The Use of Frogskin as a Biological Dressing for Temporary Cover of Burn Wounds

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16.1 Introduction

Most burns experts will agree that third degree burn wounds should be excised by the end of the first postinjury week. The wound should be closed if one is to minimize or avoid the deleterious effects of the hypermetabolic changes which occur after a major burns injury. Early excision and grafting has significantly decreased morbidity and mortality in burns patients. In selected cases, this method offers a better chance of a faster recovery, with marked reductions in metabolic consumption, as a consequence of the prompt closure of the wound.

This method became progressively more popular after Dr. Jazenkovic reported her clinical experience in 1970 [1]. The users of this technique, however, soon realized that there was an urgent need for immediate closure of the wound, since organic losses were even greater via a non-covered, excised wound.

Although an autograft is the most desirable way to cover the excised wound, patients with extensive injury usually have very limited donor areas. Several authors have suggested alternatives for this coverage, these being that allograft (cadaver skin) is still considered the gold standard, although this option was already suggested by Brow (1942) and Artz (1955) [2, 3].

Alternatives are inter-vivo skin transplantation (allograft), xenografts and the use of other biological tissues and of industrial or bioengineered products.

There are several alternatives used around the world which may serve to cover the burn wound, ranging from honey to potato peel, to the different methods of using cultured keratinocytes, to a most diverse selection (and use) of biological tissues and bioengineered skin substitutes. Cuono (1987), Poulsen (1991) and Klugston (1991) recommended cultured epithelial cells from the patients themselves or from other donors, and bioengineered tissue usage was demonstrated as long ago as 1988 by Heimbach and in 1989 by Tompkins [4–11].

Allograft inter-vivo skin transplantation has been practiced in our country for several decades, and the major difficulty with this procedure is the difficulty of finding willing donors, usually a relative or close friend of the patient, who has to be convinced to donate skin, at rather short notice. The sudden news of a burns accident with a loved one and the emotional impact of the severity of the injury may hinder clear decisions for the closest relatives, who would be the most logical donors, delaying or making this procedure improbable in the recommended time (the first postburn week).

Cadaver skin (allograft) then becomes the most recommended way of covering an excised burn wound in large burns with limited donor sites. The difficulty with this procedure in our country is that there are only two tissue banks, which although they are open to donate skin for government insured patients (about two-thirds of our burns patients), do not have enough skin to distribute for the 31 registered tertiary care burns centers in Brazil.

In our country, xenograft transplantation becomes the next available and feasible everyday alternative, since the industrial and/or bioengineered products are extremely expensive and are usually not financially supported by most insurance companies [12–16].

Pigskin has been used worldwide for several decades with good success, by Bondoc (1971), Yang (1980), Alexander (1981) and Heimbach (1987). The idea is that the injured body will not recognize the foreign tissue for some time and that temporary adherence will lead to cure of more superficial burns and to good protection on deeper or excised wounds. The problem with this technique in our country is that it has long been unavailable due to government imposed difficulties on the legal importation of these products, making them extremely expensive, and over time most companies lost interest in importing them for commercial sales within Brazil [17–21].

As a consequence of these difficulties, we have had to look for alternatives. Locally, the most logical alterna-

