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Demonstration of technical feasibility and viability of whole eye transplantation in a rodent model

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immunosuppression;
nerve regeneration

Summary *Introduction:* Whole eye transplantation (WET) holds promise for vision restoration in devastating/disabling visual loss (congenital or traumatic) not amenable to surgical or neuroprosthetic treatment options. The eye includes multiple tissues with distinct embryonic lineage and differential antigenicity. Anatomically and immunologically, the eye is unique due to its avascular (cornea) and highly vascular (retina) components. Our goal was to establish technical feasibility, demonstrate graft viability, and evaluate histologic changes in ocular tissues/adnexae in a novel experimental model of WET that included globe, adnexal, optic nerve (ON), and periorbital soft tissues.
Methods: Outbred Sprague-Dawley rats ($n = 5$) received heterotopic vascularized WET from donors. Each WET included the entire globe, adnexa, ON, and periorbital soft tissues supplied by the common carotid artery and external jugular vein. Viability and perfusion were confirmed

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by clinical examination, angiography and magnetic resonance imaging (MRI). Globe, adnexal, and periorbital tissues were analyzed for histopathologic changes, and the ON was examined for neuro-regeneration at study endpoint (30 days) or Banff Grade 3 rejection in the periorbital skin (whichever was earlier).

Results: Gross examination confirmed transplant viability and corneal transparency. Average operative duration was 64.0 ± 5.8 min. Average ischemia time was 26.0 ± 4.2 min. MRI revealed loss of globe volume by 36.0 ± 2.8% after transplantation. Histopathology of globe and adnexal tissues showed unique and differential patterns of inflammatory cell infiltration. The ON revealed a neurodegeneration pattern.

Conclusion: The present study is the first in the literature to establish an experimental model of WET. This model holds significant potential in investigating mechanistic pathways, monitoring strategies or developing management approaches involving ocular viability, immune rejection, and ON regeneration after WET.

Introduction

Vision loss due to ocular trauma (OT) results in 2.5 million eye injuries per year worldwide, with over 1 million cases resulting in permanent visual impairment.[1,2] Globally, an estimated 32–39 million people are blind and another 191–246 million have moderate to severe visual impairment.[2,3] The global economic productivity loss for visual impairment was projected to grow from $42 billion in 2000 to $110 billion in 2020, based on per-capita gross domestic product (GDP) estimates.[4-6] The major risk factors for OT in civilians are motor vehicle accidents, sports injuries, gunshot wounds, penetrating foreign bodies and industrial or occupational accidents.[3,7,8] Combat OT due to explosive devices, projectiles, chemicals, biohazards or laser injury is devastating for deployed troops.[9,10] Between 2011 and 2016, 922 service members sustained facial and penetrating neck injuries in combat operations in Iraq and Afghanistan with 26.3% involving the orbit and 25.1% the maxilla.[11]

Regardless of etiology, OT leads to very poor visual and functional outcomes despite the best available surgical intervention.[4,12-14] The majority of OT occurs with midface injuries due to the high incidence of orbital fractures and severe globe injuries.[15,16] Midface tissue deficits involving soft tissue and skeletal components challenge the best conventional reconstructive techniques.[17-19] Reconstruction of the globe and periorbital tissues such as fat, muscle, and eyelids is far from optimal, despite the use of local and free tissue transfers and implant materials for repair.[20]

Over the past two decades, advances in immunology and microsurgical techniques have enabled the clinical realization of vascularized composite allotransplantation (VCA) including, but not limited to, extremity, craniofacial, genitourinary, tracheal or abdominal tissues, with long-term immune, sensorimotor, and graft survival outcomes.[21,22] Of the 13 face transplants that involved ocular or periocular tissue, 4 subjects were blind or had visual impairment.[23-26] More face transplants continue to be performed worldwide in blind subjects.

Akin to hand and face transplants, whole eye transplantation (WET) involves composite tissues, including muscle, nerves, vessels, bone, and skin. Hypothetically, if combined with a face transplant in a blind subject, WET can potentially allow transplantation of the entire ocular system (globe plus adnexa and ON) and can provide an intact retina with viable retinal ganglion cells (RGC) capable of axonal regeneration and reintegration into the brain. However, the success of WET is fraught with significant hurdles. The primary surgical and immunologic challenges are restoring vascularity, sustaining viability, and suppressing acute and chronic rejection of the ocular tissues. The neurologic challenges include RGC protection from the effects of ischemia and reperfusion injury, ensuring ON regeneration, neuro-integration of the donor eye with the host visual cortex, and reestablishing sensation and motor control of the ocular structures.

The last decade has seen significant advances in neuroscience involving therapies that prevent RGC death, stimulate growth of RGC axons through the ON, and reconnect RGC axons to central visual targets.[27-34] These collective advances in VCA and neuro-ophthalmology afford hope that clinical realization of WET, however audacious, may not be a totally insurmountable goal.

Our group has pioneered experimental models, procedures, and techniques for craniofacial VCA (total face, hemiface, and facial subunit transplants).[35-41] Advancing to the ultimate goal of neuro-anatomical integration and visual recovery after WET requires a thorough evaluation of promising therapies, surgical techniques, and imaging tools in reproducible experimental animal models of WET prior to clinical translation. To our knowledge, this is the first rodent WET model including periorbital soft tissues. This study aims to demonstrate technical feasibility, graft viability, and patterns of immune cell infiltration and inflammation in ocular tissues, specifically the retina and ON.

