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Techniques and Innovations in Flap Engineering: A Review

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Background: Currently, the gold standard for complex defect reconstruction is autologous tissue flaps, with vascularized composite allografts as its highest level. Good clinical results are obtained despite considerable obstacles, such as limited donor sites, donor site morbidity, and complex operations. Researchers in the field of tissue engineering are trying to generate novel tissue flaps requiring small or no donor site sacrifice. At the base of existing technologies is the tissue's potential for regeneration and neovascularization.

Methods: A review was conducted identifying relevant published articles in PubMed on the subject of flap engineering, with the focus on plastic surgery. This review article surveys contemporary technologies in flap engineering, including cell sheet technology, prefabricated flaps, and tissue engineering chambers.

Conclusions: Some of the described procedures, though not yet ready for clinical use, are certainly ready for trial in large animal models and even human studies. Tissue engineering is a promising field for the handling of large and complex tissue defects. *(Plast Reconstr Surg Glob Open 2022;10:e4523; doi: 10.1097/GOX.0000000000004523; Published online 21 September 2022.)*

INTRODUCTION

Plastic and reconstructive surgery deals with the restoration of tissue defects in the human body precipitated by different causal agents, such as trauma, infections, tumor resection, or congenital deformities. Autologous tissue flaps are currently the gold standard for these kinds of reconstructions, but carry major disadvantages such as limited donor sites, donor site morbidity, and complex and prolonged operations with their own risks.[1,2]

While attempting to overcome these obstacles, researchers in the field of tissue engineering are trying to generate novel tissue flaps requiring small or no donor site sacrifice. At the base of existing technologies is the tissue's potential for regeneration. Each method uses this characteristic through a different approach, trying to generate a well-perfused, stable, and functional tissue flap.

The basic construction units of flap engineering are extracellular matrix (ECM), viable cells, and adequate vascular pedicle and capillary network. The goal of flap construction has not yet been reached. However, vast research is being conducted, and numerous concepts are being examined, utilized, and developed in this new scientific frontier.[3,4]

The objective of this review is to survey novel technologies and summarize the recent research that has been conducted on this topic. The outline of this review was constructed according to the main technical approach. Leading methods are schematically described in Figures 1–5. Each method's current status and comparison between different approaches are described in Tables 1 and 2.

METHODS

A review was conducted identifying relevant published articles on the subject of flap engineering, with the focus on plastic surgery. Previous review articles were excluded. Articles discussing engineering of tissue grafts, scaffolds without a vascular pedicle, or wound healing with stem cell assistance were not included. This review surveys contemporary technologies in flap engineering, including cell sheet technology, prefabricated flaps, and tissue

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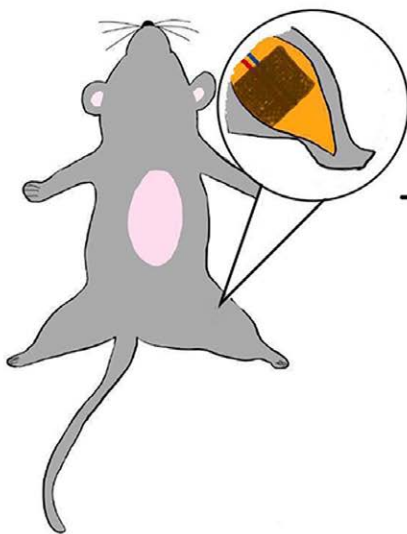
engineering chambers. Articles were arranged according to the main technique used, and evaluated according to their current status (in vitro versus in vivo and animal model versus human subjects) and in terms of future clinical applicability. (**See appendix, Supplemental Digital Content 1**, which displays a list of abbreviations, **http://links.lww.com/PRSGO/C155**.)
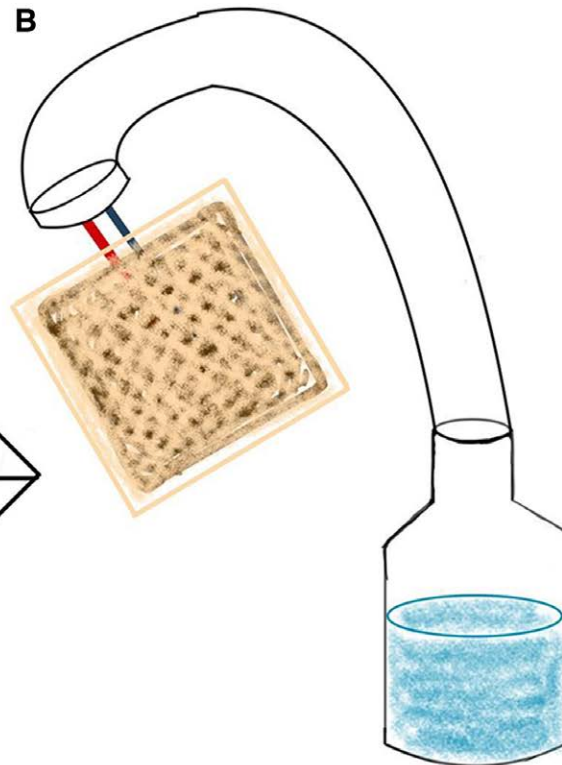
FLAP ENGINEERING TECHNIQUES

