LABORATORY STUDY

A comparison of skin storage methods for oculoplastic surgery

LELIO BALDESCHI, ANTONELLA LUPETTI, MARCO NARDI, CHRISTOPH HINTSCHICH, J. RICHARD O. COLLIN

Abstract

Purpose **To assess the level of contamination of full-thickness skin grafts stored with or without an antibiotic cover.**

Methods **Full-thickness skin grafts were harvested from 40 bilateral upper lid blepharoplasties. Before surgery the face was sterilised, the head of the patient was packed with sterile, single-use surgical drapes and the whole face was left exposed. The harvested full-thickness skin grafts were conserved in sterile containers at 4 °C for 6 days, rolled in gauze moistened with either 4 ml of sterile saline solution (group I) or with 4 ml of gentamicin solution (2 mg/ml) (group II). The degree of contamination, expressed in colony forming units (CFU), was evaluated on days 2, 3, 4, 5 and 6. Identification of the microorganisms was done to species level following standard procedures and commercial methods.**

Results **In group I 2 grafts (5%) were negative during the whole observation period while the other 38 grafts (95%) presented a degree of contamination ranging from 10^2 to 10^4 CFU. Microorganisms isolated were: *Staphylococcus epidermidis* (24 cases), *Staphylococcus aureus* (5 cases), *Staphylococcus saprophyticus* (2 cases), *Pseudomonas aeruginosa* (4 cases), *Serratia liquefaciens* (1 case) and *Klebsiella oxytoca* (2 cases). In group II, 26 grafts (65%) were negative during the whole observation time while in 14 cases (35%) a few colonies (3 to 6) of *Candida albicans* were isolated on day 2 and remained constant in number for the whole observation time.**

Conclusions **The storage of full-thickness skin graft with an antibiotic cover is more reliable than the storage of full-thickness skin graft without an antibiotic cover.**

Key words Contamination, Full-thickness skin grafts, Gentamicin, Storage methods

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Full-thickness or split-thickness skin can be harvested, stored and used as a viable graft many days later. This is particularly useful when treating patients with extensive skin loss such as after burns, so that the number of general anaesthetics required for harvesting grafts can be kept to a minimum. Any areas in which the primary grafts do not take can then be regrafted without harvesting more skin. Since skin can be grafted after a delay, it is a sensible precaution to save the skin excised in such procedures as cosmetic blepharoplasty, so that in the event of unacceptable post-operative lagophthalmos some of the skin can be regrafted if necessary. This could be required in patients with thyroid eye problems, in whom there is often more apparent than true excess skin and the situation is aggravated by proptosis and a poor tear film. Skin is often stored in saline swab in a sterile container in a fridge.[1–3] Although it is rarely needed in ophthalmic practice, clearly if it is required it is desirable that it should be as free from contamination as possible if it is to be used as a graft. This study was designed to assess the level of contamination of skin stored with or without an antibiotic cover.

Materials and methods

Full-thickness skin grafts were harvested from 40 bilateral upper lid blepharoplasties. Before surgery the face was sterilised with a povidone–iodine (polyvinylpyrrolidone–iodine complex) solution (100 mg/ml). The head of the patient was packed with sterile, single-use surgical drapes and the whole face was left exposed. All skin grafts were taken by the same surgeon under the same circumstances in the same unit.

The harvested full-thickness skin grafts were conserved in sterile containers at 4 °C for 6 days, rolled in gauze moistened with either 4 ml of sterile saline solution (group I) or with 4 ml of gentamicin solution (2 mg/ml) (group II). One graft from each patient was included in group I and the other in group II.

A small sample (5 × 5 mm) was cut from each piece of stored skin using sterile instruments in a laminar flow hood and washed in 1 ml of sterile solution on days 2, 3, 4, 5 and 6. Subsequent dilutions (1:10, $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$) in sterile saline of such suspensions were obtained. Ten microlitres of each suspension was inoculated onto blood-agar plates in order to evaluate the degree of contamination in colony forming units (CFU).

