Reviewing immunosuppressive regimens in animal models for vascularized composite allotransplantation

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Abstract

The development of vascularized composite allotransplantation (VCA) and its clinical need has led to the need for more animal models to study and perform the research required to further this specialty in terms of functional recovery and immunomodulatory improvements. Much of the animal models are reported in individual series in the literature but there has not been a review as such of these models. Here we present a compilation of the animal models reported in the literature thus far in VCA. A comprehensive review of the literature was performed for any studies which involved the use of animal models in various aspects of VCA research. The models were organized according to the type of VCA transplant, whether they were orthotopic or heterotopic, immunosuppressive regimen each study used and investigation purpose. Twenty-one facial transplant models were reported, 3 abdominal wall transplants, 4 penile transplantations, 21 uterus transplantations, 12 hindlimb transplantations and 4 myocutaneous flap transplantation animal models were reported. Primates, swine, rats, mice, rabbits, sheep and dog animal models in VCA were also reported. The most used immunosuppressive drugs are calcineurin inhibitor such as cyclosporin A and tacrolimus in these VCA animal models. They can significantly suppress lymphocyte function by blocking the phosphatase activity of calcineurin of lymphocytes. They are sometimes used combined with mycophenolate mofetil or steroids or antilymphocyte serum. The review of existing animal models will allow further research to be focused in other areas of VCA where there is a current paucity of literature. The immunosuppressive regimens used in each animal model can also be reviewed to determine which regimen works in which type of animal model which will save time and resources for future research.

Keywords: Animal models, vascularized composite allotransplantation, immunosuppressive regimens

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INTRODUCTION
Vascularized composite allotransplantation (VCA) is an up and coming clinical modality in the realm of reconstructive microsurgery. Being able to replace tissues like for like en bloc is absolutely crucial and empowers the surgeon to achieve the most optimal outcome. However, the greater goal of VCA is the ability of the reconstructive surgeon to not only restore form but also function. Functional restoration could arguably be the epitome of reconstruction where the quality of lives are improved not only from external appearance but rather also allow the patients to get back to their activities of daily living.

Trauma remains a significant burden in today’s society with many resulting in soft tissue defects. Other causes of soft tissue defects include congenital deformities and neoplastic conditions. Much of the previous methods for reconstruction include prosthesis or sequential flaps that obliterate and attempted to restore the form of a tissue defect. However, this is often inadequate and is lacking in function. VCA differs from solid organ transplantation (SOT) where tissues of varying antigenicity are transplanted en bloc. This results in issues of varying rejection rates. In particular, skin which is often a component of VCA transplants such as the hand and face has the highest antigenicity of all body tissue types[1]. As such, rejection faced by skin component is high and the recipient or patient is dependent on a high constant level of immunosuppression. Skin contains dendritic cells such as Langerhans cells that have strong immunogenic properties and it has been shown that some of these cells of donor origin reside in the epidermis decades after the transplantation[2].

Chronic immunosuppression itself carries deleterious effects in the long run. Patients face opportunistic infections and an increased risk of malignancy from the decreased immunity that is usually present to prevent and take on a surveillance role. As such, one has to carefully weigh up the pro and cons when deciding the perform VCA on a patient. The patient should also be able to finance a lifelong requirement of immunosuppressive drugs which are often costly and have a high dropout rate due to the side effects.

Much of the research at present in VCA is on better improving the safety profile of such procedures, especially with the need for the improvement in immunosuppressive regimens. By decreasing our reliance on immunosuppressive drugs, we increase the acceptability of such a procedure as the downside of immunosuppression can be deleterious. The ultimate goal in transplant science would be to achieve allograft tolerance. Tolerance to an allograft is a phenomenon where the recipient body does not recognize the foreign antigens from the donor and hence will accept the graft. Immunosuppressive drugs can hence be reduced or even omitted. In order for this process to occur, immunological manipulation and re-education of the recipient’s immune system has to occur. Several strategies already show promise in this respect and will be discussed in this article. Varying tissue types also have varying levels of inducibility with regards to tolerance formation. In particular, due to the varying tissue types of differing antigenicity in VCA, tolerance is often difficult to achieve.

