Large Antigenic Skin Load in Total Abdominal Wall Transplants Permits Chimerism Induction

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Abstract: The application of composite tissue allograft (CTA) transplants in clinical reconstruction is parallel with extended knowledge of anatomy, microsurgical skills and development of transplantation immunology. CTAs are composed of multiple tissues, some of which such as skin are highly immunogenic and cause strong immunologic responses. Strong antigenic nature of skin may be related to Langerhans cells, which are powerful antigen-presenting cells. They are leukocytes found in the epidermis as members of immunologic cascade. Large skin components of CTA transplant may cause higher antigenic load as a result of increased surface area of transplanted skin correlating with increased load of Langerhans cells. In clinical abdominal wall transplants, increased rejection episodes were reported compared with other CTA transplant. This complication may correlate to large skin component of these transplants. To evaluate correlation between large skin island flaps and immunologic responses, CTA models with large skin components should be tested in experimental studies. Here we propose a total abdominal wall (TAW) transplant model in rat to test the feasibility of TAW transplantation in 2 groups: the anatomic study and experimental transplantation group. In anatomic study, TAW flaps were elevated bilaterally on superficial epigastric vessels and replaced. The entire TAW skin islands of all flaps were viable at postoperative day 21. Dye study confirmed that TAW flap was supplied by 2 vascular pedicles. Data in this group demonstrated that this flap is composed of the largest skin island when compared with other CTA transplant models such as full face, hemiface, limb, and groin flap. In experimental group, isograft transplantations were performed between Lewis rats ($RT1^1$) while allograft transplantations were performed Lewis ($RT1^1$) donors and LBN (RT^{1+n}) recipients. All TAW transplants showed viable islands at posttransplant day 200 under cyclosporine A monotherapy protocol. Microangiography of the transplant demonstrated entire skin island was supplied bilaterally by superficial epigastric vessels pedicle. Dye studies with India ink demonstrated dye uptake in all flap components. Histologic examination also demonstrated the viability of flap tissues. High chimerism levels in peripheral blood was determined at the 7th, 21st, 35th, 63rd, and 100th posttransplant days was compared with full face, hemiface, groin flap data. It was found that TAW transplant had higher chimerism level compared with groin flap but lower chimerism than full face and hemiface allografts. TAW transplant may serve as a new experimental model with large skin component and may be useful to determine immunologic responses to tissues with high antigenic load under different immunosuppressive protocols.

Key Words: experimental total abdominal wall transplantation model

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Transplantation of abdominal wall has become a clinical reality. Although clinical abdominal wall transplant is second most frequent transplant in composite tissue allograft (CTA) transplants; there is no experimental model of total abdominal wall (TAW) transplant described recently. The abdominal wall transplant has the largest skin component compared to all clinical CTA transplants such as hand or face. Wide abdominal defect were covered successfully with this transplant.[1,2] However, frequent allograft rejection episodes were shown in these series. Although clinical or experimental studies were not performed previously to determine reason of increased rejection, it may be related to increased antigenic skin load in these transplants because powerful immunologic responses to skin component of CTA transplant may be seen compared with other components.[3,4] It is known that the skin is the most antigenic tissue in CTA models.[5,6] Furthermore, increased antigenic load may determine if CTA transplant includes skin component. Prior CTA models such as total face, hemi face, groin, and limb transplant included smaller skin component. An experimental CTA model that has large skin component to simulate abdominal wall transplant may help in giving an answer for immunologic problems related to high skin antigenic load. The purpose of this study was to introduce new total abdominal wall transplant model in rats and to monitor immunologic responses to high skin antigenic load.

MATERIALS AND METHODS

Animal Care

In total, 22 adult Lewis ($RT1^1$) (n=14) and LBN (RT^{1+n}) (n = 8) rats weighing 200–250 g were used in this

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study. The research protocol was approved by the Animal Research Committee of The Cleveland Clinic, which is accredited by the American Association for the Accreditation of laboratory Animal Care. This study was conducted according to *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health.[7] All animals were kept in individual cages in a room with standard environmental conditions. The animals were fed ad libidum. Operations were performed under cocktail of ketamine (30 mg/kg), xylazine (6 mg/kg) and acepromazine (1 mg/kg) anesthesia. Supplementary doses were given as necessary.

Surgery

Rats were divided 2 groups: anatomic studies and experimental TAW transplant group.

Anatomic Studies

Anatomic studies groups were divided 2 subgroups; A and B.

