Preservation of Canine Composite Facial Flaps Using UW Solution

Qinxiu Liu, MD; Qingfeng Li, MD, PhD; Shengwu Zheng, MD; Danning Zheng, MD

Objective: To evaluate the effect of University of Wisconsin (UW) solution on composite facial flaps in dogs to offer a preservation time limit for clinical application.

Methods: The experiment included 2 parts. In part 1, 32 half facial flaps were cold stored for 12, 24, 36, and 48 hours in UW solution (experimental group) or normal saline (control group). In part 2, 8 flaps that had been cold stored in UW solution for 24 (group A, n=3), 36 (group B, n=3), and 48 (group C, n=2) hours were autotransplanted.

Results: After preservation in part 1, the viability of each tissue type (skin, mucosa, muscle, blood vessel, nerve, and gland) in the experimental groups was better than that in the control group. Muscle viability decreased more quickly than did the viability of other tissue. In the experimental groups, the viability of all tissue preserved for 12 and 24 hours was better than that of tissue preserved for 36 and 48 hours. After 48 hours of preservation, tissue had good structure and integrity in the experimental group but showed degeneration in the control group. In part 2, the flap survival percentages were 100%, 100%, and 99.7% in group A; 93.2%, 95.7%, and 94.1% in group B; and 87.2% and 86.1% in group C. Six months after surgery, the dogs in group A showed contraction potential and corneal reflex.

Conclusion: Twenty-four hours could be considered a reference time for clinical application of UW solution flap preservation.


Many patients experience disfigurement of the entire face due to burn injuries, extensive tumor ablation, or trauma. Currently, composite facial allotransplantation is thought to be a promising new treatment for these patients. Effective preservation is a key step toward composite facial flap (CFF) survival and recovery of function. University of Wisconsin (UW) solution (Viaspan; DuPont Pharma, Wilmington, Delaware) is a well-known preservation solution that has been used for preservation in many organ and composite tissue transplantations.1-3 Our aim was to research the preservation effect of UW solution on canine CFFs to offer a preservation time limit for clinical application.

Herein, we evaluate tissue viability, pathology findings, and autotransplantation outcomes of canine CFFs preserved in UW solution. The experiment included 2 parts: part 1 was in vitro research on tissue viability histologic changes. Part 2 was an in vivo study that included an autotransplant model and nerve and muscle function recovery evaluation.

METHODS

Twenty-four adult beagle dogs of both sexes weighing 8 to 15 kg were used in this study. All animals were kept and treated in compliance with the guidelines of the National Institutes of Health, and the experiment was approved by the Shanghai Association For Laboratory Animal Sciences.

PART 1: TISSUE VIABILITY AND PATHOLOGY RESEARCH

Harvest and Preservation of CFFS

Thirty-two half facial flaps including scalps, ears, eyelids, conjunctivae, parotid glands, and mimetic muscles were aseptically harvested from 16 dogs while the animals were under general anesthesia according to procedures previously described (Figure 1).4 The skin was incised deep to the subplatysma in the anterior neck, deep to the subfacial muscles in the facial region, and deep to the periosteal plane in the nasal and frontoparietal region. In the neck region, dissection was continued above the occipitomandibularis muscle to the angle of the mandible, preserving the external jugular vein and its main branches; the external maxillary vein, anterior jugular vein, and internal maxillary vein were ligated and transected. The occipitomandibularis muscle was transected to expose the external carotid artery and its main branches. The superficial temporal artery and posterior auricular artery were included in the flap; and the external maxillary artery, internal maxillary artery, and lingual artery were ligated and transected. The external carotid artery canal was detached, and the external carotid was kept within the flap. The facial nerve was transected near the stylomastoid foramen. Finally, the external carotid artery and external jugular vein...
were divided. The flap supported itself by the external carotid artery and received the external jugular vein as the return vessel. Penicillin G (200,000 U), regular insulin (40 U), and dexamethasone (16 mg) were separately added to 1 L of normal saline (control group) or UW solution. The proper caliber of tube was inserted about 0.3 to 1.0 cm into the external carotid artery. We then perfused the CFFs with normal saline or UW solution at 4°C from the external carotid artery until the solution emerging from the external jugular vein became clear and light. The perfusion pressure was 80 cm H2O. Next, the flaps were preserved in normal saline or UW solution at 4°C for 12, 24, 36, and 48 hours (16 flaps in each group and 4 flaps at each preservation time).

