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Attachment of Peptide Growth Factors to Implantable Collagen

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Ingrowth of fibrovascular tissue from the woundbed into collagen-based dermal substitutes and survival of cultured epithelium after transplantation may be enhanced by attachment of heparin binding growth factor 2 (HBGF2) and epidermal growth factor (EGF) to collagen. Biotinylation of collagen and the growth factors allows immobilization of HBGF2 and EGF by high affinity binding of tetravalent avidin. Biotinylated HBGF2 and EGF (B-GF) were exposed to complexes of biotinylated collagen (B-COL)–avidin (A) and detected with peroxidase-labeled avidin (AP) followed by chromagen formation on nitrocellulose paper. Binding of biotinylated HBGF2 and EGF was specific (*, $P < 0.05$), proportional to the concentration of biotinylated collagen, and resistant to ionic (NaCl) displacement. Data are expressed as mean percentages of maximum binding ± SEMs:

Condition	% Maximum binding HBGF2	% Maximum binding EGF
(1) B-COL, A, B-GF, AP	95.5 ± 2.3	96.2 ± 5.2
(2) COL, A, B-GF, AP	40.7 ± 2.6*	16.7 ± 1.0*
(3) B-COL, A, GF, AP	19.6 ± 1.7*	1.8 ± 0.4*
(4) B-COL, A, B-GF, P	9.3 ± 0.7*	8.4 ± 0.5*
(5) Condition 1, 0.15 *M* NaCl	77.1 ± 1.4*	58.6 ± 1.8*
(6) Condition 1, 1.00 *M* NaCl	79.5 ± 2.8*	65.6 ± 4.3*

Growth response of cultured human epidermal keratinocytes to HBGF2 (population doubling time, PDT = 0.70 population doublings (PD)/day) confirmed the retention of mitogenic activity after biotinylation (PDT = 0.80 PD/day). Specific binding of biotinylated HBGF2, EGF, or other biologically active molecules (antibiotics, NSAIDs) to implantable collagen may provide a mechanism for positive therapeutic modulation of wound healing, including repair of full-thickness skin wounds with cultured cell–collagen composite grafts.

INTRODUCTION

Large thermal or chemical burns, traumatic degloving injuries, or pathologic skin conditions all require a large amount of skin graft for wound closure. Early coverage of wounds after full-thickness excision of abnormal skin is critical to reduce fluid losses, to decrease microbial colonization, to reduce the metabolic rate, and to control pain associated with these wounds [28, 29]. Fundamental requirements of wound closure include restoration of a desquamating epidermis and of vascular perfusion sufficient to support the long-term survival of the epidermis.

Split-thickness skin autograft, meshed [41] or unmeshed, remains the conventional standard for wound closure because it satisfies the requirements for skin substitutes [37]. However, in extensive injuries, skin autograft is often not available, and multiple reharvesting of donor sites is required to complete wound closure. Limitations to split-thickness skin autografting include additional trauma from skin harvesting, repeated surgeries, protracted hospitalizations, and unacceptable functional and cosmetic outcome.

Alternatives to split-thickness skin have been studied, but experimental skin substitutes [4, 17, 18, 25, 30, 31, 36, 42] have not yet proven clinical and economic advantages compared to skin autograft. Dermal–epidermal skin substitutes that are prepared *in vitro* [4, 12] may be distinguished from split-thickness skin graft in the mechanism by which each is vascularized after placement onto a wound. Dermal–epidermal skin substitutes that are applied in a single application must become vascularized across the entire thickness of the dermal substitute to allow survival of the epidermal substitute on the surface. By comparison, split-thickness skin graft contains a vascular network that joins to ingrowing blood vessels by anastomosis near the graft–wound junction, [19, 32, 35]. This mechanistic difference is perfusion of skin substitutes, and skin autograft, may contribute significantly to the survival of the grafted epithelium. Therefore, to stimulate vascularization, an angiogenic compound is added to the collagen base of a dermal–epidermal skin substitute [12].

Heparin binding growth factor, type 2 (HBGF2, also known as "basic fibroblast growth factor") has been shown to be a very potent mitogen and chemoattractant for capillary endothelial cells [21]. Biochemical analysis shows that HBGF2 is a 155-amino acid protein with a molecular weight of approximately 16 kDa [1]. HBGF2 has both immediate and long-term effects on the morphologies and growth patterns of many other cells of mesodermal and

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neuroectodermal origin, including human melanocytes [27, 39]. This compound has been shown to initiate cell proliferation and greatly reduce the average cellular doubling time. HBGF2 has been shown to increase the synthesis and function of fibroblasts and myofibroblasts and to control the deposition of collagen and proteoglycans [20]. It also stimulates the reepithelialization of the epidermis detached from the dermis after blister induction [22] and has been shown to be mitogenic to cultured human keratinocytes [34, 40]. The variety of biologic effects of HBGF2 on numerous cell types has led to the acceptance that HBGF2 is an archetypal growth factor. This compound has been purified from a wide range of tissues including pituitary, brain, hypothalamus, retina, adrenal gland, thymus, corpus luteum, kidney, placenta, and solid tumors [23].