A brief history of VCA
VCA has come a long way since its first conception back in AD 348. It has always been a goal of mankind to be able to replace like with like where allograft transplantation en bloc of a gangrenous leg of an elder church sacristan was performed by two brothers known as the miracle of Cosmas and Damian[3]. Previously known as composite tissue allotransplantation (CTA), VCA in the past started off with transplantation between identical twins which obviated the need for immunosuppression, which is the bane of VCA and is a focus of intense research at present.

The first-hand allotransplantation was performed in 1964 in Ecuador where a first generation drug regimen was provided. This included steroids and azathioprine initially. However, the hand allograft still was rejected 2 weeks later. Allografted tendons had been performed using non-vascularized techniques to
replace lost or nonfunctional upper extremity flexor tendons but end results were unacceptable due to the lack of viability of the grafts resulting in rupture as well. With the limited knowledge in immunological manipulation and the adverse effects that happened, further VCA cases were put on hold. It was not until the discovery and development of cyclosporin A during kidney transplantation that it was applied to VCA in the 1980s where immunosuppression finally became more effective. The first successful hand transplant then was carried out in 1998 in France. However, the patient refused to adhere to the immunosuppressive regimen due to personal reasons and compliance issues and hence the arm was again amputated almost 3 years after surgery. The first vascularized tendons were performed by Guimberteau et al.\cite{4} where two allotransplantations of digital flexor tendon apparatus were collected from a living nonrelated donor and from a deceased donor. The tendons were then revascularized using the recipient’s ulna vessels and ultimately received acceptable using multiple doses of cyclosporin A\cite{5}. The first successful face transplant occurred in 2005\cite{6} and since then, several countries have followed suit.

An overview of clinical VCA cases to date

Only a few specialized centers in the world with the capability and infrastructure for performing a VCA procedure. As such, an important source of data is the International Registry on Hand and Composite Tissue Transplantation (IRHCTT), which is a voluntary registry that collects clinical information on VCAs. The most recent report of the IRHCTT was published in 2010 and provides follow-up data on 49 hand transplants in 33 patients. Thus far, there have been 89 hand transplants performed since 1998. The United States currently has the largest number of cases, followed by China and Poland.

TYPES OF VCA ANIMAL MODELS REPORTED

Face transplant models

A variety of animal models have been used in VCA experiments with the majority being orthotopic face transplants. The animal models were performed in animals such as primates, swine, sheep, canine, rabbit, rats and mice. Different compositions of face allograft comprising of bone, nerve and soft tissue in each animal model have been reported in the literature which has varying levels of antigenicity. As such, each report has used varying types of immunosuppression, which is also dependent on the response of each animal type and to the type of immunosuppressive drug. The transplantation of each allograft can be considered orthotopic if the graft replaces the original site of the donor, i.e., the face, or heterotopic if the allograft is placed in a distant site different from the original area. Orthotopic transplants in these animal models are mostly for assessing not only the rejection process but also the functional restoration of the allograft. Heterotopic allografts, however, are used more for assessing the degree of rejection but normally do not carry an assessment of functional recovery.

In a primate model, heterotopic transfer of a facial transplant including the mandible was transferred from MHC mismatched M fascicularis monkeys. Anti-thymocyte globulin (ATG) was used as an induction regimen with tacrolimus and rapamycin in combination as a maintenance regimen.

Two reports using swine and sheep models were used with facial allografts including bone. However, no immunosuppression was used in these models and was more for the surgical technique of producing such models.

