생인공피부를 이용한 독성 반응 시험

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Use of Cultured Bioartificial Skins as in vitro Models for Cutaneous Toxicity Testing

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Abstract

Cytotoxicity assays using artificial skins have been proposed as in vitro alternatives to minimize animal ocular and dermal irritation testing. Accordingly, the responses of artificial skins to the well-characterized chemical irritants toluene, glutaraldehyde, and sodium lauryl sulfate (SLS), and the non-irritant polyethylene glycol were studied. The evaluation of the irritating and non-irritating test chemicals was also compared with the responses observed in human dermal fibroblasts and human epidermal keratinocytes grown in a monolayer culture. The responses monitored included an MTT mitochondrial functionality assay. In order to better understand the local mechanisms involved in skin damage and repair, the production of several mitogenic proinflammatory mediators, interleukin-1α, 12-HETE, and 15-HETE, was also investigated. Dose-dependent increases in the levels of IL-1α and the HETEs were observed in the underlying medium of the skin systems exposed to the two skin irritants, glutaraldehyde and SLS. The results of the present study show that both human artificial skins can be used as efficient in vitro testing models for the evaluation of skin toxicity and for screening contact skin irritancy.

Introduction

Cytotoxicity assays using monolayer cultures have been proposed as in vitro alternatives to minimize animal ocular and dermal irritation testing [1]. Many of these systems are well defined and can be used in conjunction with objective and quantifiable endpoints. Conventional submerged monolayer cultures have several inherent limitations, however, including susceptibility to test samples at concentrations far below those required to induce irritation in vivo. Furthermore, in order to expose cells in a culture to a given test sample, the sample must be solubilized in the cell culture medium. A large number of substances that come into contact with the skin, including topical formulations, are not suitable for testing using these systems [2].

Recent advances in cell culture techniques have led to the development of the three-dimensional reconstruction of artificial skin using living cells [3]. This artificial skin consists of a collagen matrix containing dermal fibroblasts that is overlaid with epidermal keratinocytes. Since the nutrients and growth factors are diffused to the epidermis through the prepared dermal equivalent, the in vitro artificial skin is grown in an environment which is similar to in vivo skin development [4]. Such artificial skin has already been used in experiments to investigate the nature of skin diseases, such as psoriasis [5], as well as in studies on skin grafting [6]. As an in vivo model of skin, artificial skin may prove to be useful in other areas, for example, it may form the basis for a method that can predict cutaneous toxicity.

In addition, the use of animals for safety evaluation is increasingly being criticized from an ethical point of view, therefore, it has become necessary to develop alternative test methods which do not use animals. It has been reported that the European Community directive, that requires that effects claimed for cosmetic actives must be validated using non-animal procedures, has stimulated the use of in vitro models for pharmacotoxicological trials [7]. The development of artificial skin and the extent of the epidermal differentiation attained in artificial skin with the appearance of a stratum corneum, thereby allowing for the topical application of test samples, suggests that artificial skin may provide a realistic model.

The release of eicosanoids and cytokines is a critical signal in the cascade of events leading to edema and erythema in vivo and cultured keratinocytes have been shown to release these mediators in response to chemical irritants [8]. Elevated levels of proinflammatory mediators have been detected in a variety of dermatoses including those induced by primary contact irritants. Monocytes are a major secretary cell with the capability of secreting more than 100 different molecules. Among these secretary products are numerous polypeptide molecules that are central to the initiation, amplification, and resolution of an inflammatory response. Of these monokines, interleukin-1 (IL-1) is possibly the most well studied. The production of IL-1 by activated monocytes/macrophages plays an important

role in the early events of inflammation, which are collectively called "acute phase responses". IL-1 is a very important chemical mediator in the acute inflammatory phase of wound healing [9-11].

Another inflammatory mediator that may be involved in the stimulation of epidermal mitosis is 12- and 15-hydroxyeicosatetraenoic acid (12-HETE and 15-HETE) [12]. The ability of metabolites of arachidonic acid to mediate this type of localized vascular response in human skin suggests that the cell membrane may be a site of interaction for many cutaneous irritants [13]. Many of the lipoxygenase metabolites of arachidonic acid have been implicated in dose-related erythematous responses and are potent chemotactic agents [8]. The expression of lipoxygenase activity within artificial human skin and the release of lipoxygenase-induced arachidonic acid metabolites may thus prove to be a sensitive and relevant measure of in vivo cutaneous toxicity.