Cell Sheet Technology

The first step in flap engineering is the construction of simple vascularized, possibly cultured, or regenerated skin substitutes that can serve as cutaneous pedicled flaps and be utilized to cover large skin defects. In 2015, Fujisawa et al[5] described a novel technique for extracorporal skin flap fabrication. Until then, exogenous materials for skin restoration consisted of cultured patient epidermis, characterized by a poor aesthetic outcome, rough texture, and short durability. Another solution was cultured epidermal cells on a collagen-fibroblast structure to form a thicker sheet, resembling natural skin. The drawback of these techniques was the absence of a distinct vascular pedicle that could be transposed as a flap with immediate postimplantation perfusion.
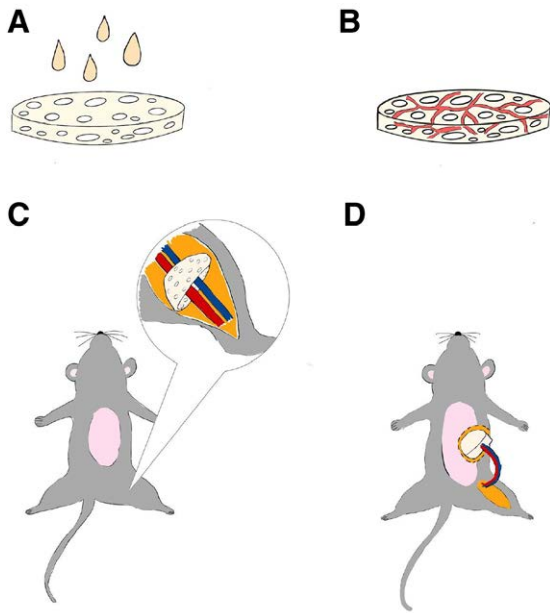

Takeaways

Question: Autologous tissue flaps are currently the gold standard for complex defect reconstructions, but carry major disadvantages such as limited donor sites, donor site morbidity, and complex and prolonged operations with their own risks.

Findings: Researchers in the field of tissue engineering are trying to generate novel tissue flaps requiring small or no donor site sacrifice.

Meaning: Tissue engineering is a promising field for solving large and complex tissue defects and will hopefully make native tissue sacrifice obsolete in the future.

Fujisawa et al sandwiched femoral blood vessels in between two layers of artificial dermis, in vivo, which were inserted into a porous chamber. The content of this chamber was then harvested with its nourishing vessels and connected ex-vivo to a perfusion tube in a bioreactor. Simultaneously, it was overlaid with an epidermal sheet cultured from animal cells. After 3–10 days, the fabricated flaps showed good keratinization and a structural similarity to native skin (Fig. 1).

This method, however, is still inapplicable for clinical implementation, as the vascular beds are harvested,

Fig. 1. Cell sheet technology. A, Vascular bed fabrication: pedicle between two sheets of artificial dermis. B, Connection to perfusion bioreactor and transplantation of an epidermal sheet onto the vascular bed.

Fig. 2. Prefabricated flaps. A, Cells seeded onto a scaffold. B, Formation of a capillary network. C, Implantation around a vascular pedicle. D, Flap transposition.

sacrificing their nourishing vessels. Also, the size of the produced flaps is not indicated, and this method may present a surface area limitation toward live-scale reconstructions.

PREFABRICATED FLAPS

Prefabrication is the implantation of a vascular pedicle under a cutaneous flap to construct a new axial flap through the process of revascularization.[6] This contrasts with prelamination, where composite grafts are implanted in a site with an existing vascular pedicle and later transferred to a recipient site.[7,8] In 2004, Staudenmaier et al[9] attempted to create a prefabricated flap consisting of different tissue types. First, chondrocytes were seeded on a scaffold for in vitro cultivation. Second, a microvascular anastomosis was made between a native artery and vein, creating a vessel loop that was implanted under a random skin flap, thus creating a neovascularized axial flap. Eventually, bioengineered cartilage was implanted under this flap. Analysis showed a good connection between the vessel loop and random vasculature, creating a well-perfused flap. Cartilage constructs showed stable and elastic properties, resembling hyaline cartilage, with robust blood supply.

