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Skin regeneration, repair, and reconstruction: present and future

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Summary
Background Large skin defects caused by trauma (e.g., burns) or due to other reasons (e.g., tumor-related skin resections) require sufficient skin replacement. The constant improvement of innovative methods of skin replacement and skin expansion mean that even burn victims with more than 80% body surface burned have a realistic chance of survival. Due to these new developments, not only has survival rate increased, but also quality of life has increased tremendously over the past decades.
Methods The aim of this review is to present an overview of current standards and future trends concerning the treatment of skin defects. The main focus is placed on the most important technologies and future trends.
Results Autologous skin grafting was developed more than 3500 years ago. Several approaches and techniques have been discovered and established in burn care and plastic surgery since then. Great achievements were made during the 19th and 20th centuries. Many of these old and new techniques are still part of modern burn and plastic surgery. Today, autologous skin grafting is still considered to be the gold standard for many wounds, but new technologies have been developed, ranging from biological to synthetic skin replacement materials.
Conclusion Today, old and new technologies are available which allow us new treatment concepts. All this has led to the reconstructive clockwork for reconstructive surgery of the 21st century.

Keywords Reconstructive surgery · Burn surgery · Skin grafts · Fish skin · Skin replacement

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Introduction

Large skin defects caused by trauma (e.g., burns) or due to other reasons (e.g., tumor-related skin resections) require a sufficient skin replacement. The constant improvement of innovative methods of skin replacement and skin expansion technologies mean that even burn victims with more than 80% body surface burned have a realistic chance of survival. However, due to these new developments, not only has the survival rate increased, but also the quality of life has increased tremendously over the past decades.

Both in the case of extensive third-degree burns and other extensive skin losses, it has been shown that pleasing functional and cosmetic results cannot be achieved through conservative measures, but only through surgical procedures and modern skin replacement strategies.

In the past, deep burn wounds (grade 2b, grade 3) were routinely covered with autologous skin grafts directly after necrosectomy. Today, new technologies are available which allow us new treatment concepts. All this has led to the reconstructive clockwork for reconstructive surgery of the 21st century (Fig. 1).

The idea of the reconstructive clockwork is to mirror the integral parts of various reconstructive echelons serving the one goal of addressing the defect, the

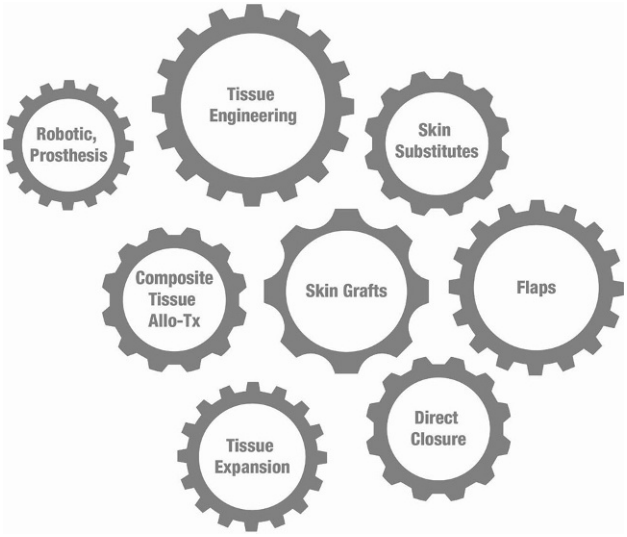

Fig. 1 Reconstructive clockwork

function, the deformity, or all of them in combination [1].

Materials and methods

The aim of this review is to present an overview of current standards and future trends concerning the treatment of skin defects. The main focus is placed on the most important technologies and future trends.

Results

Skin transplantation

Skin grafts are classified as either split thickness or full thickness based on the strength of their associated dermal portion. If the skin is removed with the dermis completely included, it is called a full-thickness skin graft. A "typical" split-thickness skin graft is thinner and includes only parts of the dermis. The skin appendages located in the deeper dermal layers remain at the site of removal and provide the resources necessary for the defect to heal ([2]; Fig. 2).

Autologous full-thickness skin grafts

The full-thickness skin graft has proven to be the best choice, both functionally and cosmetically, for covering burned areas on the face, hands, and over large joints in particular, since the strong dermal component prevents excessive scarring with subsequent shrinkage.