This work studied the responses of artificial skins to the well-characterized chemical irritants toluene, glutaraldehyde, and sodium lauryl sulfate, and the non-irritant polyethylene glycol. The evaluation of the irritating and non-irritating test chemicals was also compared with the responses observed in human dermal fibroblasts and human epidermal keratinocytes grown in a monolayer culture. The responses monitored included an MTT mitochondrial functionality assay. In order to better understand the local mechanisms involved in skin damage and repair, the production of several mitogenic proinflammatory mediators such as interleukin-1α, 12-HETE, and 15-HETE was also investigated.

Materials and methods

Cell Culture

Human normal skin cells were aseptically isolated from a circumcised neonatal foreskin at the Cha General Hospital (Bundang, Korea). The epidermis and dermis came loose by incubation in 0.9 units/ml dispase (Gibco BRL, Grand Islands, N.Y., U.S.A.) in a culture medium without serum for 16 h at 4°C. The normal fibroblasts were isolated from the dermis by 2 mg/ml type I collagenase (type I, 1.6 units/mg solid, Sigma Chemical Co., St. Louis, Missouri, U.S.A.) and cultured routinely in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C in a 5% CO_2 incubator. The keratinocytes were isolated from the epidermis by a 0.25% trypsin solution and grown in a Keratinocyte-Serum Free Medium (K-SFM, Gibco BRL) consisting of an MCDB 153 medium supplemented with insulin (0.005 g/l), hydrocortisone (0.074 mg/l), triiodothyronine (0.0067 mg/l), bovine pituitary extract (BPE, 50 mg/l) and an epidermal growth factor (EGF, 5 μg/l) at 37°C in a 5% CO_2 incubator [14].

Dermal Equivalent and artificial skin

Dermal equivalent (DE) cultures were prepared from acid soluble type I calf skin collagen (C9791, Sigma Chemical Co.) according to a modification of the method described by Bell et al. [3] and Yang et al. [15]. 1×10^5 cells/ml of human fibroblasts were seeded into a mixed solution containing seven volumes of a 3 mg/ml collagen solution, two volumes of 5 × DMEM, and one volume of 0.05 N NaOH including 2.2 % sodium bicarbonate and 200 mM Hepes buffer solution. The human dermal fibroblasts contracted the collagen gel into a fibrillar connective tissue-like dermal equivalent after 7 days of incubation within the culture medium at 37 °C with 5% CO_2 in air.

The in vitro production of the artificial skin (AS) began by casting the DE onto a 3 μm porous polycarbonate membrane of a culture insert (Nunc TC Insert, Nalge Nunc International, Naperville, IL U.S.A.). After the DE was cultured for 7 days, the epidermal keratinocytes were applied to the surface at an initial density of 1×10^5 cells/cm^2. The construct was submerged for 7 days of incubation under a K-SFM medium containing EGF and BPE inside and outside the culture insert. The developing multilayered AS was then cultured for 2 weeks at the air-liquid interface by removing the inside medium of the insert. The underlying culture medium was switched to a 10% FBS supplemented K-SFM medium plus DMEM (1: 1) omitting the EGF.

The AS was fixed using 10% neutral buffered formalin (Sigma Chemical) for 2 h at 4°C. The fixed samples were then embedded in paraffin, and 5 μm-sectioned paraffin ribbons were stained with hematoxylin and eosin.

