What Criteria Should be Used for Designing Artificial Skin Replacements and How Well do the Current Grafting Materials Meet These Criteria?

SPEAKER

JOHN F. HANSBROUGH, M.D.

Early coverage of the burn wound remains an important goal. Even temporary closure of the wound, be it partial- or full-thickness in depth, can result in a decrease in metabolic rate, reduction in fluid losses, decrease in pain, and decreases in colonization and invasion of the wound by microorganisms. In addition, wound coverage promotes the rate of epidermal and dermal repair processes (1), and can also delay the appearance of granulation tissue (9). However, the identification and development of completely satisfactory skin substitutes remain elusive goals. To begin the discussion: what are the characteristics to look for regarding an artificial skin and what is the status of currently available skin substitutes? Included will be research performed during the last year involving wound coverings and a review of the research involving cultured epidermal cells as an eventual coverage for the burn wound.

As far as the need for artificial skin replacements, three types of wounds need to be covered. These are, number one, the donor site, where coverage may promote healing and minimize discomfort, number two, partial-thickness wounds, for which artificial materials can protect the wound and promote rapid epithelialization with minimal inflammation and scar formation; and number three, and most important, excised, full-thickness

wounds, where temporary coverage is often required prior to autografting. In the future, hopefully, synthetic skin replacements will be developed which will allow for final wound coverage and avoid the need for subsequent autografting.

The ideal properties of skin substitutes have been discussed before (5, 9). It has become clear that both physical and biologic properties of these materials must be considered (5), if a completely satisfactory material is to be developed. The most important criteria merit discussion here.

The material should adhere to the burn wound soon after placement on the wound surface. The material should control evaporative fluid losses from the wound and also avoid the build-up of fluid between the wound and the dressing material. It should be flexible, and should be durable and resistant to tearing. The material should act as a barrier to microorganisms and prevent bacterial invasion from the external environment into the burn wound, as well as limit the growth of microorganisms already present in the wound. It should not provoke an inflammatory or foreign body reaction in the burn wound, which may lead to granulation tissue.

The material should have safety. It should be sterilizable and should be non-toxic. If the material is only a temporary wound covering, it should be easily removable and not cause damage upon removal. The material should be cost effective, which is going to be, of course, much more important in coming years.

The list of available materials has not changed markedly in the past 5 years (3, 9). As far as biologic dressing materials for temporary wound coverings, viable human homograft, either fresh or cryopreserved, remains the standard for comparison, as evidenced by the proliferation of skin banks in this country. Amniotic membrane is still used by a number of groups (10) but lack of durability and availability and high vapor transmission remain major problems.

Porcine heterograft, either fresh or frozen, continues to be used for temporary wound coverage, although some have questioned its utility (2, 7, 9). Problems with pigskin include poor adherence and infection control, early rejection, and occasional incorporation into the wound. The efficacy of pigskin in controlling infection may be improved by the incorporation of antimicrobial agents such as silver nitrate into the material. Various tissue biologic derivatives such as collagen sheets, mats, fabric, or sponges have been studied as wound dressings. Although many collagen materials adhere well initially to the wound, collagen is not an effective burn wound covering for several reasons. Collagen stimulates the development of granulation tissue and elicits a chronic inflammatory response before being biodegraded (6). Collagen sheets are not elastic and dislodge with shear stress. Also, collagen dressings fail to control the growth of microorganisms in the wound (2, 8).

Finally, there are available the synthetic and compos-

ite materials which have been intensely studied in the last few years. Solid silicone membranes fulfill some of the criteria for wound coverings, and are particularly valuable because of their control of water vapor transmission. Silicone membranes alone fail to control infection in the burn wound. Various plastics such as the polyurethanes (e.g., Op-Site) and polyvinyl chlorides, in various physical forms—foams and sheets, solid or microporous—are also partially effective but generally fail to adhere to the wound or control bacterial growth.

The composite materials offer a useful alternative to wound coverings. These materials generally combine two different substrates in order to achieve the overall qualities desirable in an artificial skin covering; generally a porous material is used against the wound with a semipermeable membrane on the exterior. One of the first of the composite dressings used was cotton gauze bonded to silicone membrane (9).