Keratinocyte growth is of obvious importance to epithelialization of cultured skin substitutes. Epidermal growth factor (EGF) is a peptide hormone that exerts a generalized stimulatory effect on colonies of keratinocytes as evidenced by accelerated proliferation, migration, and keratinization [2]. EGF is synthesized in the acinar cells of the human submaxillary and duodenal glands [14]. EGF has been studied biochemically and is known to be a 49-amino acid protein with a molecular weight of 6 kDa [26].

Acceptance and persistance of skin substitutes that contain cultured cells may be optimized by deliberate stimulation of angiogenesis and epithelialization after placement onto wounds. To address this hypothesis, collagen of a dermal–epidermal skin substitute [12] is modified biochemically by covalent addition of biotin-*N*-hydroxysuccinimide (BNHS) [6, 13] that is detected with avidin–peroxidase. The present study demonstrates the biotinylation of HBGF2 and EGF and their attachment to biotinylated collagen that may be implanted in the form of a hydrated, crosslinked sponge containing glycosaminoglycan (GAG). Both HBGF2 and EGF are also shown to retain their mitogenic activity for cultured human keratinocytes after biotinylation. The biotinylated collagen–GAG sponge can serve as a substrate and vehicle for cultured cells and peptide growth factors. Together, these components can form a human skin substitute consisting of cultured human keratinocytes and fibroblasts, biopolymers, and immobilized peptide growth factors.

METHODS

Materials. Comminuted bovine collagen was obtained from USDA Eastern Regional Facility (Philadelphia, PA). The following reagents were obtained from Sigma Chemical Co. (St. Louis, MO): biotin-*N*-hydroxysuccinimide (BNHS), approximately 98%; avidin, type VII alkaline phosphate, 35% protein; horse radish peroxidase (HRP), Type VI salt free powder, 325 units/mg; avidin–HRP, Type VI, 80% protein, 50–150 units peroxidase/mg protein; diaminobenzadine tetrahydrochloride (DAB); hydrogen peroxide, 30% solution; and pepsin, from porcine stomach mucosa, 3100 units/mg protein. Spectropor dialysis tubing, molecular weight cutoff approximately 3500, and nitrate nitrocellulose paper (0.22) were obtained from Fisher Scientific (Tustin, CA). Heparin binding growth factor 2 (human recombinant) was obtained from Synergen, Inc. (Boulder, CO). Epidermal growth factor (extracted and purified biochemically) was obtained from GIBCO (Grand Island, NY). The equipment used was the Virtis Unitop 800 lyophilizer and the Sorvall Superspeed RC-5B.

Biotinylation of collagen and growth factors. Bovine collagen, HBGF2, and EGF were biotinylated by the formation of an amide linkage between the *N*-hydroxysuccinimido group of the BNHS and the ϵ-amino group of lysine residues on proteins as adapted from Boorsma [15]. Pepsin-digested bovine collagen was biotinylated as previously described [6].

Biotinylated HBGF2 was prepared from a solution of 2×10^{-5} *M* HBGF2 (300 μg of HBGF2 in 1.0 ml of Hepes-buffered saline, HBS). This represented approximately 2×10^{-8} mole HBGF2 total. Thirty microliters of a 0.1 *M* BNHS solution (34.1 mg of BNHS in 1.0 ml of dimethylformamide, DMF) was added to achieve a 100:1 molar excess of biotin. The mixture was allowed to react in an ice bath for 1 hr followed by a 24-hr dialysis at 4°C against several changes of phosphate-buffered saline (PBS), *p*H 7.6. The biotinylated HBGF2 was then lyophilized overnight. Three hundred micrograms of dry powder representing biotinylated HBGF2 was recovered and 600 μl of HBS, was added to achieve a final concentration of 0.5 mg/ml of biotinylated HBGF2. EGF was biotinylated by the same method, maintaining the same molar ratios and the same final concentration of 0.5 mg/ml of biotinylated EGF.

Spot test procedure. The spot test procedure, adapted from Graham and Karnovsky [25], was very similar to that described in detail in previous reports from this laboratory [6, 13]. To summarize, biotinylated and nonbiotinylated collagen (0.52%, w/v) were diluted 1:3, 1:10, and 1:30, and 3 μl of each dilution was applied to demarcated areas on nitrocellulose (NC) paper. In the labeled avidin to bridged avidin and biotin (LA-BRAB) procedure, 5 μl of 1 mg/ml free avidin was applied to each spot and allowed to incubate for 30 min. After incubation, spots were washed, and 5 μl of 0.5 mg/ml biotinylated growth factor was applied to each spot and allowed to react for 30 min. After incubation and washing, 5 μl of 100 μg/ml avidin–HRP was applied over each spot and allowed to incubate for 30 min. After a final wash, NC paper was flooded with a chromagen solution containing 0.5 mg/ml diaminobenzadine (DAB) with 0.01% H_2O_2 in 0.05 *M* Tris buffer and allowed to develop for 3 min. The reaction was quenched with excess 0.05 *M* Tris buffer, *p*H 7.6.