The limiting factor for use of full-thickness skin grafts, however, is the fact that the removal sites for full-thickness skin grafts always have to be primarily closed; thus, mostly only smaller grafts are available [2–4].

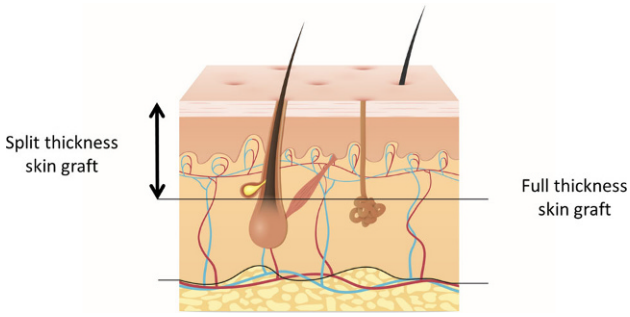

Fig. 2 Split-thickness skin graft, full-thickness skin graft (↔)

Combined reconstruction using a split-thickness skin graft combined with dermal replacement material (matrix, scaffold)

In the case of full-layer skin defects in functionally important regions (e.g., hands), combined skin reconstruction using a split-thickness skin graft (often non-meshed) in combination with a dermal matrix is often used. There are currently several matrices available (e.g., Matriderm® [MedSkin Solutions Dr. Suwelack AG, Germany] and Integra® [Integra Life Sciences, Germany], PolyNovo® [Polynovo Limited, Australia]) [5, 6].

Autologous meshed split-thickness skin grafts (meshed graft)

With the lattice split-skin graft, a defined mesh-like perforation is produced on a roller in combination with a corresponding template using a special arrangement of parallel knives on a roller, which leads to a relative increase in the area of the transplant. Split-skin grafts are particularly useful where large burned areas can only be covered with a remnant of healthy skin. An expansion ratio of 1:1.5 to 1:3 is preferably selected. With larger expansion ratios, the Meek graft is superior to the mesh graft in terms of healing and expansion [7, 8].

Meek technique

In 1958, Meek described a dermatome with which the split skin obtained can be cut into small square islands of equal size. In the 1990s, this method was modified in connection with an easy-to-use transplantation method, which made it possible, in one step, to not only cut the split skin layer, but also to expand it in ratios of up to 1:9 after applying it to a cork and silk support and to transplant. This method, which is somewhat easier to use, has now become established in many burn centers because of the mathematically favorable use of the enlargement factor and is preferred to the mesh graft for very large burns and other skin defects. This grafting technique has also become established for the coverage of chronic wounds [2, 3, 7–10].

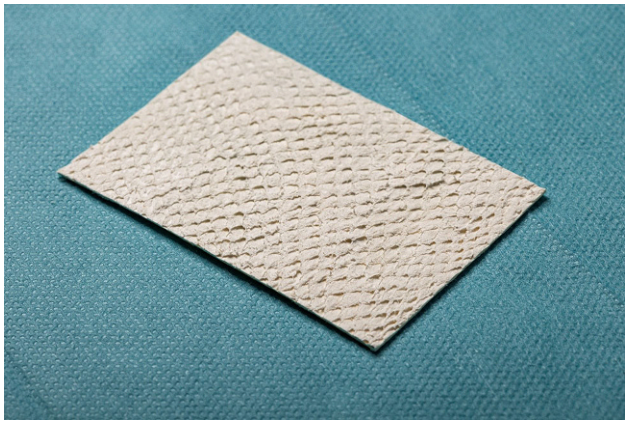

Fig. 3 Acellular fish skin (Kerecis ®)

Alternate methods

The use of standard surgical methods depends on the availability of a sufficiently large area of undamaged skin as a donor area for transplantation. In order to circumvent this limitation, efforts have focused on finding alternative methods so that patients with more than 70% of the body surface burned have a realistic chance of survival [9].

Allogeneic transplants (allografts)

If there are not enough donor areas available, allogeneic transplants can be used temporarily as a temporary skin replacement. Allogeneic transplants became more widespread when the so-called sandwich technique was used, in which widely meshed autologous transplants are covered with less widely meshed allografts [2, 10–12].

Xenogeneic transplants (xenografts)

Since the mid-1950s, especially in China, pigskin has often been used to temporarily cover large wound areas. After transplantation, the xenograft initially finds a nutritive connection to the basal wound bed. The dermis is initially revascularized, but then usually quickly dissolved and replaced by collagen structures. Especially in countries where allogeneic transplants are not used for ethical reasons, temporary wound covering with xenografts is still an important procedure today. These grafts are not only used in the case of severe burns, particularly in situations where donor sites are scare, but have been used for the treatment of other acute and chronic wounds [2, 3, 13].