Over the past year the use of the composite material Biobrane (Hall-Woodroof) (8) has been investigated for temporary wound coverage. Biobrane is a knitted nylon mesh covered with a very thin silicone membrane; both layers are bonded with collagen peptides to improve adherence. Biobrane has been shown to be an excellent temporary covering for both partial-thickness and full-thickness wounds and has many of the desirable characteristics of a temporary skin covering, particularly properties of water vapor transmission and adherence. It appears to control bacterial proliferation in clean, debrided wounds and appears to be associated with minimal inflammation in the burn wounds. The combination of antimicrobial agents with Biobrane may prove to control bacterial growth and make this material more useful for contaminated wounds.

As mentioned previously, native collagen is not an effective wound covering material. Dr. Burke and Dr. Yannas have developed an "artificial skin" consisting of a collagen and glycosaminoglycan (GAG) artificial dermis covered with a Silastic "epidermal" component. This material, first and very importantly, has biochemical characteristics of controlled rate biodegradation and it is neither inflammatory nor immunogenic, and second, possesses physical characteristics, namely, proper pore size and pore orientation, that are equally important to allow for cell migration into the material and provide proper subsequent collagen fiber orientation. Vascularization of the collagen framework occurs in 3 to 5 days to form a "neodermis"; at a later time the Silastic epidermis is peeled off and the neodermis is covered with thin autograft to provide the epidermal component for the wound. Wound contracture and subsequent scar formation appear to be minimal, although long-term follow-up results are not yet available.

The limitations of this material, however, have also been discussed by Dr. Burke. The first limitation is premature loss of the Silastic layer. This leads to subsequent damage of the neodermis below, followed by the development of granulation tissue, which is undesirable, and ultimately leads to a suboptimal functional and cosmetic result. Secondly, subsequent autografting of the wounds covered with the collagen-GAG neodermis is necessary to restore the epidermal layer and achieve permanent wound closure.

The burn unit in Colorado has begun a collaborative effort with Dr. Richard Hamm and Dr. Steven Boyce of the Department of Molecular, Cellular, and Developmental Biology on the Boulder campus of the University of Colorado. This project involves the culturing of human epidermal cells to provide more permanent wound closure to the burn patient in the future. To initiate epidermal cultures, skin tissue is digested with collagenase and cells are dispersed with trypsin-EDTA to yield suspensions of epidermal keratinocytes which then are plated out and cultured under biochemically defined conditions. Most importantly, Dr. Hamm and Dr. Boyce have determined, recently, that the growth rates and the differentiation of the epidermal cells can be very precisely regulated by adjusting the culture conditions (4). Figure 1 demonstrates the effect of calcium ion concentrations on keratinocyte morphology in vitro; increasing the calcium concentration in culture has a very pronounced effect on cellular growth and differentiation. Keratinocyte cultures grown initially in lower calcium concentrations proliferate very rapidly but do not stratify significantly. By varying the calcium concentrations between 0.3 mM and 1.0 mM, rapid cell growth and desirable differentiation and stratification of the epidermal cells can be achieved. At a high calcium concentration (1.0 mM), 20% of cells will produce highly developed cornified envelopes (4), which will be important to provide biological protection when the cells are placed on the wound.

In the next group of experiments, made possible by the very generous donation of samples of the collagen-GAG membrane by Dr. Yannas to this laboratory, the growth of epidermal cells on that membrane have been studied. The area of the collagen-GAG membrane covered by cultured epidermal cells has been quantitated as follows. The keratinocyte cultures on the collagen-GAG membrane or on polystyrene were fixed in gluteraldehyde and then stained. Specimens were photographed, printed, and areas covered by the cells were excised from the photograph and weighed and the per cent area covered by the cells expressed as the weight of the area covered by the cells divided by the total weight of the photograph to give the per cent area of the membrane covered by human epidermal cells.

Figure 2 shows the effect of the culture substrates on keratinocyte growth. With optimum concentrations of calcium about 15% of the polystyrene dish was covered by cells in 12 days. The addition of human serum albumin or bovine pituitary extract to this medium does not have a significant effect on increasing the measurable cell growth.