Viability Detection

The CFF tissue viability was determined before or after storage using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Skin, mucosa, muscle, blood vessel, nerve, and gland tissue were studied, and the viability percentages in the control group were compared with those in the UW solution groups.

The MTT assay has been used for determining cell viability. The original assay procedure has been modified to assess the viability of tissue specimens. The MTT was dissolved (2 mg/mL) in phosphate-buffered saline (PBS) and filtered through a 0.45-mm filter to remove any undissolved crystals. Four-millimeter biopsy samples were obtained from the CFFs, and each sample was weighed and placed into a 6-well tissue culture plate. Two milliliters of MTT solution was added to each well, and the plate was placed on a rotating platform (100 rpm) at 37°C for 2 hours. After 2 hours, the MTT solution was removed, and the tissue was rinsed twice with 1 mL of PBS for 1 minute and then minced with surgical scissors. To extract the water-insoluble formazan, 4 mL of dimethyl sulfoxide (DMSO) was added to each well and rotated (100 rpm) for 80 minutes at 37°C. The optical density (OD) was measured at 540 nm (with DMSO as a blank) on a spectrophotometer. The viability index (VI) of the tissue sample was expressed as the ratio between the OD (at 540 nm, according to the test) of the tissue and its weight in grams:

\[
VI = \frac{OD \text{ (Measured at 540 nm)}}{\text{Grams of Tissue, No.}}.
\]

The viability percentage is the ratio of the VI of the specimens after storage and the value of the fresh samples (considered as 100% viability):

\[
\text{Viability Percentage} = \frac{VI \text{ After Preservation}}{\text{Fresh Tissue VI}} \times 100\%.
\]

Histology

All fresh and preserved samples were processed for histologic analysis. All specimens were fixed in 10% formalin, embedded in paraffin cut into 4-µm sections, and stained with hematoxylin-eosin.

**PART 2: AUTOTRANSPLANT MODEL**

Based on the results of part 1, we selected different preservation times at which tissue viability percentages were high and between which statistically significant viability percentage differences existed \((P < .05)\). After the flaps were harvested, the wound was wrapped with oil gauzes, chloromycetin gauzes, dry gauzes, and cotton pads. Before replantation, the flaps were flushed via the vessel with 300 mL of Ringer lactate solution to remove the high-potassium preservation fluid. At first, anastomosis of the auditory canal cartilage was performed to fix the flap. Next, end-to-end neurorrhaphy of the facial nerve was performed by a skilled surgeon with 7-0 nylon sutures. Then the anastomoses of the vein and artery were performed using standard end-to-end microsurgical technique with 8-0 nylon sutures. The external carotid artery and external jugular vein were anastomosed with their branches—maxillary artery and anterior jugular vein, respectively. Next, the occipitomandibularis muscle was reapproximated, and negative-pressure drainage and rubber-tissue drainage were placed. Finally, the muscles and skin were closed in layers. A pressure dressing was placed, completing the procedure. Postoperative analgesia was provided with buprenorphine for the first two days. Antibiotics (potassium penicillin and ciprofloxacin) were prophylactically administered for 5 days after the operation. Novamin, milk fat, and a fluid supplement were administered as nutritional support for 5 days. If less than 10 mL of fluid had drained after 24 hours, the drain was removed; if not, the drain was removed 5 days after the operation.

