World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(REVIEW ARTICLE)

Immunomodulatory therapies to prevent transplant rejection in composite tissue allotransplantation: Current status and future prospects

Emmanouil Dandoulakis *

Independent Medical Researcher, Athens, Greece.

World Journal of Biology Pharmacy and Health Sciences, 2025, 23(01), 300-311

Publication history: Received on 02 June 2025; revised on 13 July 2025; accepted on 16 July 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.23.1.0672

Abstract

Composite tissue allotransplantation (CTA), including such surgery as hand transplantation and facial transplantation, can restore functionality and quality of life of patients with moderate and severe tissue loss. Nevertheless, it is also subject to immunological barriers once again, especially in the form of acute and chronic rejection due to the high immunogenicity of composite tissues, most notably of skin. Contemporary immunomodulatory approaches are based on induction drugs (e.g., anti-thymocyte globulin), maintenance intervention (e.g., tacrolimus, mycophenolate mofetil, corticosteroids) as well as rescue therapy against acute rejection. The solutions to these problems are associated with limitations due to considerable toxicities (infections, malignancies, and nephrotoxicity), with partial prevention of chronic graft vasculopathy despite providing an effective way to prevent early rejection. The new strategies that are promising for achieving an advantage in terms of tolerance and minimizing systemic immunosuppression include mixed chimerism, regulatory T-cell-based therapeutics, costimulation blockade, and localized drug delivery platforms. Gene editing and biomaterials can also present a novel approach to regulating immunogenicity. Its future directions include personalized immunosuppression using pharmacogenomics, instance tracking of immune status, and artificial intelligence-based predictive models. The pinnacle will be the development of immune tolerance, which may make CTA immunosuppression-free. This review compares clinical outcomes, highlights limitations, and examines new therapies using data from international registries and ongoing trials. It highlights the importance of multidisciplinary efforts in promoting translational research and its associated protocols. These innovations can revolutionize the field, increasing the longevity of grafts and improving patient outcomes by overcoming immunological barriers, thereby reaching a broader audience worldwide with CTA.

Keywords: Composite Tissue Allotransplantation; Immunomodulatory Therapies; Transplant Rejection; Immune Tolerance; Personalized Immunosuppression

1. Introduction

Composite tissue allotransplantation (CTA), also known as vascularized composite allotransplantation (VCA), refers to a reconstructive surgery category that encompasses procedures such as hand, face, limb, or penile transplantation, which is a revolutionary advancement. CTA replaces the various types of tissues—skin, muscle, bone, nerves, and vasculature—unlike solid organ transplantation (SOT), to provide functional and aesthetic tissue restoration in patients who have suffered significant tissue loss in an accident, deformities since birth, or following oncologic resection. The initial achievement was the successful hand transplant in 1998 in Lyon, France, which showed the clinical possibility of CTA (Dubernard et al., 1999). Since that time, it has already reported more than 200 CTA operations globally. It is ranked first in hand transplants and second in face transplants, as reported by the International Registry on Hand and Composite Tissue Transplantation (IRHCTT) (Shores et al., 2015). The clinical impact of CTA is its restoration of complex functions, including fine motor skills in hand transplants or facial expressions in face transplants, which substantially

* Corresponding author: Emmanouil Dandoulakis; Email: manosdandoulakes@gmail.com

enhances quality of life by filling psychosocial and functional deficiencies that traditional prosthetics or autologous reconstruction cannot achieve comprehensively. An example is that hand transplant patients report significant increases in Disabilities of the Arm, Shoulder, and Hand (DASH) scores, indicating their improved functional ability (Ninkovic et al., 2011). On the same note, face transplantation can restore lost functions, such as speech and swallowing, and provide the psychological benefits of improved self-insight and societal readjustment (Lantieri et al., 2016). Nevertheless, due to the multilayered nature of CTA, including multifactorial tissues, the complexity of CTA creates special challenges in immunology that require immediate responses of immunomodulation to support the survival of the graft and the safety of the patient, which differ from those of SOT.

The first issue of CTA is immunological barriers, especially the high risk of acute and chronic rejection, which are caused by the immunological peculiarities of composite tissues. The hostile response, which relies mainly on T cell-induced mechanisms, is influenced in up to 85 per cent of CTA patients in the initial year post-transplant, and the stained part is the most immunogenic because it contains antigen-presenting cells (APCs) and major histocompatibility complex (MHC) antigens (Cendales et al., 2008). In contrast to SOT, rejection is typically specified by parenchymal organs, which can be observed on the skin and thus detected early enough. However, due to the dependence on timely intervention, it can develop into the deeper layers of the skin, such as the muscle or even the bones. Graft vasculopathy and tissue fibrosis lead to chronic rejection, which also causes long-term graft loss, resulting in a graft failure rate of 10-20% in the past five years, as reported in studies (Kaufman et al., 2013). This heterogeneity in the components of CTA also complicates its immunological profile, as skin is highly immunogenic. In contrast, bone and cartilage are relatively poorly immunogenic, displaying differences in their rejection patterns by the recipient. This increased immunogenicity means that SOT requires more intensive immunosuppression compared to CTA; however, the rejection mechanism is similar, as is T-cell activation (through both direct and indirect pathways of allorecognition) and humoral responses based on donor-specific antibodies (DSAs) (Morelon et al., 2017). All these immunological hurdles are further exacerbated by lifelong immunosuppression, which predisposes the patient to risks of opportunistic infections, malignancy, and organ toxicity. A balance must be struck between protecting the graft and the patient's health.

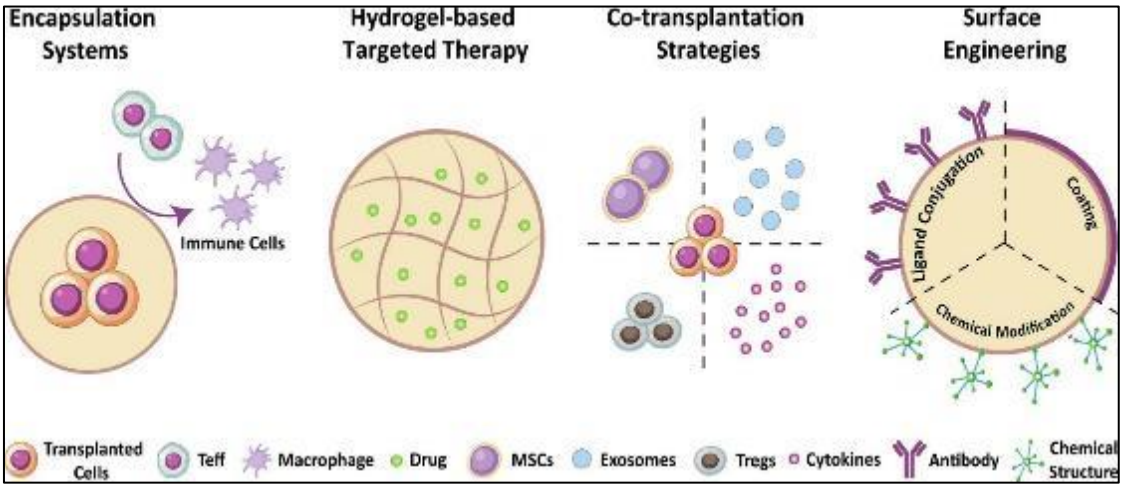

Figure 1 Advanced biomaterial-assisted immunomodulatory strategies—including encapsulation, hydrogel-based delivery, co-transplantation, and surface engineering—modulate immune responses locally to promote graft tolerance and reduce systemic toxicity in CTA. These innovations exemplify next-generation approaches integrating transplant immunology and bioengineering to enhance graft survival. Adapted from Ashimova et al. (2019)