Acellular fish skin Acellular fish skin grafts (Fig. 3) have several advantages in comparison to other xenografts of porcine and bovine origin. Acellular fish skin grafts can be stored at room temperature and have a shelf life of 3 years. Due to the particularly gentle process of decellularization and preservation, the protein and matrix structure of marine omega-3 wound matrices are extremely similar to the structure of human skin (Fig. 4). Its structure remains intact and enables the ingrowth of cells and capillaries (Fig. 5). Beside this, acellular fish skin grafts are extremely rich in omega-3 fatty acids. These grafts have anti-inflammatory and anti-infective properties, too. Therefore, omega-3 wound matrices seem to be suited for the treatment of complicated acute and chronic wounds [14–21].

Cell culture and tissue engineering

The surgical standard methods have their limits in terms of effectiveness in people who have been burned extensively, since the remaining unburned residual skin resources as donor areas are reduced to a minimum depending on the extent. The development and improvement of new cultivation methods and the introduction of transplantable and resorbable biomaterials using so-called tissue engineering offers a potential way out of the dilemma [22–24].

The aim is in vitro generation of tissues that are able to permanently replace specific tissue losses with comparable biomechanical and biochemical quality.

Specifically, the epidermis was the first organ or biological structure that could be successfully cultivated under in vitro conditions and transplanted in vivo [25–28].

These successes have made it possible, especially during the past 30 years, to treat patients with burns covering more than 60% of the body surface successfully [9]. Today, cultivated allogeneic cell transplants

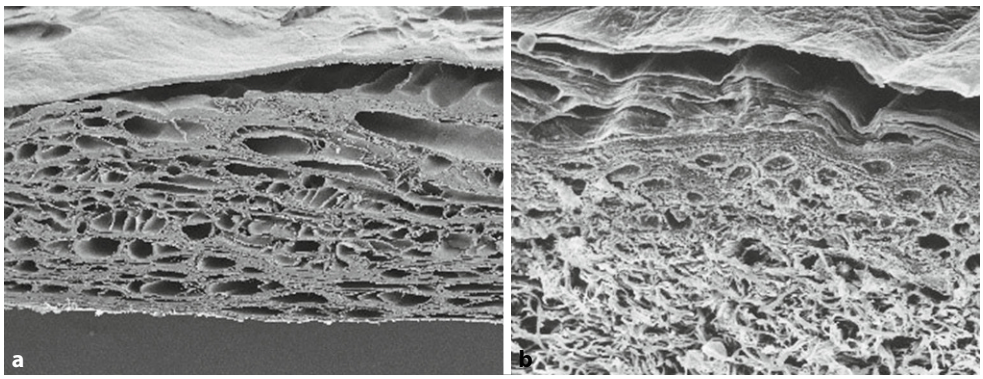

Fig. 4 Direct comparison of fish (**a**) and human skin (**b**): similar 3D structure

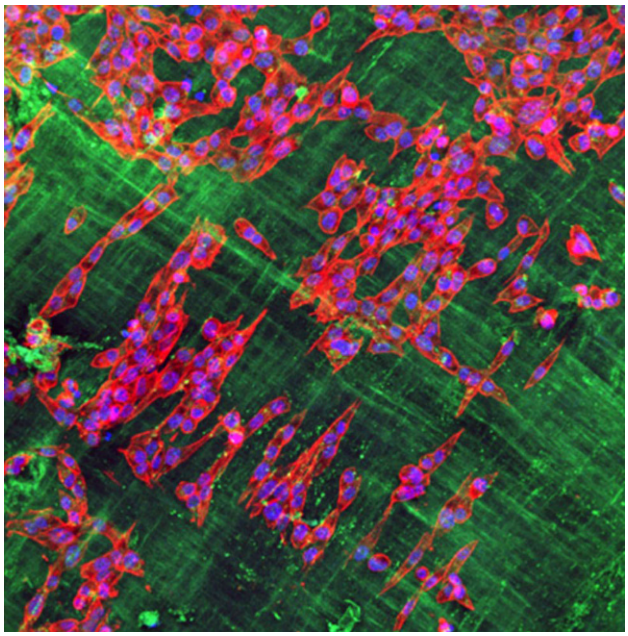

Fig. 5 Image of fibroblast cells and acellular fish skin (Kerecis®; Island) under confocal fluorescence microscopy. Kerecis® fluoresces *green*. NucBlue bound to the fibroblast nucleus is *blue* and Alexa-Fluor 546 Phalloidin bound to F-actin in the cytoplasm is *pink*. As well as interacting with the two-dimensional structure as shown in the image, the cells populate the three-dimensional spaces in the fish skin medical device throughout its whole depth

and autologous cell transplantation kits are commercially available.