**Calculation of Skin Survival Percentages of the CFFs**

An assessment of flap survival percentage was undertaken at 7 days following the operation. A viable flap was characterized by warm, pink, hair-bearing skin. Nonviable flaps were characterized by a dry, hard, hairless eschar. Nonviable flaps were characterized by a dry, hard, hairless eschar. The entire flaps were drained in transparent paper, and the necrotic areas were marked. Thereafter, the drawings were photographed using a digital camera mounted on a tripod and placed 20 cm from the drawings. These digital images were imported into a computer and analyzed with Image-Pro Plus 6.0 software (Media Cybernetics Inc, Bethesda, Maryland). Percentage survival was determined by the following equation:

\[
\text{Percentage of Survival Area of the Flap} = \frac{\text{Survival Area of the Flap}}{\text{Area of the Entire Flap}} \times 100\%.
\]

All observations and calculations were performed by the same individual, who was blind to the experiment.

**Electromyelography**

The flaps included intact nervus auricolopalpebralis and relatively intact orbicularis oculi muscle. Therefore, electromyelography of the orbicularis oculi muscle was performed to observe the functional recovery results of nervus auricolopalpebralis at 3 and 6 months following the operation. Electromyelography was performed using a Keypoint electric muscle-inducing apparatus (Medtronic, Copenhagen, Denmark). The stimulating electrode was placed at the exit of the facial nerve from the stylomastoid foramen, and the recording electrode was inserted into the orbicularis oculi muscle. A single rectangular electrical stimulation of 0.2-millisecond duration and 5-mA intensity was applied. The latency and amplitude of the compound muscle action potential (CMAP) waves were compared between the experimental and control sides of the animals.
Clinical Evaluation

We evaluated the eyelid closure reflex 12 weeks after surgery and every 2 weeks thereafter to observe the contraction of the orbicularis oculi muscle and thereby monitor the regeneration of the nervus auriculopalpebralis.

STATISTICAL ANALYSIS

The data were analyzed with SPSS software, version 11.0 (SPSS Inc, Chicago, Illinois). Statistical analysis was performed by t test for comparison of the 2 treatment groups. For multiple groups, 1-way analysis of variance was used. In part 2, the Kruskal-Wallis test was used because of the small sample. Data were considered statistically significant at \( P < .05 \).

RESULTS

VIABILITY CHANGES

The changes in tissue viability percentage in the 2 groups are shown in Figure 2. The tissue viability in the control group decreased more rapidly than that of the UW group. The viability of muscle and nerve tissue fell more rapidly than that of other tissue, and skin viability decreased slightly. After 48 hours of preservation in normal saline, the mean (SD) percentage of tissue viability for skin was 46.39% (3.44%); mucosa, 35.36% (7.01%); muscle, 18.46% (7.35%); blood vessel, 34.17% (2.48%); nerve, 20.44% (4.67%); and gland, 40.45% (1.36%). However, after 48 hours of preservation in UW solution, the mean (SD) tissue viability for skin was 87.02% (1.63%); mucosa, 84.97% (8.03%); muscle, 77.05% (4.09%); blood vessel, 82.94% (7.18%); nerve, 80.92% (6.47%); and gland, 85.05 (5.71%). Viability comparison between the 2 groups showed significant differences in tissue preserved for 12, 24, 36, or 48 hours (\( P < .05 \)). In the experimental groups, viability percentages in all tissue types showed no difference between 12 hours and 24 hours. Skin and mucosa viability decreased after 36 hours of preservation, and other types of tissue viability percentages decreased after 24 hours of preservation.

HISTOLOGIC CHANGES

After 24 Hours of Preservation

In the control group, the boundary between acinus cells appeared unclear; the space between muscle bundles was enlarged; and slight edema could be seen in the other kinds of tissue. In the UW solution group, there was little change in the flap compared with fresh tissue.