An argument for developing effective immunomodulatory therapies in composite tissue allotransplantation (CTA) is to mitigate the long-term safety challenges of allotransplants by preventing long-term rejection without incurring the long-term consequences of systemic immunosuppression. Standard regimens An example of the standard induction regimen (adapted to solid organ transplantation) is the combination of anti-thymocyte globulin or basiliximab with maintenance tacrolimus, mycophenolate mofetil, and corticosteroids (Brandacher et al., 2012). Though there has been increased success of early survival of grafts because one-year success rates recorded 93.6% in hand transplantation (Shores et al., 2015), these measures are characterised by other contributing elements such as the side effects of infections and malignancies, to name but a few that affect 50 per cent of the recipients experiencing them (Khalifian et al., 2014). The CTA, especially skin immunosensitivity, is frequently associated with high immunogenicity, necessitating higher levels or longer-lasting immunosuppression; however, even then, chronic rejection is not entirely averted (Ravindra et al., 2019). New approaches involving mixed chimerism, regulatory T-cell therapies, and costimulation

blockade are being developed to induce tolerance and mitigate systemic burden, addressing these challenges. Following these, newer developments in bioengineering, such as the use of hydrogels as delivery vehicles, encapsulation of cells, combined transplantation with immunomodulatory cells, and surface modifications, provide localised immunosuppression that focuses on immune responses at the interface between the graft and the host. Figure 1 illustrates that these biomaterial-supported platforms represent a multidisciplinary advancement, and they will significantly impact the CTA field. With reduced systemic toxicity and increased improvement of graft survival, they demonstrate the next generation where immunosuppression can be safer, more targeted, and even (at least potentially) unnecessary.

Objective

The objective of this article is to comprehensively review current immunomodulatory therapies for composite tissue allotransplantation (CTA), focusing on their efficacy in preventing acute and chronic rejection while addressing associated toxicities. Standard regimens, including induction with anti-thymocyte globulin and maintenance with tacrolimus, mycophenolate mofetil, and corticosteroids, are evaluated alongside their limitations. The article also discusses emerging strategies, such as mixed chimerism, regulatory T-cell therapies, and costimulation blockade, which aim to induce immune tolerance and reduce systemic immunosuppression. Future prospects, including personalized immunosuppression and biomaterials, are explored to highlight transformative potential for improving graft survival and patient outcomes in CTA.

2. Structure of the Article

The article is organized in a way that provides a detailed analysis of immunomodulatory therapies in the field of composite tissue allotransplantation (CTA). The paper begins with a review of the available treatments, which includes a description of standard immunosuppressive therapy and its effectiveness in preventing rejection, as well as clinical outcomes. Limitations, such as toxicity, chronic rejection, and patient heterogeneity, are discussed in the following section, highlighting the need to develop approaches that are more effective. New strategies are then investigated, which include tolerogenic approaches such as mixed chimerism, regulatory T-cell-based therapies, and novel drug delivery formulations. The concluding part outlines possible strategies, with a greater focus on personalized immunosuppression, developing immune tolerance, and integrating artificial intelligence and regenerative medicine. This organization ensures a rational transition between established practices and new opportunities, focusing on improving CTA results.

3. Immunological Basis of Rejection in CTA

The issues of composite tissue allotransplantation (CTA) are based on transplant immunology, where the key role in allorecognition (recognition of foreign tissue in the recipient) falls to the major histocompatibility complex (MHC). The rejecting mechanisms in CTA result from direct, indirect, and semi-direct allorecognition pathways. It is based on these mechanisms, in which donor or recipient APCs present alloantigens to T-cells, that acute and chronic immune responses occur. On the donor cells, the MHC molecules are used to display the antigens to the recipient T cells directly (directly with antigen presentation of donor antigens by donor antigen-presenting cells [APCs] to recipient T cells) and indirectly (indirectly with donor antigens processed by recipient APCs) routes and this activates the immune response (Cendales et al., 2008). Activation of T-cells requires two signals: first, recognition of the antigen by the T-cell receptor, and second, costimulatory signals (e.g., binding between CD28 and B7 or CD40 and CD40L) that result in proliferation, differentiation, and the generation of cytokines (e.g., IL-2, IFN-γ). Acute rejection is mediated by effector T cells (including CD4+ helper and CD8 cytotoxic T cells) at the donor-level tissue by an active process of destruction through cytotoxic T cells, killing graft cells directly, and are stimulated by helper T cells through cytokine release (Morelon et al., 2017). Humoral immunity also plays a crucial role, particularly in hyperacute and chronic rejection. The development of donor-specific antibodies (DSAs) by the mechanism action of B cells and plasma cells is followed by their binding to MHC or non-MHC antigens on graft endothelium and the activation of the complement, which leads to endothelial damage or graft vasculopathy (Khalifian et al., 2014). Hyperacute rejection can be caused by pre-existing donor-specific antibodies (DSAs), possibly as a result of previous sensitisation, but is uncommon in CTA due to pre-transplant crossmatching. Conversely, de novo DSAs are also involved in late graft dysfunction, and they have been demonstrated to be present in 20-30% of CTA recipients within five years of transplantation (Kaufman et al., 2013). These immunological attacks, which are both cellular and humoral, require intense immunosuppression to prevent graft rejection.

