

Cell therapies for skin regeneration: an overview of 40 years of experience in burn units

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Summary

The earliest attempts at cell therapy can be attributed to Charles-Edward Brown-Séguard (1817–1894), who sought to treat senescence and aging by injecting animal gonad shreds into his contemporaries, a practice that was widespread in late 19th century. Since then, advances in science have enabled the development of biological substitutes to restore the function of various tissues. Skin was one of the first tissues to be regenerated. For severe burns, patient survival depends on the restoration of skin function as a barrier against pathogens and control of body temperature and fluid loss. We aim here to overview the different cell therapy techniques implemented at the University Hospital of Lausanne (CHUV), one of the two Swiss national centres of highly specialised medicine for burn care. In particular, we will describe the specific indications for each of the different therapies as well as future perspectives.

Keywords: cell therapy, skin, regenerative medicine, burn

Introduction

Throughout and across history, allusions to the artificial generation of tissues and organs can be found in mythology, literature or art, such as that illustrated in the “Healing of Justinian” painting (278 AD), where the transplantation of an artificial limb by Cosmas and Damien [1] is depicted. About four decades ago, technological advances in biomedical sciences achieved the translation of this fundamental concept to practical approaches, thus giving birth to the modern fields of tissue engineering and regenerative medicine [2]. Langer and Vacanti, who are often referred to as tissue engineering pioneers, defined tissue engineering as the interdisciplinary field that applies the principles of engineering and life sciences to the development of biological substitutes that restore, maintain or improve the function of tissues or whole organs [3]. Tissue engineering has evolved from the field of biomaterials and now refers to the combination of scaffolds, cells and biologically active molecules into functional tissue constructs designed to be implanted into damaged tissue [4]. Regenerative medicine has a broader research scope that not only includes the concepts of tissue engineering, but also incorporates

the notion of self-healing, where the body uses its own systems with the help of foreign biomaterials to rebuild tissues and organs. Tissue engineering and regenerative medicine have become largely interchangeable, as both fields use cell therapy to cure complex wounds or diseases [5].

Because of their two-dimensional structure, skin and cartilage were considered good candidates to be the first tissues to be engineered or regenerated using cell-based products [6]. Although several examples of cell-based engineered tissues have been approved in the USA by the Food and Drug Administration (FDA), a lack of funding has limited their evaluation in extensive clinical trials [7]. For burn care, cell-based therapies have been implemented worldwide for many years, and improved techniques have helped to save many lives since they were first used in 1981 [8, 9].

There is a long history of using cell therapy in Switzerland. Famous pioneering examples of Swiss-based therapy were performed around 1930 by Paul Niehans, who harvested embryonic cow cells to treat illustrious figures such as Charlie Chaplin or Pope Pius XII [10]. With increased regulation for security, Swiss authorities (Federal Health Office and Swissmedic) have regulated such practices to ensure the overall safety of patients. With a high incidence of cellular therapy use (>40 per 10 million population), Switzerland had one of the highest rates of cell transplantation in Europe in 2009 [11]. Cell therapies are particularly common for skin regeneration, especially at the University Hospital of Lausanne (CHUV), which is one of only two national centres specialised in burn care. Here, we aim to give an overview of the history of the different cell therapies used for skin regeneration in the burn unit at the CHUV, and describe the techniques and the conditions under which they are used, as well as highlight the regulatory challenges and perspectives of cell therapies.

The degree of burn damage according to skin structure

The skin is composed of three layers: the epidermis, dermis and hypodermis (fig. 1). The outermost protective layer is the epidermis, which is composed mainly of keratinocytes and provides a barrier against pathogens [12]. The dermis, the layer beneath the epidermis, contains the vasculature, nerves and glands and is mainly composed of fibroblasts

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surrounded by an extracellular matrix of collagen and proteoglycan, which provides the elasticity and tensile strength of the skin [13]. Finally, the hypodermis or the subcutaneous tissue is connective tissue that attaches the skin to bone and muscles, and is mainly composed of adipose tissue. Burn severity is classified according to the depth of the wound and layers of skin that are effected: a first degree burn involves only the epidermis, second degree superficial is a wound of the upper half of the dermis, second degree deep involves almost all of the dermis, and third degree burns reach the hypodermis. When a burn wound is second degree deep / third degree, the vasculature is destroyed and the body loses its capacity to self-regenerate. Surgical procedures combined with cell therapies thus become indispensable.