Materials and methods

This preliminary study was carried out in strict accordance with Institutional Animal Care and Use Committee and American Association for Laboratory Animal Care guidelines. Following institutional ethical board approval, 8- to 12-week-old male outbred Sprague-Dawley rats ($n = 10$, 5 donors, 5 recipients), weighing between 200 and 250 g, were used in the experimental study. The rats were anes-

thetized using intraperitoneal xylazine (Rompun, 2% solution; Bayer, Leverkusen, Germany) and ketamine (Ketalar, 30 mg/kg, Parke-Davis, Morris Plains, NJ).

Surgical procedures

All surgical interventions were performed under sterile conditions and general anesthesia. Microvascular anastomoses and neurorrhaphies were performed using a high-resolution operating microscope (Zeiss OP-MI 6 SD; Carl Zeiss, Goettingen, Germany).

Donor procedure

Following a midline incision, the submandibular gland was excised after ligation of its pedicle. The sternocleidomastoid muscle was released from sternoclavicular and mastoid insertions and excised. Two main branches of the common carotid artery (internal and external carotid arteries) were exposed. The anterior and posterior bellies of the digastric muscle were excised, the omohyoid muscle was cut, and the greater horn of the hyoid bone was excised to improve access to the external carotid artery and its branches. Starting with the common carotid artery, the dissection was performed distally, and all branches of the external carotid artery and internal carotid artery were tied and dissected except for the superficial temporal artery and facial artery. Flap elevation was continued to the facial region to include the earlobe and periorbital area. In the facial region, the plane of dissection was deep to the SMAS layer and was extended into the periorbital areas. Hemostasis was accomplished by bipolar cautery, and sub-periosteal dissection was continued into the orbital apex. The ON was identified, exposed, and included in the flap by transecting at the orbital apex, retrograde to the site of entry of the ophthalmic artery into the substance of the ON. Finally, donor flaps containing the earlobe, orbital contents with periorbital soft tissues (periorbital fat, extraocular muscles), and facial skin were elevated on a common pedicle based on the common carotid artery (superficial temporal artery, facial artery) and the external jugular vein (Figure 1(a)). Each donor flap was perfused with heparinized sodium lactate solution prior to transplantation. Following procurement, the perfusion of the composite WET flap was confirmed by on-table contrast angiography.

Recipient procedure

The common carotid artery and the external jugular vein were prepared in a manner similar to the donor and the WET flap was transferred to the anterior neck area of the recipient rat (Figure 2). First, end-to-end anastomosis was performed between the recipient and donor external jugular veins using interrupted 10/0 nylon sutures (Ethicon, Johnson & Johnson, New Jersey, US). Next, end-to-side anastomosis was performed between the recipient and donor common carotid arteries using continuous 10/0 nylon sutures. Graft perfusion was confirmed by restoration of the retinal red-reflex. The greater auricular nerve (GAN), a sensory nerve, was identified and dissected. The proximal (cranial) stump of the GAN was cauterized and buried into the remnant of the sternocleidomastoid. The distal stump of the GAN was prepared for neurorrhaphy with the donor ON stump with 11–0 epineural sutures (Figure 1(b)). Flap inset was accomplished with interrupted 5–0 chromic sutures (Figure 3(a)). Finally, a central tarsorrhaphy was performed on the WET flap for corneal protection by using 8/0 nylon sutures (Ethicon, Johnson & Johnson, New Jersey, US). The rats were given Transgel (Charles River Laboratories, Inc., Wilmington, MA) and a powder form of rat chow (LabDiet, St. Louis, MO) to ease oral consumption and to maintain proper weight gain in the postoperative period. All transplant recipients received buprenorphine (0.05 mg/kg) subcutaneously for postoperative analgesia every 12 h for 72 h. A 5-ml bolus of lactated Ringer's solution was intraperitoneally administered immediately after donor flap transplantation, followed by once daily until POD 3 for hydration. After transplantation, animals received cyclosporine A (CsA-Sandimmune, Novartis, Basel, Switzerland) monotherapy, tapered from 16 mg/kg/day (started post-operative day [POD] 0) to 2 mg/kg/day over 21 days, and maintained at the same level thereafter until POD 30. The technical and procedural steps in the donor and recipient are detailed in (Supplemental Figure 1(j)–(l)).

Posttransplant evaluations

Clinical monitoring of WET recipients

Following WET, recipients were evaluated daily in the immediate postoperative period for graft loss, arterial or venous compromise, hematoma or seroma formation and general health. Ocular structures were visually monitored daily for signs of inflammation such as corneal clouding, corneal neovascularization, iris injection, dilatation of limbal capillaries or iris vessels and phthisis. The anterior chamber was evaluated for cell debris or infiltrates. In addition, the periorbital skin flap was monitored for clinical signs of rejection, including erythema, edema, desquamation, and necrosis based on the Banff classification of VCA. This study was observational in nature and no specific immunological evaluations were performed apart from the histopathological examination of transplanted tissues to assess differential patterns of inflammation.