Biotinylated collagen and biotinylated growth factors

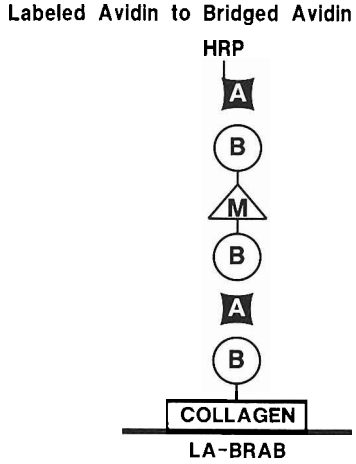


FIG. 1. Schematic representation of the labeled avidin to bridged avidin and biotin (LA-BRAB) procedure. Free avidin (A) has four binding sites for biotin and can conjugate two to four biotinylated compounds (i.e., collagen and M = molecules; e.g., growth factors). Horseradish peroxidase (HRP) bound covalently to avidin allows for detection of the complex through a chromagen reaction.

(HBGF2, EGF) were coupled using tetravalent avidin as a link. The protocol was referred to as the labeled avidin to bridged avidin and biotin procedure (Fig. 1). The protocol represents a modification of the LAB and BRAB protocols as discussed in a companion report [13]. The chromatic spots were measured and quantitated with a digital reflectance photodetector instrument [33] as described in a companion report [13]. Maximum binding (100%) was defined as the measured intensity of chromatic spots formed by direct application and drying of avidin–HRP onto nitrocellulose paper, followed by reaction with DAB and H_2O_2. Each spot test contained internal controls for 100% maximum binding.

Elution of biotinylated growth factors. To test whether the biotinylated collagen–avidin–biotinylated growth factor complex could be disrupted as a function of ionic strength of the aqueous medium, incubations in isotonic and hypertonic salt solutions were performed and tested for reduction of staining. Following the 30-min incubation of biotinylated growth factor to each spot, the NC paper was submerged and incubated in 0.15 *M* NaCl (isotonic osmolarity) or 1 *M* NaCl (hypertonic osmolarity) for 1 hr at 37°C. The NC paper was then rinsed, and staining with avidin–HRP was performed as described above (see Spot Test Procedure).

Clonal growth assay. To demonstrate retention of mitotic activity by the growth factors after biotinylation, clonal growth assays of human keratinocytes (HK) [10] were performed with biotinylated HBGF2 and biotinylated EGF in the culture medium. HK were inoculated at a density of 25 cells/cm^2 (500 cells per 60-mm petri dish) (Lux 5220, Miles Scientific, Naperville, IL) containing constant conditions of 5 ml MCDB with 0.2 m*M* Ca^{2+}, supplemented with insulin (5 μg/ml) and hydrocortisone (0.5 μg/ml). Cellular growth rate was measured after 12 days of incubation and expressed as population doublings per day. HK growth rate in biotinylated HBGF2 (1, 10, 100 ng/ml) was compared to growth in 30 ng/ml HBGF2. HK growth rate in biotinylated EGF (5, 15, 50 ng/ml) was compared to growth in 5 ng/ml EGF. Cell morphology was also noted.

Data collection and statistical analysis. Data points for all spot tests are expressed as means plus or minus standard errors of the means in triplicate from duplicate experiments. Experimental groups were compared pairwise with Student's *t* test, and statistical significance was assumed if $P < 0.05$. Growth rate determinations are expressed as the average values of three experimental cultures from single experiments.

RESULTS

Labeled avidin to bridged avidin to biotin (LA-BRAB) spot test. As previously described, a four-part complex was sequentially formed from: (1) biotinylated collagen, (2) free avidin, (3) biotinylated growth factor, and (4) avidin–HRP.

Dilution curve. Growth factor binding as a function of substrate concentration by serial dilution is shown in Fig. 2. Figure 2A shows the effect of dilution of biotinylated collagen on the binding of biotinylated HBGF2. Binding intensity is greatest (95.5%) on undiluted, biotinylated collagen. Serial dilution of biotinylated collagen of 1:3, 1:10, and 1:30 results respectively in decreased binding of 83, 50, and 33% of maximum. Although some nonspecific chromagen formation is seen, differences in binding intensity ranging from 55% at undiluted concentration to 35% at a 1:30 sample dilution can be attributed to biotinylation of the collagen substrate. Figure 2B shows comparable results for a decrease in binding of biotinylated EGF that is specific and dependent on the concentration of biotinylated collagen. Binding of chromagen was statistically significant ($P < 0.05$) for biotinylated HBGF2 and EGF at 1:1, 1:3, and 1:10 dilutions of biotinylated collagen, compared to nonbiotinylated collagen.

Reaction specificity. The LA-BRAB attachment mechanism is specific. If biotin or avidin is omitted from individual components of the reaction, the conjugation of growth factors to the collagen is reduced dramatically. Figure 3 shows the relative binding intensity of HBGF2 in four different reaction conditions. Figure 3A shows binding specificity of biotinylated HBGF2 to biotinylated collagen. Binding of 95.5% of maximum (left-most bar) occurs if all four compounds that contain biotin or avidin are present. Reduction of binding to 40% (bar second from left) occurs if nonbiotinylated collagen is the primary binding substrate. Nonbiotinylated HBGF2 results in 19% maximal binding (bar second from right) demonstrating the importance of biotinylation of the growth factor in

