4 The Bioengineered Organoid Skin in Plastic and Reconstructive Surgery

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4.1 Introduction

The development of tissue engineering has given us the possibility of treating organic pathologies, whether congenital or acquired. Langer and Vacanti, in their article titled "Tissue Engineering" in 1993, defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and sciences of life in order to develop biological substitutes that restore, maintain or improve the function of a tissue or an organ" [10].

Tissue engineering can be considered one of the most promising fields in the new chapter of modern surgery, so-called regenerative surgery or inductive surgery.

Tissue engineering uses two principal methods:

- *In vivo:* stimulating self cells to regenerate as a reaction to the appropriate biomaterials or growth factors; or restoring a proper function using gene therapy.
- *Ex vivo:* using cells able to grow in cultures, seeded in a scaffold (a three-dimensional matrix of support made up of hyaluronic acid, collagen, chitosan or synthetic materials, in the case of culture of fibroblasts or keratinocytes), which can later be implanted in the host.

This is the most widely used and promising approach, generally called "tissue engineering." It therefore includes the use of biomaterials such as scaffold (support matrix) and living cells. Moreover, biologically active molecules can be added such as growth factors in order to facilitate the growth and/or the differentiation of the cellular components.

In this way it is possible to take advantage of the structural and mechanical characteristics of the biomaterials and of the biological characteristics of the cells and/or bioactive molecules in order to obtain substitutes able to interact with the host organism to compensate for a lack of function or to modulate biological phenomena (e.g., growth and tissue reactivity).

A choice can be made between heterologous type cells that are from various species, allogeneic type cells that are from the same species but taken from different organisms, or autologous type cells that are from the same organism. Autologous cells are preferred because they do not cause an immune reaction from the host against the implant, avoiding the use of immunosuppressive agents, which are known always to have many adverse effects, often of a serious nature [8].

Different maturative stage cells can be used, such as differentiated tissue specific cells, isolated progenitor cells isolated from specific tissues such as the stem cells of the bony marrow or at least the embryonic stem cell derivatives from the blastomere [2, 5]. Autologous cellular cultures have found a remarkable use in the field of reconstructive plastic surgery, and particularly the culture of autologous fibroblasts and keratinocytes for the repair and reconstruction of widespread loss of cutaneous substance. In our department we have developed a clinical method for the application in vivo of a scaffold in which both fibroblasts and autologous keratinocytes are present and arranged to form an organotypical co-culture that could simulate the architecture of the normal skin, so as to reduce convalescence and surgical times in patients affected by widespread loss of substance, particularly in those affected by giant congenital nevi.

4.2 Indications

The bioengineered skin can be used in all cases in which a widespread loss of cutaneous substance is present. These can be classified, based on their etiology, into wounds caused by: burns, trauma, surgery, and vascular causes.

4.3 Biomaterials

Tissue engineering cannot considered without also including the development of biomaterials. These are indispensable for the culture of the cells in a three-dimensional environment, so that they produce a geometrical organization similar to that existing in the tissue of origin. The esters of hyaluronic acid (HYAFF®) are examples of the latest generation of biomaterials. These are semisynthetic biomaterials produced by the chemical modification of natural polymers with the purpose of improving their mechanical characteristics, manageability and degradation times. This type of modification is generally made in polymers of saccaridic origin because of their elevated hydrophilicity, which renders them suitable for the production of scaffolds for cell culture. Their development has been carried on to the production of reabsorbable sheets used like matrices of support for cellular growth and differentiation in vitro, like a substrate for the transport and delivery of cell cultures and for three-dimensional tissue reconstruction (three-dimensional sheets that simulate the normal structure of the extracellular matrix of the tissues).

4.4 Cellular Bioengineered Cutaneous Substitutes

The idea of recreating the skin in vitro has always fascinated the bioengineers, who have been engaging in this difficult enterprise for some considerable time. There is an urgent clinical requirement for such methods: annually 6,000 persons in Europe are hospitalized for third degree burns. Similarly, every year approximately 800,000 diabetic patients in the same geographic area consult doctors for ulcers of the foot that are difficult to heal; approximately 1,500,000 patients suffer from chronic ulcers of another nature; and more than 3,000,000 patients suffer from decubitus sores.

In 1989 Gallico et al. were the first to propose a technique they already used in the treatment of burns patients, based on the use of keratinocyte autografts cultivated in vitro, for the treatment of giant congenital hyperpigmented nevi [6]. The removal of the nevi was performed by excising deep to the fascial plane and an area corresponding to 6.9 % of the body surface was removed at each procedure. The grafted areas underwent a contraction, which was greater in the softest regions, so the method was not used on flexor surfaces; moreover, in many cases the epithelialization was only partial. In 1990 Matsuda and Suzuki used artificial dermis to treat giant congenital nevi and obtained good results [14].

In 1999 Carsin et al. obtained pleasing results utilizing autologous epithelial culture for the treatment of 30 patients affected by widespread acute burns [3].