The critical importance of prevascularization was demonstrated by Mandlik et al,[10] who tried to prefabricate a full-thickness skin flap. They used a nonseeded scaffold
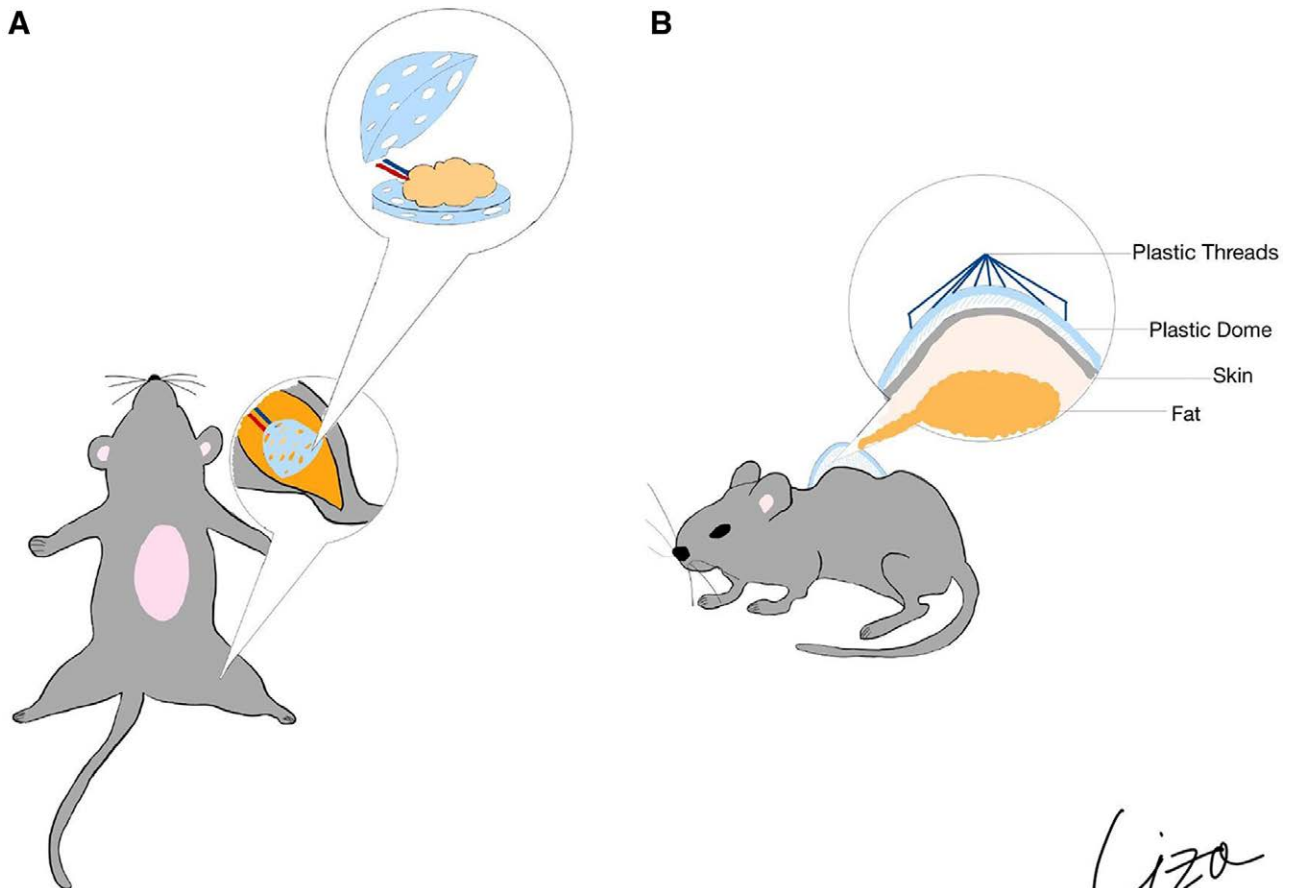
Fig. 3. Tissue engineering chambers. A, Traditional tissue engineering chamber. B, External suspension device.
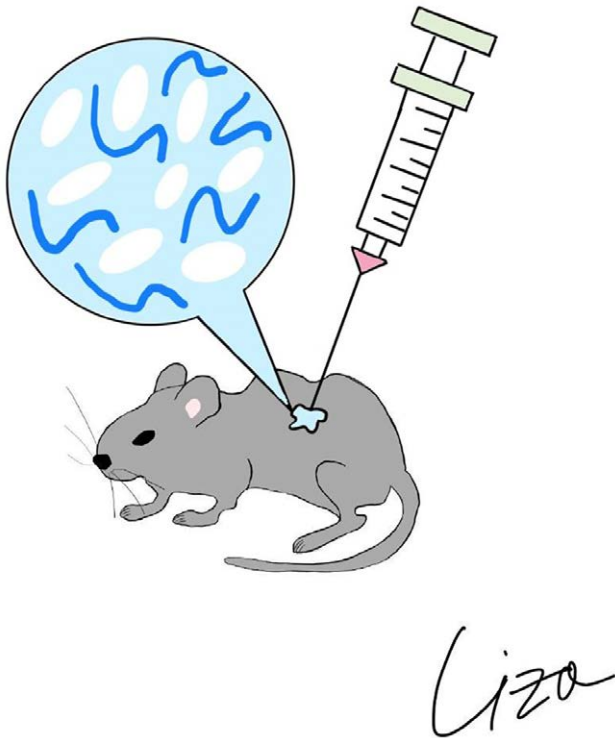
Fig. 4. Hydrogels. Composite nanofiber-hydrogel scaffolds injected into study animals' backs.