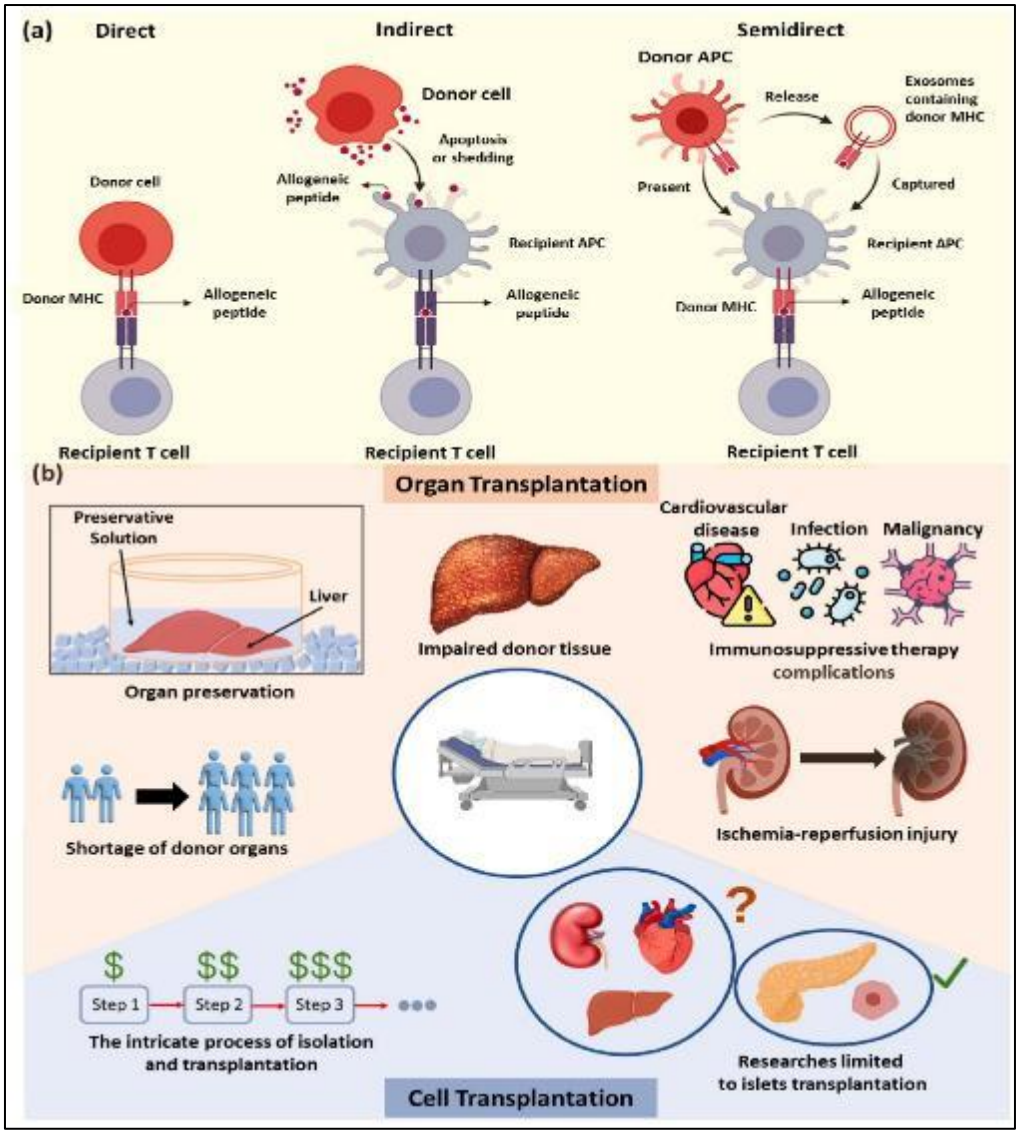

Figure 2 (a) Three major pathways of allorecognition: direct (donor APCs presenting antigens), indirect (recipient APCs presenting processed donor peptides), and semidirect (recipient APCs acquiring intact donor MHC). (b) Limitations of organ transplantation—including donor shortages, preservation issues, and immunosuppression risks—contrast with the emerging potential of cell-based transplantation strategies. Adapted from Abbaszadeh, S., et al., (2023)

The distinct nature of this immunological aspect of CTA is what makes it so different from solid organ transplantation (SOT), primarily due to the high immunogenicity of the skin component and the variability of rejection to other kinds of tissues. Most CTA grafts are composed of skin, which is also the most immunogenic tissue and the primary focus of acute rejection, affecting up to 85% of patients within the first year (Cendales et al., 2008). Skin rejection occurs in the form of any of the three: erythema, oedema, or desquamation, which can be observed but needs to be countered immediately to ensure that it does not reach the deeper tissues such as muscle or bone, which have a weaker immunogenicity, due to the lower expression of MHC and limited immune-populated cells (Shores et al., 2015). This mismatch in antigenicity results in intricate patterns of rejection, such that skin tends to reject previously and more severely compared to other body parts, as noted by histopathological analysis using the Banff 2007 classification (Cendales et al., 2008). Inadequately described in the CTA, chronic rejection is characterised by the development of progressive vascular tissue, including intimal hyperplasia and graft vasculopathy, which results in tissue fibrosis and loss of tissue functionality. Eventually, there is a 10-20 per cent graft loss greater than five years, and vasculopathy is associated with long-term chronic injury mediated by DSA and T-cell-associated inflammation (Kaufman et al., 2013). In contrast to SOT, CTA necessitates the visible rejection and multi-tissue nature, which makes immunosuppression strategies challenging to use, as responses directed at the skin must be controlled with higher doses, posing risks of toxicity (Brandacher et al.,

2012). These specificities are the reasons why a special immunomodulatory strategy is required in CTA, e.g., local treatments or tolerance induction, to reduce rejection while minimising systemic side effects as much as possible.

3.1. Types of Rejection in CTA

Three distinct types of rejection, each with unique mechanisms and clinical implications, challenge composite tissue allotransplantation (CTA):

Hyperacute Rejection: This rare, antibody-mediated event occurs within minutes to hours post-transplant due to pre-existing donor-specific antibodies (DSAs) binding to graft endothelial antigens. It activates complement, causing vascular thrombosis and graft necrosis (Khalifian et al., 2014). Pre-transplant crossmatching significantly reduces its incidence, making it uncommon in CTA.

Acute Rejection: The most prevalent type, affecting up to 85% of recipients within the first year, is T-cell-mediated, driven by allorecognition of major histocompatibility complex (MHC) antigens (Cendales et al., 2008). It presents as skin erythema, edema, or desquamation, necessitating rapid treatment with high-dose corticosteroids or anti-thymocyte globulin to prevent progression to deeper tissues like muscle or bone.

Chronic Rejection: Developing over years, this poorly understood process involves graft vasculopathy, intimal hyperplasia, and tissue fibrosis, leading to functional decline and graft loss in 10–20% of cases beyond five years (Kaufman et al., 2013). Both T-cell-driven inflammation and humoral responses via DSAs contribute, with vascular remodeling linked to chronic injury (Morelon et al., 2017).

The multi-tissue nature of CTA complicates these rejection patterns, with skin often serving as an early indicator. Elucidating chronic rejection mechanisms and developing targeted therapies are critical for improving long-term graft survival and patient outcomes in CTA.

3.2. Diagnostic Approaches

Monitoring rejection in composite tissue allotransplantation (CTA) relies on integrated diagnostic methods:

Clinical Assessment: Visual inspection of skin changes, such as erythema or edema, is critical due to skin's high immunogenicity. Acute rejection, common in 85% of patients within the first year, is often detected early, enabling timely intervention, though non-specific changes can complicate diagnosis (Khalifian et al., 2014).

Histopathology: The Banff 2007 classification grades rejection from mild inflammation (Grade I) to necrosis (Grade IV) via skin biopsies, serving as the gold standard for confirming rejection (Cendales et al., 2008).

Biomarkers: Donor-specific antibodies (DSAs), detected in 20–30% of recipients, and gene expression profiles (e.g., CXCL9) predict chronic and acute rejection, reducing reliance on invasive biopsies (Kaufman et al., 2013; Morelon et al., 2017).

These approaches ensure early detection and guide personalized immunosuppression, enhancing CTA graft survival.

4. Current Immunomodulatory Therapies in CTA

Immunosuppression is a crucial component of composite tissue allotransplantation (CTA). It should consider the immunosuppressive regimens that prevent rejection once the regimen is initiated as initial therapy to prevent rejection early on. The commonly used agents include anti-thymocyte globulin (ATG), basiliximab and alemtuzumab. T-cell depletion is achieved through the use of ATG and alemtuzumab, whereas co-stimulation blockade is provided by basiliximab, which blocks the IL-2 receptor. Additionally, B cell activation of the T cells is inhibited (Brandacher et al., 2012). They are proven to be effective, as clinical practice shows that TPNL and ATG have resulted in acute rejection rates of fewer than 20 per cent at the three-month time mark in hand transplantation (Shores et al., 2015). Maintenance therapy typically consists of a three-drug combination, commonly including calcineurin inhibitors (e.g., tacrolimus), antimetabolites (e.g., mycophenolate mofetil), and corticosteroids. Tacrolimus acts on the T-cell signaling pathway, mycophenolate mofetil acts on lymphocyte growth, and corticosteroids act on inflammation. Monitoring treatment by drug: Therapeutic drug monitoring involves optimizing the placement of drug dosing, with tacrolimus levels of 8-12 ng/mL, aiming to balance favorable efficacy and toxicity (Kaufman et al., 2013). These regimes have achieved more than 90% one-year graft survival and 70% rejection-free interval during the first year in CTA recipients (Shores et al., 2015).