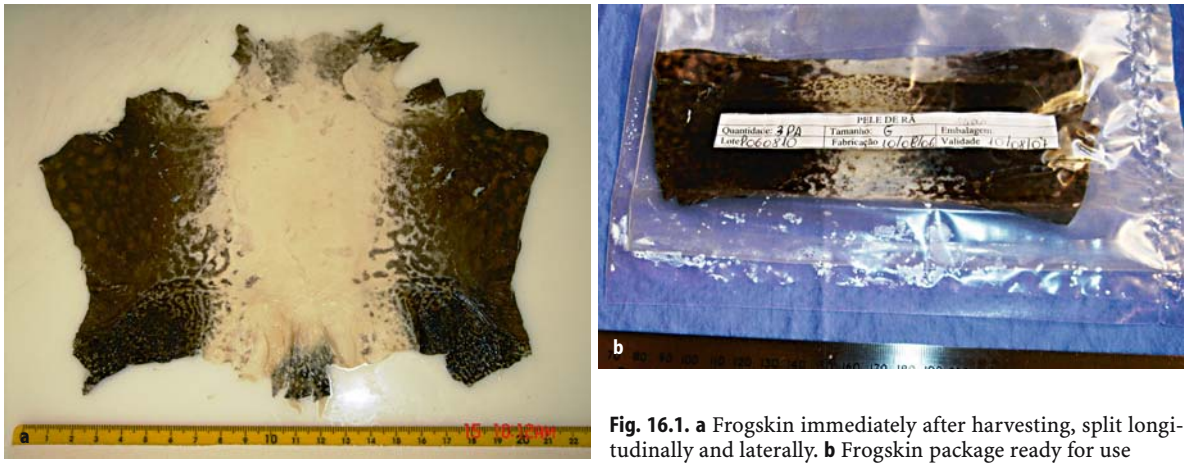

Fig. 16.1. a Frogskin immediately after harvesting, split longitudinally and laterally. **b** Frogskin package ready for use

tive was to attempt the use of frogskin as a temporary cover for these wounds, since there was practically an unlimited supply of these animals in our region due to the large number of commercially raised frogs which are slaughtered for their meat, which is sent out to several countries in Asia and also to the USA and some countries in Europe.

We originally tried frogskin on full thickness burns in Wistar rats, and it turned out to be a most useable biological dressing, with the added advantage of having its own antibiotic and other active substances within its skin. Magainine was described by Zaziass as a dipeptide with antibiotic properties which would be present within the structure of the skin of the frog. We use the skin from the frog known as *Rana catesbiana* (Shaw), also called the bullfrog [22].

Although we had this idea originally and independently in 1989, when searching the literature later, it was found that the use of frogskin had already been attempted by Fowler in 1899 and by Ricketts in 1890. These surgeons used live frogskin placed directly over a burn wound [23, 24].

Based on previous experiences of other authors with irradiated tissue, we are now also running a study where the "traditional" frogskin (prepared fresh and then kept frozen), which has been in use since 1989, is compared to freshly prepared frogskin which is irradiated and kept on a shelf at room temperature. If the comparison shows similar results with the irradiated skin, the advantage would be the elimination of the need for conservation at low temperatures, which, from the public health point of view, would make its use more widespread even in the most remote areas where the maintenance of products in a freezer may be a cost issue [25].

16.2 Materials and Methods

The frog will usually yield a piece of skin with an average size of 120 cm^2, which is prepared in our laboratory by a proprietary method, making it sterile/aseptic, when it can be immediately used or saved in a regular freezer for future use. It is used in full thickness wounds, after excision, where it is removed when autograft becomes available for definite cover as donor areas or more superficial wounds heal. It is also used as a biological dressing for deep second degree burns.

Frogs 180 g in weight are killed at the frog farm, and the skin of the body ("below" the neck) and extremities is removed as one would remove a glove from a hand. It is split on one of the sides longitudinally and divided into 1-kg sublots, which are kept refrigerated at 2 °C. In the laboratory, the skin is cut into a rectangular shape, usually yielding a fragment of 15 × 8, which is meticulously cleansed and prepared sequentially on sterilizing and detoxifying solutions. The "extra" pieces of irregular skin are also prepared for use around fingers or smaller wounds (Fig. 16.1a, b). "Prepared" skins are kept on sterile vacuum packed bags in a –20 °C freezer.

When needed, the skin is thawed and applied directly to the wound, covering it completely, as with any other graft. Its adherence is believed to be a function of fibrin bridges and the naturally occurring reactions of the wound to a graft. Fine mesh gauze may be applied with or without topical agents and the dressing is changed every 2 days.

Non-adherent skin fragments are substituted by fresh ones and the dressing replaced in a similar fashion. Xenograft loses its adherence as epithelial tissue or granulation tissue is formed on the wound. It can also be removed for definite autografting of the excised wound. In our routine, wounds are excised sequentially on the 2nd, 4th and 6th days postburn – the wound is covered with frogskin