In the first group A, anatomic dissections were performed to assess vascular anatomy and determine skin island border of TAW in Lewis (RT1[1]) rat (n = 4). Skin island was planned measuring approximately 12 × 8 cm over the abdominal region and this diameter included total abdominal wall dimensions (Fig. 1A). Border of skin island extended from xiphoid process and arcus costa to pubic tubercle and inguinal ligaments and between 2 anterior axillary line. Following incision of the skin island, total abdominal flap was elevated over the anterior abdominal wall muscle. Superficial epigastric vessels (SEVs) were dissected bilaterally from the lower border of flap to its origin at the femoral artery. Femoral vessels were ligated from distal region of SEVs origins and divided during flap elevation. All branches of femoral vessels were ligated and dissection of the vascular pedicle was continued from distal to proximal direction up to inguinal ligament (Fig. 1B–D). To demonstrate skin island perfusion with one or 2 SEVs, India ink was applied using same technique of Indian ink injection study in experimental group (n = 2). Next silicon sheet was placed under the flap to prevent contact with vascular supply of underlying tissues (Fig. 1E). Next all flaps were reinserted in the abdominal region (n = 2). Right pedicles of selected flaps were transected to check whether one pedicle of vascular supply was sufficient for flap survival (n = 2). All animals were observed until postoperative day 21.

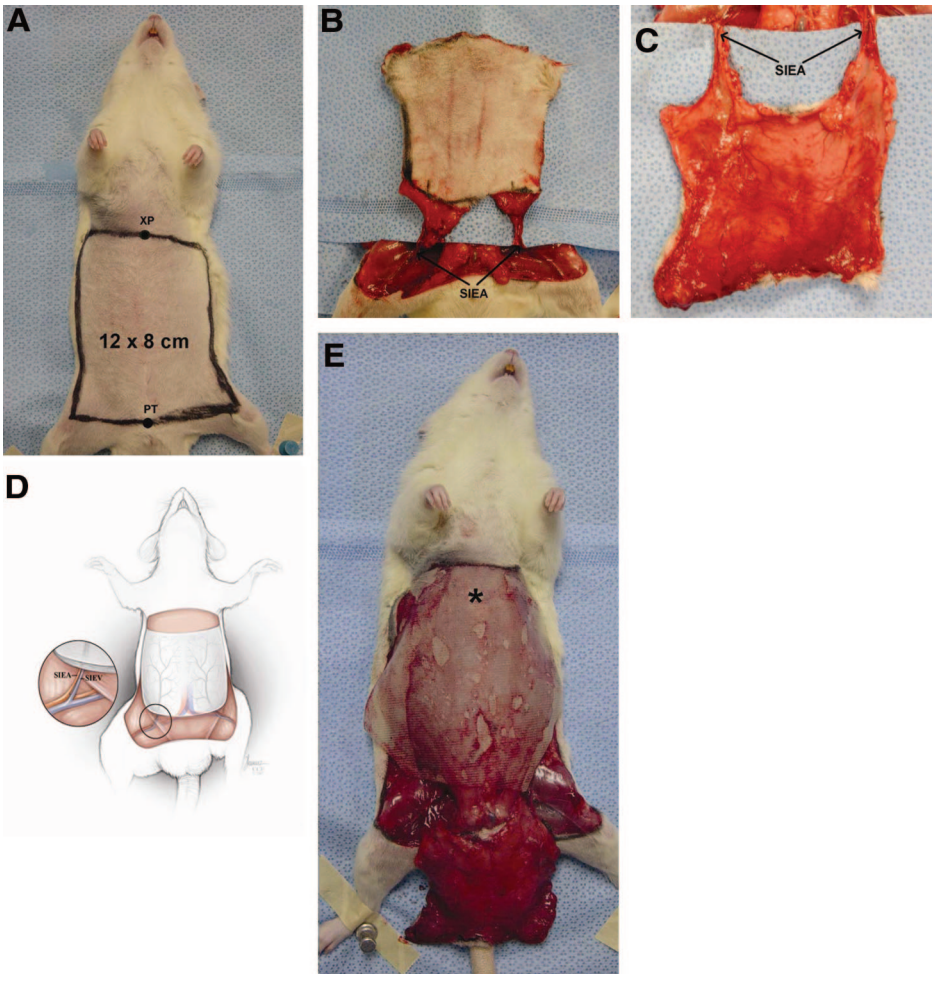


FIGURE 1. A, Skin island designed over anterior abdominal wall muscles. B, TAW flap harvested on SIEA bilaterally. C, SIEAs on posterior view of TAW flap. D, Schematic presentation of TAW flap. E, The silicon sheet (*) lying over anterior abdominal muscle from the xsphoid process to pubic tubercle. TAW, total abdominal wall; SEA, superficial epigastric artery; SEV, superficial epigastric vein; XP, xsifoid process; PT, pubic tubercle.

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In the second group B, diameters of total abdominal wall skin were compared with previous CTA models, which included skin component. For this purpose, hemi face, full face, groin, and limb transplant models were performed in Lewis ($RT1^1$) rats (n = 2). Entire skin component was harvested from limb transplant model. Next total cross-section areas of skin components in all flaps were measured using millimetric graph paper and digital photography were taken.