Magnetic resonance imaging (MRI)

Recipients were anesthetized with a 2%/98% isoflurane/oxygen gas mixture and underwent MRI on POD 1 and POD 30 after WET with a 3-Tesla (3T) MRI (Achieva, Philips Medical System, Best, Netherlands) equipped with a dedicated, small animal transmit-receive coil (35 mm $\times$ 38 mm diameter). Tissue viability and perfusion as well as orbital volume changes were assessed at baseline and POD 30. T1- and T2-weighted fat-suppressed and non-fat-suppressed thin slices were obtained. Images were reviewed (with appropriate post-processing) using multi-planar reformatting (MPR), maximum intensity projection (MIP), and volume rendered (VR) techniques. The native eye (internal control) and transplanted eye were identified and regions of interest (ROI) were delimited for automated orbital volume quantification using a Philips Extended MR Work Space 2.6.3.2. All scans were blind-read by an experienced neuroradiologist.

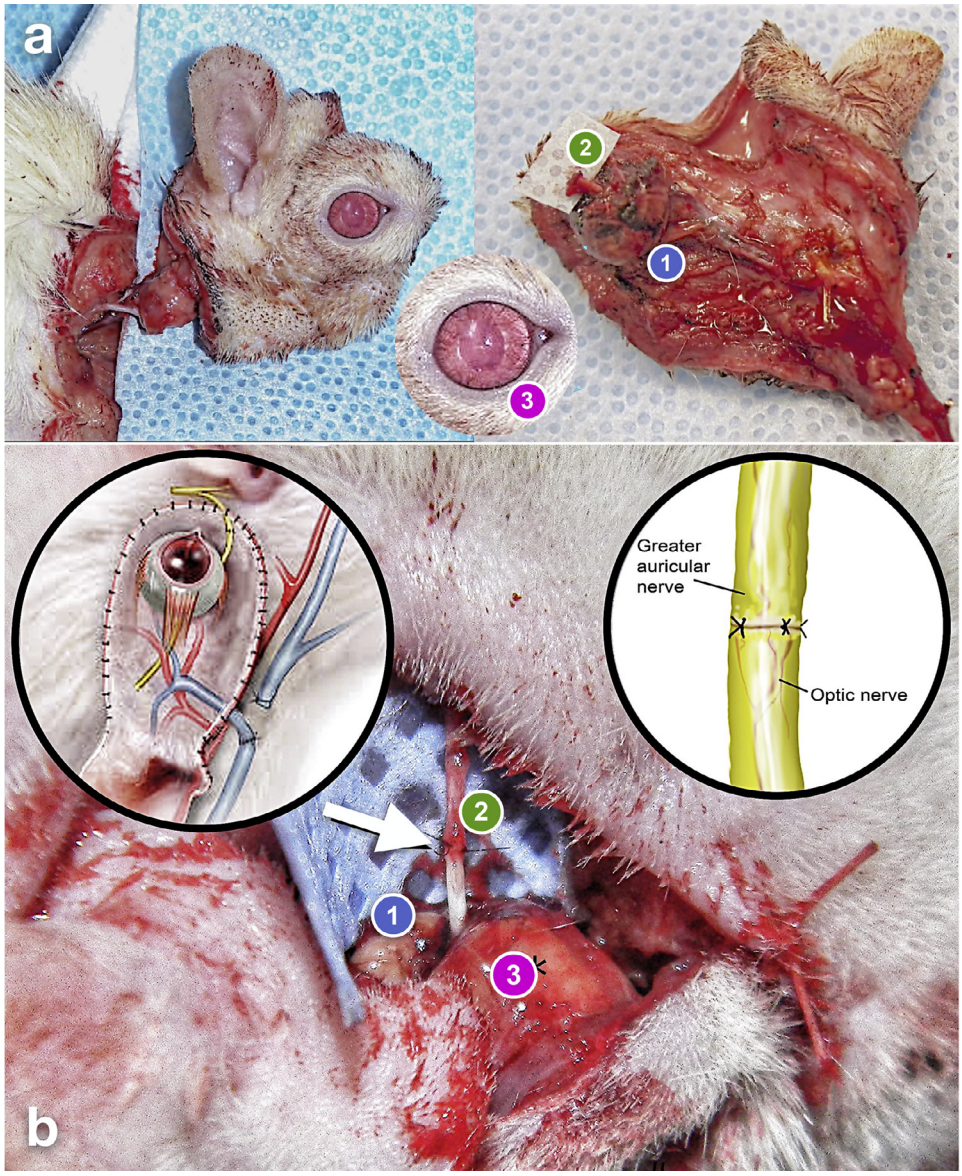

Figure 1 Technical and procedural considerations of WET flap elevation and neurorrhaphy.
(a) The donor WET flap is elevated following subperiosteal dissection of the bony orbit to expose the globe [1] and the ON is transected at the orbital apex [2]. The retinal red reflex is demonstrated in the donor eye after flap elevation [3]. (b) Coaptation of the ON [1] to the GAN [2] in the recipient rat prior to flap inset with globe [3].

X-Ray contrast angiography for flap viability
All animals underwent angiography under fluoroscopic control to verify vascular patency and perfusion of flaps prior to WET. An angiocatheter was used to inject 0.1–0.2 mL of iohexol 300 mg/mL (Omnipaque; GE Healthcare, Milwaukee, WI) diluted to a 50% concentration with normal saline. Fluoroscopy and angiography were performed using a surgical imaging C-arm system (OEC Elite, GE Healthcare).