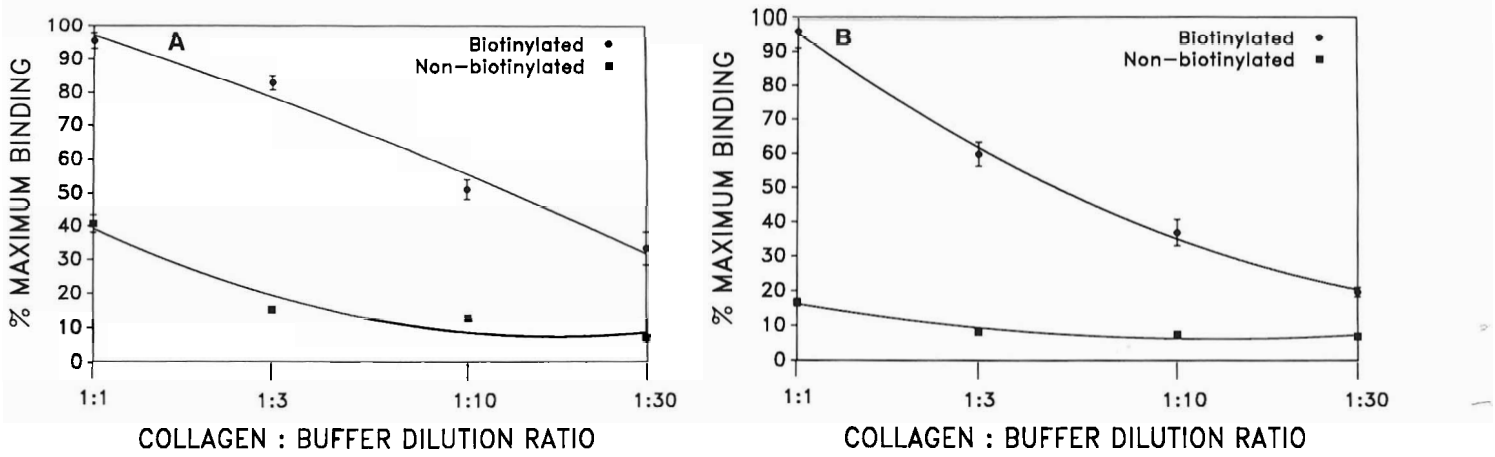


FIG. 2. Dilution curve for binding of biotinylated growth factors to increasing dilutions of biotinylated collagen. Binding of growth factors decreases as a function of concentration of biotinylated collagen (●) or nonbiotinylated collagen (■). Differences in binding between biotinylated and nonbiotinylated collagen are statistically significant ($P < 0.05$) for pairwise comparisons of substrate at each dilution. (A) Biotinylated HBGF2 curve. Binding of HBGF2 to biotinylated collagen decreases from 95% for undiluted biotinylated collagen to 33% for a 1:30 dilution of biotinylated collagen. Nonbiotinylated collagen shows 40% maximum binding for undiluted substrate, decreasing to 9% at a dilution of 1:30. (B) Biotinylated EGF curve. Binding of EGF to biotinylated collagen decreases from 96% for undiluted biotinylated collagen to 19% for a 1:30 dilution of biotinylated collagen. Nonbiotinylated collagen shows 18% maximum binding for undiluted substrate, decreasing to 8% at a dilution of 1:30.

the reaction. If HRP is not avidinylated (right most bar) only 9% of maximal binding and chromagen formation is seen.

Similar results are seen in Fig. 3B in which biotinylated EGF was tested. Binding of 96% of maximum (left-most bar) for all biotin or avidin reagents is reduced to 17% with nonbiotinylated collagen (bar second from left), to 2% with nonbiotinylated EGF, and to 8% with nonavidinylated HRP. These deletion studies illustrate the specific binding involved in biotin–avidin conjugates.

Each test for specificity with each growth factor was statistically significant if compared to its respective complete reaction. Controls with deletion of free avidin from the reaction were not performed. This condition results in minimal binding of biotinylated growth factors, followed by washing and addition of avidin–HRP which binds directly to the biotinylated collagen and produces a false positive test.

Elution of biotinylated growth factors. If incubated in a salt (NaCl) solution for 1 hr before exposure to avidin–HRP and chromagen formation, elution of biotinylated growth factors from the biotinylated collagen–avidin