However, if these cells are cultured at a low inoculation density of 25 cells per square centimeter on collagen-GAG membranes, there is no measurable growth of these
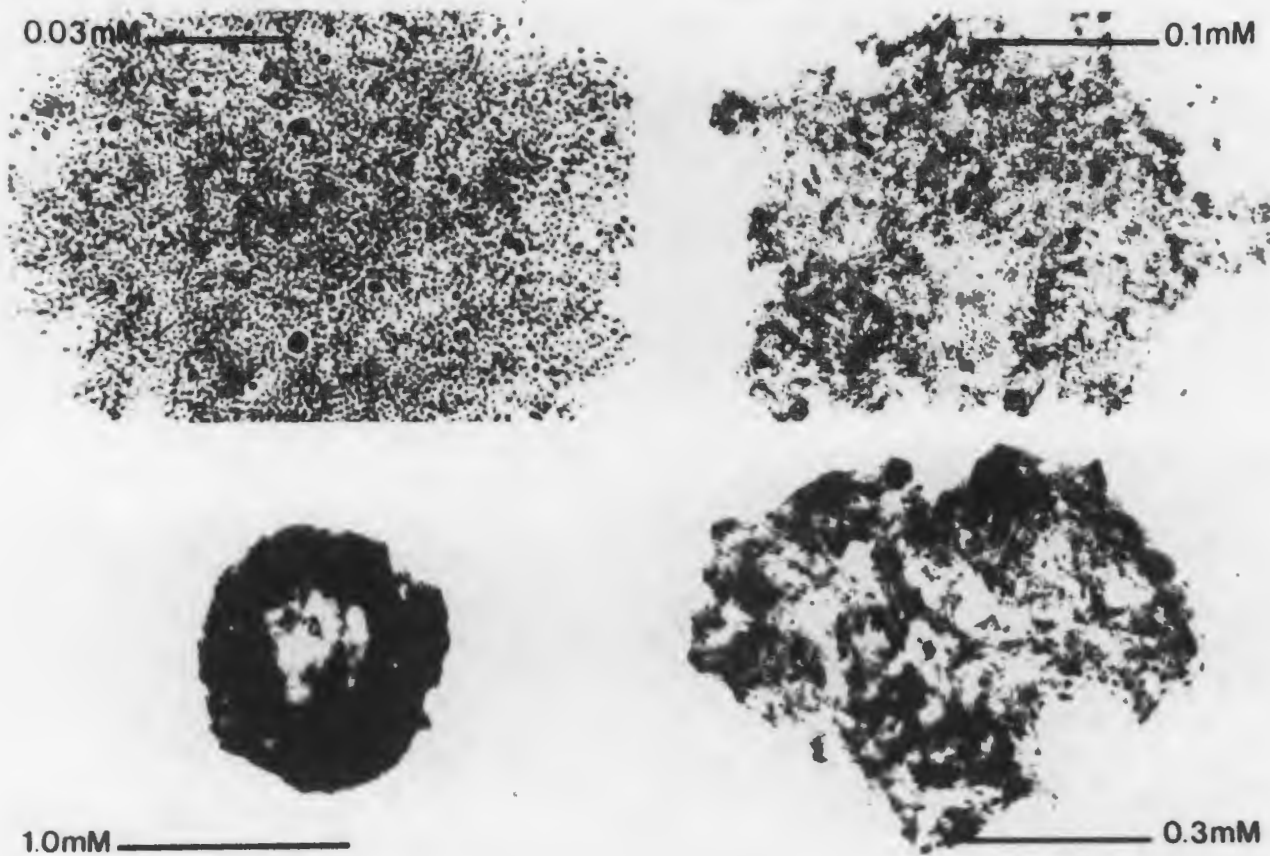
FIG. 1. Effect of different calcium concentrations in defined medium on human keratinocyte colony morphology. Calcium concentrations used were: 0.03 mM, upper left; 0.1 mM, upper right; 0.3 mM, lower right; 1.0 mM, lower left. 0.03 mM and 0.1 mM calcium produce unstratified keratinocyte colonies, while 0.3 mM and 1.0 mM calcium produce increased colony stratification as represented by darker histological staining. The apex of the cellular colony grown in 1.0 mM calcium is covered with cornified envelopes that have excluded the stain from the colony center. Scale bar = 1 mm.
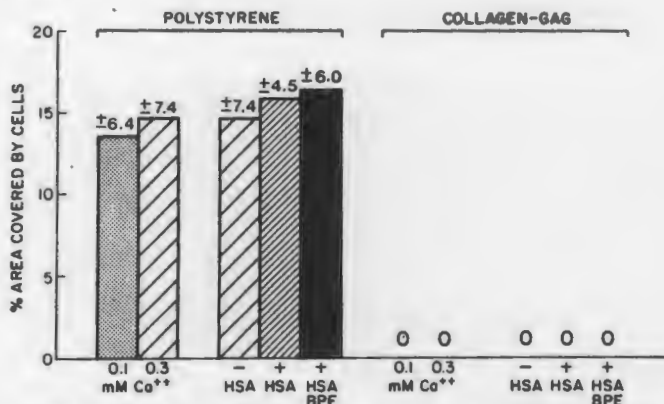