Histopathology of WET
The study endpoint was 30 days or clinical signs of Banff Grade 3 rejection in the periorbital skin (whichever was earlier). For-cause periorbital skin biopsies were not performed. At euthanasia, recipients underwent intracardiac perfusion with saline followed by 4% paraformaldehyde (PFA). The entire WET flap with periorbital soft tissues, globe, and ON was rapidly dissected, and tissues were post-fixed for 2 h in 4% PFA and paraffin embedded. Sagittal and coronal sections of each sample were obtained at 5 μm thickness. Sections including periorbital skin, periorbital fat, and extraocular muscles from both transplanted and native eyes were mounted on Superfrost Plus slides and examined by routine histology with hematoxylin and eosin stain (HE). All slides were read in a double-blind manner by a trained ophthalmic pathologist.

Statistical analyses
Statistical analysis was performed with SAS for Windows V. 9.4. The volumetric change in the globe was calculated as percentage decrease of the baseline volume (measured on

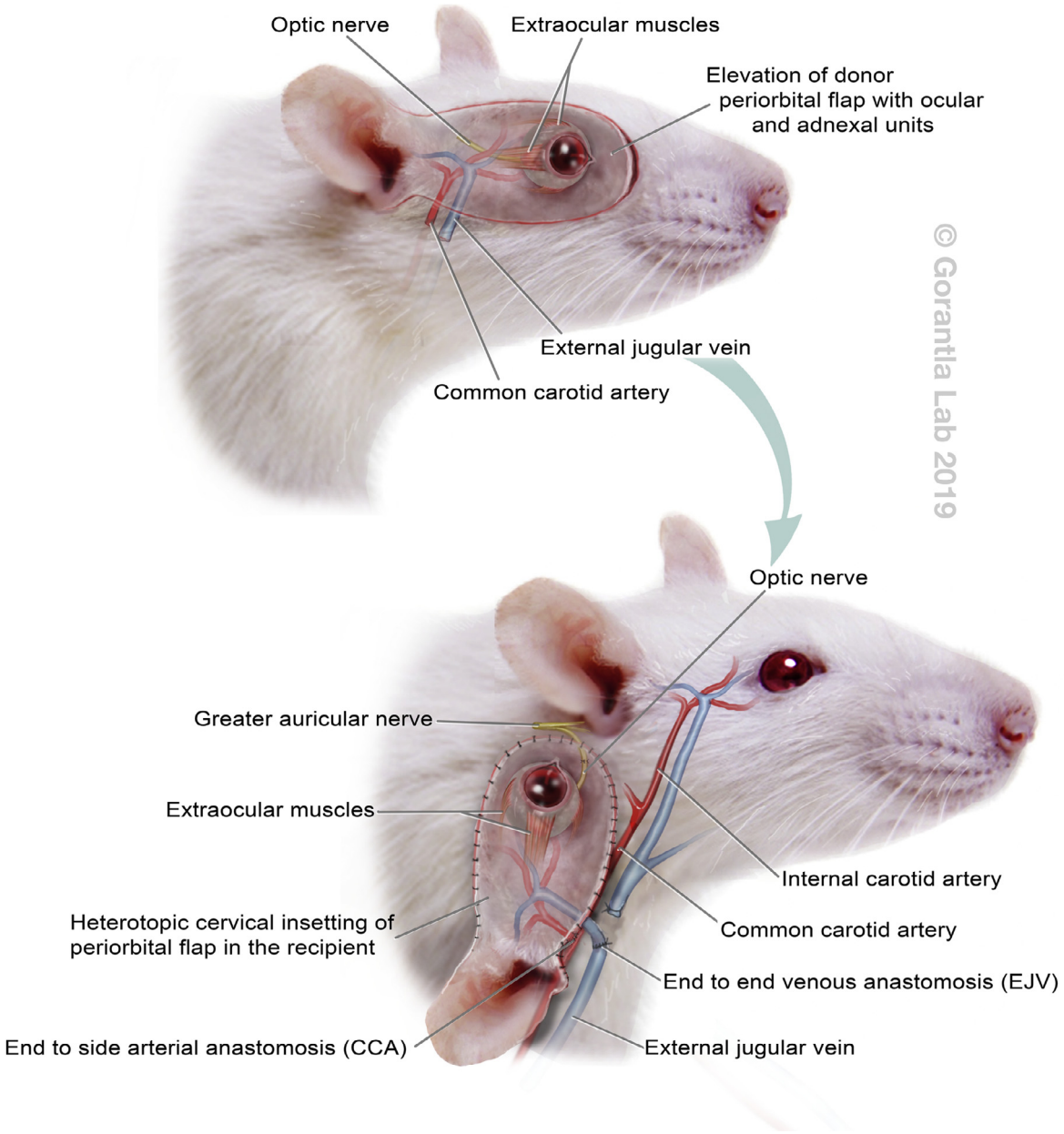

Figure 2 Schematic Representation of Donor and Recipient Operation.
The donor flap includes globe, adnexa, optic nerve, and periorbital skin (Upper Panel). Location of WET flap inset with vascular anastomoses and site of neurorrhaphy of the optic nerve to the great auricular nerve is shown (Lower Panel).