Some Italian authors such as Uccioli et al. (in 2002) and Travia et al. (in 2003) have obtained good results with the treatment of patients affected by acute burns, chronic ulcers from pressure or diabetes, and with the treatment of extended lack of substance provoked by trauma, using cultures of fibroblasts and autologous keratinocytes [15, 16].

Organotypical co-culture of keratinocytes and fibroblasts, containing collagen hydrogel, have been used in vitro to study several aspects of the epithelial-mesenchymal interactions in epidermal regeneration and morphogenesis. These conventional systems have shown, however, some defects such as a limited survival, an insufficient resistance to the contraction and an anchorage deficit of the epidermis to the matrix of collagen [12]. In addition, for clinical applications, it is preferable to avoid a matrix of xenogeneic collagen because of the imminent danger of infective or immunological complications.

In 2004 Paul Ehrlich published results on the implantation of sheets of a co-culture of fibroblasts and keratinocytes expanded on a scaffold composed of native collagen in rats, showing a remarkable performance in the acceleration of wound healing through the liberation of numerous growth factors [1].

Recently, Judith Hohlfeld (in August 2005) published in an authoritative international review the results of a clinical trial based on the use of a scaffold of collagen repopulated with fetal cutaneous cells from a donor for the treatment of young burns patients, obtaining encouraging results but with too small a number (only eight) of patients examined [9].

4.4.1 Bioengineered Autologous Organotypical Skin (Figs. 4.1 – 4.3)

The production of autologous organotypical co-culture we have utilized is based on a non-complex method [12]. The patient is seen at the day hospital for blood and instrumental preoperative tests. At the same time, a skin biopsy of about 6 cm^2 is taken from the right groin and sent to the laboratory [Fidia Advanced Biopolymers (FAB), s.r.l., Italy] specializing in the production of skin substitutes.

The technique for preparing organotypical cultures is based on this method: briefly, skin specimens are enzymatically digested to separate epidermis from dermis. The fibroblasts and keratinocytes obtained are propagated for subsequent passaging, after which the cells are seeded to produce the skin substitute [11].

The skin equivalent is produced starting with a scaffold (a non-woven pad measuring 64 cm^2) constituting a benzyl ester of esterified hyaluronic acid (HYAFF 11), especially a partial ester of hyaluronic acid [3]. A quantity of 2×10^5 fibroblasts/cm^2 was seeded on the HYAFF

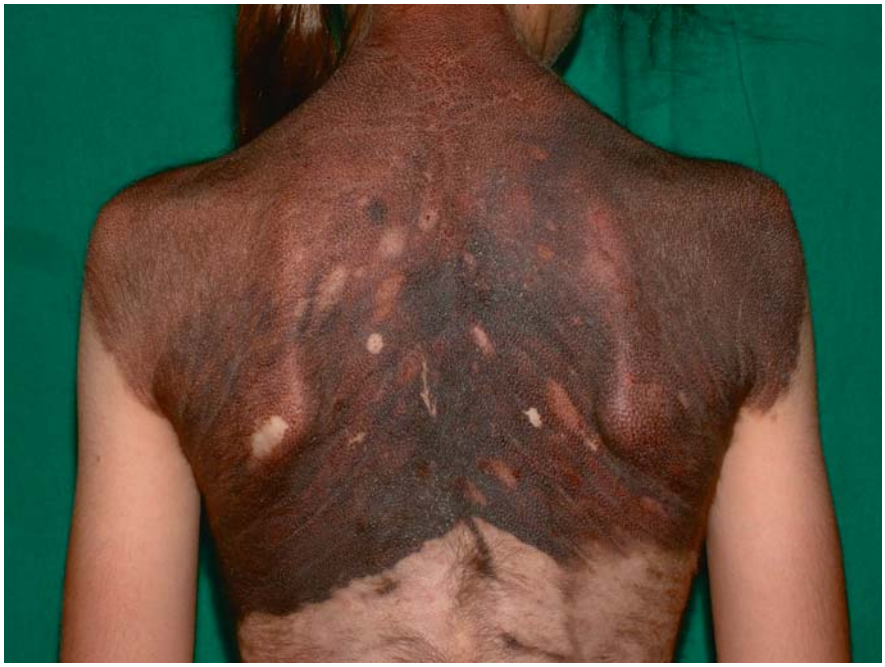

Fig. 4.1. Seven-year-old girl with giant congenital nevus (preoperative view)

