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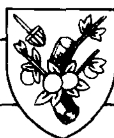
BRITISH JOURNAL OF  PLASTIC SURGERY

Skin graft storage and keratinocyte viability

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SUMMARY. **The viability of human split skin grafts stored in four solutions has been assessed by monitoring the percentage of viable keratinocytes in the stored grafts.**

Skin grafts stored in RM$^+$ (Ready Mix) tissue culture medium remained more viable than those stored in Hartmann's, Marshall's or saline solutions. By day 10 (postoperative), the percentage of viable keratinocytes of those grafts stored in RM$^+$ was around 85%, compared to a value of around 10% for the other media. By day 30, RM$^+$ achieved a value of around 60% keratinocyte viability compared to a value approaching 1% in the other storage media under investigation.

RM$^+$ provides mitogens, nutrients, growth factors and physiological pH, all of which are important factors for successful skin graft storage.

Autologous and allogenic skin graft preservation and storage may, at times, be indicated and both may benefit the burned patient who has limited donor site availability.[1] Adequate skin storage may reduce the number of harvesting procedures required to achieve skin cover to a single operation and the stored skin can be applied when required as a bedside dressing without anaesthesia.

Skin can be preserved by three techniques:–

1. Refrigeration with saline or with nutrient media at 4°C.[2,3]
2. By freezing (pre-treatment with glycerol or DMSO) and storing at temperatures below 0°C.[4]
3. Freeze-drying or lyophilisation and storage at room temperature.[1]

It is interesting to note that de Martigny in 1913 noticed that skin preserved for several days in cold storage gave better results than when used without hypothermic preservation; and that Carrel (1912), when discussing the tissue-culture of pieces of skin, said "when they cease to proliferate I place them in cold storage for several days in a state of latent life, then when I replace these tissues in a medium suitable for their culture I have observed a new and active growth of cellular proliferation".[3] There is also a possible relationship between refrigeration and an increase in the power of skin to withstand infection by some organisms, according to Matthews.[3]

In the United Kingdom most skin grafts are stored by refrigeration at 4°C with the skin grafts laid on tulle gras wrapped in saline moistened gauze.

The objective of this research is to study what extent the viability of stored skin grafts can be affected by the storage media, viability being assessed by monitoring the percentage of viable keratinocytes. When storing any living tissue, the destructive effects of hypoxic metabolism must be controlled. A tissue removed from its blood supply will die unless cellular metabolic activity is decreased or nutrients are provided. By reducing metabolism and providing nutrients, viability can be further improved. Cell metabolism is reduced by cooling and the ideal medium in which the skin is stored should supply electrolytes and nutrients in physiologic concentration and be buffered against the acid metabolites constantly produced by the graft.

Methods and materials

Split thickness skin grafts 2 cm^2 to 4 cm^2 were harvested from 24 patients undergoing routine surgery for skin tumours, burns, skin lacerations, etc. The mean age was 46 years (range 12–80 years). The 24 patients were randomly divided into 4 groups (I–IV). Each group contained six patients. The skin grafts were taken from the thigh in the majority of cases and a few from the abdomen (following abdominoplasty). They were laid on tulle gras, outer-side downwards and folded with dermis to dermis contact. The skin grafts were then stored at 4°C in swabs moistened with the storage solution. Four different storage solutions were used (Table 1).

RM$^+$ is a universally used medium for culturing of keratinocytes. The term RM$^+$ (Ready Mix) was used by the Department of Dermatology at the Royal London Hospital, where the medium is prepared under aseptic conditions. This is the same medium described by Wu *et al.*[6]

The skin grafts in the moistened swabs were put in dry sterile containers and sent to the laboratory on the day of the operation, to be studied for keratinocyte viability at varying times following harvest.