The cell type in a cell-based therapy

For a further understanding of the intricacies of cell therapy approaches in skin regeneration for burns, we first introduce the definitions of various cell types. Several cell types from different stages of development that have different properties can be used in a cell-based therapy (fig. 2).

Stem cells can be of embryonic or adult origin. *Embryonic stem cells* (ESCs) are derived from the inner cell mass of a blastocyst and are pluripotent; they have the ability to differentiate into all types of cells [14]. ESCs are multipotent at later stages of development (can develop into a limited number of cell types in a particular lineage or tissue [15]). Directing a uniform differentiation of ESCs into the desired cell type with *in vitro* cell culture is challeng-

ing, as they require intense treatment with growth factors to be fully and successfully differentiated; undifferentiated ESCs carry a risk of creating tumours when transplanted [16]. *Adult stem cells* can be multi- or unipotent cells that reside in tissue-specific niches for tissue regeneration [17]. However, adult stem cells are difficult to identify as they have a similar morphology to normal fibroblast-like cells and require specific surface proteins to be distinguished [18].

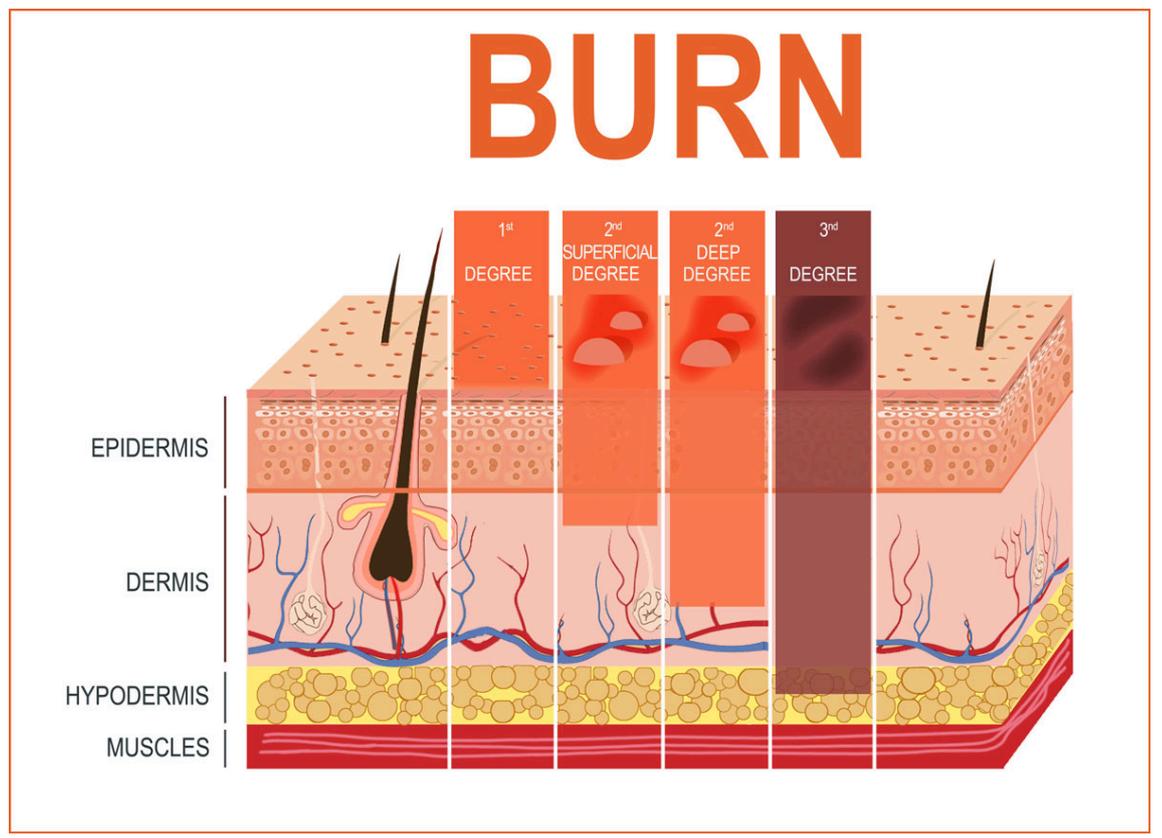
Progenitor cells, unlike stem cells, are cells of fetal origin that are already specific to each organ, but are not fully differentiated [19]. Progenitor cells have a higher regenerative potential than other cells, minimise scarring and grow rapidly in culture [20]. Also, progenitor cells maintain a stable expression of markers specific to skin without additional growth factors during cell culture, unlike stem cells [21].

