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Transplantation of cultured human keratinocytes in single cell suspension: A comparative in vitro study of different application techniques

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ABSTRACT

Transplantation of autologous cultured keratinocytes in single cell suspension is useful in the treatment of burns. The reduced time needed for culture, and the fact that keratinocytes in suspension can be transported from the laboratory to the patient in small vials, thus reducing the costs involved and be stored (frozen) in the clinic for transplantation when the wound surfaces are ready, makes it appealing. We found few published data in the literature about actual cell survival after transplantation of keratinocytes in single cell suspension and so did a comparative in vitro study, considering commonly used application techniques. Human primary keratinocytes were transplanted in vitro in a standard manner using different techniques. Keratinocytes were counted before and after transplantation, were subsequently allowed to proliferate, and counted again on days 4, 8, and 14 by vital staining. Cell survival varied, ranging from 47 to >90%, depending on the technique. However, the proliferation assays showed that the differences in numbers diminished after 8 days of culture. Our findings indicate that a great number of cells die during transplantation but that this effect is diminished if cells are allowed to proliferate in an optimal milieu. A burned patient's wounds cannot be regarded as the optimal milieu, and using less harsh methods of transplantation may increase the take rate and wound closing properties of autologous keratinocytes transplanted in a single cell suspension.

1. Introduction

Cultured keratinocytes have been used for about 20 years in the treatment of burns and other cutanous wounds. In 1975, Rheinwald and Green [1] described a reliable method of culturing human epidermal cells in stratified and coherent layers, and so cultured epidermal sheet autografts became available to complement autologous split thickness skin grafts in treating major burns or other large wounds (Fig. 1A) [2]. However, producing confluent grafts of keratinocytes puts heavy demands on laboratory skills, comprises manual labour, and is expensive, which limits the use of the autografts in many ways. The sheets are only 8–10 cells thick, which make them fragile and difficult to handle, and means that they have to be placed on a supportive backing material to be possible to transfer from laboratory to patient (Fig. 1B). When the autografts have been detached from the culture vessel, they must be transplanted the same day, which requires the wound surfaces to be ready for grafting at the same time as the autografts are ready to be transplanted. Because the

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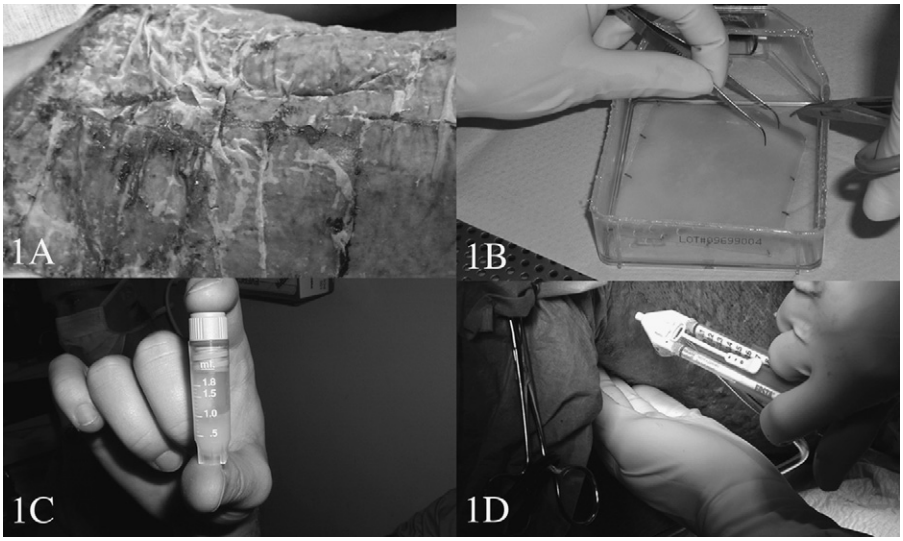