After 48 Hours of Preservation

In the control group, the fibers in connective tissue of the skin and mucosa ranged in porosity, and the tissue was slightly edematous. The tissue around the hair follicle was hyalinized, and cell edema could be seen. Enlargement of the intercellular space of muscle and other tissue edema was more evident. In the UW solution group, there was no significant change in the flaps. The fibers of skin and mucosa were arranged tightly, and the space between muscle bundles was not enlarged.

AUTOTRANSPANT RESULTS

Based on the results of part 1, CFFs cold stored in UW solution for 24, 36, and 48 hours were autotransplanted. The dogs were subgrouped by preservation time: group A, 24 hours (\( n = 3 \)); group B, 36 hours (\( n = 3 \)); and group C, 48 hours (\( n = 2 \)). The survival percentages of the CFFs were 100%, 100%, and 99.7% in group A; 93.2%, 95.3%, and 94.1% in group B; and 87.2% and 86.1% in group C (Table 1). The Kruskal-Wallis test showed significant differences between these groups (\( P < .05 \)). The necrosis regions were in 3 main sections: most were in the front neck, followed by the lower eyelid, and then at the ear tip (Figure 3). We gave no special treatment to the ear necrosis but just removed the necrotic part. At the lower eyelid and neck, the necrotic parts were removed, and the adjoining normal tissue was liberated to cover the raw surface.

Electromyelography

Electromyelography of 1 dog in group C was not obtained because of too large an area of necrosis at the lower eyelid and failure of the reconstruction. New CMAPs were detected. Compared with the nonexperimental side, the
CMAP latency of the orbicularis oculi muscle on the experimental side was extended, especially 3 months after the operation, and the wave amplitude was decreased. Six months after transplantation, the extension of CMAP latency had decreased (Table 2).

Clinical Evaluation

Corneal reflex could be seen in all the dogs of group A 6 months after the operation and improved gradually. One dog in group B showed corneal reflex occasionally 6 months after the operation, and no dog showed corneal reflex until 9 months after operation in group C.

COMMENT

Face transplantation offers a potential solution for disfigured patients with conditions that cannot be adequately addressed by conventional reconstructive surgery procedures. Ethical concerns and issues of the surgical challenge and immunosuppression have been discussed in the literature, but CFF preservation has received little attention. Maintaining the CFF in a viable condition is the critical factor for successful transplantation.

Currently, optimal perfusion and preservation of the flap improve the ischemic and hypoxic tolerance. The method of quick harvest and simple cold storage is widely used. Proper perfusion allows for an unobstructed vascular net because hypothermia slows the metabolism of organs and tissue and reduces oxygen and energy consumption, both of which are beneficial to maintaining viability. Ploeg et al10 note that the ideal cold storage solution should (1) prevent enlargement of tissue space and ischemia-reperfusion injury during perfusion; (2) offer a precursor for synthesis of adenine nucleotides; and (3) maintain the stability of the internal environment. They invented the UW solution, which had been widely used in the preservation of organ and tissue flaps.

Herein, our aim was to research the preservation effect of UW solution on canine CFFs to determine a preservation time limit for clinical application. The experiment protocol attempted to mimic the human model, and therefore, the CFF had the following characteristics:

1. The harvested flap removed a large section from the scalp to the neck and across the external ear, lengthwise eye, and the whole face transversally.
2. The anatomic structures of the CFF included several mimetic muscles, nerves, the internal carotid artery, internal jugular vein, external carotid artery, and external jugular vein.
3. The flap included the scalp, ear, conjunctiva, eyelids, parotid gland, and tissue such as skin, mucosa, muscle, blood vessels, nerves, and glands. It met the same requirements as single-organ allotransplantation: after blood circulation was resumed, the tissue could survive well and had good potential for concrescence and recovery of function.
4. The flap was rich in muscle, which was the most sensitive tissue to ischemia and hypoxia.
5. The flap contained at least 1 nerve-muscle system to allow us to evaluate the recovery of nerve and muscle.
6. The aim of composite facial allotransplantation was to reestablish expression and appearance on the basis of conserving the blood supply system. This required an integral harvest and rebuilding of a “skin-muscle-nerve” expression system with a high level of operation. All of these set a high demand on the perfusion and preservation of the CFF.