Specialised cells are fully differentiated cells that are specific to each tissue. For skin, there are two main cell types, keratinocytes and fibroblasts, as well as melanocytes, Merkel cells and Langerhans cells [22].

In cell therapy, *autologous* transplants are where the cell donor and recipient are the same person. For *allogeneic* transplants, the cell donor and recipient are different individuals. One of the specialisations at the CHUV is the use of both autologous and allogeneic progenitor cells for the regeneration of skin in burn patients (fig. 3).

The use of progenitor cells has been part of the Federal Organ Transplant Programme since 1991 and is registered with the Swiss Federal Office of Public Health. Progenitor

Figure 1: The different degrees of burn wounds according to skin structure.



cells do not induce a rejection reaction, unlike other sources of allogeneic cells [23], which means that it could be possible to create a universal cell bank allowing the treatment of several patients from only one organ donation [24]. Hundreds of biological bandages can be applied to the patient within 24 hours, whereas an autologous cell therapy requires a biopsy for each patient and culture for several weeks (3–7, depending on technique).

Cell therapies utilised at the CHUV burn unit

Cell therapy has a long history at the burn unit of the CHUV: the first cultured epithelial autograft (CEA) was introduced into clinics as early as in 1985. For CEA, a biopsy of the patient's epidermis (approximately 10 cm²) is taken and digested to obtain keratinocytes. Cells expand on a feeder layer and form stratified cell sheets in 14–21 days when cultured in the appropriate medium. This thin layer of skin (keratinocyte sheet) can then be transferred

on Vaseline gauze and applied topically to the patient for wound closure [25] (fig. 4a). In exceptional cases (1–2 cases per year), where the patient's condition does not allow for a long delay in treatment, it is possible to apply keratinocytes by spray instead of as a sheet in order to reduce preparation time [26].

Cultured dermal-epidermal autograft (CDEA), which was implemented in the mid-1990s, is based on the principle of CEA, but allows re-creation of a thicker skin-graft, because a double layer composed of autologous fibroblasts and keratinocytes is used [27]. CDEA provides a better clinical outcome, with a skin graft having more functional mechanical properties than a CEA. However, CDEA is less commonly used since it requires a full thickness biopsy and longer culture times, typically 6–7 weeks. CDEA is therefore used exclusively in severely burned patients for whom hospitalisation duration is longer and multiple surgical and grafting operations are needed. It is worth mention-

Figure 2: Different stages of development. In Switzerland, the use of human fetal cells and adult cells is part of a Federal Transplantation Programme (blue), whereas the use of human cells at earliest stage requires a license (red).