MRI). The mean, standard deviation, minimum, maximum, and median values were presented as descriptive statistics. Spearman correlation coefficient was used to establish the correlation between the ischemia time and changes in orbital volume. A P value < 0.05 was considered statistically significant.

Results

Gross clinical evaluation after WET

Successful WET transplantation was accomplished in all animals, with 100% graft survival for 30 days (Figure 4). One subject partially scratched the facial flap at postoperative day 7. This was surgically repaired as the wound defect affected only the periorbital skin tissue. The average operative time for the preparation of the WET allograft was 64.0 ± 5.8 min (median: 65; range: 55–70 min). The average ischemia time of the allograft was 26.0 ± 4.2 min (median: 25; range 20–30 min). No signs of arterial or venous compromise were noted on clinical examination. The skin component of the WET flaps did not show any clinical signs of rejection during the duration of follow up (Supplemental Figure 2(a)). No signs of erythema, edema, desquamation, necrosis or hair loss were noted. The red-reflex of the retina was preserved until the end point, indicating intact perfusion of the transplants.

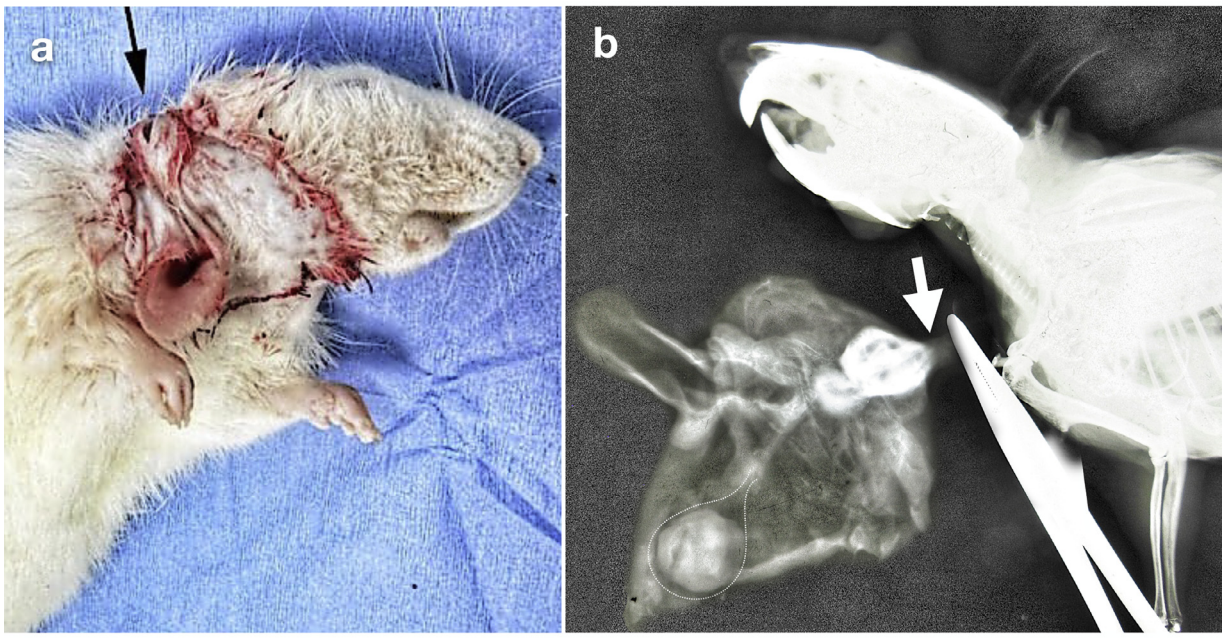

Figure 3 WET Flap Inset and X-Ray Perfusion Angiography.
(a) Flap inset in recipient. Transplanted eye is indicated by the arrow. (b) X-ray angiography of the WET flap to confirm viability after transplantation (arrow). Perfused donor eye in WET flap is shown (white lasso outline).

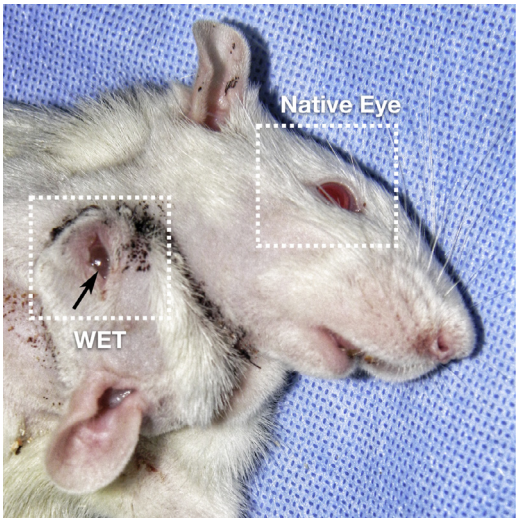

Figure 4 Clinical Appearance of WET.
WET recipient at POD 30 demonstrating viable flap and transplanted eye (arrow).