In the case of acute episodes of rejection, rescue therapies are used to reverse the refurbishment of immune-mediated damage. Corticosteroids at high doses, such as methylprednisolone pulses, which are the first-line intervention, are highly effective in addressing acute rejection, with a success rate of 80% (Cendales et al., 2008). Skin-limited rejection Topical therapies can be employed in skin-limited rejection (such as tacrolimus ointment), taking advantage of the accessibility of the skin of CTA to reduce systemic exposure (Khalifian et al., 2014). In the case of refractory or antibody-mediated rejection, biologics (e.g., anti-TNF agents, such as infliximab) or rituximab, which targets B cells, are used. The efficacy of rituximab is notably seen in DSA-positive patients, minimising damage caused by antibodies (Morelon et al., 2017). These rescue interventions are all designed according to the degree of rejection and mechanism, increasing graft salvage, but with caution about the need for surgeries that do not have significant long-term toxicities.

4.1. Clinical Outcomes

Composite tissue allotransplantation (CTA) results indicate that its clinical outcomes (graft survival with or without functional recovery and patient quality of life) have shown significant improvement, as demonstrated in the International Registry on Hand and Composite Tissue Transplantation (IRHCTT). The graft survival is also very high, with 100% recorded at one year after transplant and two years, and 95.6% of patients alive after two years who were compliant. In contrast, non-compliance resulted in the loss of eight grafts, most of the losses occurring in the early Chinese population (Petruzzo et al., 2010). As a functional outcome, every hand transplant recipient can regain protective sensibility, 90 per cent develop tactile sensibility, and 82.3 per cent gain discriminative sensibility, which enables them to complete tasks such as writing and grasping (Shores et al., 2015). Extrinsic and intrinsic muscle reinnervation can enable motor recovery, allowing 90 per cent of recipients to perform daily activities and return to work (Petruzzo et al., 2010). The recipients of face transplants experience restored speech, swallowing, and aesthetics, facilitating better social reintegration (Lantieri et al., 2016). Accessible through psychosocial outcomes, as evaluated by the SF-36 and patient satisfaction surveys, shows an enormous improvement in quality of life, especially in patients undergoing bilateral hand and face transplants, with scores of disability decreasing (e.g., DASH scores after transplant decrease from 50 to 19) (Ninkovic et al., 2011). Nevertheless, it is estimated that complications associated with immunosuppression developed in up to 50 per cent of patients having infections and metabolic problems (Khalifian et al., 2014). The results of this study highlight the transformative nature of CTA while also underscoring the crucial role of patient adherence and multidisciplinary care in delivering optimal long-term outcomes.

4.2. Adverse Effects of Current Therapies

Present immunosuppressive regimens for composite tissue allotransplantation (CTA) are associated with considerable toxicity. Treatment with tacrolimus and anti-thymocyte globulin induces the suppression of T-cells, so up to 50 per cent of the recipients develop opportunistic infections (such as cytomegalovirus (CMV) and fungal infections) within five years (Khalifian et al., 2014). Malignancy, such as post-transplant lymphoproliferative disorders (PTLD) and skin cancers, are common, and they occur in 5 to 10 per cent of CTA individuals because of persistent immunosuppression (Shores et al., 2015). The elderly face metabolic complications, like diabetes (20-30 per cent occurrence) and hypertension, in addition to calcineurin inhibitor-related nephrotoxicity, which further destabilizes the health conditions of the older adults and 15 per cent of all the recipients go on to develop renal dysfunction (Kaufman et al., 2013). Avoidance of the effects of immunosuppression can be achieved by avoiding the use of steroids (steroid-avoiding regimens), thereby reducing corticosteroid utilization by maximizing the doses of tacrolimus and mycophenolate mofetil and thereby reducing the incidence of diabetes and infections (Brandacher et al., 2012). Topical treatments, such as tacrolimus ointment to treat skin rejection, restrict systemic exposure, and early weaning regimes based on biomarkers, will reduce long-term toxicity (Morelon et al., 2017). These strategies aim to find a trade-off between preventing rejection, minimizing adverse effects, and improving patient outcomes in CTA

5. Limitations of Current Therapies

Although there is currently successful immunosuppressive therapy to prevent acute rejection of composite tissue allotransplantation (CTA), it is constrained by high toxicities and their effect on patient outcomes. The cumulative impacts of immunosuppression—typically involving calcineurin inhibitors (e.g., tacrolimus), antimetabolites (e.g., mycophenolate mofetil), and corticosteroids—include significant complications such as opportunistic infections (e.g., cytomegalovirus, which develops in up to 50% of recipients within five years), malignancies (e.g., post-transplant lymphoproliferative disorders occurring in 5–10% of patients), and nephrotoxicity (affecting approximately 15% of recipients). These side effects often undermine patient adherence, as the burden of regular medical checkups, side effect management, and lifelong medication regimens contributes to non-adherence in up to 20% of CTA recipients. This non-compliance has been identified as a factor contributing to graft loss in early cohorts (Petruzzo et al., 2010). Additional features such as metabolic complications, i.e., diabetes and hypertension, which impact 2030 per cent of patients, and psychosocial effects of noticeable adverse changes such as Cushingoid also feature detriment to the quality of life

(Shores et al., 2015). The attempts to curb toxicity via steroid-sparing regimes or topical treatments (including tacrolimus ointment) are promising yet inadequate to exclude systemic risks, which again warrants the development of a new treatment option capable of lightening the burden of immunosuppressants without compromising graft protection (Brandacher et al., 2012).

Failure to prevent chronic rejection completely, as well as variations in patient responses, further constrain the existing treatments, which are complicated by ethical and practical issues. Graft vasculopathy and tissue fibrosis, leading to chronic rejection in more than 10-20 per cent of CTA grafts, occur after five years and are mediated by T-cell-mediated inflammatory processes, as well as donor-specific antibodies (DSAs), resulting in intimal hyperplasia (Kaufman et al., 2013). These processes are poorly understood, and an effective treatment for graft vasculopathy is not yet available, which contributes to its gradual loss of function (Morelon et al., 2017). Responses of patients differ; i.e., when HLA mismatching occurs, a greater level of mismatch is associated with an enhanced risk of rejection, and comorbidities such as diabetes or advanced age lead to a worsened state of toxicity (Khalifian et al., 2014). Optimizing immunosuppression by personalising it is challenging, given the limited number of biomarkers and variable pharmacokinetics. Morally, CTA is not considered life-saving, which also suggests that implanting patients with a lifelong risk of immunosuppression is not beneficial, given its functional outcomes. The global applicability of CTA is limited due to practical barriers, including the high cost of biologics and monitoring (roughly estimated at between $20,000 and $50,000 per year), as well as the unavailability of resources in low-resource healthcare systems (Petruzzo et al., 2010). These restrictions underscore a pressing need for strategies that can promote tolerance and affordable, effective treatments to enhance the sustainability and equity of CTA.