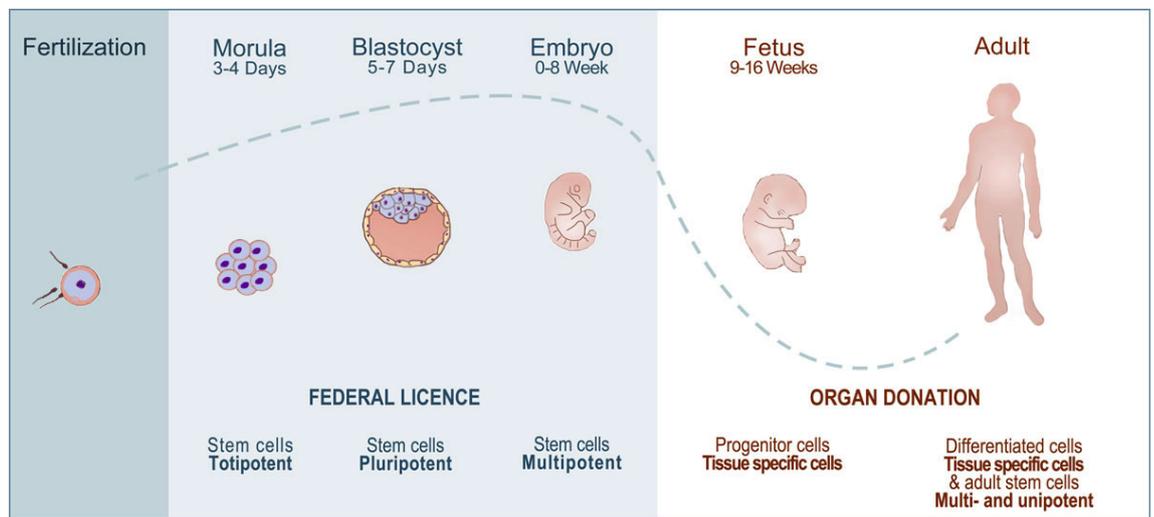
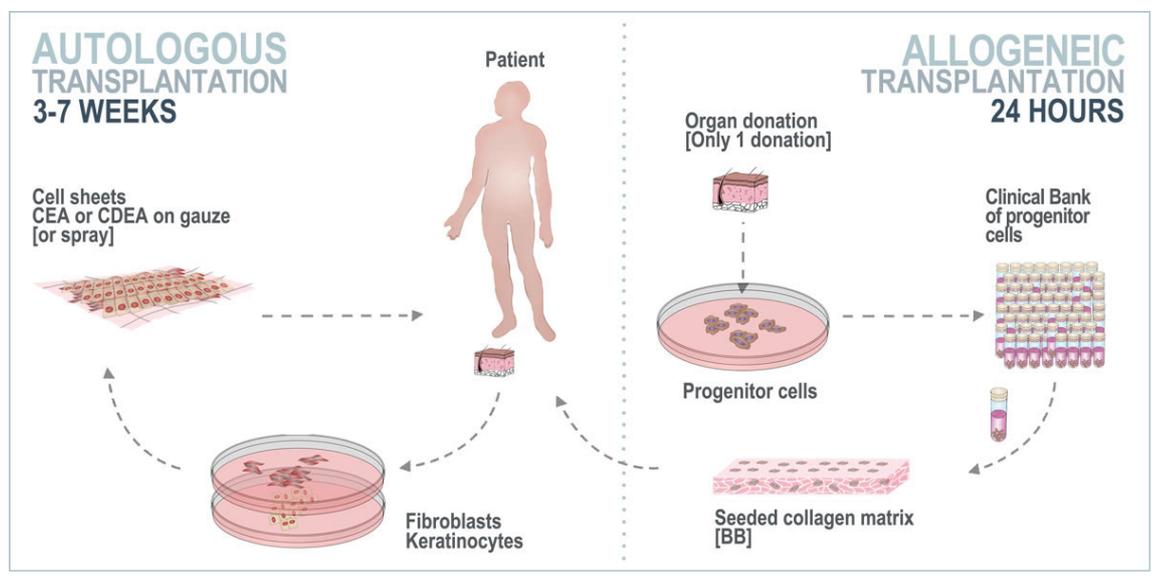


Figure 3: Autologous and allogeneic cell therapy performed at the CHUV Burn Centre. Cell culture is required for each patient for autologous transplants, whereas for an allogeneic transplant, a single organ donation creates a cell bank that can treat multiple patients and significantly reduce the time-to-treatment. CEA = cultured epithelial autograft; CDEA = cultured dermal-epithelial autograft; BB = biological bandage



ing that systematic prophylactic use of antibiotics prior to a skin autografting is not recommended, to avoid promoting resistance; however, in the case of a cell-based therapy such as CEA or CDEA, it is common practice at CHUV to prepare recipient sites 2 days prior to grafting by a daily application of gentamicin and/or terbinafine, according to the targeted pathogen.

During the 3–7 weeks required for preparation of CEA and CDEA, patients are covered with temporary skin substitutes to prevent fluid loss, reduce infection risk and decrease pain by nerve covering [28]. Over the years, temporary skin substitutes have included xenogeneic decellularised porcine skin, allogenic cadaveric human skin and synthetic dressings [29], all of which carry some risk of rejection and disease transfer. Furthermore, the frequent integration of temporary covers by the recipient renders ablation difficult with painful eschars [27]. In early 2000, a new allogenic cell therapy was developed in the Regenerative Therapy Unit at the CHUV: use of biological bandages made of human progenitor skin fibroblasts seeded onto biodegradable equine collagen scaffolds (fig. 4b). The function of these bandages was to replace other temporary dressings that had many clinical complications. These bioactive bandages allow simultaneous enhancement of skin regeneration, which is most likely related to their release of growth factors and cytokines [30]. The first clinical trials, begun in 2000, demonstrated that biological bandages provided efficient treatment of second degree superficial and deep burns without autografting, showing that progenitor skin cells may have great potential to treat burns and, eventually, acute and chronic wounds of other types

[20]. Biological bandages have since been used routinely at the CHUV, particularly for paediatric burns.