on top of a vessel loop and transferred it as a flap. All flaps underwent necrosis in 72 hours. This proved that a large nourishing vessel is insufficient for prefabricated flap durability. A preformed capillary network is a crucial component for flap survival.

Naujokat et al[11] performed a trial of bone tissue engineering using a scaffold implanted in the omentum. Bone scaffolds were soaked in bone marrow aspirate and bone morphogenetic protein and wrapped with either collagen membranes or autogenous periosteum. This layer served both as a shield to the scaffold and a supportive structure for bone regeneration. Constructs were implanted in omental flaps of study animals. Previous research has showed that omental tissue is rich in pluripotent cells and has a great potential for regeneration.[12] These studies showed positive results of new bone formation, enhanced by periosteal flap wrapping. Alas, the procedure requires at least two operations with access to the intraabdominal cavity, which may be intolerable in humans.

Shandalov et al[13,14] used a three-dimensional (3D) biodegradable scaffold seeded with myoblasts, fibroblasts, and endothelial cells (ECs) in different combinations, to create muscle tissue. Following in vitro culturing and formation of a capillary network, the graft was wrapped around a study animal's artery and vein. Only 1 week after grafting, all grafts showed good vascularization. When tissue was transferred to the recipient site, best flap characteristics were observed in the group with all three cell types. Despite excellent results in survival and mechanical performance, the flap lacked similarity in size to clinically relevant defects, which require an enlargement of scale by 200–400 fold. A

schematic illustration of flap prefabrication is shown in Figure 2.

Zhang et al[3] used collagen-chitosan scaffolds seeded with human adipose-derived stem cells (ASCs) and polymer microspheres containing vascular endothelial growth factor (VEGF), as a source for neovascularization. The VEGF group showed a larger quantity of fat tissue compared with controls. This study achieved the creation of adipose tissue and ECM in vivo and improved vascularization through VEGF exposure.

Stem cell type was also evaluated as a promotor of flap construction. Freiman et al[15] proposed seeding scaffolds with the combination of human adipose microvascular endothelial cells (HAMECs) and fat tissue-derived mesenchymal stem cells (MSCs). Based on previous research, HAMECs have a better potential to promote angiogenesis than large-vessel ECs, while MSCs contribute to microvascular density. Flaps of the bicellular study group demonstrated greater tensile strength compared with both monoseeded groups. This study proved superiority of seeding scaffolds with two types of stem cells over a single type of cell infusion.

An attempt to construct an autologous free flap was made by Kong et al.[16] Biodegradable scaffolds were seeded with human-induced pluripotent stem cell-derived endothelial cells (ECs) and human vascular smooth muscle cells (hvSMCs) in vitro. As soon as 24 hours postseeding, a capillary network was formed in the scaffold. After in vivo implantation, an integration between human capillaries from the cell-seeded scaffold and mouse native pedicle was observed. This inosculation proved that an in vitro human-derived vascular network could be integrated in vivo and produce a prefabricated free flap. However, it is not yet known how durable the created capillaries are.

A further advance toward complex defect repair was made by Redenski et al.[17] They attempted to fabricate a de novo flap consisting of both bone and soft tissue. First, synthetic scaffolds were seeded with a combination of dental pulp stem cells and HAMECs to induce prevascularization in vitro. Then, scaffolds were implanted around vessels in vivo, and a decellularized bone scaffold was imbedded. Formed tissues showed good vascularization and integration with host capillaries. Finally, the composite flaps were rotated and mobilized to a recipient site for bone defect repair. In this study, besides regeneration of the bone defect, constructs were also enveloped by newly formed soft tissue. This was the result of a proregenerative environment, which supported muscle fiber regeneration and penetration from adjacent tissues. Muscle fibers were organized in the newly formed tissue, without caving in the bone defect. This approach to repairing complex tissue defects is promising and should be further investigated on larger models, to be relevant to clinical use.