The present study combined in vitro (part 1) and in vivo (part 2) experiments. The results of part 1 provided grouping data for part 2. In the control group, tissue viability decreased quickly, and the degraded tissue could not be used for transplantation. In the experimental groups preserved in UW solution for 12 and 24 hours, the tissue viability percentages in all tissue types showed no differences. In part 2, the flaps stored in UW solution for 24, 36, and 48 hours were studied.

In the literature, dogs that received livers preserved for 48 hours in UW solution survived for 5 or more days with excellent liver function.11 Ploeg et al10 proved that after 72
hours of storage in UW solution, canine kidney flap survival was 100%, with normal function. Naka et al2 showed that UW solution was effective in preserving hearts and lungs for 24 hours in a canine transplantation model. Wahlberg et al12 demonstrated that dogs receiving pancreas flaps that had been cold stored for 72 hours in UW solution retained normal function for at least 28 days. Furthermore, UW solution has been widely used in tissue storage for hand, limb, abdominal wall, and joint flaps.13

University of Wisconsin solution has been used to preserve several types of tissue. Wilson et al14 used UW solution to preserve cadaver vessels for 24 hours, and the vessels could still release relaxing factor (nitrogen oxide), and smooth muscle cells were still sensitive to epinephrine. Yoshikawa et al15 used a cavitary 2-layer cold store method (UW solution/perfluorochemical combination) to preserve small intestines for 24 hours; after transplantation, the mucosal injury and cell apoptosis were significantly alleviated, and mucosal restoration was morphologically complete within 4 days. Ikeguchi et al16 placed peripheral nerves in UW solution for 4 weeks before transplantation into recipient Lewis rats. Nerve regeneration was similar to that of fresh tissue morphologically, and the electrophysiologic results were equal to those of fresh isoflaps. Norden et al17 once reported 87% survival of limbs stored in UW solution at 4°C for 24 hours and then transplanted onto a fresh rat, with adenosine triphosphate levels being 33% of those of the fresh limb. The ischemic time of the first transplanted hand was 12.5 hours.13

In the present study, we found that as storage time increased, the CFF survival area percentage decreased. The blood vessel network was extensive, and the diameter of the pedicle was smaller than that of a kidney or liver, and so perfusion was both difficult and time-consuming, possibly owing to partial blood capillary blocking. The necrosis of the neck portion, lower eyelid, and ear—all of which are located at the distal end of flap—might be related to the difficulty of perfusion. In addition, the necrosis of the lower eyelid might also be related to the narrow pedicle. The ear, containing little soft tissue, tended to display dry necrosis.

Ischemic and hypoxic tolerance is worst for muscle tissue and second worst for nerve. Skin has the highest tolerance. Therefore, the ischemic and hypoxic tolerance is better in fingers than in limbs because fingers have no muscles. The time limit for the reattachment of a severed limb is less than 6 to 8 hours; preservation at low temperatures will extend that time. Datiashvili and Chichkin18 were the first to successfully reattach a severed shank 42 hours after an injury in 1992, and the function of the lower limb recovered well a year and half after the procedure. The CFF in the present study contained several mimetic muscles and nerves, so its endurance time to ischemia and hypoxia was short. The vessels of the CFF were thinner than those of the liver, kidney, and pancreas. In addition, blood capillaries were numerous, and the area to be perfused was large. In conclusion, it was a difficult flap to perfuse. The survival requirements of muscle are quite high because mimetic muscle function recovery depends on the various expressions accomplished by the cooperation of numerous fine muscles. Therefore, maintenance of the muscles’ viability was essential for the survival of the CFF.