Exposure of Test Substances and MTT assay

Four test chemicals glutaraldehyde (Jin Chem., Ansan, Korea), sodium lauryl sulfate (SLS, Fischer Scientific, Pittsburgh, PA, U.S.A.), toluene (Fischer Scientific), and polyethylene glycol (PEG, M.W. 400, Wako Pure Chemical Industries, Osaka, Japan) were used as the exposing substances. The human fibroblasts and keratinocytes were seeded into 12-well plates at 1×10^5 cells per well in 1.0 ml of each cell culture medium for 24 h at 37°C in a 5% CO_2 incubator. Before the test chemical exposure, the medium was aspirated from the fibroblast or keratinocyte monolayer culture, then 1.0 ml of the medium plus 80 μl of the indicated concentration of the test chemical was dispensed into the 12-well plates. The cultures were incubated for 24 h at 37°C in 5% CO_2, then the medium was aspirated from the cultures and 1.0 ml PBS (phosphate buffered saline) containing 0.33 mg MTT/ml (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide, Sigma Chemical Co.) was dispensed into each well [1]. The cultures were incubated for 4 h, then the PBS solution was aspirated, and the monolayers were extracted in 1.0 ml isopropanol (Oriental Chemical Co., Seoul, Korea), and acidified with 0.04 N HCl (Junsei Chemical Co., Tokyo, Japan) for 5 min at room temperature. The absorbances of 3.0 ml aliquots were read at 570 nm in an UV-VIS spectrophotometer (Smart plus 2605, Young

Hwa Co., Seoul, Korea).

The human dermal equivalent and artificial skin were cultured onto 25 mm inserts in 6-well plates containing the culture medium. The culture medium was removed and new media were added into the outer section of the inserts. 80 μl of the test chemical was added to the center of each surface of the dermal equivalent and artificial skin using water as the vehicle as shown in Fig. 1. The cultures were incubated for 24 h at 37°C in 5% CO_2, and then the medium was aspirated from the cultures and 1.0 ml PBS containing 0.33 mg MTT/ml was dispensed into each well. The cultures were incubated for 4 h, then the PBS solution was aspirated and extracted for 2 h at room temperature in 3.0 ml acidified isopropanol. The absorbances of 3.0 ml aliquots were read at 570 nm.

Assay for Interleukin-1α production

IL-1α determinations were performed using an enzyme-linked immunoassay [1]. A capture antibody (anti-human IL-1α, R&D Systems Inc., Minneapolis, MN, U.S.A.) was coated for 16 h at room temperature, and then the plate was washed with a washing buffer of PBS containing 0.05% Tween 20 (Sigma Chemical). 300 μl of a blocking buffer was added along with PBS containing of 1% BSA (Sigma Chemical), 5% sucrose (Junsei Chemical), and 0.05% sodium azide (Sigma Chemical) then the plate was incubated for 1 h at room temperature, and washed with the same washing buffer. The standard or sample were injected into the plate, incubated for 2 h at room temperature, and then washed. 100 μl of a detection antibody (biotinylated anti-human IL-1α, R&D Systems Inc.) was added then and the plate was incubated for 2 h and washed. 100 μl of Streptavidin HRPO (Caltag Laboratories, Burlingame, CA, U.S.A.) and was added then the plate was incubated for 20 min. After washing, 100 μl of a substrate solution (TMB, Sigma) was added and the plate was incubated for 30 min. 50 μl of 1 M H_2SO_4 was added as a stop solution and the optical density was at 450 nm in a 96-well plate reader (Ceres UV900 HDi, Bio-Tek Instruments, Frederick, MD, U.S.A.).

Assay for Hydroxyeicosatetraenoic acid (HETE) production

The lipoxygenase metabolites of arachidonic acid such as 12- and 15-HETE were analyzed using solid-phase extraction and a high-performance liquid chromatography analysis (HPLC) according to the modified method of Eskra et al. [16]. 5 ml of the medium including 1 ml of the standard or sample and 4 ml of fresh media from the treated artificial skin was mixed with 5 ml of ice-cold methanol. The precipitated proteins were removed by centrifugation at 10,000 g for 10 min. The supernatant was then adjusted to pH 3.5 with 1 M HCl. The HETEs that had been released into the medium were isolated and purified on C18 bond-elute reverse-phase minicolumns (Sep-Pak catridge, Waters Chromatography, Millipore Co., Milford, MA, U.S.A.). The cartridges were washed with 10 ml of methanol, followed by 10 ml of water. The 5 ml samples were loaded and the cartridges washed with

10 ml of water and 10 ml of 20 % methanol. The extracted metabolites were eluted with 10 ml of 80% methanol and the eluate dried in a vacuum oven. The extracts were then redissolved in 20 μl of 80% methanol and injected into HPLC (Waters), using a Spherisorp C18 column (3 μm, 6 A, Waters) with a mobile phase of methanol/water/trifluoroacetic acid/triethylamine (80/20/0.1/0.05). The HETEs were detected at 235 nm using a Waters 486 tunable absorbance detector.