A major component of flap survival is its vascular supply. A crucial step toward fabricating an implantable vascular network was made by Szklanny et al.[18] Using 3D printing with biological inks, they have created constructs with vessel-mimicking scaffolds seeded with ECs, and microsurgically connected them to animals' native vessels. Vasculature remained patent for 2 weeks and maintained blood flow. This work showed the ability to create a fully
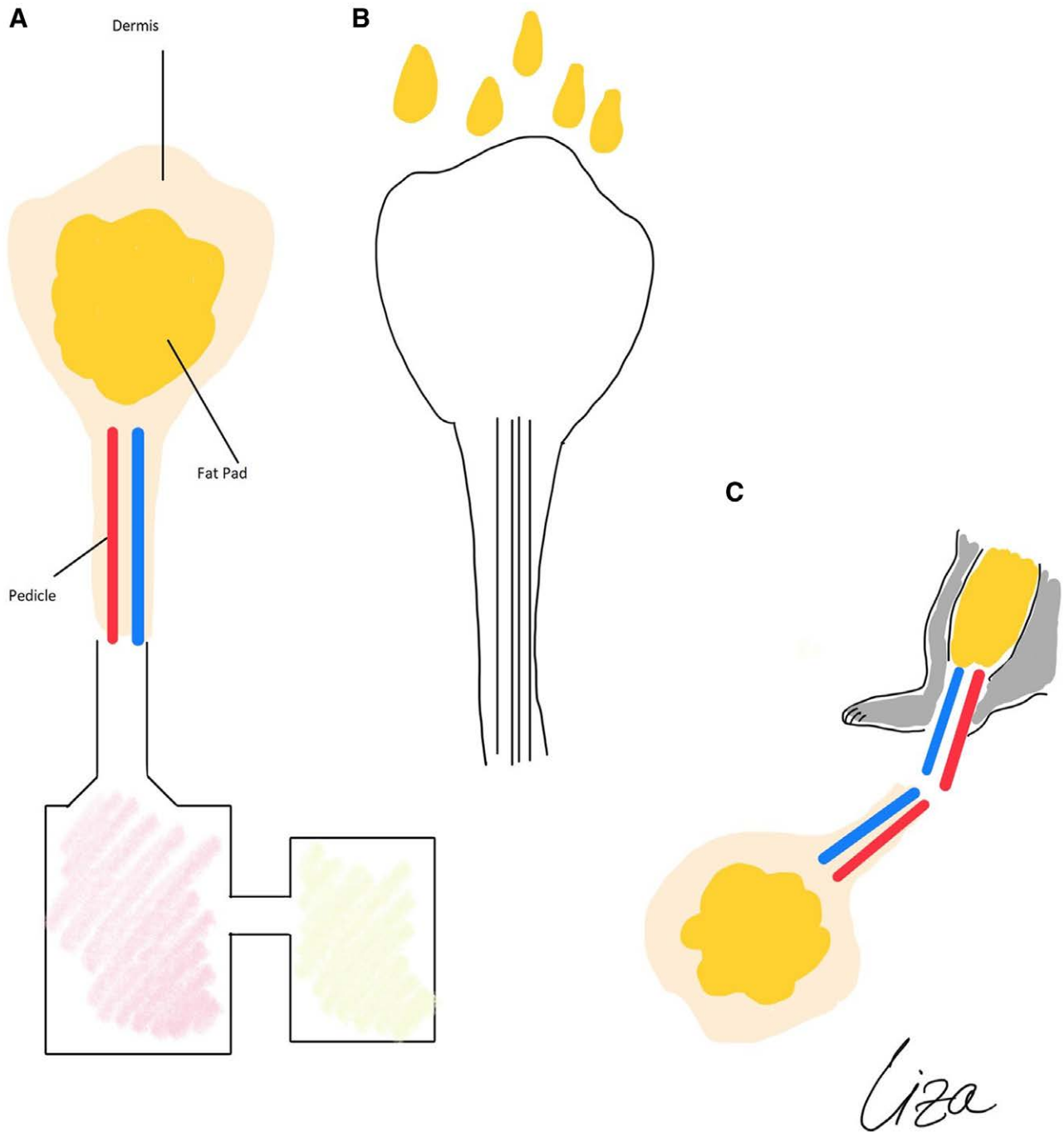
Fig. 5. Flap decellularization. A, Flap perfusion with decellularizing solutions. B, Recellularization with stem cells. C, Microsurgical flap transplantation.

vascularized bioengineered flap, anastomosed to a native host artery.