A standard sterile technique was employed throughout the procedure. Each skin graft was cut into four equal pieces with one piece analysed on day 0 (day of operation) and a piece each for days 10, 20 and 30. Unused pieces were stored on the moistened swab at 4°C. The piece to be studied was washed in Versine

Table 1 Solutions used for Storage of Skin Grafts

Group	*Storage solution*	*Contents of solution*	
I	Hartmann's moistened swab [physiological solution]	Sodium	131 mM/L
		Potassium	5 mM/L
		Calcium	2 mM/L
		Chloride	111 mM/L
		Bicarbonate (as lactate)	29 mM/L
II	Marshall's moistened swab[5] [donor organ preservation fluid]	Sodium	80 mM/L
		Potassium	80 mM/L
		Magnesium	40 mM/L
		Sulphate	40 mM/L
		Citrate	55 mM/L
		Mannitol	150 mM/L
III	Saline moistened swab	0.9% Sodium chloride	
IV	*RM$^+$ moistened swab (tissue culture medium)	***3 parts of Dulbecco's Modification of Eagles Medium (DMEM)	
		**1 part of HAMS F_{12} Medium	
		10% Foetal Calf Serum (FCS)	
		Epidermal Growth Factor (EGF)	10 ng/ml
		Hydrocortisone	0.4 μg/ml
		Cholera toxin	10^{-10} M
		Transferrin	5 μg/ml
		Lyothyronine	2×10^{-11} M
		Adenine	1.8×10^{-4} M
		Insulin	5 μg/ml

* RM$^+$ = Ready Mix.
** DMEM and HAMS F_{12} are two synthetic tissue culture media which provide inorganic salts, vitamins and amino acids.

Fig. 1

Figure 1 – Trypan blue method. Dead cells stained blue (dark colour), viable cells remained clear of the dye.

(EDTA), a chelating agent for divalent ions Mg^{++}, Ca^{++} (trypsin inhibitors). The washed piece of skin was transferred to a 0.25% trypsin solution and incubated at 37°C for approximately 90 min. After trypsin digestion the epidermis and dermis were separated with syringe needles, liberating keratinocytes as a single cell suspension *i.e.* the cells were not clumped together. The liberated epidermal cells included basal cells, suprabasal cells and the corneocytes from the stratum corneum. The cells were pelleted by centrifuging at 1,000 rpm for 5 min and suspended in 1 ml of Dulbecco's Modification of Eagles Medium (DMEM) and 10% Foetal Calf Serum (FCS).

Equal volumes of cell suspension (100 μl) and 0.4% trypan blue were mixed and loaded onto a haemocytometer to count the cells. Dead cells were stained blue by the trypan blue, whereas viable cells remained clear of the dye as their membranes were intact (Fig. 1). Each of the 96 pieces from the 24 skin grafts were studied in this way on the appropriate day, except for three pieces (all belonging to one patient) which were stored in RM$^+$ and showed fungal contamination from day 10 (postoperative) (Table 5).

All results were statistically analysed using the standard "2-way analysis of variance".

Results

There was a marked difference in keratinocyte viability between skin grafts stored in RM$^+$ and those stored in the other storage solutions (Tables 2, 3, 4, 5).

By day 10 there was a marked drop in the percentage of viable keratinocytes in skin stored in Hartmann's, Marshall's and saline compared to a value around 85% of viable keratinocytes of those grafts stored in RM$^+$. By day 30, RM$^+$ achieved a value of around 60% keratinocyte viability compared to a value

Table 2 Percentage of Viable Keratinocytes for Skin Stored in Hartmann's Solution

Patient	*Day 0*	*Day 10*	*Day 20*	*Day 30*
1	97.2	6.0	8.0	0.0
2	97.0	28.0	1.8	1.7
3	95.3	15.4	1.3	0.0
4	95.4	1.9	1.3	0.0
5	87.8	5.0	0.0	0.0
6	85.6	1.3	0.0	0.0
Avg	93.1	9.6	2.1	0.3

Table 3 Percentage of Viable Keratinocytes for Skin Stored in Marshall's Solution

Patient	*Day 0*	*Day 10*	*Day 20*	*Day 30*
7	87.4	7.9	5.3	0.0
8	83.4	11.0	8.7	0.0
9	88.6	8.4	2.8	0.0
10	90.7	22.5	0.0	0.0
11	56.2	6.1	0.0	0.0
12	88.1	7.2	4.3	0.0
Avg	82.4	10.5	3.5	0.0

Table 4 Percentage of Viable Keratinocytes for Skin Stored in Saline Solution

Patient	*Day 0*	*Day 10*	*Day 20*	*Day 30*
13	73.1	2.1	0.4	5.4
14	57.0	32.0	10.2	2.3
15	76.5	1.2	2.1	0.0
16	71.2	4.8	7.6	0.5
17	49.1	7.7	1.7	0.0
18	89.1	1.2	3.9	0.0
Avg	69.3	8.2	4.3	1.4

Table 5 Percentage of Viable Keratinocytes for Skin Stored in RM$^+$ Solution

Patient	*Day 0*	*Day 10*	*Day 20*	*Day 30*
19	91.1	85.2	54.4	76.2
20	91.1	70.1	83.0	71.7
21	94.6	91.5	57.3	27.3
22	92.3	90.8	29.4	50.0
23	93.9	92.0	80.8	75.0
24	97.9	C	C	C
Avg	93.5	85.9	61.0	60.4

C = Contaminated.