Results and discussion

The results of this study demonstrate that it may be feasible to use a skin equivalent as an in vitro model for investigating injury by potentially irritant topical preparations. The human skin monolayer cell culture, dermal equivalent, and artificial skin were all exposed to toxicants according to the protocol shown in Fig. 1. The submerged monolayer cultures were exposed to the test chemicals through dispersion into the medium solution for 24 h. In the dermal equivalent and artificial skin, the toxicants were exposed directly onto the air-exposed surface using culture inserts for 24 h. The culture media were only fed through the outer space of the inserts by removing the media of the inner side of the inserts.

The epidermal layer of the cultured artificial skin was stratified and organized after 14 days of exposure to the atmosphere. A layer of cuboidal basal keratinocytes was firmly attached to the fibroblast-containing dermal layer. The stratum spinosum and granulosum was three to four cell layers thick, as shown in Fig. 2. The epidermis was covered by stratum corneum layer. The morphological features of a differentiating epidermal permeability barrier could be identified in the upper stratum layer. The fully developed artificial skin expressed a broad range of protein products typically found in differentiated human epidermis [17], and the expression of proteins such as cytokeratin 19 and involucrin was observed, as reported in a previous publication [14].

In order to evaluate the potential differences in the cytotoxic susceptibility, the responses of the cells in the artificial skin were compared with the responses of the cells in the monolayer culture when exposed to a known chemical irritant. The potential contribution of the epidermis in the artificial skin was assessed by comparing the response of the artificial skin with that of the fibroblasts in the dermal equivalent collagen layer. In this study, the various cell systems were exposed to varying concentrations of glutaraldehyde, SLS, toluene, and PEG (Fig. 3). Glutaraldehyde is currently the most widely used reagent, however, glutaraldehyde cross-linked biomaterials induce local cytotoxicity [18]. SLS is a commonly applied surfactant and has been used as a model-irritant in both in vivo and in vitro studies [19], toluene is a solvent inducing cell necrosis, and PEG is used as a cosmetic ingredient and hydrophilic ointment. The extent of cytotoxicity induced after a 24 h exposure period was qualified using an MTT assay. The dose-response profiles showed that the artificial skin appeared to

be more resistant to the cytotoxic effects of the irritants than either the dermal equivalent or the cells in the monolayer culture. As shown in Fig. 3, the MTT conversions of the cultured artificial skins exposed to glutaraldehyde and SLS were much higher than those of the dermal equivalent and the monolayer cells. With exposure to toluene, the MTT conversion of the artificial skin also was higher than the dermal equivalent, however, the cells in the monolayer were not irritated by the toluene due to its non-polarity and insolubility in an aqueous medium. As expected, the non-irritating test substance, PEG did not inhibit the MTT conversion in the artificial skin and dermal equivalent exposed to this substance at full strength for 24 h. However, test with the substances PEG and toluene, the MTT conversion of the monolayer cell test did not correlate with the dermal equivalent or artificial skin. The results of Fig. 3 (a) to Fig. 3(d) show that the cells in the monolayer systems were inadequate for the cytotoxic test thus the cultured artificial skin was a more effective test system. The magnitude of the response differences between the artificial skin, the dermal equivalent and the cells in the monolayer culture was expressed as the interpolated effective concentration of the applied substance that inhibited 50% of the MTT conversion (EC_{50}). The interpolated EC_{50} values for the MTT conversion were compared with previously published threshold doses for skin irritation (Table 1). The artificial skin was exposed for 24 h to a number of well-characterized primary contact chemical irritants at varying concentrations. The EC_{50} values for the cell systems exposed to glutaraldehyde for 24 h were 0.31% for the artificial skin, 0.056% for the dermal equivalent, 0.021% for the human fibroblasts, and 0.003% for the human keratinocytes. Accordingly, the doses of glutaraldehyde required to inhibit the MTT conversion by 50% in the artificial skin were 15- and 100-fold higher than the values for the fibroblasts and keratinocytes, respectively. When compared with the dermal equivalent, the artificial skin was 5.5-fold more resistant to glutaraldehyde-induced toxicity. Compared with the negative control, the MTT conversion was reduced by 50% in the artificial skin exposed to 0.308% SLS for 24 h. For the four test chemicals listed, the rank order and absolute EC_{50} values both showed a general correlation with the in vivo threshold doses and previously published values.