TISSUE ENGINEERING CHAMBER

Tissue engineering chamber (TEC) techniques promote flap growth and tissue regeneration in vivo. The technique uses a perforated dome-shaped hollow chamber inserted in vivo around a tissue flap (Fig. 3A). Besides providing a protected space for flap growth, mechanical forces inside the TEC encourage angiogenesis and thereby adipogenesis. These flaps can be transposed and inset later for defect or organ reconstruction.

In 2011, Findlay et al[19] used a TEC filled with small-volume (5 mL) fat flaps, implanted on an arteriovenous ligated pedicle. Inclusion of a vascular pedicle increased the success in flap growth, reaching a fat tissue volume of over 50 mL. However, they also showed that the TEC promotes the presence of a fibrous capsule around the flap, that even though it regresses over time, may still be an obstacle in a clinical setting.

Table 1. Current Stages of Described Techniques

Modality	Current Stage of Experiments	Ready for Human Experiments?
Cell sheet technology	In vivo (combined with ex-vivo stage), small animals (rats)	No
Prefabricated flaps	In vivo (combined with ex-vivo stage), small animals (rabbits)	No
TEC	In vivo, human studies	Yes
External suspension TEC	In vivo, small animals (rabbits)	No
Hydrogels	In vivo (combined with ex-vivo stage), small animals (rabbits)	Yes
Decellularized flap matrix	In vivo (combined with an ex-vivo stage), large animals (pigs), cadaveric studies	No

Doldere et al[20] tested the long-term survival of such flaps. They showed good flap survival over a 20-week period, both when left in situ and when transferred to a recipient site after 10 weeks.

Tanaka et al[21] also tested the long-term durability of TEC-produced flaps. Twelve-week-old flaps were extracted from their chambers, transferred on their pedicle, and followed for 5 months in vivo. The flaps maintained their shape and volume, increased in density, and underwent further maturation.

An important finding of this study was the presence of lipid droplets and fibroblast-like cells with internal lipid accumulations in the arterial wall. Another observation was that adipose tissue expanded from the center of the flap to its periphery, that is, from the pedicle outward. These two findings combined are suggestive of the presence of circulating progenitor cells that are able to differentiate into adipocytes and also assist in the formation and growth of adipose tissue, without the need for exogenous stem cell implantation into the chamber.

To elevate the TEC model, Morrison et al[4] implemented this technique for breast reconstruction in humans. Although all flaps showed good vascularity, a thick capsule restraining flap growth was formed. The authors proposed different solutions, each with its own risks. The use of TEC was proved to be applicable on human patients but still requires further research for its realization.

Mechanical traction along with relative hypoxia stimulates angiogenesis and adipogenesis in a TEC. To demonstrate this concept, Lee et al[22] performed an experiment using tractable chambers. This experiment supports the hypothesis by which fat flaps placed in receding chambers develop additional adipose tissue. Nonetheless, the origin of the additional adipocytes was not characterized, and whether true de novo adipogenesis occurs is yet to be determined.

To reduce foreign body reaction to silicone TECs, several solutions were proposed. Luo et al[23] used nanofibrous meshes on the inner side of TECs, resulting in thinner capsules, less inflammation, and larger flaps. Qin et al[24] implanted a biodegradable film on the inner surface of TECs, separating native tissue from the silicone chamber, which also led to doubling in flap size and to a thinner, less constraining capsule. Faglin et al[25] used a bioabsorbable polymer to produce degradable TECs. The maximal volume of absorbable TEC flaps did not differ from the traditional ones.

A further challenge to the TEC method was made by Lei et al.[26] They inserted ligated vessels with fat grafts into a chamber, using fat grafts instead of less available native flaps. This study demonstrated that ligated vessels can serve as a nourishing source to fat flap creation inside a TEC. Thus, this approach may enable the implementation of the TEC technique to irradiated or largely dissected areas, when native fat flaps are not available, for standard TEC regeneration.

Chang et al[27] made an attempt at dissection-free, that is, without isolation of a distinct vascular pedicle, adipose tissue engineering. Lack of dissection limits the trauma needed to promote adequate host response for regeneration. An integration of TEC with growth factors and novel substrates was made to overcome this problem. Gelatin cryogel (GC) was coated with a polydopamine layer, with the GC mimicking adipose tissue stiffness, and the polydopamine aiding in platelet immobilization. PRP and ADSCs were scattered over the scaffolds to promote regeneration. Chambers were implanted subcutaneously in rats, without tissue or vessel dissection, with an opening providing contact with a native fat pad. After 12 weeks, GC remainders were fully absent. Newly formed adipose tissue was structurally similar to native fat tissue, with a mature vascular network inside. This method can promote the TEC technique toward human application, eliminating the need for flap and vessel manipulations.