Experimental Group

Two subgroups were prepared as isograft and allograft transplant. Isograft transplants were performed between Lewis ($RT1^1$) rats (n = 8). Total abdominal wall transplants were elevated as described above and femoral vessels were transected under the inguinal ligament. For recipient vessels, keeping bilateral femoral arteries and veins as long as possible, they were transected before the bifurcation at popliteal region. Total abdominal wall skin (approximately 12 × 8 cm) was resected from recipient rat to make defect in proportion to donor flap size. Femoral arteries and veins of donor and recipient were anastomosed using end-to-end technique with 10/0 nylon. TAW flap was sutured into created defect with absorbable suture material (4/0 Vicryl, Ethicon, a Johnson & Johnson company, Somerville, NJ). In allograft transplant group (n = 8), TAW flaps were transplanted from LBN (RT^{1+n}) donor to Lewis ($RT1^1$) recipients. To avoid seroma complication that we observed in the first 2 experiments (Fig. 2A), a few suture were placed with absorbable suture material (4/0 Vicryl, Ethicon) between flap and anterior abdominal wall to decrease the dead space and provide close contact (Fig. 2B). For immunosuppression protocol, Cyclosporine A monotherapy was used with tapered dosage (from 16 to 2 mg/kg/d).

Assesment of Flap Viability

Direct Observation

Viability of the skin island was followed by daily observation of both iso and allograft transplants by the same surgeon. The survival of the TAW skin transplant was evaluated during postoperative follow-up period by gross inspection of the flap color, edema, erythema, hair loss, necrosis compared with normal skin.

Microangiography

Microangiography was performed to delineate the vascularization of the total abdominal wall flap in 2 animals in isograft transplant groups on posttransplant day 15. After cannulating the common carotid artery, 50 mL of the mixture (at 70°C) that was made of 50 g of silver oxide, 5 g of gelatin, and 100 mL of the isotonic was injected with a syringe. The flaps were stored at 4°C for 4 hours. Then flaps underwent radiography with soft x-ray machine, using Microvision-C mammography film.

Indian Ink Injection Studies

In 2 isograft transplants, viability of flaps was evaluated by the presence of dye in the vessels after India ink injection. Following cannulation of the common carotid artery with a 24-gauge catheter, 5 mL of India ink were injected. The flap was harvested and stained with hematoxylin-eosin, and examined under light microscopy for the presence of India ink in blood vessels of the skin and subcutaneous tissue.

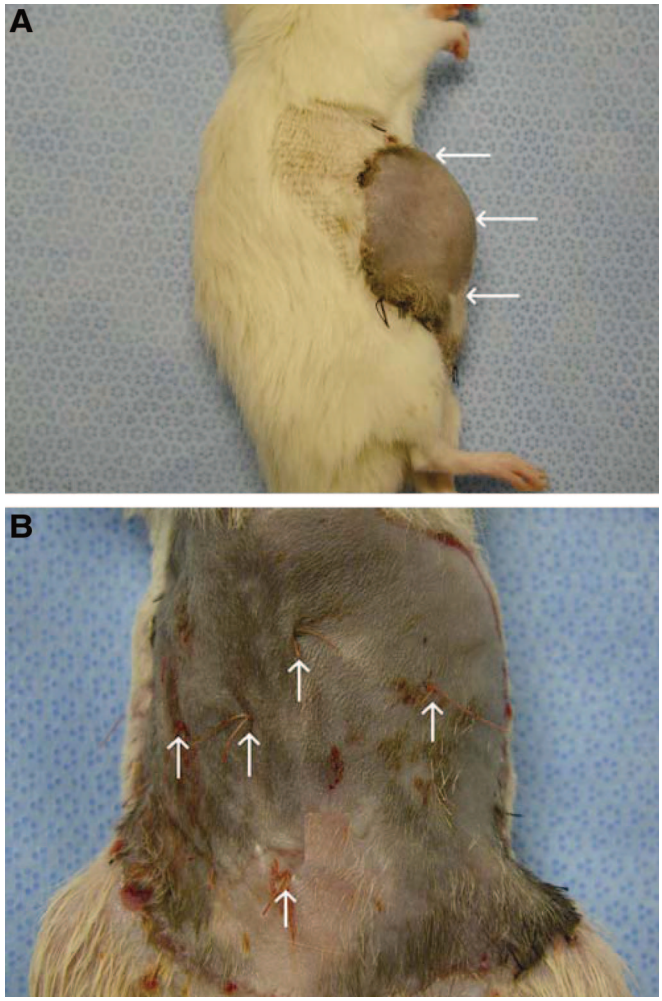


FIGURE 2. A, Expanded flap with seroma (white arrows) at posttransplant 2nd week. B, Sutures (white arrows) for fixing the flap with abdominal muscle.

Histology

On posttransplant day 100, 2 animals in allograft group were euthanized via intracardiac lidocaine injection under general anesthesia. Flaps were harvested and placed in 10% buffered formalin. The skin sections were stained with H&E for evaluation.