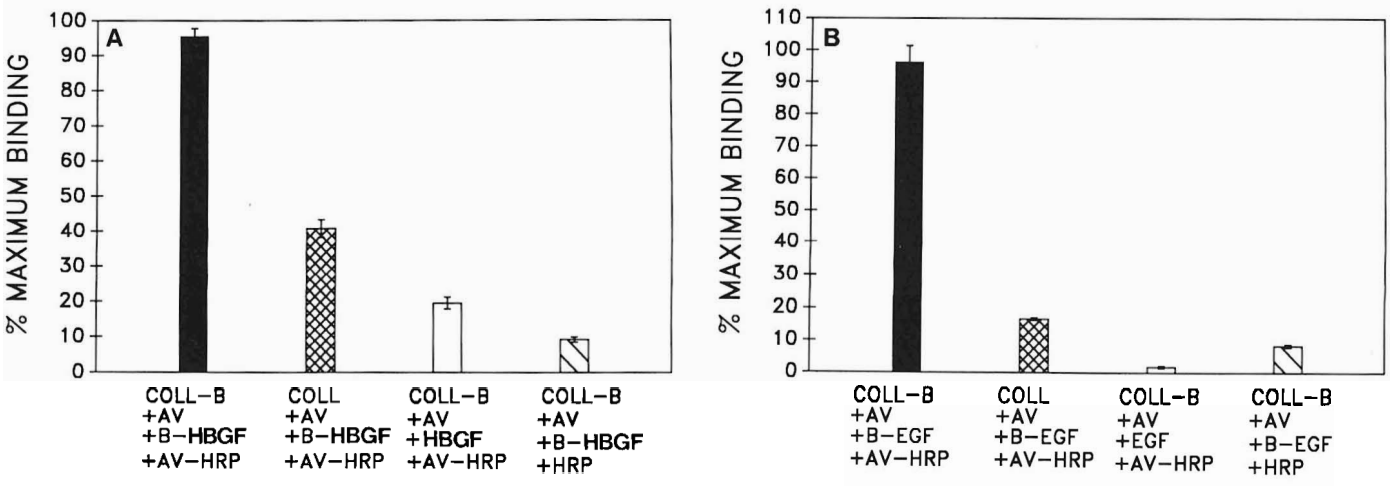


FIG. 3. Specificity of binding reaction. Specific binding of biotin–avidin conjugates is demonstrated by comparison of the test reaction with reactions using nonbiotinylated collagen, nonbiotinylated growth factors, or nonavidinylated HRP. Complete reactions with biotinylated collagen growth factors and avidin–HRP show statistically significant differences ($P < 0.05$) in comparison to each reaction in which nonbiotinylated or nonavidinylated reagents were used. (A) HBGF2 experiments: complete reaction, 95% binding (solid); nonbiotinylated collagen, 40% (cross-hatched); nonbiotinylated HBGF2, 20% (open); nonavidinylated HRP, 9% (diagonal). (B) EGF experiments: complete reaction, 96% binding (solid); nonbiotinylated collagen, 17% (cross-hatched); nonbiotinylated EGF, 2% (open); nonavidinylated HRP, 8% (diagonal).

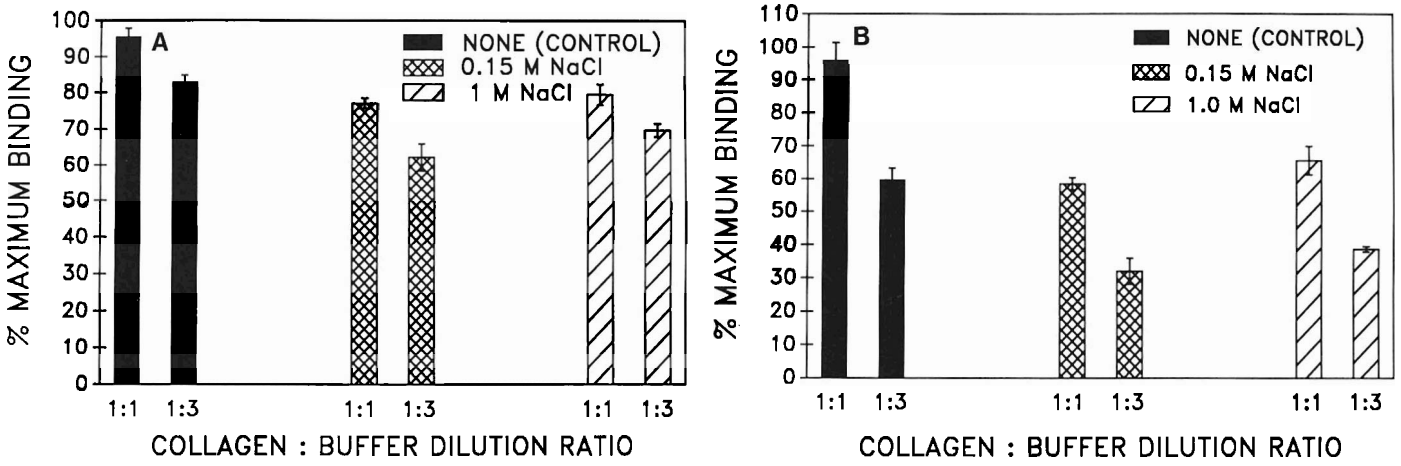


FIG. 4. Elution of biotinylated growth factors from biotinylated collagen–avidin complex. Biotinylated collagen (undiluted or 1:3 dilution) was reacted with avidin and biotinylated growth factors and treated with 1-hr incubations at 37°C in 0.15 *M* NaCl (cross-hatched bar), 1.0 *M* NaCl (diagonal bar), or no incubation (solid bar). (A) Biotinylated HBGF2, reduction in binding by approximately 20% due to salt elution. (B) Biotinylated EGF, reduction in binding by approximately 30–40% due to salt elution.

complex was observed. Compared to the maximal staining of undiluted and 1:3 dilutions of a control group (complete reaction/no salt incubation), the staining of biotinylated HBGF2 was reduced at both the 0.15 and 1 *M* NaCl incubations by less than 20%, as shown in Fig. 4A. In contrast, Fig. 4B indicates that under the identical conditions, the staining of biotinylated EGF is reduced by 30–40% of the nonincubated control. The concentration of the salt bath (0.15 *M* vs 1.0 *M*) does not seem to have a significant impact on the degree of elution of the biotinylated growth factor. The reduction in staining seen after incubation does indicate that the biotin–avidin link, although strong under most conditions, can be disrupted by salt elution. Biotinylated HBGF2 showed less reduction in staining than biotinylated EGF. This may be due to their differences in structure. HBGF2 has 12 lysine residues [1] that are believed to be biotinylated, but EGF has just three lysine residues [26]. Presumably, the greater the number of biotinylated side groups the less susceptible the biotinylated compound is to elution. In both cases, however, greater than 50% of the biotinylated growth factors is retained in the complex as would be expected under physiologic conditions.