Four canine models were used in mismatched donors to beagle dog recipients. All reports were orthotopic and involved a hemifacial transplantation. With these reports, 2 reports utilized cyclosporine and steroids as maintenance immunosuppression. Two other reports used tacrolimus as maintenance immunosuppression and with 1 report using tacrolimus only for 7 days. One report in a rabbit model used a face and scalp transplantation model with no immunosuppression.
Eleven rat animal models for face transplant were reported in the literature. Nine of the reports were allografts and 2 were syngeneic. Ten reports were orthotopically transferred and 1 with heterogenic transplantation. Various face transplant components were reported ranging from ear, scalp, face, mystacial pad or mandible with tongue transplantation. A combination of cyclosporin A or tacrolimus was used in these animal models. Four of these reports had nerve coaptation which looked at the functional recovery in allograft especially using mystacial pad transplantation.

Two reports of murine orthotopic face transplant were reported with either a hemiface or ear allograft. No immunosuppressive regimens were used in these reports with more focus on the surgical technique of transferring an ear or hemiface. The information is presented in Table 1.

**Abdominal wall transplantation models**

Abdominal wall transplantation comprising of various tissue types also constitutes a vascularized composite allotransplantation model. All reported models thus far have been carried out in rats across MHC mismatched rats from Brown-Norway to Lewis rats. The abdominal wall transplants were orthotopic with 2 hemi-abdominal wall transplants and 1 with the inclusion of a hindlimb transplant. One report had a total abdominal wall allograft transplanted. Anti-lymphocyte serum was used in 2 of the reports for induction therapy. Two reports utilized cyclosporine and 1 in combination with adipocyte derived stem cells intravenously. The models do not include all nerve anastomoses and mixed chimerism all at once. The information is presented in Table 2.

**Penile transplantation models**

Penile allograft transplantation models have been described in four articles, all of which have been performed in rats. Two studies were syngeneic rats, 1 of which was orthotopic and 1 heterotopic. These studies were focused on the surgical model and being syngeneic grafts, no immunosuppression was used. Anastomosis of the penile artery and vein was key in each model and ensuring the conduit of the urethra was restored. The other 2 studies used allografts and heterotopically transplanted penile grafts. One of the studies used tacrolimus and the other cyclosporin A. The information is presented in Table 3.

**Uterus transplantation models**

Uterus transplantation has been touted as a method of restoring fertility but functionally must perform as required. Three articles report uterus transplantsations in primates, 7 in sheep, 2 in rabbits, 6 in rats and 3 in murine models. The function of the transplanted uterus was tested in rabbits, rats and mice which were successful in 3 of the studies. In primate uterus transplantation, various types of immunosuppressive regimens were used including tacrolimus, mycophenolate mofetil and methylprednisolone as maintenance regimes. Another protocol utilized ATG as an induction agent followed by tacrolimus and corticosteroids as maintenance. The information is presented in Table 4.

**Hindlimb transplantation models**

Hindlimb transplantation has been a model to mimic hand transplantation where components of bone, muscle, nerve, fat and skin are included in a hindlimb. The animal models demonstrated here to explore the feasibility of modulating the immunosuppressive regimen in improving the viability of hindlimb transplants. When transplanted orthotopically, they also serve as a model to assess the functional recovery of the hindlimb when used for gait. The nerve recovery is crucial in improving the function of the transplanted allograft. The information is presented in Table 5.

**Myocutaneous tissue transplantation models**

Soft tissue alone with varying tissue types including fat, connective tissue and muscle are collectively known as myocutaneous flaps in free flap transplantation. The varying antigenicity of the tissue types is what
constitutes the unique response directed against vascularized composite allotransplantations. Two swine models were reported with the use of gracillis myocutaneous flaps and fasciocutaneous flap transfers. One study had no immunosuppression and another had total body radiation with cyclosporin A maintenance therapy. One study utilized the transfer of the rectus abdominus myocutaneous flaps in syngeneic beagles without any immunosuppression as a model. One study utilized a combination of heart transplantation with an
abdominal musculocutaneous flap. The combination of two models is particularly interesting which confers a high degree of morbidity in the animal. In the rat study, maintenance was carried out with cyclosporin A after the inclusion of the heart transplantation. The information is presented in Table 6.