Immunologic Evaluation

Flow cytometry was used to evaluate the presence of donor-specific chimerism for donor MHC class I ($RT1^n$) antigen in the peripheral blood of Lewis abdominal wall transplant recipients during observation time at day 7, 21, 63, and 100 posttransplant. Donor chimerism was assessed with combinations of conjugated mouse antirat mAb $RT1^{n\text{-FITC}}$

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(for MHC class I of donor cells $RT1^n$, clone MCA156; Serotec, UK) with mAb specific for T-cells: $CD4^{-PE}$ (clone OX-35), $CD8a^{-PE}$ (clone OX-8), B-cell $CD45RA^{-PE}$ (clone OX-33) and monocytes, granulocytes $CD11_{b/c}^{-PE}$(clone OX-42) (BD Bioscience Pharmingen). After incubation, samples were lysed and fixed with 1% PFA solution. Negative control panels were tested and included isotype-matched antibodies (IgG_1^{-FITC}/IgG_2^{-PE}) and phosphate-buffered saline samples. Analysis was performed on 1×10^4 cells using FACS SCAN (BD Bioscience Pharmingen) and Cell Quest software. All results of flow cytometry at posttransplant period were compared with data of hemiface and groin flaps. These data were taken from our previously study and this studies were used same immunosuppressive protocol.[8,9] Blood chimerism level of limb allotransplant were not added this study. Because these transplants include vascularized bone marrow and this component may caused different immunologic result compare with CTA transplant models without vascularized bone marrow.

RESULTS

Anatomic Studies

At postoperative 21 day, all skin islands were viable for total abdominal wall flaps which were based bilaterally on SEV pedicles (Fig. 3A). Half of skin islands were necrotic in the remaining flaps, which had only one pedicle (Fig. 3B). In dye studies, total and half of skin island of TAW flap which had 2 and 1 SEVs, respectively, were stained with India ink (Fig. 4A, B). These results demonstrated that of TAW flaps bilateral SEVs for total flap viability. In measurement of skin islands of CTA different models, skin diameters were found from the largest to smallest diameter as 27.92 cm^2, 12 cm^2, 6.72 cm^2, 5.84 cm^2, and 5.20 cm^2 for TAW, total face, limb transplant, hemiface, groin, respectively (Table 1) (Fig. 5).

Experimental Group

Direct Observation

On postoperative follow-up period (at posttransplant 100 day) of skin island of all flaps in isograft and allograft transplant groups was completely viable (Fig. 6A, B).

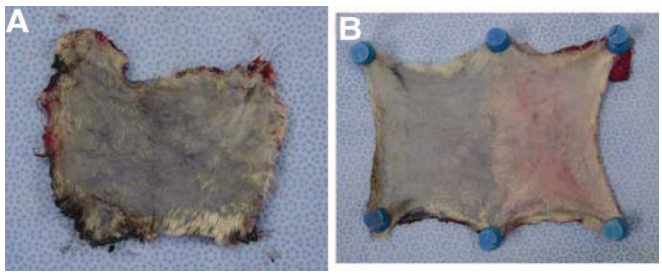


FIGURE 4. View of skin island of abdominal wall. Stained whole (A) and half of skin island (B) in TAW, which had 2 and 1 SIE vessels, respectively.

TABLE 1. Comparing of Skin Surface Area of CTA Transplants

CTA Transplant Model	Surface Area (cm^2)
TAW	27.92
Total face	12
Limb transplant	6.72
Hemiface	5.84
Groin	5.20

CTA indicates composite tissue allograft; TAW, total abdominal wall.