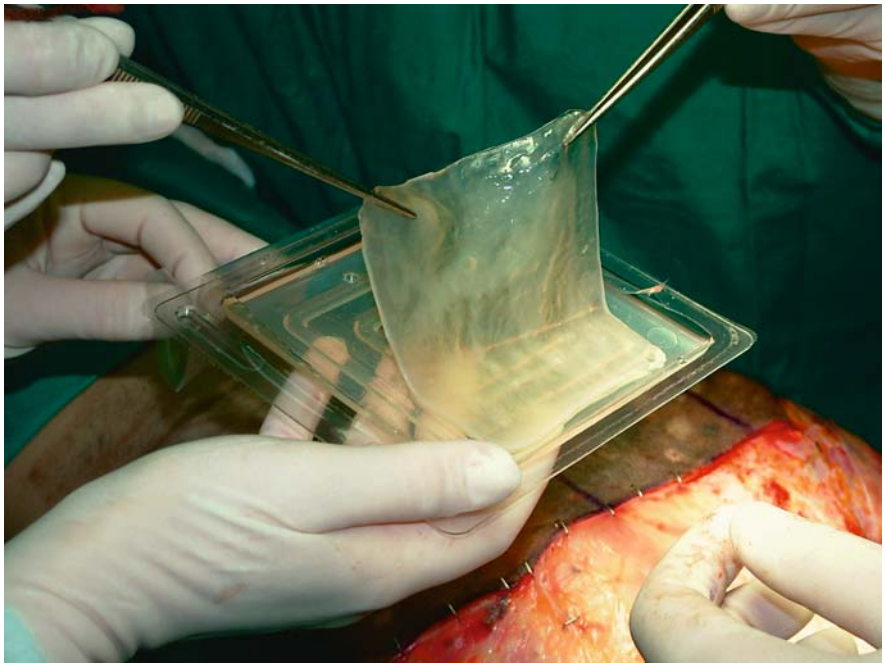

Fig. 4.2. A sheet of autologous organotypical skin substitute based on HYAFF

11 scaffold, resuspended in fibrin gel (Tissucol Baxter). Subsequently, 2×10^5 keratinocytes/cm^2 resuspended in culture medium were seeded on the surface of the skin equivalent. After about 4 days incubation, during which time the cells were kept submerged in the culture medium, the level of the latter was lowered so as to expose the surface of the cells to the air. Incubation at the air-liquid interface promoted development in vitro of a keratinized epidermal surface with a stratum corneum analogue [7].

The applications were performed in ten patients affected by widespread post-traumatic and iatrogenic loss of substance and were extended on a variable surface area of from a minimum of 64 cm^2 to a maximum of approximately 400 cm^2, with a degree of integration of between 40 % and 90 % of the grafted area. At the follow-up, carried out at time intervals of from 6 months to 2 years, a perfect stabilization of the new skin, with a progressive improvement of the scars and the absence

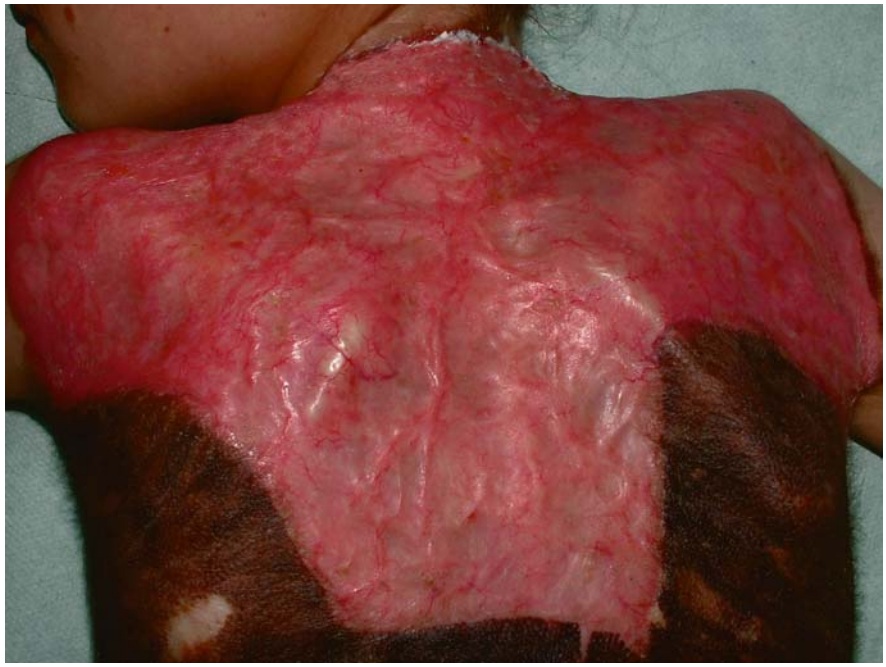

Fig. 4.3. Seven-year-old girl with giant congenital nevus after the application of autologous organotypical skin substitute based on HYAFF (postoperative view, 6 months follow-up)

of alterations of the cellular turnover of the more superficial layers, was observed.

4.5 Conclusions

The results obtained from in vivo experimentation of organotypical co-culture of fibroblasts and keratinocytes on a scaffold of HYAFF 11 demonstrate the possibility of using complex structures in human patients, produced in the laboratory and composed of an artificial part ("scaffold") and a cellular mixed autologous component. The reduction of the hospitalization times and the possibility of carrying out only one surgical procedure are the elements that encourage and stimulate us to persevere in overcoming the technical difficulties. An important next step in tissue bioengineering will be the production of a cutaneous substitute with combined dermis and epidermis arranged with other cellular components such as the endothelial vessel cells, to produce a capillary vessel net that will ensure adequate nourishment to the new tissue. The vascularization is very important in the complete integration of cutaneous substitutes as well as for all types of grafts [13].

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