Cultured autologous epidermis

Transplantation of a cultured epidermal membrane of autologous keratinocytes (cultured epidermal autografts, CEA) was the first successful clinical use of a cultured organ component. Applied cultured epidermis transplants usually consist of three to five cell layers. However, the transplants are very fragile and hard to handle. Another problem is the lack of a dermal component in case of third-degree burns. In order to counteract this problem, the development of dermal analogs of different compositions has been promoted and used clinically with success [2, 23, 24].

Cell suspensions

In 1895, the first successful transplantation of scraped keratinocytes suspended in autologous wound serum was performed. However, this technique was not initially able to establish itself, because of a lack of suitable carrier substances. The use of allogeneic keratinocyte suspensions aims primarily to utilize the paracrine-secreting activity of the cells. In areas with a burn degree of 2a to 2b, the re-epithelialization of the remaining skin appendages can be stimulated and the time until healing can be shortened. The same technology can be used to treat split-skin donor areas, where this possibility of using allogeneic cells is intended to ensure that the donor areas are available again more quickly [2, 3].

Cultured cells

The combination of cultured autologous keratinocytes on alloplastic or mixed synthetic/biological materials as dermal regeneration matrices has been investigated by different groups. In the 1980s, Yannas and Burke produced a skin equivalent by centrifugation of primarily trypsinized keratinocytes and fibroblasts in a collagen–glycosaminoglycan matrix (C-GAG), which healed completely after transplantation in guinea pigs [29–31]. Today, this and other matrices have been increasingly used for this purpose, also in humans [23, 24].

In spite of the tremendous advances in skin tissue engineering, a “complete” tissue-engineered skin substitutes is not yet available. Current substitutes are mainly composed of keratinocytes and fibroblasts, but still lack some of the functional components such as nerves, adnexal structures, and pigment cells. [22].

Synthetic materials

In addition to biological materials, more and more purely synthetic materials are on the market. In addition, various polymers/polymer composites (including polycaprolactone, PCL; polyurethane, PU; silicones; polylactic acid compounds PLA/PGLA) and “natural” materials such as silk proteins and bacterial cellulose are used for research and clinical purposes [32–34].

Synthetic skin replacement materials should replicate the functions of the natural extracellular matrix as far as possible. These include influencing cell proliferation, cell migration, and cell differentiation. The following factors should be considered in the development and production of synthetic biomaterials: composition and suitability (biocompatibility), biodegradation in vitro and in vivo, production and shaping as well as availability, batch-to-batch variability, production under physiological conditions (e.g., temperature, pH), and easy processing and application in the clinic. The materials should also have physiological properties that are as similar as possible to those of the skin, such as elasticity or biomechanical stability, and provide a 3D structure for tissue regeneration.

Common manufacturing methods

Common methods for the production of biomaterials are freeze drying, salt leaching, gas foaming, and electrospinning. Freeze drying (lyophilization) is a gentle technique for drying sensitive valuable materials (such as proteins) and can be used effectively for the production of collagen mats, for example. A porous 3D structure is created that can either be populated with endogenous cells or allow endogenous cells to grow in from the surrounding tissue and ECM. Salt leaching and gas foaming are techniques in which salt