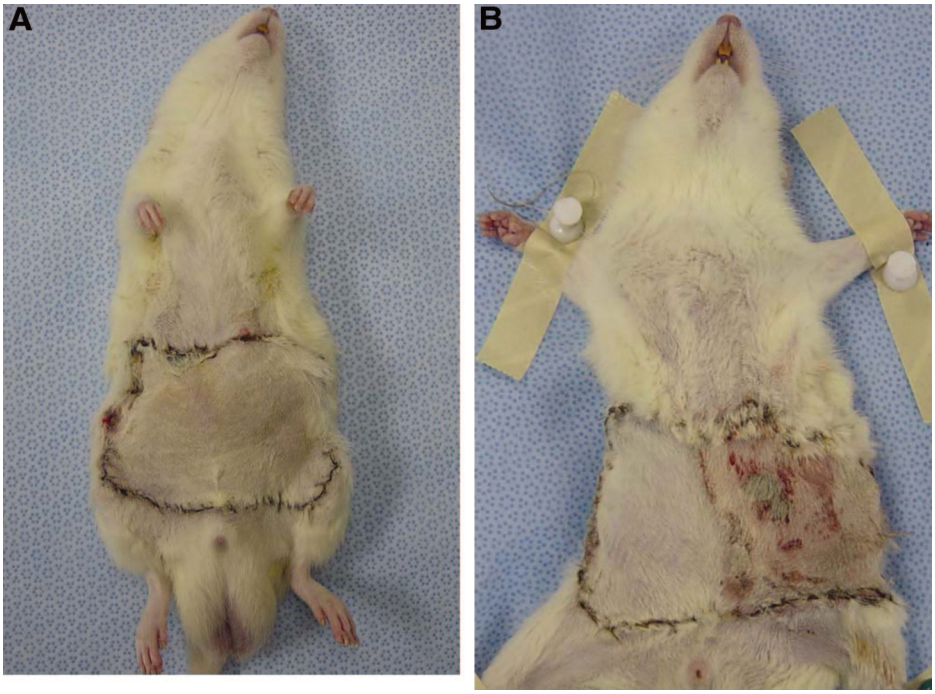


FIGURE 3. A, Total viable skin on intact bilaterally pedicle. B, Necrotic skin area on the transected pedicle side.

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Microangiography

Microangiography revealed the vascular supply in flap as provided by the pedicles (Fig. 7).

Indian Ink Injection Studies

Dye study with India ink demonstrated ink uptake by the vessels in skin, confirming perfusion via bilateral pedicle (Fig. 8).

Histology

Histologic examination of TAW allotransplants at post-transplant day 100 were not determined rejection sign such as blood cell extravasation, edema, necrosis, and pigment incontinence. Normal skin and subcutaneous tissue architecture were protected and viable epidermis and dermis layers of skin and intact subcutaneous tissue were determined (Fig. 9).

Immunologic Evaluation

At day 7 posttransplant, the mean value of total donor chimerism was assessed at 6.7% for $RT1^n$ positive cells.



FIGURE 5. Comparing skin islands of CTA transplants.

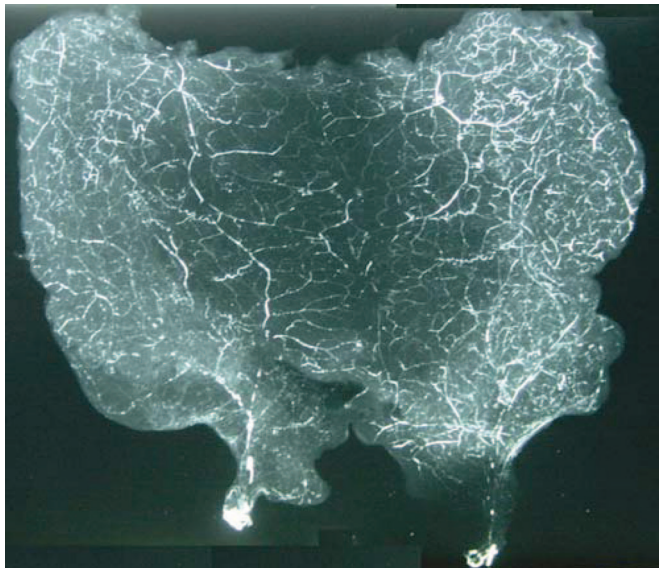


FIGURE 7. Microangiographic analysis reveals courses of pedicles.

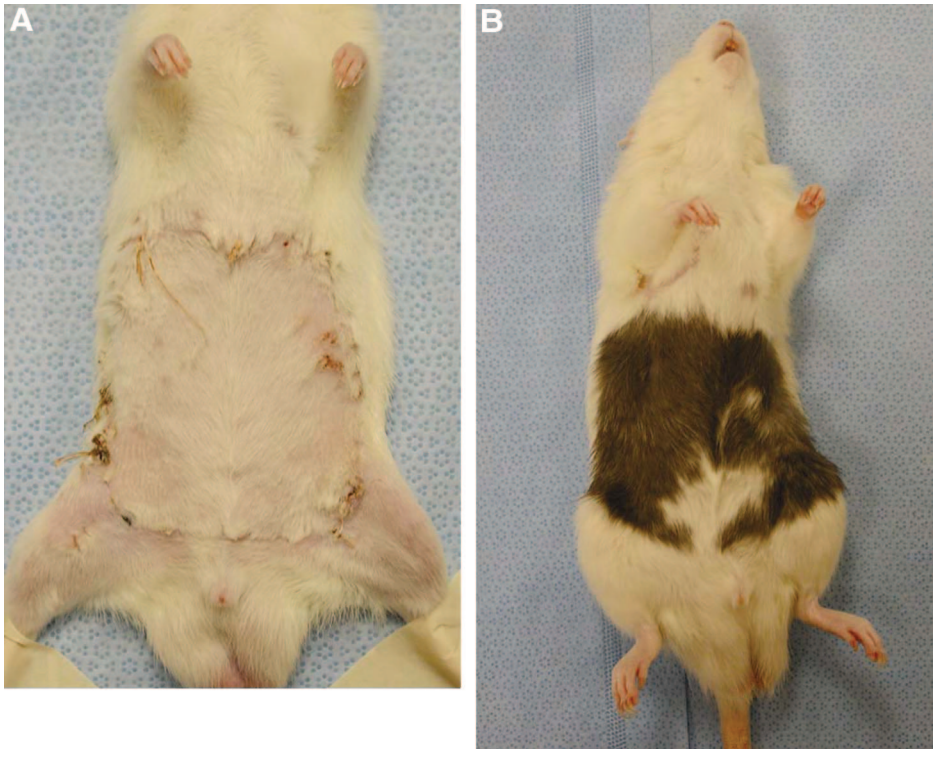


FIGURE 6. Viable skin islands of isograft (A) and allograft (B) transplants at late follow-up period.