Growth rates of human keratinocytes. Biotinylated HBGF2 or EGF stimulated growth of cultured keratinocytes in biochemically defined culture medium. Figure 5A compares HK growth rate by HBGF2 before and after biotinylation. Nonbiotinylated HBGF2 (30 ng/ml) stimulates a cellular growth rate of 0.70 population doublings (PD/day). Additions of 1, 10, or 100 ng/ml stimulate respectively growth rates of 0.52, 0.73, or 0.80 PD/day. Therefore, the process of biotinylation does not reduce

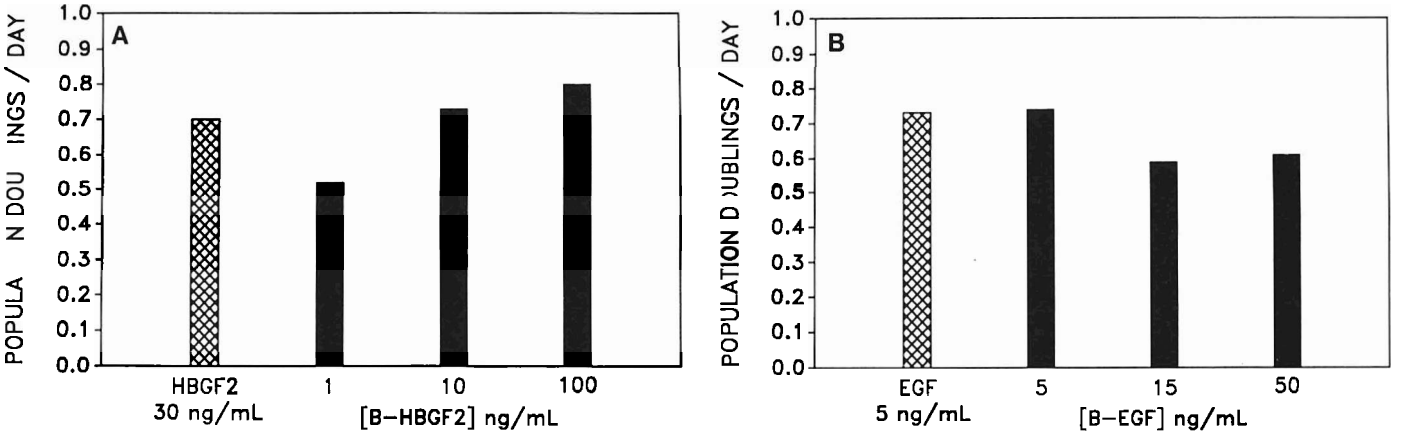


FIG. 5. Growth rate of cultured human epidermal keratinocytes (HK) in medium supplemented with biotinylated and nonbiotinylated growth factors. No growth was obtained in medium without either HBGF2 or EGF. (A) Biotinylated HBGF2 at concentrations ranging from 10–100 ng/ml stimulates growth equivalent to nonbiotinylated HBGF2. (B) Biotinylated EGF at 5 ng/ml stimulates HK growth equivalent to nonbiotinylated EGF.

appreciably the mitogenic activity of HBGF2 for cultured human keratinocytes. Figure 5B compares HK growth rate by EGF before and after biotinylation. Nonbiotinylated EGF (5 ng/ml) stimulates a cellular growth rate of 0.73 PD/day. Additions of 5, 15, or 50 ng/ml stimulate respectively growth rates of 0.74, 0.59, or 0.61 PD/day. No growth was measured in the absence of both HBGF2 and EGF (not shown). Therefore, the process of biotinylation does not reduce appreciably the mitogenic activity for cultured human keratinocytes of either HBGF2 or EGF. Retention of biological activity is critical to delivery of peptide growth factors or other biologically active molecules for therapeutic purposes.

HK colony morphology. Figure 6 shows differences between HK colony morphology in culture with EGF or HBGF2 after biotinylation. HK grown in biotinylated EGF form colonies with dispersed perimeters (Fig. 6A), similar to clonal growth of HK in nonbiotinylated EGF under comparable medium conditions [12]. Morphologies of individual cells in the colonies are also irregular, varying in shape from polygonal to moderately fusiform. These morphologies imply some degree of cell migration during the incubation period. Substitution of biotinylated EGF with biotinylated HBGF2 produces compact cell colonies with distinct perimeters composed of individual cells with a more regular and polygonal shape (Fig. 6B). These morphologies imply less cell migration during the incubation period. Mitotic HK (refractile, spherical cells) are seen in cultures containing each growth factor.