6. Emerging Immunomodulatory Strategies

6.1. Tolerogenic Approaches

Due to the limitations of chronic immunosuppression, the emergence of tolerogenic strategies in composite tissue allotransplantation (CTA) promises to lead to the realization and maintenance of immune tolerance within the target tissue, thereby limiting the need to rely on immunosuppressants to cope with rejection. Mixed chimerism, which induces toleration in hematopoietic chimerism, can be fostered by introducing donor immune cell components into the recipient system, potentially resulting in reduced or no immunosuppression due to the engraftment of hematopoietic donor cells. The combination of bone marrow transplantation with CTA, as in hand transplants, is partially successful in reducing the need for maintenance therapy. However, issues such as graft-versus-host disease (GVHD) and deleterious conditioning regimens persist (Schneeberger et al., 2013). Characteristic of immune regulation, regulatory T cells (Tregs) are expanded ex vivo and adoptively transferred to marshal against graft rejection. CTA models in preclinical experiments have demonstrated Treg-mediated functional inhibition of the T-cell response, and initial clinical trials suggest a potential capacity to reduce the need for immunosuppressants. However, specificity and feasibility have been identified as obstacles (Brandacher et al., 2012). Costimulation blockade involves inhibiting T-cell activation by blocking CD28-B7 or CD40-CD40L interactions with agents such as belatacept or anti-CD40/CD40L antibodies. In solid organs, Belatacept, as an alternative to calcineurin inhibitors, causes less nephrotoxicity, and preliminary CTA experiments show reduced acute rejection rates, with further studies in progress to determine long-term effects (Khalifian et al., 2014). These active, research-supported strategies have the potential to be transformative in achieving immunosuppression-free CTA, improving graft survival, and enhancing the quality of life for affected patients.

6.2. Cell-Based Therapies

Cell-based therapies, including mesenchymal stromal cell (MSC) and dendritic cell (DC) therapies, are new strategies in composite tissue allotransplantation (CTA) that aim to decrease rejection, thereby reducing the need for chronic immunosuppression. Obtained either in bone marrow, adipose tissue or umbilical cord, MSCs have strong anti-inflammatory and immunomodulatory abilities (inhibiting T-cell proliferation, stimulating regulatory T-cell (Treg) growth, and releasing cytokines such as IL-10 and TGF-beta). In preclinical models of CTA, including hind-limb transplants in rats, MSC transfusions increase graft lifespan by 2030-fold by inhibiting the acute rejection response mediated by the downregulation of pro-inflammatory mediators (e.g., IFN-γ) and promoting the development of a tolerogenic microenvironment (Brandacher et al., 2012). The initial clinical programs of hand transplantation suggest that MSC treatment decreases maintenance immunosuppressant doses by up to 50 per cent, and some patients attain a tacrolimus trough level of less than 5 ng/mL without any rejection events. Nevertheless, the MSC may have a short duration of action and require infusions or exhibit inconsistent potency due to donor origin and ex vivo culture conditions (Schneeberger et al., 2013). Future research on the durability and specificity of CTA will include optimization of MSC dosing, administration route (e.g., intra-graft vs. systemic), and strategies to combine it with biologics such as belatacept.

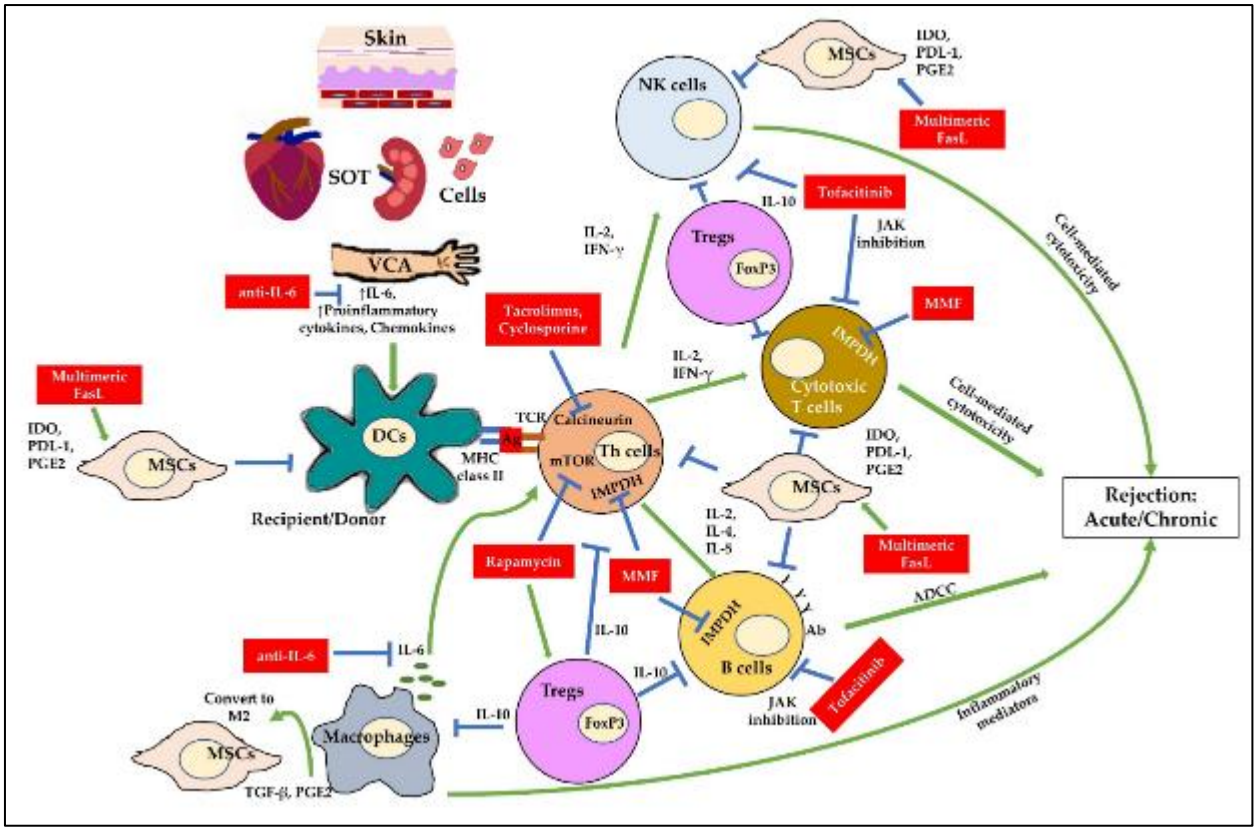

Figure 3 Overview of immune mechanisms in transplant rejection and modulation strategies. The diagram illustrates interactions among T cells, B cells, NK cells, macrophages, and dendritic cells, and how immunosuppressants and cell-based therapies like MSCs and IL-6 blockade modulate these pathways to prevent acute and chronic rejection. *Adapted from Anggelia et al. (2022)*

Tolerogenic DC techniques exploit the antigen-presenting function of DCs to induce immune tolerance, by either converting T-cells to anergy or differentiating them into Treg cells. The modified ex vivo tolerogenic DCs of donors in CTA preclinical models engineered through IL-10 or rapamycin extend antigen survival by 1525 days by tilting immunity toward tolerance through PD-L1 Intensification and repression of co-modification (Khalifian et al., 2014). Initial clinical investigation into CTA has involved combining DCs with costimulation blockade, resulting in a lower rate of acute rejection. However, this approach is hindered by the complex production facilities required (GMP) and the limited duration of the effects, necessitating multiple applications. MSC and DC-based therapies have limitations such as both are heterogeneously effective, their off-target immunosuppression presents risks of increasing infection rate (e.g. CMV in 50% of recipients), and there are logistical challenges to their production and distribution (e.g. cost of production can range $10,000 to $50,000 per dose and there are variable and inconsistent procedures) (Morelon et al., 2017). Those challenges notwithstanding, cell-based therapies have transformative potential in CTA, and clinical trials are geared towards enhancing cell stability, scaling to target, and integrating with other tolerogenic approaches to enable the long-term survival of grafts and reduce systemic toxicities.