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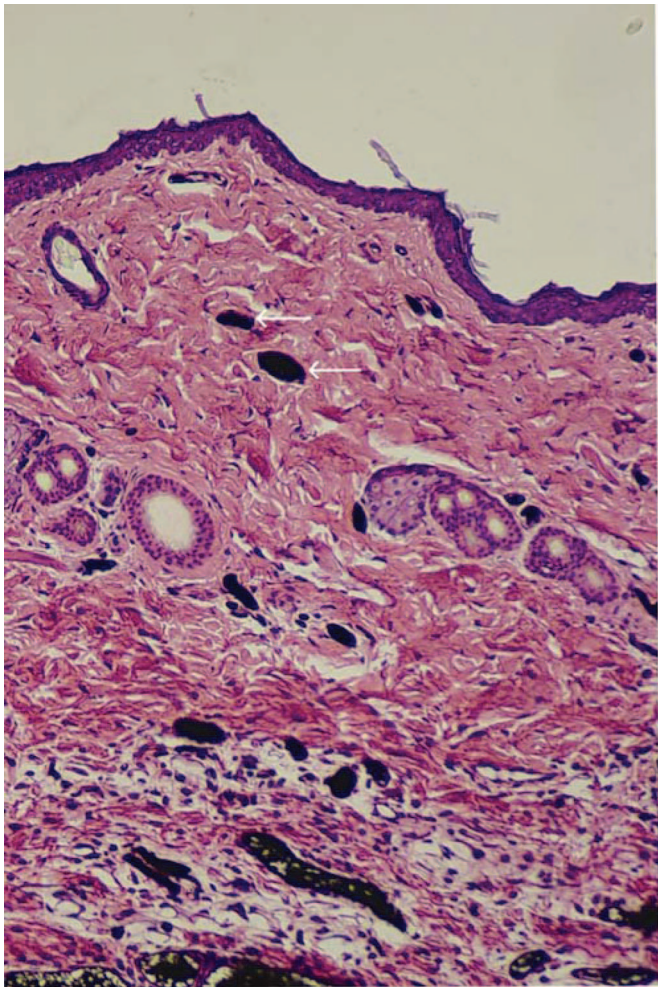


FIGURE 8. Histologic examination of flap skin indicates perfusion by presence of ink in vessels (arrows).

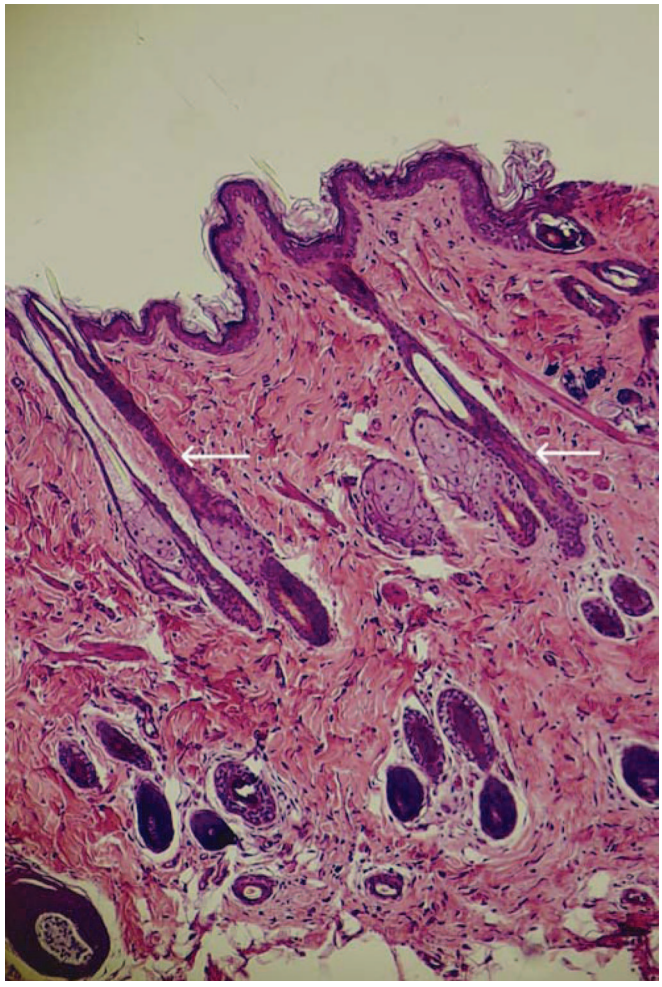


FIGURE 9. Histologic section. Skin and subcutaneous fat tissue component of flap on postoperative day 100 demonstrates viability of each segment (arrows, hair follicles).