Beginning in the early 2000s, use of platelet rich plasma [31] was integrated into patient care. Local application of platelet rich plasma has been shown to improve healing in donor sites [32] (fig. 4c).

It is worth mentioning that a new Federal Act on Transplantation of Organs, Tissues and Cells came into force in 2007. This involved implementation of good manufacturing practice (GMP) procedures for cell therapy preparation [33]. Thus, a cell production centre was initiated as part of the CHUV's 2009–2013 strategic plan. Since 2015, all types of cell therapy are manufactured at the Cell Production Centre, which is an accredited GMP facility in Switzerland for cell therapy production, not only for clinical trials but also for routine preparation of cell therapies for burn patients. A summary of the different cell therapies for burns can be found in table 1.

Different indications for a cell therapy in burn patients

When burns are deep (second and third degree burns), the skin loses its ability to regenerate and a skin graft is required. Split-thickness autografting is still the gold standard for the treatment of deep burn wounds [27]. This consists of taking from the patient epidermis with a small portion of the dermis in order to cover the burn wound (the donor site heals spontaneously by re-epithelialisation because of the remaining dermis portion). However, for severely burned patients, when the damaged surface is too

Figure 4: Different types of cell therapy used at the CHUV Burn Centre. [a] Cultured epidermal autograft and cultured dermal-epidermal autograft (CEA and CDEA) – keratinocytes are cultured on feeder cell layers for cell expansion, fibroblasts are retrieved from dermis explants (left panel). In the case of a CDEA, parallel culture of fibroblasts is done, and the keratinocytes are applied on the fibroblast layer after expansion. Finally, the cell sheet (middle panel) is washed with PBS and applied on patient with gauze (right panel). [b] Biological bandage (BB) – a vial from the cell bank (left panel) is thawed, and cells are seeded on a collagen matrix and incubated overnight in culture medium (middle panel), then washed and applied to patient (right panel). [c] Platelet rich plasma (PRP) – blood is collected, centrifuged in a two-step process to isolate platelets, which are then applied to the patient.



large to be covered by autografting, cell therapy is needed. The appropriate type of cell therapy depends on the total body surface area (TBSA) and the depth of the burn. A CEA strategy is used for adult burn patients with a deep burn surface that exceeds 40–50% of the TBSA. The surgeon may also decide to use a CDEA for the areas where he/she considers it to be necessary, such as the hands or other body areas that require a certain functionality. However, this is only done in patients where it is known that hospitalisation will be for a long time, permitting cell culturing (generally for very severe burns).

Biological bandages serve as a temporary cover, allowing the time to prepare CEAs and/or CDEAs. They can also be used to cover donor graft sites to stimulate wound closure and in combination with a mesh graft in a “sandwich-like” configuration. Likewise, platelet rich plasma is used in burns to stimulate and accelerate the healing of wounds with large surfaces.

For paediatric burn patients at the CHUV, it is standard to use biological bandages to cover burns of 10% TBSA or more, since this is the definition of a severe burn in paediatric patients. Every 2 days the biological bandages are replaced by new ones, and this process is normally repeated up to four times. After 10 days, the surgeon assesses the wound closure and decides if a skin autograft is required for surface areas that are not completely closed. Most paediatric burns at CHUV are second degree superficial burns that are appropriately treated with biological bandages, with complete wound closure usually within 10 days, thus decreasing the necessity of skin autografting. In addition, CEA and CDEA can also be used in paediatric burn patients (>40% TBSA), but these are fortunately rare cases in the CHUV. A summary of the different cases for cell therapies used can be found in [table 2](#).