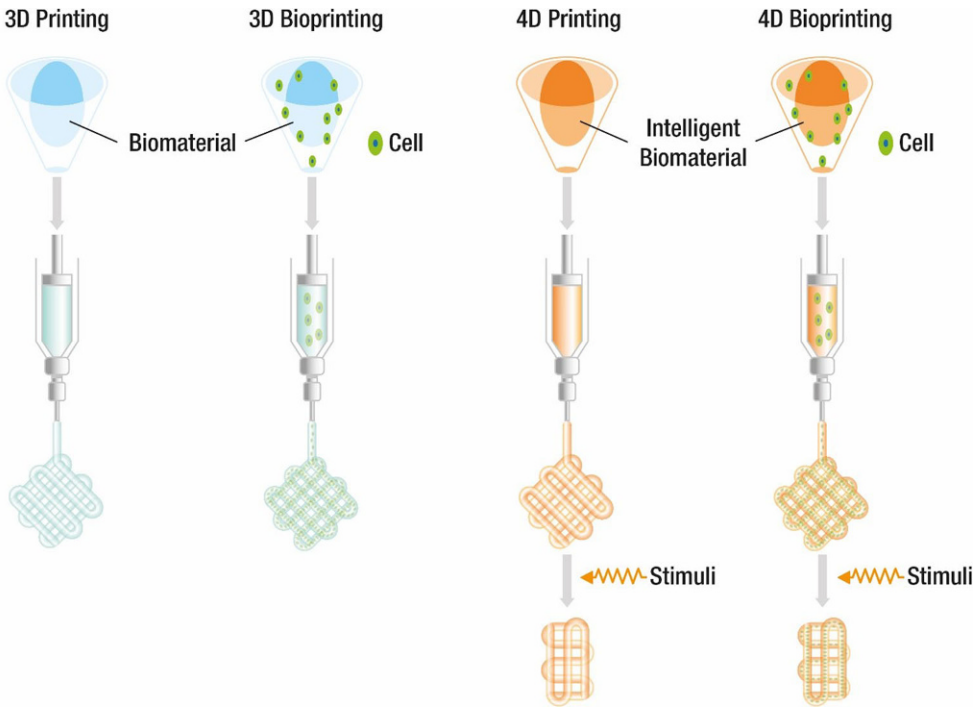

Fig. 6 Three- and four-dimensional printing technologies

crystals or gas (e.g., CO_2) are deliberately introduced into the material mixture and later released. This is how porous 3D membranes are created. With electrospinning, natural (e.g., collagen) or synthetic polymer solutions (e.g., PCL) can be spun into very thin fibers (nanometers to microns) in an electric field. These fibers (e.g., polymer, collagen) can also be processed as bundles as well as mats.

Three-dimensional printing In spite of the tremendous advances in skin tissue engineering, a "complete" tissue-engineered skin substitute is not yet available. Therefore, skin substitutes that replace the entire function of the skin are urgently required. From this perspective, 3D printing technology, bioink, and artificial skin bioprinting technologies that imitate the skin structure and microenvironment have gained immense attention. [35–38].

Beside the therapeutic impact, 3D bioprinting has the potential to serve as a platform for studying tissue development and homeostasis and for modeling diseases in pharmaceutical testing [35]. Bioprinting seems to be a technology that could overcome the gap between grafts and skin substitutes.

As very briefly described, 3D bioprinting seems to be very promising, but the next step is already being taken: 4D bioprinting, where the fourth dimension is transformation. It is the 3D printing of smart, stimuli-responsive biomaterials to create constructs that emulate the dynamic processes of biological tissues and organs.

Imagine, for instance, that instead of having to 3D print a skin graft for a burn victim, with all the entailed complexity, you could 4D print a basic skin graft that would, once implanted on the patient, vascularize itself, develop all nerve endings, take on the patient's complexion, and even grow hair if on the head. In a way, 4D bioprinting is to medicine what artificial intelligence is to computer science (Fig. 6; [35–38]).

Discussion

Autologous skin grafting was developed more than 3500 years ago. Several approaches and techniques have been discovered and established in burn care and plastic surgery since then. Great achievements were made during the 19th and 20th centuries. Many of these old and new techniques are still part of modern burn and plastic surgery. Today, autologous skin grafting is still considered to be the gold standard for many wounds, but new technologies have been developed, ranging from biological to synthetic skin replacement materials [2–4, 9, 39–41]. In spite of the tremendous advances in skin tissue engineering, a "complete" tissue-engineered skin substitute is not yet available and there is a need for new innovations and developments [42, 43]. One of these promising new technologies is 3D and 4D bioprinting [35–37].

Today, old and new technologies are available which allow us new treatment concepts for patients suffering from large skin defects. All this has led to the reconstructive clockwork for reconstructive surgery of the 21st century [1], and the clock will made more complex by new technologies.

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Conflict of interest L.-P. Kamolz, P. Kotzbeck, M. Schintler, and S. Spendel declare that they have no competing interests.

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