During the entire follow-up period, chimerism declined and at day 100 posttransplant revealed 1.31% for $RT1^n$ positive cells (Fig. 10). When comparison of blood chimerism levels of TAW, hemiface and groin flap, chimerism levels were found from the highest to lowest as hemiface, TAW, and groin flap for all period (Fig. 11).

DISCUSSION

Severely damaged abdominal wall due to injuries, repeated laparotomies, ostomy construction, wound infections, tumors, and enterocutaneous fistulae causes wide abdominal wall defects often seen in clinical practice. In these situations, the use of prosthetic material and modified plastic surgery approaches may solve the problem. Unfortunately, there are severe cases in which standard approaches are not possible. Transplantation of abdominal wall allograft could be an effective way to address this problem. It allows for primary closure of the abdomen, avoids the potential morbidity of exposed viscera, and permits early mobilization and rehabilitation of these patients.[1,2] But one-third of patient in abdominal wall series have presented with episodes of rejection.[10] This rate is higher compared with previous clinical CTA transplants such as hand transplant.[11] CTAs are histologically heterogenous, composed of different tissues types (skin, muscle, bone, bone marrow, lymph nodes, nerve, and tendon), and express different immunogenicity of transplanted elements. Hierarchy of antigenicity of CTA was demonstrated in experimental model and showed that skin is the most antigenic tissue and requires higher immunosuppression.[5,6] Vascularized muscle component of limb allografts may induce cell-mediated response greater than the skin, however, muscle as a single component is less antigenic than skin.[5] Furthermore in a large animal model study, tolerance was developed to the musculoskeletal elements of CTA, but the skin compartment was rejected or had no immuntolerance.[3,4] The skin and in particular the epidermis have important role in immune reactivity. Key players in that regard are epidermal Langerhans cells.[12] These cells are a heterogeneous population of leukocytes with efficient immunostimulatory capacity and they present antigens for recipient T-cells. Langerhans cells migrate out of the allograft to the draining lymph nodes

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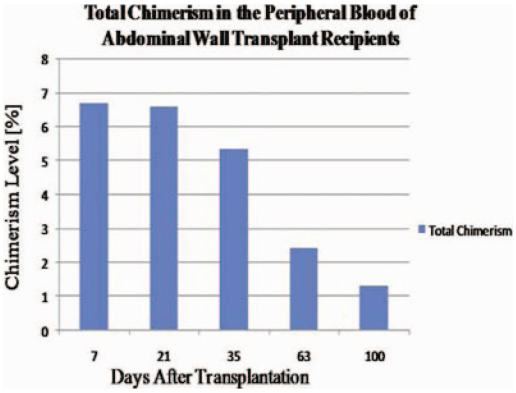


FIGURE 10. Total chimerism levels of TAW transplant.

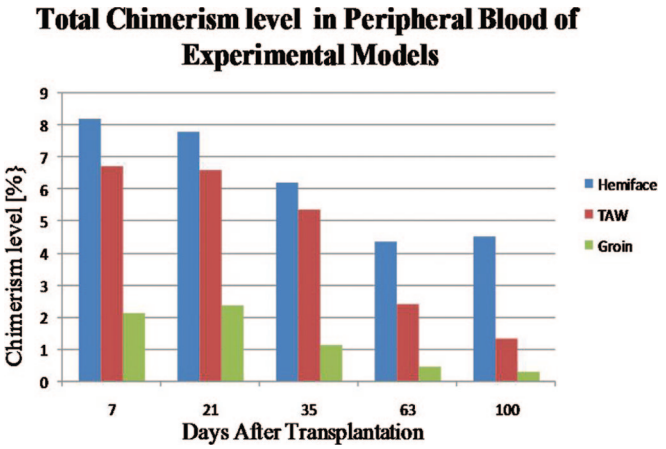


FIGURE 11. Comparison of total chimerism levels of experimental CTA models.