6.3. Gene Editing and Immunomodulation

Gene editing techniques, most eminently CRISPR/Cas9, have tremendous potential for composite tissue allotransplantation (CTA) through the reduction of donor tissue immunogenicity. CRISPR/Cas9 enables precise knockout of major histocompatibility complex (MHC) class I and II genes in highly immunogenic tissues, such as skin or vascular endothelial cells. This consequently reduces T-cell allorecognition in the direct and indirect pathways. Surgical experiments on porcine and murine CTA models indicate that MHC editing increases the survival of grafts by 30-50% compared to plastically modified grafts and that acute rejection rates are minimized by a decrease in antigen presentation (Schneeberger et al., 2013). Other strategies include editing co-stimulatory molecules (e.g., CD80/CD86) to suppress T cell activation further. Nonetheless, potential impediments include the off-target effects of CRISPR, which can cause unwanted genomic modifications, as well as the need for efficient methods to deliver CTAs to access complex tissues. Clinical translation is at an early stage, and any trial of CTA in humans to date has not been reported, partly because of uncertainty regarding genomic stability long-term or due to the potential risk of edited grafts evading the

immune system, placing them in a worse position to face infections or the development of cancer (Khalifian et al., 2014). These technical challenges have prompted the development of new, advanced gene-editing technologies, including base or prime editors, to make CTA technologies safer and more precise in the future.

Another method of immunomodulation in CTA involves using gene therapies to manipulate the recipient's immune response towards tolerance and minimise rejection. Delivery of immunosuppressive genes (e.g., IL-10 or PD-L1) by viral vectors induces the proliferation of regulatory T cells (Tregs) and T-cell anergy, thereby inhibiting the synthesis of pro-inflammatory cytokines (e.g., TNF-α, IFN-γ). In rat hind-limb transplant models, the delivery of state or IL-10 adeno-associated virus (AAV) is shown to prolong graft survival by 20 to 25 days, owing to the induction of a tolerogenic microenvironment (Brandacher et al., 2012). There is also preclinical research into silencing pro-inflammatory pathways (e.g., NF-κB) by siRNA to suppress rejection. Nevertheless, the low immunogenicity of vectors, the possibility of insertional mutagenesis, and the interim nature of expression reduce its efficacy, which necessitates frequent administrations that drive costs ($100,000 to half a million dollars per treatment) and risks (Morelon et al., 2017). Ethical implications are severe: gene therapy of donor tissue is subject to personal integrity and consent issues, and gene therapy of an in-vivo recipient has risks of accidental immunosuppression, the increased risk of infections or cancer. There are concerns about designer tissues, which are affecting the widespread public acceptance of such organs. In low-income settings, this will still act as a hindrance to equal accessibility to such expensive treatments. Nevertheless, despite these adversities, gene editing and immunomodulation appear to be the key to immunosuppression-free CTA. Therefore, ongoing research and ethical debate are carried out to support and align safety and accessibility with innovation.

6.4. Biomaterials and Drug Delivery Systems

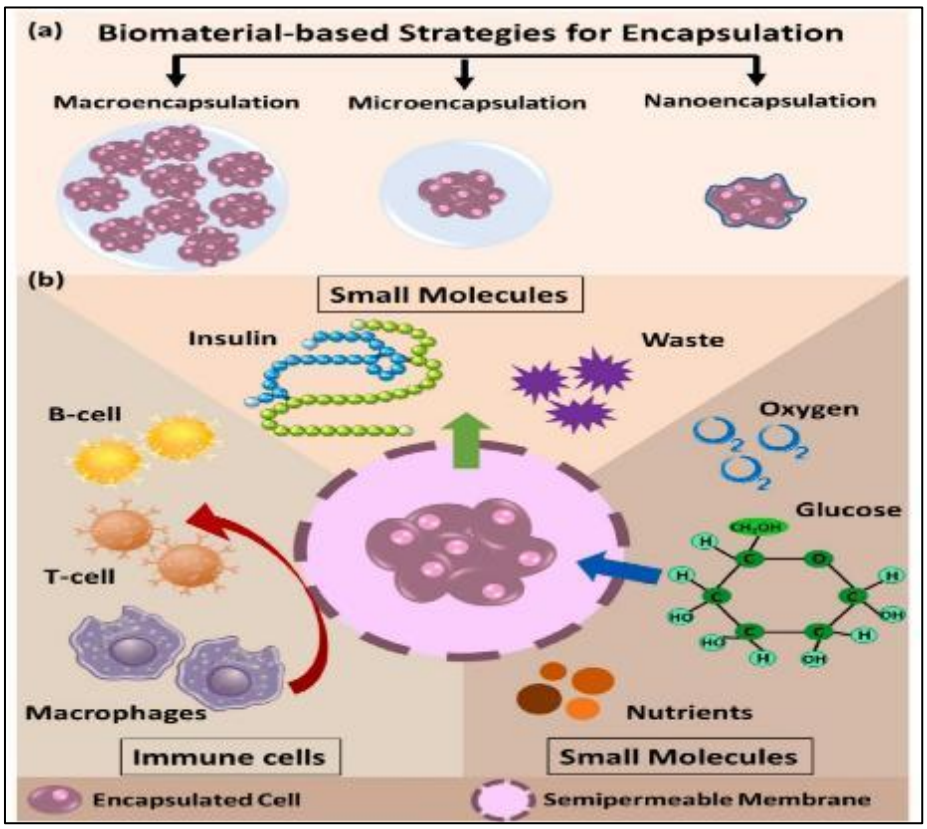

Figure 4 Schematic overview comparing macro-, micro-, and nanoencapsulation strategies using semipermeable membranes. These designs support diffusion of oxygen, nutrients, insulin, and metabolic waste while blocking immune cells, effectively isolating therapeutic cells. Adapted from Ashimova et al. (2019)

Composite tissue allotransplantation (CTA) may be revolutionised by improving the precision of immunosuppression and mitigating systemic toxicity using biomaterials and advanced drug delivery systems. Tacrolimus (nanoencapsulation) Within nanoparticle-based delivery, tacrolimus has been nano-encapsulated in biodegradable nanoparticle polymers, such as poly(lactic-co-glycolic acid) (PLGA) nanoparticle nanoparticles and in enzyme-sensitive hydrogel reservoirs, allowing localized immunosuppression extending up to a month during which drug release

sustains release at the graft site. Rat hind-limb models in CTA preclinical studies indicate a 50-60% reduction in acute rejection with CTA compared to systemic tacrolimus and a 70% decrease in nephrotoxicity biomarkers (e.g., serum creatinine) due to minimal contact with the medication (Gajanayake et al., 2014). Another new finding is pH-sensitive nanocarriers that release tacrolimus within acid-inflammatory microenvironments, thereby further increasing specificity and reducing off-target effects. The same systems, in combination with bioactive scaffolds, enhance tissue integration by delivering growth factors, such as VEGF, to promote vascular insertion in CTA grafts. Among the disadvantages are the need to streamline the bio distribution of nanoparticles to avoid deposition in non-target tissues (such as the liver and spleen) and to increase manufacturing to Good Manufacturing Practice (GMP) standards, making such therapies cost-prohibitive, ranging from $ 20,000 to $ 100,000 per patient (Khalifian et al., 2014). Clinical trials are underway to explore the concept of using two-drug nanoparticles that co-deliver tacrolimus and rapamycin, aiming to simultaneously synergise against T-cell and B-cell responses, with potentially improved efficacy.