DISCUSSION

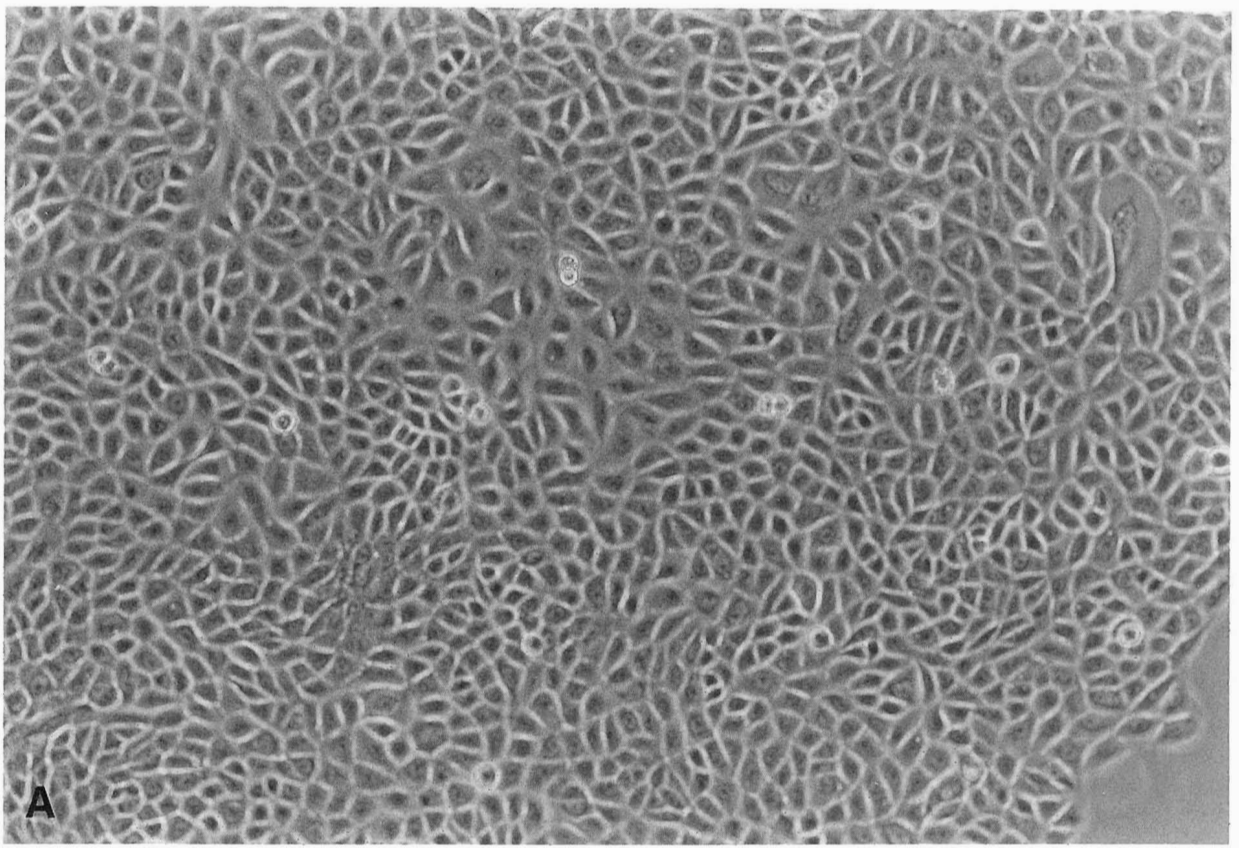
Implantable collagen that contains potent mitogens such as HBGF2 or EGF may beneficially influence the wound healing process to promote vascularization and epithelialization. If configured into a thin sponge, the implantable collagen can serve as one component of a dermal substitute that also can act as a culture substrate for human epidermal keratinocytes and dermal fibroblasts [6, 7, 12]. Histologically, this composite material resembles split-thickness skin in its total thickness and compartmentalization of cellular components. However, acceptance and persistence of skin substitutes on wounds also require rapid vascularization from the woundbed and long-term, regulated growth and differentiated function of the cellular components of the implants. Satisfaction of these requirements in the absence of infection can be expected to promote tissue regeneration by primary wound healing and to reduce scar formation. Control of infection is the other significant factor in wound healing that can be addressed by use of implantable collagen as a drug delivery device. Antimicrobial agents may also be attached to collagen by the same mechanism of biotinylation [13] used with HBGF2 and EGF in this report. Determination of toxicity of antimicrobial drugs, carried by the collagen, to cultured cells contained in the implant must also be considered [16].

Stable, covalent bonds form during the biotinylation reaction between biotin-*N*-hydroxysuccinimide and lysine residues of proteins. Numerous biological proteins including antibodies, antigens, and enzymes have been biotinylated without appreciable loss of biologic activity or specificity. Any loss of activity has been presumed to result from the presence of one or more lysine residues in the active site [38]. Biotinylation permits utilization of the natural affinity of biotin and avidin to bind tightly together otherwise nonassociable compounds. Avidin, a glycoprotein derived from avian egg white, possess four binding sites with extremely high affinity, ($K_D \sim 10^{-15}$ M), for biotin [3]. Therefore, after two biotinylated compounds have been joined by multiple biotin–avidin bridges, elution of the biotinylated compounds is dependent not only on the affinity of single biotin–avidin bridges, but also on the number of bridges between the molecules. HBGF2 has 12 lysine residues to be biotinylated, and EGF has only 3 biotinylation sites. This difference in biotinylation sites for each molecule may account, in part, for the elution of biotinylated EGF greater than that of biotinylated HBGF2 from biotinylated collagen.