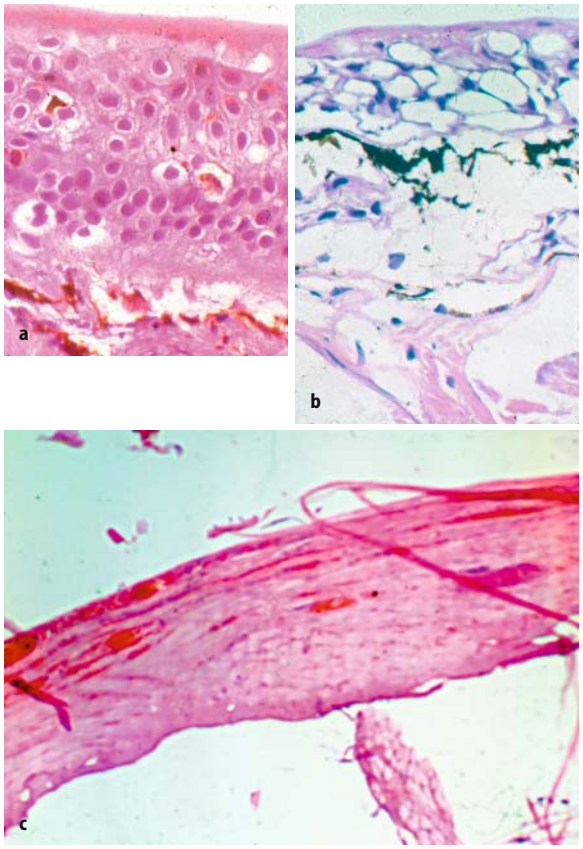

Fig. 16.2. a Photomicrograph of fresh frogskin, demonstrating a thick and well organized epidermis; absence of papillae with a dermal gland at the center of the figure. Muscle tissue can be identified in the lower portion of the image. H&E, ×400. **b** Photomicrograph of frozen frogskin, demonstrating a thinner epidermis, due to "thickening" of the dermis due to dislodgement of several of its cells as a consequence of vacuolization, probably filled with water crystals. H&E, ×400. **c** Photomicrograph of a frogskin specimen removed from a wound 12 days after application. Note the complete loss of cellular identity, probably as a consequence of progressive desiccation of this tissue. H&E, ×400

on day 2, and on day 4 another area is excised and covered with frogskin and the area originally covered with frogskin on day 2 has the xenograft removed and is autografted on day 4 and so on, as long as donor areas are available. If not, the wound is kept covered with frogskin until there is enough skin available to autograft it.

Candidates for this method of coverage are patients with excised wounds, deep second degree burns, donor areas and those with electrical injury. The biological dressing is also especially efficient in covering noble tissues such as nerves and tissues which may become exposed in deeper wounds, such as electrical injury wounds or in surgical wounds from fasciotomies and escharotomies.

For this chapter, we have considered the use of this material from 1 January 1990 up to 31 December 2005.

16.3 Results

In the last 16 years, the Pronto Socorro para Queimaduras in Goiânia has seen 188,578 burns patients. Of this population, this method of treatment was applied to 9,240 cases (4.9%) of partial thickness burns; it was used in 3,205 cases of excision (1.7%) and was also used frequently in donor areas, being applied to 4,615 patients (2.4%). The average adherence time was 9.8 days (2–20 days).

Examination of fresh frogskin under the microscope will demonstrate a relatively thick epidermis, similar to that in human skin or pigskin, and a dermis less thick than the epidermis, the opposite of human skin or pigskin. Frozen frogskin biopsy will indicate a thinner epidermis, with several dermal cell layers with vacuoles apparently filled with water crystals. The basal lamina is very evident and there is a cleft dividing the dermal stroma from the muscular plane, probably with function on the skin movement. The stratum corneum is apparently absent. An "older" specimen, removed from the wound at 12 days, will show a rather leathery appearance, with complete loss of cellular identity (Fig. 16.2a–c).

Second degree burns can greatly benefit from the use of this biological dressing, including a great deal of comfort and a practically painless wound for the patient (Fig. 16.3a–d).

Fig. 16.3. a Second degree burns covered with frogskin. **b** Same patient, 12 days after frogskin application. **c** Same patient, removing frogskin. **d** Same patient, after removal of frogskin, showing healed wound

Fig. 3 (*Cont.*)

Excision and grafting is commonly performed in our institution as depicted above. Frogskin can be removed at any time for definite autografting. Our excision cases are excised to fascia in 1 %, to deep dermis in 19 %, and to fat in 80 % (Fig. 16.4a–f). Donor areas are also preferentially covered with frogskin (Fig. 16.5).

16.4 Discussion

The benefits of early excision and grafting have already been demonstrated by several authors. This method of treatment has become our method of choice for deep partial burns which may have evolved to deeper lesions and for full thickness burns, infected or not, aiming at early closure of the wound [26–31].