The differences between the responses of the monolayer cultures and the artificial skin were attributed to the differences in the method of the sample application for the two cell systems. In the monolayer cultures, the toxicants were solubilized in the cell culture medium whereas, in the dermal equivalent or artificial skin, the toxicants were applied directly to the air-exposed surface. Accordingly, the artificial skin, which presents an epidermis at an air-liquid interface, allows for the topical application of test chemicals. In the monolayer cultures, the cells are exposed to a single concentration of the test substance present in the overlying medium and this concentration remains constant during the exposure period. In contrast, in the artificial skin, the applied dose passes from the exposed surface into the test system with a flux governed by a number of factors, including the cross-sectional area of exposure, the concentration of the test substance applied, and the diffusivity of the test substance in the

artificial skin [1]. Although all the foregoing factors are relevant when a substance is applied to skin, other factors may explain why the response of the cells in the artificial skin differed from that of the monolayer cultures. For example, it is known that fibroblasts organized in three-dimensional lattices differ intrinsically from cells grown in a monolayer, exhibiting more complete biosynthetic and morphological properties compared with those grown in vivo [14]. Elevating the epidermis to an air interface is crucial for the appearance of a multilayered stratum corneum with lamellar granule extrusion structures for generating lipid and keratin profiles approximating those of skin. The stratum corneum is the primary permeability barrier to the passage of substances through mammalian skin [20] In this study, the increased resistance to glutaraldehyde- and SLS-induced cytotoxicity in the artificial skin, when compared with the monolayer cultures or the dermal equivalent, may have been due to the presence of a stratum corneum. An epidermal structure with stratum corneum layer serves as a selective permeability barrier thereby regulating the diffusion of applied substances to the underlying susceptible cells.

The dose-response profiles of IL-1α induction are shown in Fig. 4 for the dermal equivalent and artificial skin exposed to varying concentrations of two dermal irritants, glutaraldehyde and SLS. The production levels of IL-1α in the human skin systems were increased with exposure to the toxicants, however, the maximum IL-1α peaks of the artificial skins were detected at significantly greater toxicant concentrations those that of the dermal equivalents. The levels of IL-1α detected in the underlying medium of the dermal equivalent reached a peak of 600 pg/ml with exposure to 0.1% glutaraldehyde for 24 h (Fig. 4a). While the IL-1α production of the artificial skin exhibited a peak of 400 pg/ml with 1.0% glutaraldehyde (Fig. 4b). After exposure to SLS (Fig. 4 c, d), the IL-1α peak of the artificial skin was detected with a 1.0% SLS concentration, which was significantly greater than the peak concentration of 0.4% toxicants in the dermal equivalents. The IL-1α levels in the underlying medium were elevated after exposure to the two dermal irritants yet decreased after the application of the peak concentrations of the irritants. These results may reflect the reduced ability of damaged cells to release cytokine [21].

As another proinflammatory mediator, the HETE-production of the artificial skin was monitored. 12- and 15-HETE were expressed in the artificial skin with various concentrations of toxicants, as illustrated in Fig. 5. As the glutaraldehyde and SLS concentration increased, the production of HETEs also increased. These results are similar to those previously reported for cultured human keratinocytes grown on a 3T3 feeder layer [22] and for human skin fibroblasts [23]. The primary route for the metabolism of arachidonic acid in cultured artificial skin appears to be by the lipoxygenase pathway. This stable arachidonic acid metabolite has been shown to play a central role in the down-regulation of lipoxygenase activity and in the levels of proinflammatory mediators. However, it is possible that the release of total arachidonic acid may be a better parameter to monitor in routine screening, therefore,

work is currently in progress to clarify this point. Accordingly, the release of HETEs from artificial skins may prove to be a useful indicator of potential irritancy in human skin. The total HETEs levels seen in Fig. 5a and b were the sum of the 12-HETE and 15-HETE production levels. Elevated IL-1α and HETEs levels have also been previously reported in irritated skin from human and rodents with primary contact dermatitis [24-26].