Magnetic resonance imaging (MRI) for viability and orbital volume assessment

Orbital volume measurements performed at POD 30 after WET showed a reduction in the vitreous volume in the transplanted eye as compared to baseline (POD 1). The average volume decrease in the transplanted eye was 36.0 ± 2.8% (median: 36% range 33–39%) at post-transplantation day 30 (Supplemental Figure 3). Baseline vitreous volume in the native eye was 16.68 (±1.43) mm^3. There was evidence of vitreous contraction with symmetrical reduction in globe dimensions. We did not observe an irregular contour deformation in the allograft eyeball as might be observed with phthisis due to changes in the anterior chamber volume. There was no correlation between the ischemia time and volume loss of the eye (Spearman rho: 0.527, p:0.362). The lens of the transplanted eye retained its clarity and anatomy (compared to native eye) and showed normal MR signal density. There was, however, a significant signal change in periorbital soft (extraocular muscle and fat) tissues on POD 1 with evidence of postoperative edema and surgical inflammation. T2-weighed fat-suppressed MR images revealed signal improvement on POD 30 confirming overall reduction of tissue inflammation.

X-ray contrast angiography for flap viability

All animals demonstrated excellent perfusion of WET flaps on fluoroscopic contrast angiography (Figure 3(b)).

Histopathological assessment after WET(Figure 5)

The skin component of the WET flaps at the end point showed no inflammatory cell infiltration (Supplemental Figure 2(b)). Corneal sections demonstrated preservation of overall structural integrity of epithelial and endothelial components and stroma (Figure 5(a)). The most prominent feature in the transplanted cornea after WET was dense inflammatory cell infiltration of the corneal stroma with no evidence of neovascularization. In two animals, the outer squamous cell layer of the corneal epithelium was disrupted; this may be due to chronic irritation (dryness, tarsorrhaphy) of the transplanted eye. Scleral sections showed intact scleral stroma. In comparison to the cornea, there were almost no inflammatory cells in the sclera. The

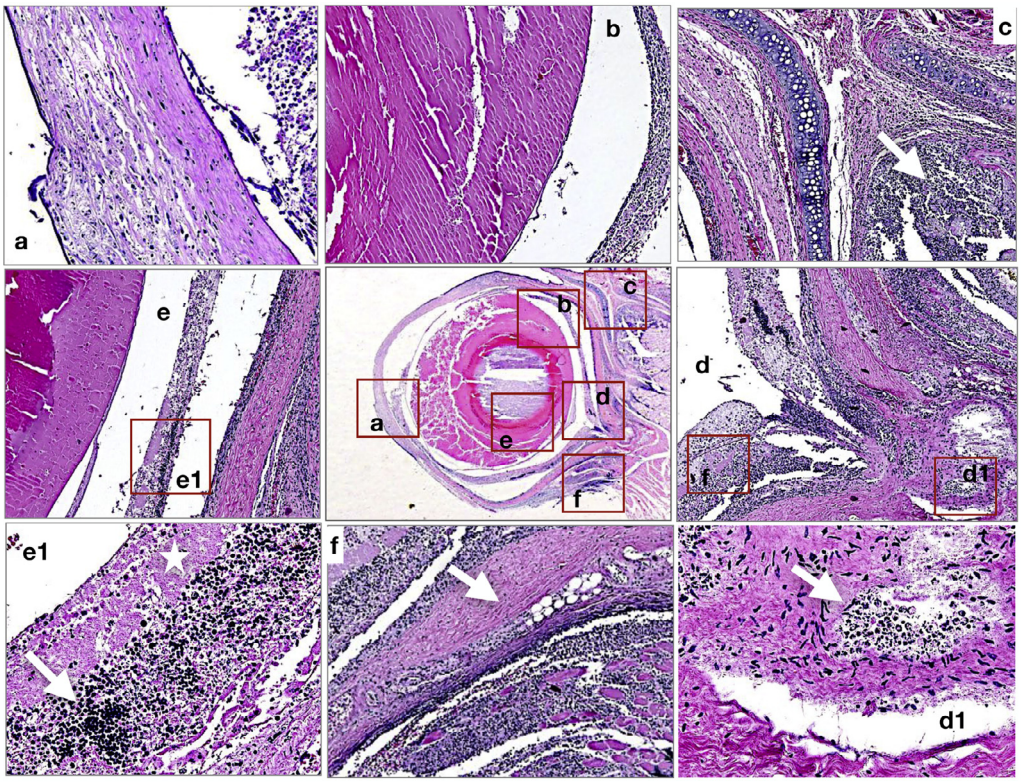

Figure 5 Histopathological Findings in the Transplanted Globe and Adnexa.
(a) Cornea: The integrity of epithelial and endothelial components and stroma is well preserved in WET. The outer squamous cell layer of the corneal epithelium in some animals was disrupted. Note the dense immune cell infiltration of the corneal stroma and well-preserved Descemet's membrane. No corneal neovascularization is evident. Upper right shows polymorphonuclear or monocytic infiltrates in the anterior chamber. (H&E, 10X).
(b) Lens: The capsular integrity, and contour of the lens are maintained in WET without inflammation barring some histologic artefacts. (H&E, 20X).
(c) Tarsal Plate: There is evidence of dense infiltration of immune cells around the tarsal plate and in the Harderian Gland (arrow). (H&E, 20X).
(d) Lamina Cribrosa: There is widespread infiltration of the retina in the area of the lamina cribrosa with zones of polymorphonuclear and monocytic infiltrates in the optic nerve head. (H&E, 20X). (Inset d1, (H&E, 40X).
(e) Retina: The transplanted retina shows diffuse immune cell infiltration (H&E, 20X). The inner limiting membrane is preserved but the retinal ganglion cell layer is necrotic (inset e1, asterisk, H&E, 40X). The outer nuclear layer (arrow) contains viable neurosensory cells, but there is significant macrophage and polymorphonuclear infiltration.
(f) Sclera and Extraocular Muscles: The structural integrity of the scleral stroma is maintained in WET with no inflammatory cells. However, the extraocular muscles show diffuse and dense infiltration (H&E, 20X, arrow).