FIG. 2. Effect of culture substrate on human keratinocyte growth in vitro. Growth is compared on substrates of polystyrene versus collagen-GAG dermal membrane. Left two values of each substrate group represent growth in defined medium with 0.1 mM or 0.3 mM calcium as indicated. Right three values represent growth in defined medium with 0.3 mM calcium plus undefined supplements, human serum albumin (HSA) at 1 mg/ml, and bovine pituitary extract (BPE) at 70 μg/ml. Inoculation density, 25 cells/cm²; incubation period, 12 days. Standard deviation of data points for each growth condition is shown at the top of each bar.
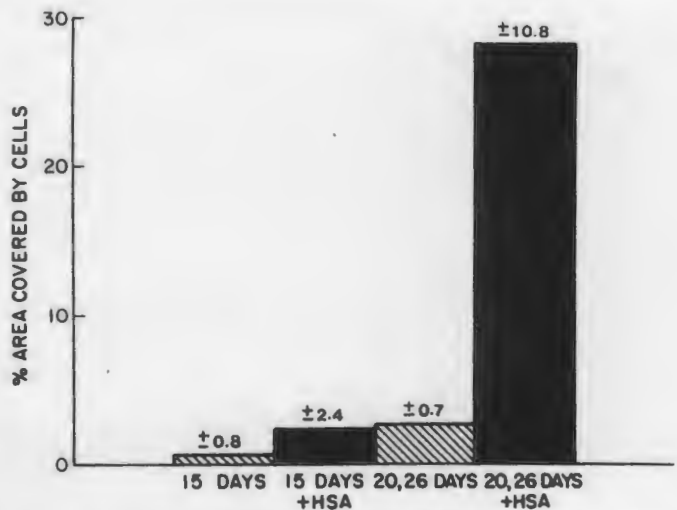

FIG. 3. Effects of incubation period and human serum albumin (HSA) on keratinocyte-collagen-GAG composites. All cultures grown in defined medium with 0.3 mM calcium. Incubation periods and cultures containing 1 mg/ml HSA as indicated. Standard deviation of data points for each growth condition is shown at the top of each bar.