**CONCLUSION**

The summary of the findings in this article demonstrates the various VCA models reported in the literature before. In order to carry our further experiments and determine the future of allotransplantation, animal models summarized in this article will hopefully shed light on the future directions for research and where

### Table 3: Penile animal models

<table>
<thead>
<tr>
<th>Allo-transplantation</th>
<th>Approach</th>
<th>Graft</th>
<th>Regimen</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>SD19 autotransplant</td>
<td>Original region</td>
<td>Penis</td>
<td>No immunosuppression</td>
</tr>
<tr>
<td>Rat</td>
<td>SD19 autotransplant</td>
<td>Transferred to groin region</td>
<td>Penis</td>
<td>No immunosuppression</td>
</tr>
<tr>
<td>Rat</td>
<td>BN to LEW</td>
<td>Heterotopic</td>
<td>Penis</td>
<td>Tacrolimus 0.6 mg/kg/day maintained</td>
</tr>
<tr>
<td>Rat</td>
<td>Lew-BN to LEW</td>
<td>Heterotopic</td>
<td>Penis</td>
<td>CSA 16 mg/kg/day tapered to 2 mg/kg/day in 4 weeks, then maintained</td>
</tr>
</tbody>
</table>

BN: Brown Norway; LEW: Lewis; CSA: cyclosporin A; SD 19: Sprague-Dawely rats

### Table 4: Uterus animal models

<table>
<thead>
<tr>
<th>Allo-transplantation</th>
<th>Approach</th>
<th>Graft</th>
<th>Regimen</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primate</td>
<td>M. fascicularis monkey autotransplant</td>
<td>Uterus</td>
<td>No immunosuppression</td>
<td>[35]</td>
</tr>
<tr>
<td>Primate</td>
<td>Mismatched M. fascicularis monkey</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>Tacrolimus 0.3 mg/kg/day, MMF 20-10 mg/kg/day, and methylprednisolone 10-2 mg/day maintained</td>
</tr>
<tr>
<td>Primate</td>
<td>Mismatched olive baboons</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>ATG 10 mg/kg induction, followed by tacrolimus 0.1 mg/kg/day, Corticosteroids 60-5 mg/kg and MMF 50 mg/kg</td>
</tr>
<tr>
<td>Sheep</td>
<td>Swedish wool sheep autotransplant</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>No immunosuppression</td>
</tr>
<tr>
<td>Sheep</td>
<td>Sheep autotransplant</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>No immunosuppression</td>
</tr>
<tr>
<td>Sheep</td>
<td>Sheep autotransplant</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>No immunosuppression</td>
</tr>
<tr>
<td>Sheep</td>
<td>Mismatched sheep</td>
<td>Heterotopic</td>
<td>Whole uterus</td>
<td>CSA 2-5 mg/kg/day maintained and prednisone 2 mg/kg/day for 2 weeks</td>
</tr>
<tr>
<td>Sheep</td>
<td>Mismatched Romney marsh sheep</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>ATG 50 mg induction, followed by tacrolimus 0.02 mg/kg/day, methylprednisolone 40 mg/day and MMF 1.5 g/day</td>
</tr>
<tr>
<td>Sheep</td>
<td>Mismatched limousine sheep</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>CSA 10 mg/kg/day and MMF 3 g/day, both on POD 7, 14, 28, 42, 56, methylprednisolone 40 mg on POD 1-7</td>
</tr>
<tr>
<td>Rabbit</td>
<td>NZW allotransplant</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>Prednisolone 10 mg was given for 3 days following the &quot;spikes&quot; alongside an increase in tacrolimus dose from 500 to 1 g twice/day</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Mismatched NZW</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>Tacrolimus 500 μg twice daily postoperatively; embryo transfer</td>
</tr>
<tr>
<td>Rat</td>
<td>LEW syngeneic</td>
<td>Heterotopic</td>
<td>Uterus</td>
<td>No immunosuppression</td>
</tr>
<tr>
<td>Rat</td>
<td>LEW syngeneic</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>No immunosuppression</td>
</tr>
<tr>
<td>Rat</td>
<td>LEW syngeneic to DA</td>
<td>Heterotopic</td>
<td>Whole uterus and ovaries</td>
<td>No immunosuppression</td>
</tr>
<tr>
<td>Rat</td>
<td>BN to LEW</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>CSA 10 mg/kg/day maintained</td>
</tr>
<tr>
<td>Rat</td>
<td>BN to LEW</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>Tacrolimus 0.5 mg/kg/day pump maintained</td>
</tr>
<tr>
<td>Rat</td>
<td>Virgin Dark Agouti to virgin LEW</td>
<td>Orthotopic</td>
<td>Uterus</td>
<td>Tacrolimus 0.5 mg/kg/day maintained; male SD rats of proven fertility were used for mating</td>
</tr>
<tr>
<td>Murine</td>
<td>F1-hybrids of inbred female C57BL/6 X CBA/ca syngeneic</td>
<td>Heterotopic</td>
<td>Right uterine horn and the cervix</td>
<td>No immunosuppression; embryo transfer</td>
</tr>
<tr>
<td>Murine</td>
<td>B6 syngeneic</td>
<td>Heterotopic</td>
<td>Ovarian</td>
<td>No immunosuppression</td>
</tr>
<tr>
<td>Murine</td>
<td>F1-hybrids of C57BL/6 X CBA/ca to B6</td>
<td>Heterotopic</td>
<td>Right uterine horn and the cervix</td>
<td>CSA 20 mg/kg/day</td>
</tr>
</tbody>
</table>