Ongoing projects in cell therapies and perspectives

Skin wounds are major contributors to global disease burden, affecting millions of people worldwide [34]. This burden is expected to increase, with serious consequences in the future as over half of all chronic and acute skin wounds become infected [35]. Such infections are particularly worrisome in the context of the rise in resistance of bacterial pathogens to antibiotic treatments [36, 37]. In this context, research efforts are ongoing at the CHUV to develop a new generation of biological bandages with antimicrobial properties to treat bacterial infections topically. For biological bandage production, the use of collagen scaffolds for progenitor cell support may be more prone to infection because of their high hydroxyproline content, which is a source of nutrients for opportunistic bacterial pathogens such as *Pseudomonas aeruginosa* [38]. In 2016, Abdel-Sayed and co-authors showed that coupling the collagen matrix with a certain class of antimicrobial peptide dendrimers not only inhibited the growth of multi-drug resistant *Pseudomonas aeruginosa*, but also could have pro-angiogenic effects, which are involved in improved wound healing [39]. A preclinical study testing the safety and efficacy of these new class of bandages is currently ongoing. Meanwhile, the CHUV also has other successful cell therapy endeavours. For instance, in 2018 the first patient was treated with a matrix-assisted autologous chondrocyte transplant. This was the first clinical protocol for cell therapy to be accepted in Switzerland with full GMP processing in association with the Cell Production Centre of the CHUV. Cell therapy research is also undertaken in Zurich, where the second national centre of “highly specialised medicine” for burn care management is situated; an exam-

Table 1: Summary of different cell therapies used at the CHUV Burn Centre.

Type of therapy	Description	Type of biopsy	Type of cells (type of graft)
CEA	Cultured epithelial autograft	Epidermal	Keratinocytes (autologous)
CDEA	Cultured dermal-epithelial autograft	Epidermal and dermal	Fibroblasts and keratinocytes (autologous)
BB	Biological bandage	No biopsy: off-the-shelf cell bank	Human progenitor fibroblasts (allogenic)
PRP	Platelet rich plasma	Blood	Platelets (autologous)

Table 2: Different indications for the use of cell therapies at the CHUV Burn Centre, as well as the average number of patient per year that benefited from each therapy for the past 5 years. The time delay before receiving a cell therapy varies depending on the type of treatment: the need for a cell culture step considerably prolongs the preparation time before transfer to the patient. Note that biological bandages and platelet rich plasma can be transferred within 24–72 hours and less than 24 hours, respectively, after preparation.

	Adult	Child	Use and indication	Burned surface	Duration of preparation	Number of patients/year
Cultured epithelial autograft	+	+	Applied directly on wounds Applied in “sandwich” with skin mesh grafts On autograft donor sites	>40–50%	2–3 weeks*	11
Cultured dermal-epithelial autograft	+	+	Very rare cases, for long hospital stays only	>70–80%	6–8 weeks	2
Biological bandage	+	+	Temporary cover on areas needing further grafting On autograft donor sites	Starting from: >20% for adults; >10% for children	24–72 hours	9
Platelet rich plasma	+	–	For any type of burn On autograft donor sites	>1–2%	<24 hours	115

* This preparation time can be reduced to 1 week if cultured epithelial autograft is delivered by spray. In general, the spray alternative is used when the condition of the patient does not allow longer wait periods.

ple is experimentation of bio-printing in order to one day be able to produce skin for patients in some sort of skin factory, in a fast, accurate and efficient way.

The European Directives and Regulations (2001/83/EC and 1394/2007) classified cell therapies as “Advanced Therapy Medicinal Products” (ATMP) as of 2008, which imposes industrial GMP requirements, as well as marketing authorisation, for cell therapy production in hospital settings. This in turn has increased the costs considerably and could lengthen the process of introducing new treatments for burn patients [33]. Hence, it is important to discuss with health authorities about hospital exemptions to allow compassionate use of cell therapies, without the requirement for marketing authorisation, to make these customised and life-saving treatments available to patients [40–42]. From January 2019, revisions of the Therapeutic Products Act (Stage 2), which aim to simplify and facilitate patient access to medicinal products and improve conditions for biomedical research, have come into force. In particular, this allows a 2-year authorisation for the “compassionate use” of cell therapies provided that a clinical study has been approved in Switzerland prior to the request. Scientific research continues to advance therapeutic possibilities in tissue engineering and regenerative medicine, even as current possibilities for patient care are hampered by the hurdles of GMP procedures, increased administrative load and costs. It is of course in the best interest of physicians and scientists that these hurdles are removed by the competent authorities to ensure that patients have access to the best treatment options and with the highest security at all times.

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