Biological effects *in vivo* of growth factors attached by biotinylation to implantable collagen have not yet been investigated. However, animal and human studies from this laboratory [8, 9, 15] indicate that degradation of the collagen–GAG biopolymer substrate of the composite skin substitute requires 4–8 weeks. If avidin-bound biotinylated growth factors are assumed not to elute from biotinylated collagen after implantation, then the rate of delivery of the growth factors will be determined by the rate of degradation of the collagen substrate. If elution occurs, then the rate of peptide delivery will depend on both the rates of elution and collagen degradation. The elution rates are not known, and may be either linear or nonlinear, and will probably be affected by the number of biotin–avidin–biotin bridges that join subject compounds to implanted collagen. Further studies of delivery mechanism and delivery rate are warranted to better understand and regulate the biological effects of biotinylated compounds administered to wounds by attachment to implantable collagen.

The present studies demonstrate that the peptide growth factors, HBGF2 and EGF, can be biotinylated and conjugated to biotinylated collagen with tetravalent avi-

FIG. 6. Effect of biotinylated growth factors on HK colony morphology. (A) HK colonies formed in medium supplemented with 30 ng/ml of biotinylated HBGF2. Cell colonies have continuous perimeters and cell shape is uniform and polygonal. (B) HK colonies formed in medium supplemented with 5 ng/ml of biotinylated EGF. Cell colonies have dispersive perimeters and cell shape varies from polygonal to fusiform.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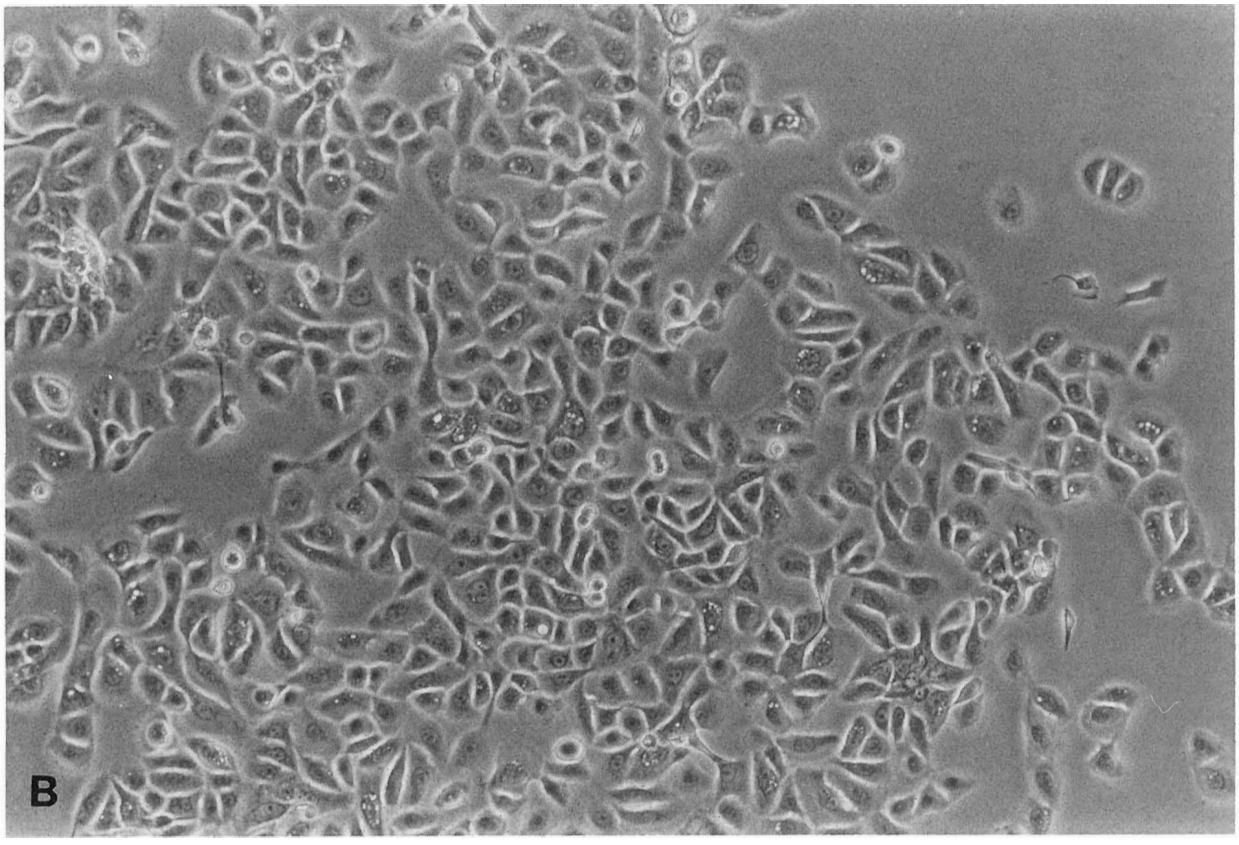
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din. These factors retain biological activity after biotinylation and are stimulatory to growth of human keratinocytes *in vitro*. Keratinocyte growth is fundamental to reepithelialization of open wounds and, together with fibrovascular ingrowth, constitute the essential components for stable closure of skin wounds. Addition of other biologically active compounds may also allow, in the foreseeable future, specific regeneration of nerve, pigment, and epidermal adnexi (hair follicles, sweat and sebaceous glands) in healing skin wounds to restore complete anatomy and physiologic function of human skin after full-thickness injury.

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