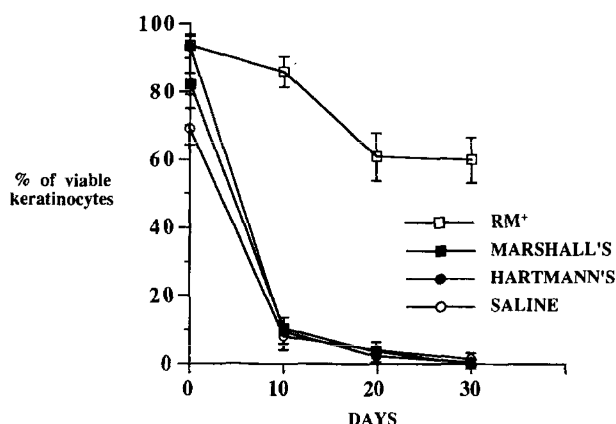

Figure 2—Average keratinocyte viability for skin grafts stored in the four solutions.

approaching 1% in the other storage solutions under investigation (Fig. 2).

The confidence limits (*i.e.* the 95% chance of the viability falling within that range) for the four solutions were: Hartmann's (2.84% to 49.76%), Marshall's (0.64% to 47.56%), Saline (0 to 44.23%) and RM$^+$ (43.04% to 89.96%). The null hypothesis (there is no relationship between the viability of the stored skin and the storage solution) was rejected. From this study RM$^+$ appears to be better than other solutions in maintaining cell viability.

Discussion

Previous work has reviewed storage of split thickness skin grafts in different media. Tissue culture media as RPMI-1640 (Roswell Park Memorial Institute-1640) were able to maintain viability of the stored skin for up to 22 days.[7] McCoy's 5A medium + 10% serum maintained skin viability up to 30 days.[1] However, the results of these studies concentrated on graft take (mainly using animal models), where many factors may affect the take, *i.e.* the storage medium is not the only variable. We believe that studying keratinocyte viability is a reliable indicator of the effect of different storage solutions on the viability of human skin grafts.

The trypan blue method is a simple method of studying viability purely by the condition of the cell membranes, *i.e.* if the membrane is intact it is termed viable. It does not indicate the ability of the cells to divide and proliferate.

The tradition of storing skin in saline soaked swabs should be reviewed, as saline lacks nutrients and has no acid buffering properties. Tissue culture media such as RM$^+$ should be of great value, as they provide the necessary mitogens, nutrients, growth factors and physiological pH, all of which are important factors for successful skin storage. EGF is a polypeptide which delays senescence of the multiplying cells by maintaining them in a state further removed from terminal differentiation. This effect is revealed by a greater ability of the cells to survive subculture and to initiate new colonies. It is an extremely potent mitogen for fibroblasts.[8] Cholera toxin (provided by RM$^+$) is the most effective agent known to increase intracellular cyclic AMP, which greatly improves the growth of cultured human epidermal cells. In the presence of the toxin, the ratio of small proliferating cells to large terminally differentiating cells is increased. Cholera toxin is not toxic[9] up to concentrations 10^{-8} M and is maximally effective in promoting multiplication at 10^{-11}–10^{-10} M which is the same concentration provided by RM$^+$.

In our study antibiotics were not added to RM$^+$, as this medium is already used universally for culturing of keratinocytes without the inclusion of any antibiotics. Antibiotics can be used to avoid contamination of stored skin if necessary.

RM$^+$ costs more in comparison to the other 3 storage solutions (£41 per litre), but such a difference may be offset by more successful graft take in patients requiring late or secondary skin grafting.

This *in vitro* study showed that RM$^+$ is a better medium in maintaining cell viability of stored human split thickness skin grafts. Future research could include the assessment of autograft take in the clinical situation.

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