extraocular muscles and periorbital fat showed inflammatory cell infiltration with no evidence of contraction bands (Figure 5(f)). The lens of the transplanted eye was normal in contour and dimensions, with the exception of some histologic artefacts (Figure 5(b)). The retina revealed significant inflammatory cell infiltration with total loss of RGCs in the ganglion cell layer of the inner retina and necrosis of the inner nuclear layer with preservation of the inner limiting membrane (Figure 5(e)). Compared to native eyes, the ON in transplanted eyes showed macrophage infiltrates, axonal disruption and connective tissue disorganization with no evidence of regeneration (Supplemental Figure 4). Transverse histologic sections of the ON obtained through the orbital apex showed structural integrity of extraocular muscles, Harderian glands, cranial nerves (III, IV and VI), and periorbital adipose tissue (Supplemental Figure 4).

Discussion

Neither the concept nor the idea of WET is novel. Early attempts date back to 1885 when Chibret in Paris transplanted a rabbit eye into a young girl who lost her left eye.[42] However, this bold attempt failed in the face of formidable hurdles surrounding the survival, function and immunology of the transplanted eye.

Almost a century later, in 1978, the National Eye Institute called for a "thoughtful and thorough" laboratory effort into the overriding challenges for this endeavor.[43,44] To date, there are no successful reports on WET in an adult mammalian animal model.[44-46] Except for evidence from corneal transplants, there is no knowledge of immune responses after WET. Our present study is the first in the literature to establish a small-animal adult mammalian experimental

WET model that could help address the gaps in our knowledge of eye transplantation.[43] Our immediate goals were to optimize the surgical approach, confirm successful perfusion/viability, and preliminarily document the histopathologic patterns of the early immune response of ocular and adnexal tissues to eye transplantation.

Although it has been argued that an orthotopic approach is essential for examining RGC survival and ON axon regeneration under the immunologic burden of WET, we strongly believe that the viability and immunologic responses after WET must be first explored discretely in their own merit. A heterotopic model can accomplish this goal without confounders and specifically study the unique effects of immunosuppressive drugs on rejection in WET and the role of ocular immune privilege in modulating rejection responses (Supplemental Table 1).

In comparison to the orthotopic model (Supplemental Table 1), our heterotopic model had minimal surgical complications, with no physiologic stress on recipients or transplanted tissues and allowed for evaluation of viability as manifested by graft survival outcomes.[47] In our experience, we believe that the technical complexity and concomitant recipient morbidity of the orthotopic the WET model may indeed confound the interpretation of immunologic or neurofunctional outcomes as well as challenge independent replication or validation across ophthalmology and neuroscience laboratories. A recent report in Nature on the "crisis of reproducibility in research" confirms that more than 70% of researchers are unable to reproduce experiments/techniques from other labs and more than 50% fail to reproduce their own experiments.[48] Our heterotopic WET model is technically feasible and does not demand extraordinary microsurgical skills, allowing for predictability and ease of reproducibility across operators and laboratories.

Taken together, a complex orthotopic model for WET is neither critical nor optimal for mechanistic studies of the immunologic rejection of ocular tissues/adnexae. In fact, the broad immune manifestations of WET may not differ significantly with an orthotopic vs. heterotopic location of the graft, although this is worthy of investigation.

Histologic alterations of ischemia and reperfusion injury (IRI)-induced retinal damage are critically dependent on the duration of ischemia. The severity of damage after reperfusion directly correlates with the duration of ischemia.

Orthotopic approaches to WET necessitate an ischemia time averaging around 1.5-2 h.[47] The extensive dissection associated with exenteration in the recipient leads to considerable intra/perioperative morbidity or mortality due to blood loss and compounds the ischemia. Rodent studies of ischemia tolerance of the retina have shown that while 30 min of ischemia followed by reperfusion did not result in histologic changes, 60-90 min of ischemia induced moderate to severe retinal edema associated with neutrophilic infiltration. Such retinal edema did not improve even after 24 h.[49] In our study, although total ischemia time was less than 30 min, we found marked inflammatory cell infiltration of the retina with loss of the inner cellular layer, features that are commonly seen in ocular IRI, possibly due to disruption of the blood-retinal barrier.[50] Studies have showed that extravasation or transmigration of leukocytes from vessels is one of the predominant events in ocular IRI and the inhibition of such migration is protective against this phenomenon.[51,52] The polymorphonuclear leukocytes and macrophages that infiltrate reperfused ocular tissues following ischemia, generate excessive nitric oxide (NO) through upregulation of Nitric Oxide Synthase 2 (NOS-2). Microglia in turn, use NOS-2 to kill RGCs.[53] Together, IRI can individually or in combination, negatively impact survival, functional, and immunologic outcomes of WET. Although we did not see evidence of retinal edema on histology, it is possible that the reperfusion injury may have caused some degree of RGC death, given their sensitivity to ischemia. This is because the retina and choroid are tissues with a blood flow and oxygen demand that exceeds that of the brain.[54-56]