An additional manipulation of the method was introduced by Debels et al,[28] TECs with vessel loops, fat grafts, and acellular hydrogel (Adipogel). Although adipocyte and ASC death was noted in early stages, by 12 weeks, the tissue contained live adipocytes, which were not survivors of the original graft, but newly formed cells. Adipogenesis has most likely derived from circulating, surrounding tissue or adventitial precursor cells, stimulated by the chamber and its contents. Although not providing enough nourishment for graft survival, it effectively stimulates neovascularization and adipogenesis, which, in turn, construct a newly formed viable flap.

EXTERNAL SUSPENSION TEC

Two challenges to the TEC technique are the limited volume of tissue that can be grown inside and the foreign body response to the synthetic chambers. For these reasons, Wan et al[29] generated an adipose tissue flap using an external suspension device (Fig. 3B). As the initial trigger for angiogenesis and adipogenesis is postulated to arise from the local destruction of the flap-preparing surgery, researchers assumed that further implantation of an internal device is not necessary. After creating an adipose flap in rabbits, external chambers were placed around the flap area and attached to the animals' skin. Chambers were held under known tension and adjusted to keep it at a constant level. Traditional TECs served as controls. After 4 weeks, all devices were removed, the flaps remaining in place.

Tissue analysis revealed that in both groups, the flaps grew well, even after device removal. Tissue in the study

Table 2. Comparison of Described Techniques

Modality	Use of Biomaterials	Use of Stem Cells	Time to Flap Maturation	Advantages	Limitations	Future Directions
Cell sheet technology	Artificial dermis, epidermal sheet	No	~30 d	Good aesthetic outcome	Sacrifice of large vessels needs to be buried in vivo for 3 weeks before ex-vivo development, unknown surface is for success	Use of a single-vessel pedicle, large surface area experiment
Prefabricated flaps	Bioscaffold	Optional	1–6 wk	Ability to create a complex tissue flap, robust vascularization	Inflammatory response to the scaffold	Large animal models, Biodegradable scaffolds
TEC	Polymer chamber	Optional	6–16 wk	Ability to create large-volume flaps, good vascularization, ability to control shape	Inflammatory response to the TEC, fibrous capsule formation	Biodegradable TECs, use of biocompatible membranes
External suspension TEC	Polymer chamber	No	4–40 wk	Large-volume flaps, low invasivity, ability to reshape flap	Need for an external device, regular tension adjustment	Large-animal studies
Hydrogels	NHC	Yes	2–3 wk	Possible percutaneous administration, minimally invasive, minimal inflammatory response	Small tissue volumes	Large animal and human application, combination with autologous tissue reconstruction
Decellularized flap matrix	Decellularized tissue	Yes	3 wk–3 mo	No need for autologous donor tissue, preserved 3-dimensional and microscopic architecture	Incomplete removal of nuclear material, loss of pedicle patency	Improvement of vascularization and pedicle patency, immunocompetent animal studies

group was significantly larger and softer at any time point (over a twofold difference) throughout the entire period, while the control group exhibited a somewhat tough tissue, which softened over time. The control group had an initially thicker capsule around the device, which may have limited nutrient supply to the flap and limited its growth. The hallmark of external suspension TEC is the evasion of the foreign body response.

HYDROGELS

Hydrogels are water-swollen polymeric materials that are porous and maintain a 3D structure. They are popular and promising scaffold matrices due to their elastic properties. Alas, their disadvantage lies in their relatively high density and small pore size, which limits regeneration by cellular infiltration.

In an attempt to overcome these limitations, Li et al[30] generated a Nanofiber-Hydrogel Composite (NHC) trying to mimic the microarchitecture and mechanical properties of soft tissue ECM. This study demonstrates the utilization of the body's natural regenerative potential, with scaffold support, even when tested without exogenous cellular or growth factor injection (Fig. 4). It may serve as a biodegradable scaffold for regeneration, gradually replaced by native soft tissue. As this technique can be administered percutaneously, it may be a well-tolerated outpatient procedure for filling relatively small soft tissue defects. Perhaps, the addition of exogenous components could make this method appropriate for larger-volume defects.

An attempt to perfect the hydrogel technique was made by Nie et al[31] They mixed decellularized adipose tissue hydrogel with adipose tissue-derived extracellular vesicles and injected them subcutaneously in an animal model. The addition of adipose vesicles with their rich biological content improved angiogenesis and adipogenesis. These vesicles are derived from different cells in the fat tissue, such as adipose cells, vascular ECs, fibroblasts, etc. The functional similarity of the vesicles to their maternal cells may be key to the improved vascularization and tissue growth.