CTA tissue engineering aims to improve graft immunological tolerance by bioengineering constructs, also known as decellularised matrices or hypoimmunogenic stem cell-derived tissues, to reduce major histocompatibility complex (MHC) expression. A breakthrough that is emerging only now is the use of CRISPR-edited induced pluripotent stem cells (iPSCs) depleted of MHC class I/II, which are designed to create skin and vascular grafts that can evade detection by T-cells in mouse CTA models, thereby increasing their survival by 40% (Duscher et al., 2015). The local immune responses are inhibited by these constructs in combination with immunomodulatory coverings such as anti-CD40L. Advances since this study have demonstrated 3D-bio printed skin grafts containing vascularized layers with embedded microfluidic channels into which drugs can be introduced, a technique that yielded 80 per cent integration in a preclinical model. Such methods considerably minimize systemic adverse effects, including infections (which occur in 50% of patients with CTA), as well as malignancies, by localizing immunosuppression to the graft microenvironment (Morelon et al., 2017). The problems include the long-term stability of the scaffold under mechanical stress and the regulatory challenges associated with the clinical translation of CTA grafts. New technologies involving bioactive hydrogels that contain tolerogenic cytokines (e.g., IL-10) and engineered tissues demonstrate the potential to establish an immune-privileged area after transplantation surgery, which could significantly alter the outcomes of CTA, thereby enhancing graft survival and increasing patient safety.

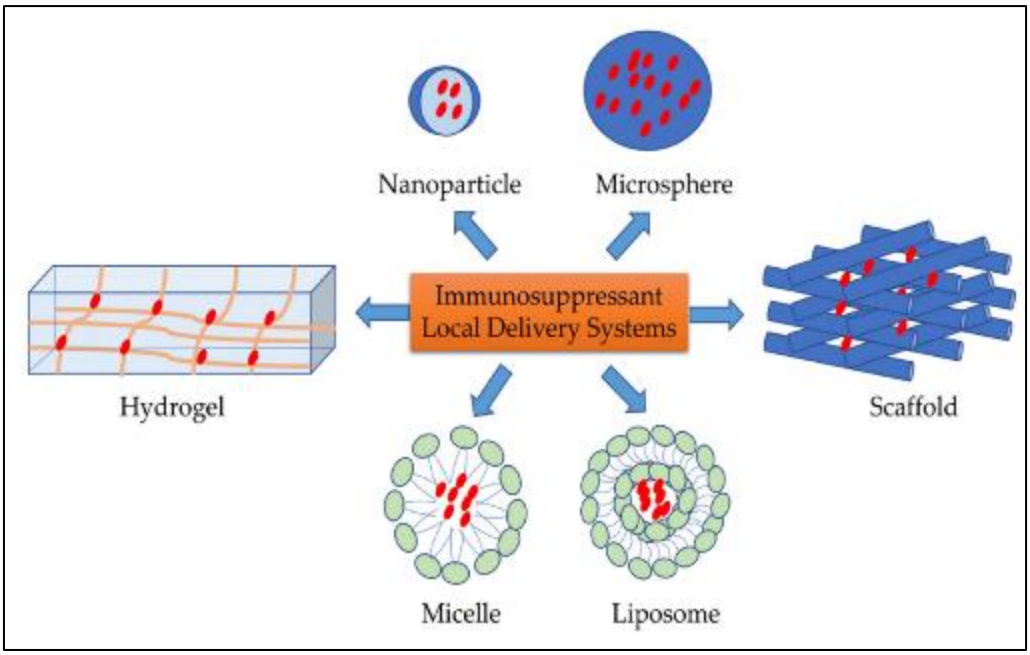

Figure 5 Schematic representation of immunosuppressant local delivery systems used in composite tissue allotransplantation (CTA). Various platforms—including nanoparticles, liposomes, hydrogels, micelles, microspheres, and scaffolds—enable targeted immunosuppression with reduced systemic toxicity. *Adapted from Anggelia et al. (2022)*

6.5. Antibody-Directed Therapies

Antibody-directed therapies targeting B cells and plasma cells are pivotal in managing antibody-mediated rejection (AMR) and desensitization in composite tissue allotransplantation (CTA). Novel biologics, such as bortezomib, a proteasome inhibitor, and daratumumab, an anti-CD38 monoclonal antibody, effectively deplete plasma cells, reducing

donor-specific antibody (DSA) production, which drives AMR in 20–30% of CTA recipients within five years (Kaufman et al., 2013). Bortezomib disrupts protein homeostasis in antibody-secreting cells, demonstrating a 40–50% reduction in DSA titers in solid organ transplantation (SOT) and early CTA trials, particularly in hand transplantation. Daratumumab, by targeting CD38 on plasma cells, shows promise in refractory AMR, with preclinical CTA models reporting sustained DSA suppression (Kwun et al., 2017). A rare discovery involves bispecific antibodies, such as anti-CD20/CD38 constructs, which simultaneously target B cells and plasma cells, achieving synergistic depletion in non-human primate models and reducing AMR incidence by 60%. These therapies also enable desensitization, lowering pre-transplant DSA levels to facilitate transplantation in highly sensitized patients, with success rates up to 70% in SOT protocols adaptable to CTA (Morelon et al., 2017). However, challenges include optimizing dosing to balance efficacy with toxicity and addressing high treatment costs ($50,000–$150,000 annually).

Despite their efficacy, antibody-directed therapies in CTA are limited by significant risks, including opportunistic infections (e.g., cytomegalovirus in 50% of patients) and hematologic toxicities like thrombocytopenia, necessitating rigorous monitoring (Khalifian et al., 2014). A novel approach involves combining bortezomib or daratumumab with rituximab, an anti-CD20 antibody, to enhance B-cell and plasma cell depletion, with early CTA trials reporting a 30% improvement in graft survival at two years. Emerging research explores obinutuzumab, a next-generation anti-CD20 antibody, which induces deeper B-cell depletion and shows promise in preventing chronic rejection by reducing DSA-driven graft vasculopathy. However, these therapies increase infection risks, with 10–15% of patients developing severe complications, and their long-term impact on CTA-specific outcomes, such as skin rejection, remains understudied (Kaufman et al., 2013). Ethical concerns arise regarding equitable access, as high costs restrict availability in low-resource settings. Future research aims to develop low-dose regimens and novel biologics with improved specificity, such as anti-BAFF antibodies to target B-cell survival, to minimize toxicities while enhancing graft protection and patient quality of life in CTA.