We found vitreous shrinkage with loss of vitreous volume on MRI in WET. There is literature evidence that inflammatory diseases of the eye of varying etiology produce opacification, liquefaction, and shrinkage of the vitreous, which may be associated with cellular proliferation and vitreous fibrosis/scarring in cases of prolonged inflammation.[57] Shrinkage of the vitreous scar can also lead to retinal detachment or RGC injury. We also found extensive axonal degeneration after WET. Similar findings were reported in ocular GVHD which may cause ON edema and/or optic neuritis/neuropathy.[58]

Although ON regeneration was not the primary goal of our current study, our WET model facilitates the evaluation of ON regeneration in the extracranial setting. Our choice of the GAN for ON neurorrhaphy, was based on the following rationale: Similar to the ON, the GAN is a sensory nerve, but unlike the ON which is a cranial nerve, the GAN is a peripheral nerve comprising branches of spinal nerves C2 and C3. The choice of the GAN as a vascularized peripheral nerve graft for coaptation with the ON segment in our WET model is supported by extensive prior work in rodent models demonstrating improved ON regeneration across peripheral nerve segments.[59-63] The importance of peripheral nerve grafts in RGC survival and ON regeneration is further supported by studies where intravitreal inflammation was induced by peripheral nerve explants.[64] Several studies support the role of such intraocular inflammation due to activated macrophages in facilitating RGC survival and axon regeneration.[29,65-67] The inflammatory response as seen in our study may indeed have a collateral positive benefit on ON regeneration in the setting of WET, a phenomenon we are currently exploring in our laboratories.

The GAN is the largest of the ascending branches of the cervical plexus, with a diameter that ranges from 1.4 to 2.0 mm with a median size of 1.7 mm.[68] Although the axonal count of the GAN is far less than that of the ON,[69] the dimensions of the ON and GAN are very similar, and the anatomical proximity of the GAN allows for easy and tension-free coaptation with the distal ON segment in the WET flap (Figure 3). In our study, we did not observe ON regeneration through the GAN. This may be attributable at least in part to the deleterious effects of IRI on RGC survival, leading to arrest of axonal regeneration across the GAN conduit. Furthermore, our study did not implement adjunctive neurotherapeutic interventions. Without such interventions, it is known that ON transection within the bony orbital canal in adult rats (as in our study) leads to death/loss of at least 90% of the RGC population by 14 days after ON injury.[70] If the ON is transected intracranially, this rate drops to 30%.[71]

The primary goal of this study was to establish a novel model of WET and preliminary evaluation of histologic outcomes. Major limitations of this preliminary, exploratory, primarily observational study included small sample size, genetic variability of outbred rats, lack of broader immune outcomes assessment (such as cytokine, chemokine or gene expression parameters), and lack of noninvasive imaging evaluation of functional ON regeneration retinal ischemic damage or loss of function. Our study did not investigate whether corneal clouding was the result of inflammation due to extrinsic nonimmune factors such as loss of protective sensation and lacrimal secretion after WET. Our primary justification in using cyclosporine (CsA) was based on prior ophthalmologic literature supporting its efficacy when used systemically in preventing corneal transplant rejection.[72,73] Indeed, it was the only agent with demonstrated efficacy in a rejection application in ophthalmology. Although tacrolimus is known for its positive effects on peripheral neuroregeneration, multiple studies have reported toxic effects on the retina and ON with the drug,including maculopathy, central retinal vein occlusion and optic neuropathy.[77–80] A limitation of using CsA in our study is that it may rarely cause ischemic retinal microvasculopathy, ON head edema, and retinal capillary/arteriolar damage.[74–76] The collateral neurotoxic effects of immunosuppressive agents such as tacrolimus and CsA must be important considerations in WET. We are currently investigating dose-response studies with tacrolimus in acute rejection after WET to evaluate the lowest dose that is immunosuppressive but not neurotoxic in ongoing research in small and large animal WET models.

Conclusion

Taken together, in this preliminary study, we successfully established the technical feasibility and viability in a novel, mammalian small animal WET model and described for the first time in the literature, unique histopathologic correlates of the immune responses in transplanted ocular tissues and adnexae. This model offers promise and potential utility in immunological, neurotherapeutic, immunomodulatory, drug delivery, imaging, and tissue engineering studies involving vascularized orbital and periorbital composite tissues.

Declaration of Competing Interest

None of the authors have any commercial associations or financial disclosures (along with all products, devices, drugs, etc., used in the manuscript) that might pose or create a conflict of interest with information presented in any submitted manuscript.

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Ethical approval

The animal studies were performed under ethical and IACUC committee approval at the Gulhane Military Medical Academy (Protocol #: 2010-18) and Wake Forest University (Protocol #: A17-136, 12/18/2017).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.bjps.2019.05.042.

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