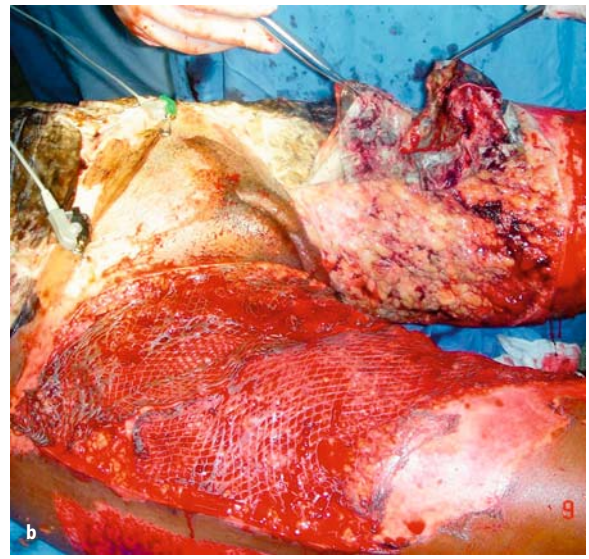

Fig. 16.4. a Third degree areas excised to fat 2 days before this picture. **b** Same patient, with the right thigh already grafted and with frogskin being removed from the left thigh. **c** Patient with full thickness burns. **d** Same patient, with wounds excised to fat. **e** Same patient, with frogskin applied 14 days before. **f** Same patient, 4 days after autografting

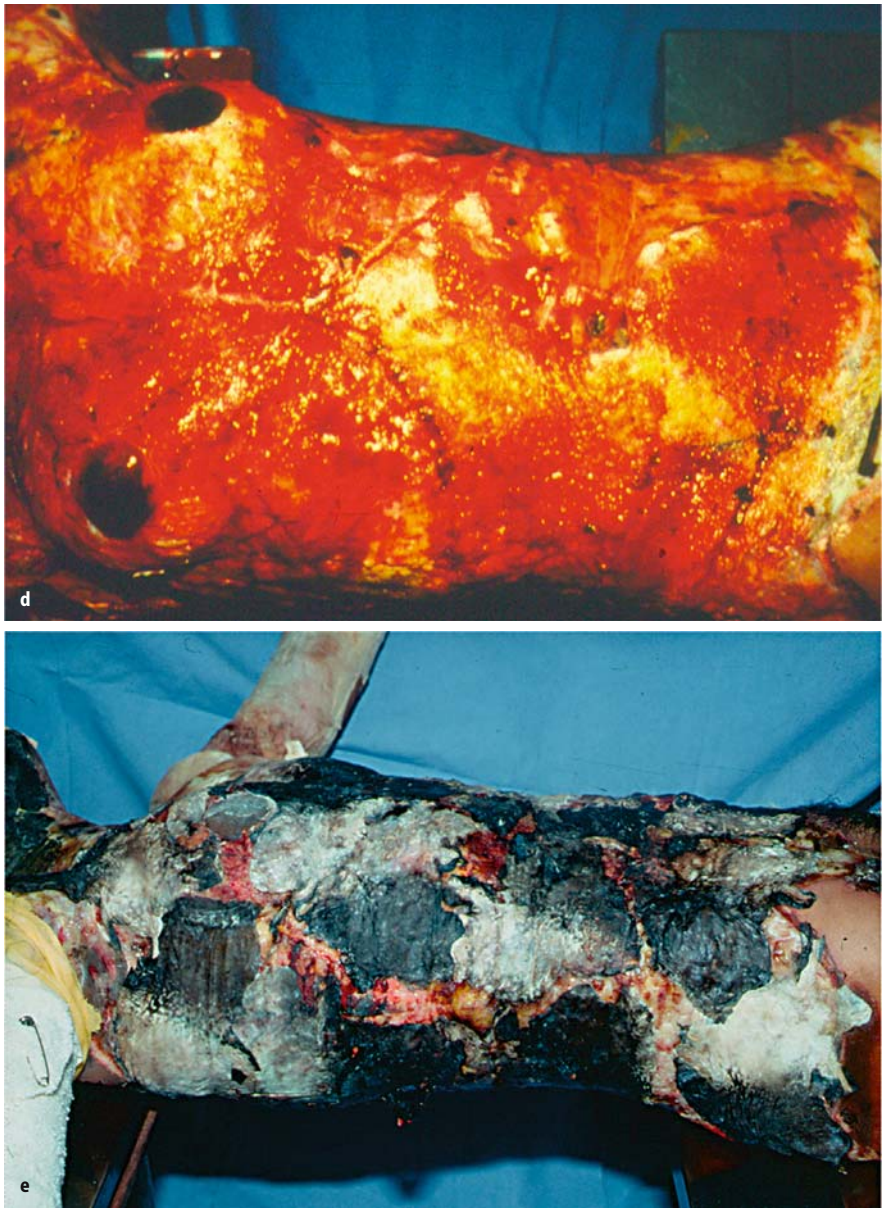

Fig. 4 (*Cont.*)