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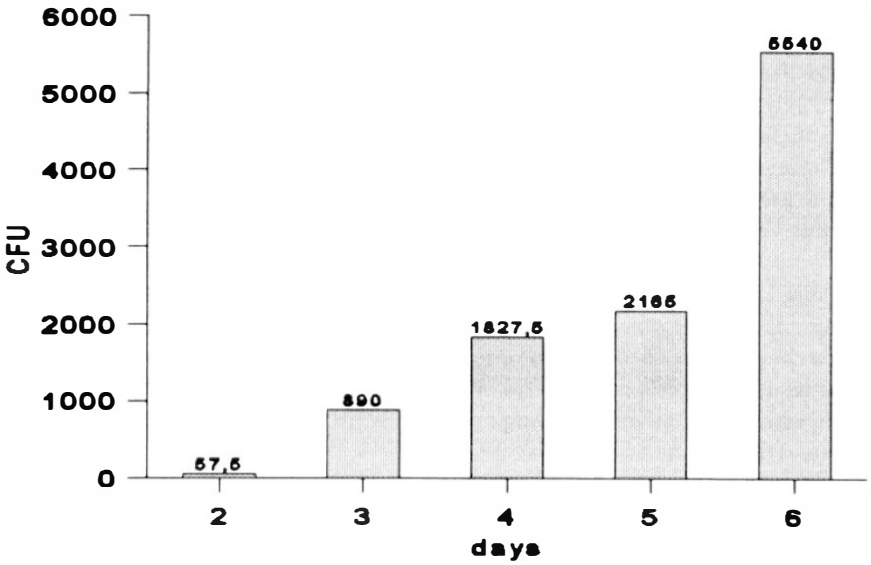

Fig. 1. *Mean degree of contamination in group I during the observation period.*

Each plate was incubated at 37 °C and evaluated after 24 h. Identification of microorganisms was done to species level following standard procedures and commercial methods: API 20 E and API-NF (Bio-Merieux, Marcy l'Etoile, France) to identify Gram-negative microorganisms; API Strep and API Staph (Bio-Merieux, Marcy l'Etiole, France) to identify Gram-positive microorganisms; Api ID 32C (Bio-Merieux, Marcy l'Etoile, France) to identify yeasts.

Results

In group I there were marked differences in the degree of contamination of the 40 grafts. Two (5%) were negative during the whole observation period, while 16 (40%) presented a constant degree of contamination (10^2 CFU) during the whole observation time. Fifteen (37.5%) developed 10^2 CFU on day 4, 10^3 CFU on day 5 and 10^4 CFU on day 6. Seven (17.5%) developed 10^2 CFU on day 2, of which 4 (10%) developed 10^3 CFU and 3 (7.5%) developed 10^4 CFU on day 3; they all developed 10^4 CFU on days 4, 5 and 6 (Fig. 1).

Several microorganisms were isolated in group I, most of which were Gram-positive (31 cases). *Staphylococus epidermidis* was the commonest (24 cases), *Staphylococcus aureus* was isolated 5 times while *Staphylococcus saprophyticus* was found twice. *Pseudomonas aeruginosa* was the commonest Gram-negative microorganism isolated (4 cases), while *Serratia liquefaciens* (1 case) and *Klebsiella oxytoca* (2 cases) were isolated more rarely.

In group II, 26 grafts (65%) were negative during the whole observation time while in 14 cases (35%) a few colonies (3 CFU in 10 cases, 6 CFU in 4 cases) of *Candida albicans* were isolated on day 2; these remained constant in number for the whole observation period (Fig. 2).

