acidic substance, and elevated lactate levels decrease the blood pH. Despite the elevated lactate levels, low bicarbonate levels with low carbon dioxide partial pressure levels and normal pH were observed till day 4 in the blood gas analysis. This situation is called respiratory compensated metabolic acidosis, and it shows the effectiveness of the buffering system within the whole blood.[24] The effective buffering system within whole blood is one of the reasons for choosing whole blood as the perfusion fluid in our model.

The blood loss related to the wide row surface area from the DIEP flap was one of the major limiting aspects of this model. Despite the strict homeostasis during flap elevation, there was a continuous 40 mL/h blood leakage from the flap through the experiment, and we had to replace 1.3 units of whole blood daily. Well-managed heparinization may prevent blood loss, but it is not easy to balance the heparinization because of the continuous loss of coagulation factors from the blood, and there is no regulatory organ like the liver connected to the system.

Despite all the negative aspects of blood loss, replacement of the blood within the system up to 8 times in a day may have also provided some benefits like removing the metabolic products, replacement of blood clotting factors, and addition of immune active cells to the system. All these benefits may have played a role on the relatively longer (mean time of 6 days) perfusion without coagulation and infection problems.

It was shown that extracorporeal tissue perfusion studies using whole blood as perfusate result in weight gain related to tissue edema by up to 20% (range, 10%–32%).[7,10,25] In our study, there was a mean rate of 38% weight gain of the flaps, which was relatively high. The long duration of perfusion in our study may be responsible for this result. We did not use any medications to reduce the edema. Tissue edema may have played a role in the disruption of tissue perfusion in the long-term.

The first article regarding flap tissue survival through extracorporeal circulation was published by Maeda et al[12] in 1993. They showed that flaps based on the thoracoepigastric vein survived for 3 days in rabbits using autologous plasma.

The first report of free flap transfer without microvascular anastomosis was published by Wolff et al[5] in 2016. They transferred a free fibula flap using extracorporeal perfusion without any microvascular anastomosis for mandibular reconstruction of 3 patients with vessel-depleted necks.[5] They did not reinfuse the venous blood for fear of infection and for direct control over the blood volume and pH. They were able to maintain the perfusion of the fibula flap with a mean perfusion rate of 1 mL/min. The DIEP flap we used had a large surface area, and we maintained the perfusion of the flaps with a mean perfusion rate of 10 mL/min. We had to replace the blood with a mean rate of 1.3 units/d of whole blood because of the 40 mL/h blood leakage from the system. If we had not reinfused the venous blood into the system, we would need additional 18 units of whole blood, which is not practically possible.

Classical long-term complications of extracorporeal perfusion are hemolysis, bacterial colonization, or systemic infection.[26,27] In this model, minimizing the row surface by tubing the flap on itself and the use of prophylactic antibiotics may have prevented bacterial infections. It should be kept in mind that this flap was perfused extracorporeally, and classical signs of infection like leukocytosis, elevated C-reactive protein levels, erythema, or fever cannot be expected. Although any soft tissue lysis, bad odor, or clinically visible bacterial colonization on the flap was not observed, direct microscopic examination of the blood or blood bacterial cultures may be more accurate for detection bacteremia/infection in this model.

There are many perfusion solutions available for extracorporeal tissue circulation studies, but whole blood remains the criterion standard for this purpose.[20] Whole blood has the capacity to carry oxygen to tissues as well as having coagulation factors and thrombocytes needed for homeostasis. It may also have some immune activity, and it has an acid/base buffer system. Thus, in our experiment, we used whole blood obtained from volunteers with the same blood type of the flap donor.

The main negative aspects of using whole blood are hemolysis and foreign surface–related thrombosis formation within the ECMO system.[21,28] In this model, we used 500 U of heparin in each unit of whole blood to prevent thrombosis formation. A relatively low flow volume within the ECMO system is also a risk factor for thrombosis, and heparinization could not be adjusted lower. Despite the heparinization, we observed thrombosis of the oxygenator in one flap, and we had to

terminate the experiment in that case. Increasing the level of anticoagulation may reduce the risk of thrombosis, but it may also increase the leakage of blood from the system. The absence of a regulatory organ like the liver connected to the system makes it hard to optimize anticoagulation.

By the end of the fourth day, venous outflow started to deteriorate, and we observed epidermal loss on the following day. This situation was correlated with the decreased arterial and venous oxygen saturation difference in the blood gas analysis. The blood gas analyses were performed every 2 hours after the first day, and in case of a decrease in pH below 7.2, sodium bicarbonate was added to system. Uncontrolled acidosis periods between blood gas measurements may have a role on tissue damage. The zone 3 of the flaps, which was relatively less perfused, showed less severe dermoepidermal detachment in the biopsies, and so overperfusion of the flaps may also be responsible for tissue damage. However, we cannot fully explain the cause of the necrosis after day 4, and additional studies are needed for this model for further prolongation of flap survival.

Absence of a control group and limited numbers of flaps were limiting factors of this study. Another limitation of this study was that, although we excised the zone 4 of the flaps and planned the flaps based on fluorescein perfusion at the beginning of the experiment, we did not show the perfusion angiographically at later stages. The presence of capillary refill all through the experiment, active bleeding from the biopsy sites, and oxygen saturation difference between the arterial and venous blood gas samples also showed the active perfusion of the flaps.

Ideal anticoagulation ratio, ideal flow rate, ideal perfusion pressure, prevention of blood loss, and cannulation-related problems (kinking-occlusion) are the main problems to be solved with this model.

CONCLUSIONS

This preliminary study shows that ex vivo perfusion of free DIEP flaps is technically feasible for at least 4 days. This is the first human model of extracorporeal free flap perfusion using ECMO device, and we hope that this model will support studies in this field.

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