BN: Brown Norway; LEW: Lewis; CSA: cyclosporin A; DA: Sprague-Dawley; MMF: mycophenolate mofetil; NZW: New Zealand White
further focus can be emphasized. Experimental animal surgical models can be difficult to perform and such research in VCA should be best collaborated with both clinicians and surgeons who can perform the difficult animal models, as well as basic scientists to further developments in this specialty.

Many of the immunosuppressive regimens used thus far involve an induction agent such as anti-thymocyte globulin or total body radiation which preconditions the host’s immune system in preparation for a chance of engraftment of donor antigens. In particular, the phenomenon of chimerism is particularly seen in VCA research where the transfer of vascularized bone marrow, in long bones in particular, mediates a constant exchange of cells such as regulatory T cells which serve to protect the allograft. A particular preference for cyclosporin A, tacrolimus and steroids were seen across each animal model - quite so due to their

<table>
<thead>
<tr>
<th>Table 5: Hindlimb animal models</th>
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<tr>
<td>Allotransplantation</td>
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<tr>
<td>----------------------</td>
</tr>
<tr>
<td>Primate</td>
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<tr>
<td>Swine</td>
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<td>Swine</td>
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<tr>
<td>Rabbit</td>
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<td>Rat</td>
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</tbody>
</table>

NZW: New Zealand White; BN: Brown Norway; LEW: Lewis; CSA: cyclosporin A; POD: postoperative day; N/A: not available; WF: Wistar-Furth; BMC: bone marrow cells; IBOMC: iliac bone osteomusculocutaneous

<table>
<thead>
<tr>
<th>Table 6: Myofasciocutaneous animal models</th>
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<tbody>
<tr>
<td>Allotransplantation</td>
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<td>----------------------</td>
</tr>
<tr>
<td>Swine</td>
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<tr>
<td>Swine</td>
</tr>
<tr>
<td>Canine</td>
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<tr>
<td>Rat</td>
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</tbody>
</table>

LEW: Lewis; CSA: cyclosporin A; TBI: total body irradiation; CD3-IT: CD3-immunotoxin; HCT: hematopoietic cell transplantation; F344: Fischer 344; WKY: Wistar Kyoto
widespread availability and immunosuppressive capabilities. They mediate and protect the allograft from being attacked by host defense mechanisms which would destroy the graft otherwise.

DECLARATIONS

Authors’ contributions

Wang AYL and Loh CYY were both involved in data collection, drafting of the manuscript, analysis of data, the second review of data, statistical analysis, ensuring data fidelity and manuscript review.

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Conflicts of interest

There are no conflicts of interest.

Patient consent

Not applicable.

Ethics approval

Not applicable.

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