Functional recovery of the flap will surely be one of the key determinants for successful face transplantation. In the present study, electromyelography at 3 and 6 months after the operation showed regeneration potential indicative of nerve regeneration. A potential explanation for the extending CMAP latency and the attenuation of amplitude was the emergence of a multiphase wave and an extension of the repolarization process that resulted from significant nonsynchronous excitation of fast and slow fibers during the early rehabilitation part of nerve regeneration. The good corneal reflex found in group A indicated that 24-hour preservation of the CFF with UW solution maintained high enough tissue viability for nerve and muscle function recovery.

In the present study, we assessed the preservation effect of UW solution on canine CFFs via measurements of viability, histologic changes, and an autotransplant model. We demonstrated that UW solution was an effective preservation solution for canine CFFs. Survival area percent-

---

**Table 2. The Results of Electromyelography After Autotransplantation**

<table>
<thead>
<tr>
<th>Group</th>
<th>Amplitude, mV</th>
<th>Latency, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dog 1</td>
<td>Dog 2</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control side</td>
<td>3.21</td>
<td>2.83</td>
</tr>
<tr>
<td>Postoperative, 3 mo</td>
<td>0.42</td>
<td>0.45</td>
</tr>
<tr>
<td>Postoperative, 6 mo</td>
<td>1.49</td>
<td>1.63</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control side</td>
<td>2.96</td>
<td>3.46</td>
</tr>
<tr>
<td>Postoperative, 3 mo</td>
<td>0.36</td>
<td>0.58</td>
</tr>
<tr>
<td>Postoperative, 6 mo</td>
<td>0.56</td>
<td>0.65</td>
</tr>
<tr>
<td>C*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control side</td>
<td>3.58</td>
<td>NA</td>
</tr>
<tr>
<td>Postoperative, 3 mo</td>
<td>0.49</td>
<td>NA</td>
</tr>
<tr>
<td>Postoperative, 6 mo</td>
<td>0.59</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.

* Of the 2 dogs in group C, only 1 could be evaluated by electromyelography owing to a large area of necrosis at the lower eyelid and failure of the reconstruction.
ages of flaps cold stored for 24 hours were more than 99%, and good corneal reflex was restored. When the preservation time was more than 24 hours (ie, 36 and 48 hours), the CFFs survived partially but without good muscle and nerve function recovery. Because the CFF was rich in muscle and nerve tissue and had high demands for functional recovery, we suggest that CFF preserved in UW solution be used within 24 hours to be acceptable for transplantation.

Accepted for Publication: November 11, 2009.
Correspondence: Qingfeng Li, MD, PhD, Department of Plastic and Reconstructive Surgery, the Ninth People's Hospital, 639 Zhi Zao Ju Rd, Huang Pu District, Shangh hai, China, 200011 (liqfliqf@yahoo.com.cn).
Author Contributions: Study concept and design: Liu, Li, and D. Zheng. Acquisition of data: Liu and D. Zheng. Analysis and interpretation of data: Liu. Drafting of the manuscript: Liu, Li, and D. Zheng. Critical revision of the manuscript for important intellectual content: Liu, Li, and S. Zheng. Statistical analysis: Liu. Obtained funding: Li. Administrative, technical, and material support: Liu, Li, and D. Zheng. Study supervision: Li.
Financial Disclosure: None reported.
Funding/Support: This study was supported by grant NCET-04-0436 from the Ministry of Chinese National Education.
Additional Contributions: Hao Jiang, MD, Tao Zan, MD, Yuping Li, BM, Rui Weng, MD, Huiyong Wang, BM, and Lujia Tang, BM, provided excellent technical support.

REFERENCES


Announcement

Visit www.archfacial.com. As an individual subscriber, you may elect to be contacted when a specific article is cited by any of the hundreds of journals hosted by HighWire. You also may sign up to receive an e-mail alert when articles on particular topics are published.

©2010 American Medical Association. All rights reserved.