and spleen and stimulate recipient T-cells directly. In contrast, indirect recognition requires that the recipient. Antigen presenting cells process the donor MHC antigens that have been continuously shed from the graft, before presenting it to the recipient T-cells in a self-restricted manner.[13,14] Although definitive data was not published before, it is thought that larger amount of skin tissue in CTA transplant may cause increasing antigenic load and increased amount of Langerhans cells. High rejection rate in clinical TAW transplants may be related to these effects, because abdominal wall flap may have larger skin component compared with other CTA transplants in clinical practice such as hand, cervicofacial flap, partial face, or ear transplant. In this study, to achieve CTA model with a large skin island the entire abdominal skin and bilateral fad pad of the rat was harvested based on the bilateral superficial epigastric artery and vein (SEAV) in our study. We demonstrated that one pedicle of SEAV is not sufficient for vascular supply of the flap. The groin flap is one of the previously described abdominal wall flaps however elevated and based on unilateral SEAV.[15] In groin flap, its vascular territory permits harvesting of only limited dimensions of abdominal wall skin. Rectus abdominis muscle flaps[16] are harvested as musculocutaneous flaps and the vascular supply of skin component of the flap is provided via skin perforators of the rectus muscle. Thus, skin component size of rectus flaps depends on rectus abdominis muscle dimension and it must be outlined with in the borders of muscle component to maintain perfusion. A TRAM flap[17] has a modified skin island design based on the rectus abdominis muscle flap and it has a horizontal skin island according to the muscle anatomy. Thus, some part of the skin island is designed outside of the rectus abdominis muscle borders, which challenges vascular supply to the skin component. In addition, the vascular pedicles of both flaps have a very small diameter and advanced microsurgical skills are required for flap transfer. Zhang et al[18] achieved only 67% survival rate in the free rectus abdominis musculocutaneous flap model, thus making models applications of this technically challenging model difficult to CTA transplant studies. In this aspect, TAW model is feasible, reliable and applicable to studies on immunologic responses of CTA with large skin component. In current study, we compared skin flap dimension of TAW with experimental models containing skin flap which were established in our laboratory including total face, hemiface, limb and groin flap transplants. We determined that TAW flap has 2 to 5 times larger skin component compared with others tested models. In addition, we compared with chimerism levels of TAW with hemiface and groin flaps to see if there is correlation between size of skin component and chimerim. Since limb transplant include tissues of different antigenic response such as muscle, bone and bone marrow in addition to skin component, this comparison of chimerism levels between vascularized limb transplant with vascularized skin transplants could be difficult to interpret. All experimental models were performed across MHC barrier same strain of animals and under the same immunosuppression protocol. Hemiface model had the highest chimerism level compare with TAW and groin flaps. Face and neck regions in rats have a very rich lymphatic network. Many lymph nodes places closely neighbor to external jugular vein especially its entering point of the flap. Furthermore subcutaneously lymph node found face region closely contact with parotid gland. Face flap was elevated over the face muscle as fasciocutaneous and its pedicle are common carotid artery and external jugular vein.[8,19] While this flap contains lymph nodes of neck, surroundings of external jugular vein, and face region. However we suggested that this high blood chimerism level of face transplant correlates with high lymph nodes load of face transplant. This is different from TAW and groin transplants because these flaps include only skin and subcutaneous fat tissue. TAW transplant without load of lymph nodes showed higher chimerism level compared with groin flap in posttransplant follow-up period. We found that similar result in different study.[20] We evaluated blood chimerism level in different sized experimental groin flap models performed in our laboratory. We found that increased skin component size of the groin flap caused increased donor chimerism levels in the recipient rat (Table 2). We suggested that level of donor hematopoetic cells which passed from allograft to the recipient may be in proportion to flap dimension. New CTA transplant model for experimental studies may be useful to

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TABLE 2. Total Level of Donor Specific Chimerism in Peripheral Blood of Recipients From Published and Unpublished Hemifaces and Groin Flap Transplantation Studies

Vascularized Skin Allograft Model	Donor-Recipient	Number	Immunosuppression Protocol	Survival (Days)	Total Chimerism Level in the Peripheral Blood (%) Days After Transplantation				
					7	21	35	63	100
Hemiface*	LBN ($RT1^{l+n}$) to LEW ($RT1^{l}$)	72	CsA tapered dose	206–440	8.2	7.8	6.2	4.3	4.5
TAW		8	CsA tapered dose	208–370	6.7	6.6	5.3	2.4	1.3
Groin†		6	CsA tapered dose	78–157	2.1	2.3	1.1	0.44	0.3

investigate the hypotheses that, there is a relation between skin size and immunologic responses. In previously described models skin islands were limited to conventional 3 × 3 cm dimensions. Here we have introduced a new TAW allograft model with largest possible vascularized skin graft with dimension of 12 × 8 cm.

We believe that the model will serve as a versatile tool to test immunologic responses to large vascularized skin allograft with the following applications.

1. This is a first model simulating the clinical abdominal wall transplant.
2. Different immunosuppressive or immunomodulatory treatment modalities for tolerance induction can be tested in this model.
3. Different experiments can be designed to test the recipient's immune responses to increased antigen load of skin.
4. The question on importance of high blood chimerim level for tolerance induction and allograft survival will be tested.

CONCLUSION

TAW transplant model in rat has consistent anatomy, straightforward surgical technique, and reliable blood supply, which are essential for the success of experimental transplantation studies. This is the first model of large vascularized skin allograft transplant in small animal such as rat. Surprisingly good allograft acceptance was achieved despite large skin surface area of transplanted TAW.

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