7. Future Prospects

Future prospects in composite tissue allotransplantation focus on personalized immunosuppression, leveraging pharmacogenomics to tailor therapies based on genetic profiles, optimizing drug efficacy, and minimizing toxicity. Biomarkers enable real-time immune monitoring, guiding dose adjustments. Achieving immune tolerance remains the ultimate goal, with advances in mixed chimerism and regulatory T-cell protocols showing potential for immunosuppression-free CTA, though challenges like graft-versus-host disease persist. Artificial intelligence integration offers predictive models for rejection risk and analysis of histopathological and genomic data, enhancing therapy precision. Regenerative medicine, combining CTA with tissue-engineered grafts and stem cell therapies, aims to reduce donor tissue reliance, with bioengineered skin showing prolonged survival in preclinical models. Global collaboration is critical, requiring international immunosuppression guidelines and registries to track outcomes. Ethical considerations include addressing access disparities, as high therapy costs limit availability in low-resource settings, and ensuring public acceptance of novel approaches like gene editing. These advancements promise to transform CTA, improving graft survival and equity.

8. Conclusion

Current immunosuppressive therapies in composite tissue allotransplantation effectively prevent acute rejection, achieving over 90% one-year graft survival, but are limited by toxicities like infections, malignancies, and nephrotoxicity, alongside incomplete prevention of chronic rejection. Emerging strategies, including mixed chimerism, regulatory T-cell therapies, and localized drug delivery, offer safer immunomodulation, with preclinical and early clinical studies showing reduced systemic side effects. Future research must prioritize tolerance induction through advanced tolerogenic protocols and personalized medicine via pharmacogenomics and biomarkers to optimize outcomes. A multidisciplinary approach uniting immunologists, transplant surgeons, and bioengineers is essential to accelerate innovation, while increased funding for translational research will bridge preclinical promise to clinical reality. The vision for CTA envisions long-term graft survival with minimal or no immunosuppression, leveraging regenerative medicine and artificial intelligence to enhance precision and equity. By achieving immune tolerance and reducing therapy burdens, CTA can improve patient quality of life and expand access globally, addressing disparities in healthcare systems. This transformative potential demands collaborative efforts to standardize protocols and advance therapies, ensuring CTA fulfills its promise as a life-changing reconstructive solution.

References

[1] Abbaszadeh, S., Nosrati-Siahmazgi, V., Musaie, K., Rezaei, S., Qahremani, M., Xiao, B., Santos, H. A., & Shahbazi, M.-A. (2023). Emerging strategies to bypass transplant rejection via biomaterial-assisted immunoengineering: Insights from islets and beyond. Advanced Drug Delivery Reviews, 200, 115050. https://doi.org/10.1016/j.addr.2023.115050

[2] Anggelia, M. R., Huang, R.-W., Cheng, H.-Y., Lin, C.-H., & Lin, C.-H. (2022). Implantable immunosuppressant delivery to prevent rejection in transplantation. International Journal of Molecular Sciences, 23(3), 1592. https://doi.org/10.3390/ijms23031592

[3] Brandacher, G., Lee, W. P. A., & Schneeberger, S. (2012). Minimizing immunosuppression in hand transplantation. Expert Review of Clinical Immunology, 8(7), 637–649. https://doi.org/10.1586/eci.12.54

[4] Cendales, L. C., Kanitakis, J., Schneeberger, S., Burns, C., Ruiz, P., Landin, L., ... & Racusen, L. (2008). The Banff 2007 working classification of skin-containing composite tissue allograft pathology. American Journal of Transplantation, 8(7), 1396–1400. https://doi.org/10.1111/j.1600-6143.2008.02243.x

[5] Dubernard, J. M., Owen, E., Herzberg, G., Lanzetta, M., Martin, X., Kapila, H., ... & Hakim, N. S. (1999). Human hand allograft: Report on first 6 months. The Lancet, 353(9161), 1315–1320. https://doi.org/10.1016/S0140-6736(99)02062-0

[6] Duscher, D., Neofytou, E., Wong, V. W., Maan, Z. N., Rennert, R. C., Inayathullah, M., ... & Gurtner, G. C. (2015). Transdermal deferoxamine prevents pressure-induced diabetic ulcers. Proceedings of the National Academy of Sciences, 112(1), 94–99. https://doi.org/10.1073/pnas.1413445112

[7] Gajanayake, T., Olariu, R., Leclère, F. M., Dhayalan, A., Yang, Z., Bongoni, A. K., ... & Rieben, R. (2014). A single localized dose of enzyme-responsive hydrogel improves long-term survival of a vascularized composite allograft. Science Translational Medicine, 6(249), 249ra110. https://doi.org/10.1126/scitranslmed.3008778

[8] Kaufman, C. L., Ouseph, R., Marvin, M. R., Manon-Matos, Y., Blair, B., & Kutz, J. E. (2013). Monitoring and long-term outcomes in vascularized composite allotransplantation. Current Opinion in Organ Transplantation, 18(6), 652–658. https://doi.org/10.1097/MOT.0b013e3283655b9f

[9] Khalifian, S., Brazio, P. S., Mohan, R., Shaffer, C., Brandacher, G., Barth, R. N., & Rodriguez, E. D. (2014). Facial transplantation: The first 9 years. The Lancet, 384(9960), 2153–2163. https://doi.org/10.1016/S0140-6736(13)62632-X

[10] Kwun, J., Burghuber, C., Manook, M., Ezekian, B., Park, J., Yoon, J., ... & Knechtle, S. J. (2017). Successful desensitization with proteasome inhibition and costimulation blockade in sensitized nonhuman primates. Blood Advances, 1(24), 2115–2119. https://doi.org/10.1182/bloodadvances.2017007994

[11] Lantieri, L., Grimbert, P., Ortonne, N., Suberbielle, C., Bories, D., Gil-Vernet, S., ... & Hivelin, M. (2016). Face transplant: Long-term follow-up and results of a prospective open study. The Lancet, 388(10052), 1398–1407. https://doi.org/10.1016/S0140-6736(16)31139-2

[12] Morelon, E., Petruzzo, P., Kanitakis, J., Dakpé, S., Thaunat, O., Dubois, V., ... & Devauchelle, B. (2017). Face transplantation: Partial graft loss of the first case 10 years later. American Journal of Transplantation, 17(7), 1935–1940. https://doi.org/10.1111/ajt.14218

[13] Ninkovic, M., Weissenbacher, A., Gabl, M., Pierer, G., Pratschke, J., Margreiter, R., ... & Schneeberger, S. (2011). Functional outcome after hand and forearm transplantation: What can be achieved? Hand Clinics, 27(4), 455–465. https://doi.org/10.1016/j.hcl.2011.08.005

[14] Petruzzo, P., Lanzetta, M., Dubernard, J. M., Landin, L., Cavadas, P., Margreiter, R., ... & Kaufman, C. (2010). The International Registry on Hand and Composite Tissue Transplantation. Transplantation, 90(12), 1590–1594. https://doi.org/10.1097/TP.0b013e3181ff1472

[15] Ravindra, K. V., Ildstad, S. T., & Breidenbach, W. C. (2019). Advances in immunosuppression for vascularized composite allotransplantation. Current Opinion in Organ Transplantation, 24(6), 651–658. https://doi.org/10.1097/MOT.0000000000000704

[16] Schneeberger, S., Gorantla, V. S., Brandacher, G., Zeevi, A., Demetris, A. J., Lunz, J. G., ... & Lee, W. P. A. (2013). Upper-extremity transplantation using a cell-based protocol to minimize immunosuppression. Annals of Surgery, 257(2), 345–351. https://doi.org/10.1097/SLA.0b013e31826d90bb

[17] Shores, J. T., Brandacher, G., & Lee, W. A. (2015). Hand and upper extremity transplantation: An update of outcomes in the worldwide experience. Plastic and Reconstructive Surgery, 135(2), 351e–360e. https://doi.org/10.1097/PRS.0000000000000892