In our service, we will excise up to 20% of the body surface area per session. Repeated procedures are performed at 2-day intervals and we prefer not to excise to fascia, since the aesthetical result may be very poor, in excess of the distal edema which may occur as a result of removal of superficial veins and lymphatics. Although there have been publications suggesting methods to evaluate the depth of the resection during an excision procedure, we prefer to graft the excised bed on the 2nd day after the original procedure, when the potential receptor area is reevaluated and there is a greater chance of autograft survival [32–34].

We try to obtain closure of the wound using the patient's own skin. When the viability of the excised area is dubious, or the patient does not have enough donor areas, we prefer to cover the wound temporarily with allograft as an inter-vivo transplantation or from a tissue bank [35].

Biological dressings decrease metabolic losses, and may prevent infection and provide more comfort for the patient. These are used as alternatives to auto- or allografting. Although several factors must be considered when choosing one of these materials, the main one is still the cost-benefit ratio. In our country, amniotic membrane is used by some services which have connections to maternity hospitals and allograft is under regulation by the Federal Government.

The Ministry of Health guidelines are not sufficient

Fig. 4 (*Cont.*)

Fig. 16.5. Frogskin in a scalp donor area, 8 days after application

to regulate the reception of allograft from the two existent tissue banks, although, even if easily feasible, there would not be enough material to cover all burns centers, making the tissue banks operational only in large cities, and also where donors are more prevalent. We obtain consent for the treatment of all of our patients [36, 37].

Pigskin is currently the most widely used xenograft around the world. It has been very well studied during recent decades, and has benefitted from several technical innovations. As with epithelial cell cultures, the use of these materials in our country is limited and very expensive. In the past few years, the introduction of bioengineered materials has opened up a new horizon in burns surgery. However, its cost is excessive, but its use can be justified in first world countries where its use may mean a saving in the overall cost of treatment, since it will definitely shorten the total treatment time [38–45].

When one compares the use of frogskin to pigskin, one can point to several advantages, mainly from the financial point of view. There is no need for the use of a dermatome to harvest the frogskin, which is used in its full thickness (about 0.5 mm). It is easy to obtain and easy to apply. The skin can be kept in any regular freezer, without any intention to maintain its viability. In 1986, Zaziass, of the National Health Institute, at Bethesda, MD, described a dipeptide, magainine, contained within the frog skin, with antibiotic properties [46].

The method we currently use to chemically render the skin aseptic or sterile will allow us to keep the frog skin in a regular freezer for up to 6 months. Adherence to the wound bed is very good, with a progressive "loss" occurring due to cure or granulation tissue formation. Early removal from tangentially excised wounds may lead to bleeding at the site, due to vascular ingrowth up to the xenograft.

As for its advantages, we can mention its practically unlimited availability, low cost and relatively easy preparation. It can be applied together with any topical

agent, and it also will prevent wound desiccation, diminishing water losses and possibly heat loss. As it "takes," it can also diminish wound contamination, creating a microambient protecting the developing tissues. It increases patient comfort in relation to the treatment area, and the patient also requires fewer blood products and less pain medication. As for its disadvantages, we can mention that it requires a very rigorous method for preparation, a sterile routine for its single use, and it cannot be resterilized. It is dead tissue, with temporary adherence or take, and it will progressively desiccate as time elapses.

How important (or not) the fact is of the dermal layer being relatively thin in the frogskin, when compared to human or porcine skin, has yet to be established. As the skin is frozen, the dermal layer structure is altered slightly by the formation of water crystals, but it is believed that adherence is a function of fibrin bridges and the other naturally occurring reactions when a graft is applied. It progressively "loses" its adherence as the wound heals or granulation tissue is formed.

Low cost, practically unlimited availability and the ease of preparation and handling are considered its main advantages. It is easily applied and its care is similar to the care of any other graft. We consider that the use of frogskin is another good option for the temporary cover of excised and deep second degree burn wounds, surgical wounds from escharotomies and fasciotomies as well as for donor areas [47].

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