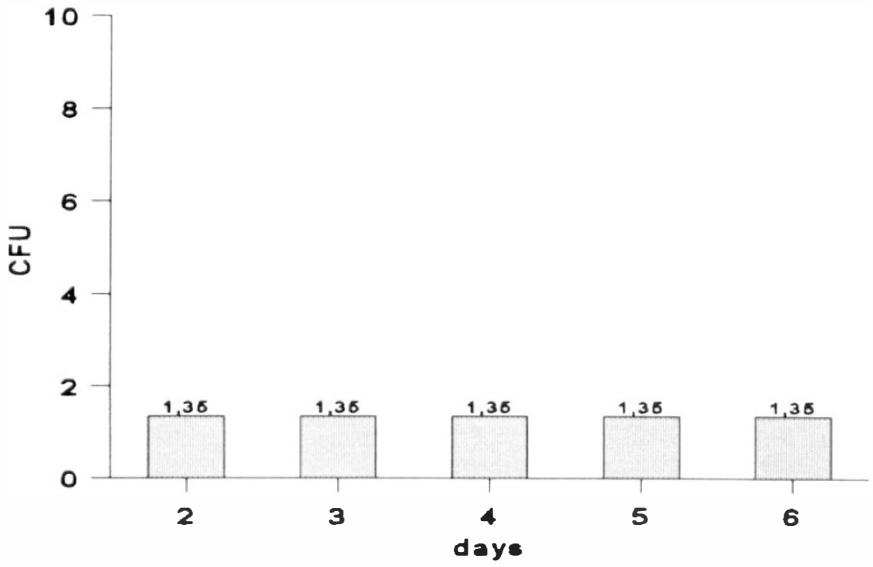

Fig. 2. *Mean degree of contamination in group II during the observation period.*

Discussion

This study demonstrates that the storage procedure used in group I is not completely reliable: 57.5% of the cases showed a high degree of contamination from the beginning; 37.5% that were negative during the first 3 days developed a high degree of contamination from day 4; and only 5% maintained sterility during the whole observation period.

In group II, a few colonies of *Candida albicans* flourished in 35% of the cases as a result of gentamicin selectively removing its competitors. *C. albicans* is not of clinical significance for healthy subjects and the use of antifungal agents is not essential in the usual storage of full-thickness skin grafts. The evaluation of all possible human diseases due to the microorganisms isolated in the present study in group I is beyond the scope of this paper; the discussion will therefore be focused mainly on the surgical, ophthalmoplastic and ophthalmic aspects.

Apart from *Serratia liquefaciens*, which occasionally occurs in clinical specimens with dubious clinical significance,[4] the other isolated microorganisms are often involved in infections complicating surgery. Skin infections caused by the coagulase-positive species *Staphylococcus aureus* are the most common human staphylococcal infections. These include folliculitis, furuncles, carbuncles, cellulitis, impetigo, scalded skin syndrome, and post-operative wound infections of various sites.[5] The reported cases of orbital cellulitis after surgery are very few, but the offending organism after surgical intervention has most commonly been *S. aureus*.[6–9]

Since the coagulase-negative staphylococci constitute a major component of our normal microflora, especially of the skin, these organisms were formerly considered to be saprophytes or of low pathogenicity for humans. Several species of coagulase-negative staphylococci are now documented as opportunistic human pathogens. Of all the species of coagulase-negative staphylococci, *Staphylococcus epidermidis* appears to have the greatest pathogenetic potential,[5] and *Staphylococcus saprophyticus* has been isolated from wound infections and septicaemia.[5]

Many species of Enterobacteriaceae, including *Klebsiella oxytoca*, commonly cause extraintestinal infections. These include wound infections.[4]

Pseudomonas aeruginosa is a common isolate from wounds and *Pseudomonas* wound infections may lead to secondary bacteraemia.[10] Although keratitis is the most common ocular infection caused by this organism, it also may cause conjunctivitis,[11] dacryocystitis,[12] orbital cellulitis,[12] endophthalmitis[13] and necrotising infection of the eyelids.[14]

The eye is surrounded by brows, lashes, lacrimal and nasal orifices that are all incapable of surgical sterilisation: the risk of infection is, for this reason, constantly present.[15] Nevertheless every effort must be made to reduce the contamination of the surgical field: infective complications in lid surgery can be sufficiently severe to interfere with or totally nullify operative results.[15]

According to the results of this study the use of gentamicin, by reducing the contamination of the grafts, improves the reliability of the simple storage method previously proposed[1–3] without any substantial difficulty. This wide spectrum antibiotic is extremely common and always available in any ophthalmic department; the minimal quantity used in this study, comparable with the antibiotic concentration in minimal culture media, is unlikely to influence the viability of the grafts adversely.

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