Fig. 1 – (A) Cultured epithelial autografts transplanted to wound surface with a polyamide-mesh backing material. Picture taken 5 days after transplantation. (B) Cultured epithelial autograft has been enzymatically released from culture vessel's bottom. Polyamide-mesh backing material (Surfasoft®) is being attached by folding the autograft's edges over the backing material and securing them with surgical micro-clips. (C) Transport and storage vial for cultured cells, e.g. cultured autologous keratinocytes. Depicted vial contains approximately 20×10^6 cultured cells ready for transplantation. (D) Autologous cultured keratinocytes from the vial in picture 1C has been aspirated into the thrombin fraction of the Tisseel Duo Quick™ tissue glue syringe and are being spray-painted on the wound surface using the Duploject™ Spray set.

transplanted epidermal sheets are quite unstable and prone to blistering, care must also be taken, not only during production and transplantation, but also during dressing of the applied grafts, mobilisation of the patient, and changing of dressings [3]. The production of stratified grafts inevitably involves some degree of maturation and differentiation, of the keratinocytes, which reduces their proliferative capacity, and this in turn may affect the take-rate and wound-healing capacity [4]. Extrinsic factors including preparation of the wound, nutritional state, and dressings used influence their success.

The attention to, and understanding of, these shortcomings have led to a progressive development of techniques of skin culture and an increased use of suspensions of single cells of keratinocytes being transplanted instead of sheet grafts. Fraulin et al. [5], in 1998, described a novel technique in which they used an aerosol device to spray epithelial cells on wounds in pigs. They noted that re-epithelialisation was quicker than in unsprayed controls. Navarro et al. [6] developed this technique further by combining it with meshed split thickness skin grafts. They reported faster healing and a better quality of cells when they were sprayed. Further advantages of suspension transplantation are the reduced time needed for culture, and avoidance of the manual labour of releasing cell-sheets from culture flasks and attaching the cell-sheets to backing materials. By culturing and transplanting the cells in a suspension rather than as a sheet, the use of enzymes like Dispase® can be avoided. Keratinocytes in suspension can then be transported from laboratory to patient in a handful of small vials (Fig. 1C) and be stored (frozen) at the clinic to be transplanted when the wound surfaces are ready [7]. The single cell suspension of keratinocytes can then be transplanted to the patient with whatever method is available such as being spray-painted on the wound surfaces with or without fibrin-glue (Fig. 1D) [8,9]. Today, transplantation of keratinocytes in a single cell suspension overgrafted with meshed allogeneic donor skin is a common approach in the treatment of burns [9,10]. Techniques used in clinics today include spraying cells, with or without the additional use of tissue or fibrin glue, painting the cell suspension with a brush, or dripping the cell suspension on to the wound bed using a syringe. At our burn unit we have used the Tissomat applicator together with Tisseel Duo Quick™ tissue glue and the Duploject™ Spray set (all from Baxter Medical AB, Kista, Sweden) to transplant cultured keratinocytes. To distribute the cells satisfactorily, pressures as high as 200 kPa must be used. This has long been thought to damage the cells, both by the passing of the spray nozzle and by the high velocity impact on to the wound bed. Harkin et al. [11] recently examined the viability of keratinocytes delivered by aerosol, using the Tissomat applicator and found that the viability after transplantation (93.7% at 70 kPa and 90% at 138 kPa) was similar to the viability of the cells just recently detached from the culture dish (94%). When they adjusted the Tissomat applicator to deliver 207 kPa, they showed that fewer cells survived (73.3%), but not significantly so.

If a brush is used to distribute the cells to the wound (the method of choice in some clinics) it probably causes shear forces that damage the cells, and may leave fibres from the brush in the wound. Drips from the cell suspension, when using a syringe, may well cause an uneven distribution of the cells, if the suspension runs off the surface, pools in cavities, and leaves some areas uncovered. The fact that surprisingly few research workers have studied the viability and survival of transplanted cells, encouraged us to design a comparative in