As there is no extrinsic vascular supply to ECM scaffolds, large defects cannot be covered and inner vascular supply is needed. Henn et al[32] implanted previously described NHCs into isolation chambers in vivo, together with an AV loop. This study proved the feasibility of creating an axial tissue flap using an injectable scaffold.

DECELLULARIZED FLAP MATRIX

The ideal tissue donors with no donor morbidity are cadaveric human allogeneic tissues. However, overcoming immune rejection is the major challenge in allotransplantation. Decellularized tissue offers one solution to this problem. Decellularization is a process performed by flushing the tissue with different solutions and buffers.[33–35]

Zhang et al[36] presented a novel technique for flap manufacturing—a decellularized skin/adipose flap. First, tissue was harvested from a euthanized animal with its vascular pedicle, irrigated, and processed so that no cells with nucleic acid material remained. This served as a scaffold. For the recellularization process, cells of human origin were added: human ASCs and human umbilical vein endothelial cells, for a 7-day culture. Later, cell-seeded scaffolds were implanted into athymic nude rats and revascularized microsurgically. Flaps were left in situ for up to 3 months. After explantation, vascular structures, including the main pedicle, microvascular network, and subdermal plexus, all remained intact (Fig. 5). This approach fulfills structural and vascular demands for a good flap with no donor site morbidity, while solving the immune response issues encountered with other allotransplantation methods.

This approach was later tested on a larger scale by Gerli et al.[37] A full upper extremity was harvested from a human cadaver and inserted into a custom-made perfusion decellularization chamber. Although good decellularization has been achieved, it must be improved to reach the gold-standard threshold.

Duisit et al[38] also performed decellularization on a complex organ—a porcine ear. After the decellularization, ear cartilage maintained its mechanical properties. Vasculature also appeared to remain intact and sustained physiologically relevant blood pressure after being implanted in vivo, and the graft did not elute an immune response. However, in their study, after reseeding, cell density remained poor.

Jank et al[39] attempted to create a full-thickness skin flap scaffold in a porcine model. Fasciocutaneous skin flaps with a dominant vascular pedicle were isolated, decellularized, and then recellularized and anastomosed to a recipient's blood supply. Flaps preserved their biomechanical properties and showed integration and neovascularization. However, full vascular perfusability was not established.

Although still far from clinical application, these experimental studies show the feasibility of creating large-scale complex bioengineered flaps, with preservation of the 3D architecture of the tissue and its elastic properties. Future studies should test the applicability of decellularization to larger animal models with higher cellular density, and, of course, the absence of an immune rejection. If successful, this strategy could form an "off-the-shelf" tissue bank for reconstructive therapy.

DISCUSSION

Several techniques in flap engineering were presented in this article. Although all appear very promising for use in defect reconstruction, not all are equally ready for clinical application. Current stages of each technique are presented in Table 1.

The decision may be complex as to the choice of the perfect flap engineering method. A good flap must fulfill many demands. Ideally, it should have abundant perfusion but no donor vessel sacrifice.[5] It must be sufficient in volume and satisfactory in terms of texture, shape, and appearance.[4,18] Also, it should not elicit an inflammatory or immune response and be well tolerated by the recipient organism.[22,31–33] Other qualities may also be desirable in the context of human application, such as relatively short production times, minimal surgical intervention, and a low complication rate. Unfortunately, no perfect method exists yet. Both types of TECs provide large-volume flaps, hydrogels can be potentially minimally invasive and suitable for outpatient settings, and prefabricated flaps have robust vascularization. Nonetheless, the same techniques may elicit an inflammatory response, provide inadequate tissue volume, or demand multiple surgical interventions. A comparison between the above-described methods is presented in Table 2.

We have some possible limitations in our review. In this review, we aim to describe the most original approaches to flap engineering, thus possibly disregarding older basic research. Additionally, tissue engineering research in disciplines adjacent to plastic surgery was not surveyed, leading to possible selection bias.

Each research group is trying to solve problems that occurred in preceding experiments and to perfect their results. Research in the field of bioengineering is progressing rapidly and is providing new and better understanding of the underlying biological processes. One must continue to be updated on the latest advancements and hope that they will be soon translated into clinical practice.

SUMMARY

Although numerous techniques exist for flap engineering, all methods rely on similar key components: ECM, cell migration, and neovascularization. However, none of the described procedures is ready for wide clinical use. Some are certainly ready for trial in large animal models and even in human studies. Tissue engineering is a promising field for solving large and complex tissue defects. Hopefully, in the future, it will make native tissue sacrifice obsolete.

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