cells on the membrane. Culture conditions have been modified in an effort to achieve growth on the membrane. Figure 3 shows the effects of the inoculation density, extended incubation periods, and the addition of human serum albumin on the keratinocyte growth on the collagen-GAG membrane. In 15 days of incubation there is limited growth. There appears to be a bit greater growth, up to about 3% of surface area covered, on the membrane
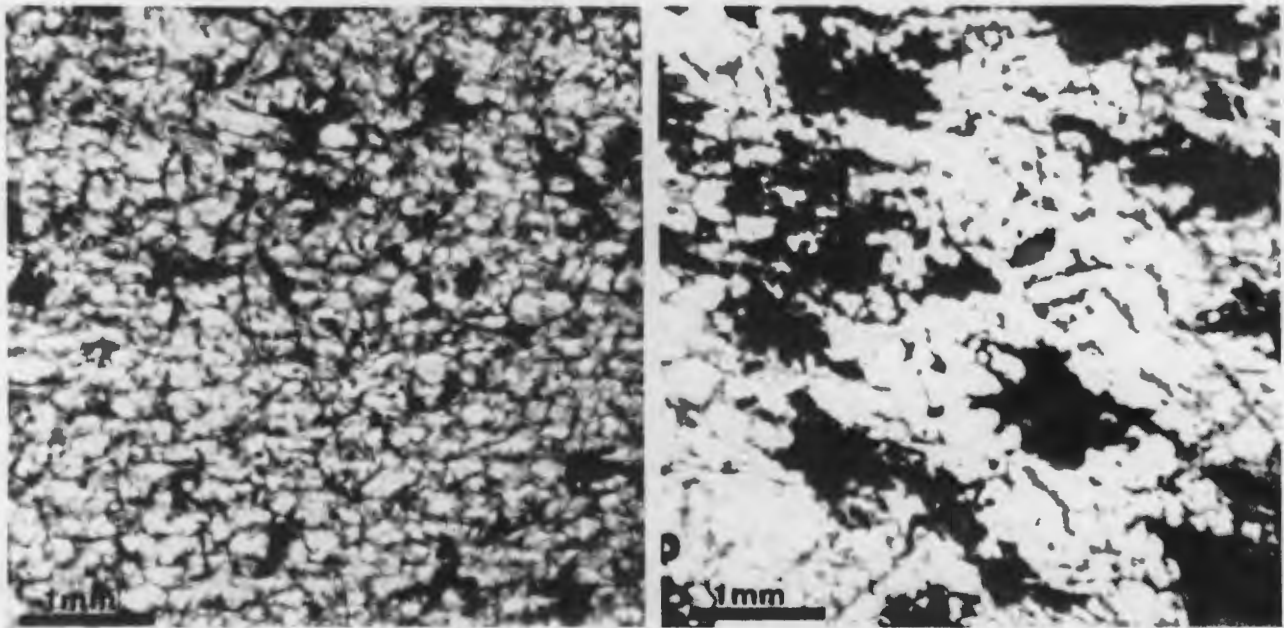
FIG. 4. Human keratinocyte growth on a collagen-GAG membrane. Cellular colonies grow larger with increased incubation period. Left panel, 15 days' incubation. Right panel, 26 days' incubation. Growth conditions: defined medium with 0.3 mM calcium plus 1 mg/ml human serum albumin; inoculation density, 1,250 cells/cm². Scale bar = 1 mm.

with the addition of human serum albumin, and this effect is much more pronounced when growth is carried out to 20 or 26 days. After these longer periods of keratinocyte growth in cultures containing human serum albumin, approximately 30% of the membrane area is covered by cells.

The fact that human serum albumin is beneficial in these experiments is noteworthy. It is suspected that the albumin or factors contained in the albumin are coating the membrane and facilitating cellular attachment and growth. In the next year it is anticipated that it will be determined exactly what factors are involved and some other defined agents, such as fibronectin, will be tested for their abilities to provide this anchoring function.

Figure 4 is a photomicrograph demonstrating human keratinocyte growth on the collagen-GAG membrane. At 15 days, colonies of cells are attached and proliferating, while at 26 days there is much more observable growth, and one can see that about 30% of the area of the collagen-GAG membrane is covered by epidermal cells.

Finally, we have observed that when the epidermal cells are inoculated onto the collagen-GAG membrane, they don't all remain on the surface. It appears that a substantial fraction of these epidermal cells migrate down into the collagen-GAG membrane, which is an undesirable result. It appears that we must make further efforts to insure that the cells grow only on the surface of the membrane.

To summarize briefly, the culture conditions that permit rapid keratinocyte growth on polystyrene are not the same conditions which are necessary to achieve growth on the collagen-GAG membrane. However, modified culture conditions in vitro, including a 50-fold increase in inoculation density, longer incubation periods, and the addition of human serum albumin, permit 25–30% of the collagen-GAG membrane to be covered by cultured keratinocytes in less than a month. Finally, the structural qualities of the collagen-GAG membrane, namely, physical pore size, pore orientation, and other qualities which permit vascular and fibroblast invasion in vivo also permit keratinocyte invasion into the material in vitro.

In conclusion, it is believed that cultured keratinocyte and collagen-GAG composites are a feasible alternative for the development of an autograft substitute. However, further modifications of the culture conditions and of the collagen-GAG membrane will be required to fully optimize keratinocyte growth on the membrane.

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