In conclusion, the results of the present study show that both human artificial skin systems can be used as in vitro testing models for the evaluation of skin toxicity and for screening contact skin irritancy. Further studies with a wide range of skin irritants are still necessary to validate these models.

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Table 1. Threshold doses EC_{50} of four chemicals tested using human artificial skin, dermal equivalent, and monolayer cells compared with thresholds reported for commercial and human skin

Test chemical	Fib*	Kc*	DE*	AS*	LSE†	Human skin†
Glutaraldehyde	0.021	0.003	0.056	0.310		
Sodium lauryl sulfate	0.071	0.053	0.190	0.308	0.24 ± 0.08	0.1-0.5
Toluene	-	-	64.1	96.1	49 ± 18	> 30
Polyethylene glycol	80	62	-	-		

* EC_{50} values are the effective concentrations in units of percentage weight that inhibited 50% of the MTT conversion compared with negative control after 24 h exposure period. Fib = fibroblasts in monolayer culture; Kc = keratinocyte in monolayer culture; DE = dermal equivalent; AS = artificial skin.

† Data from Gay et al. [1].

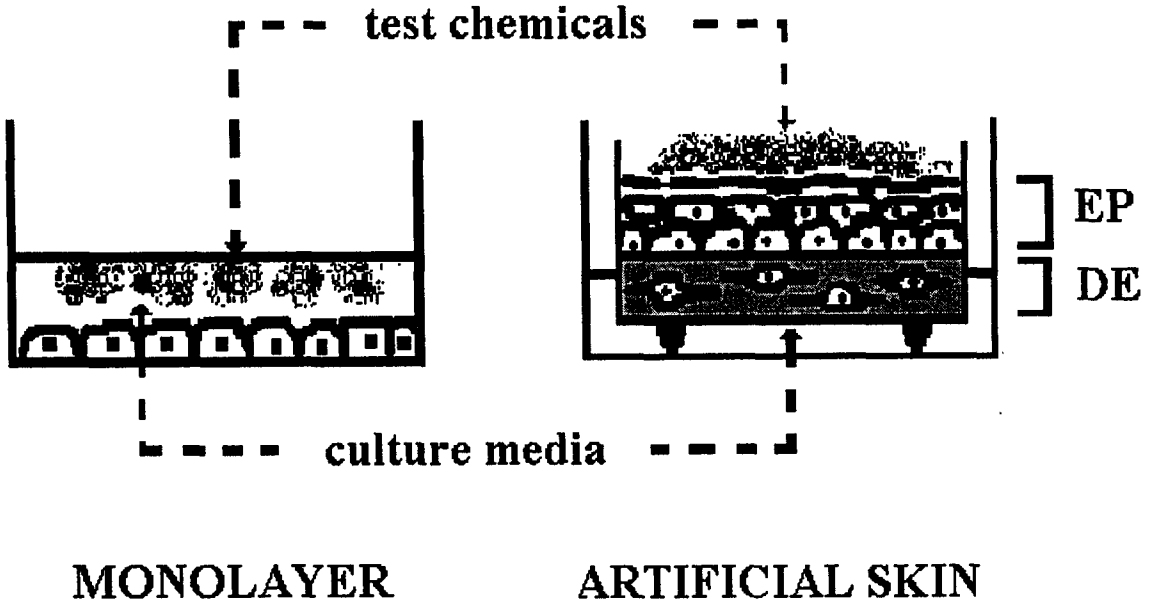


Fig. 1. Diagram of toxicant exposure systems to monolayer human skin cells and artificial skin. The submerged monolayer cultures were exposed to the test chemicals which were dispersed into the culture medium, whereas the dermal equivalent and artificial skin were exposed directly with an air-exposed surface. EP = epidermal layer; DE = dermal layer.

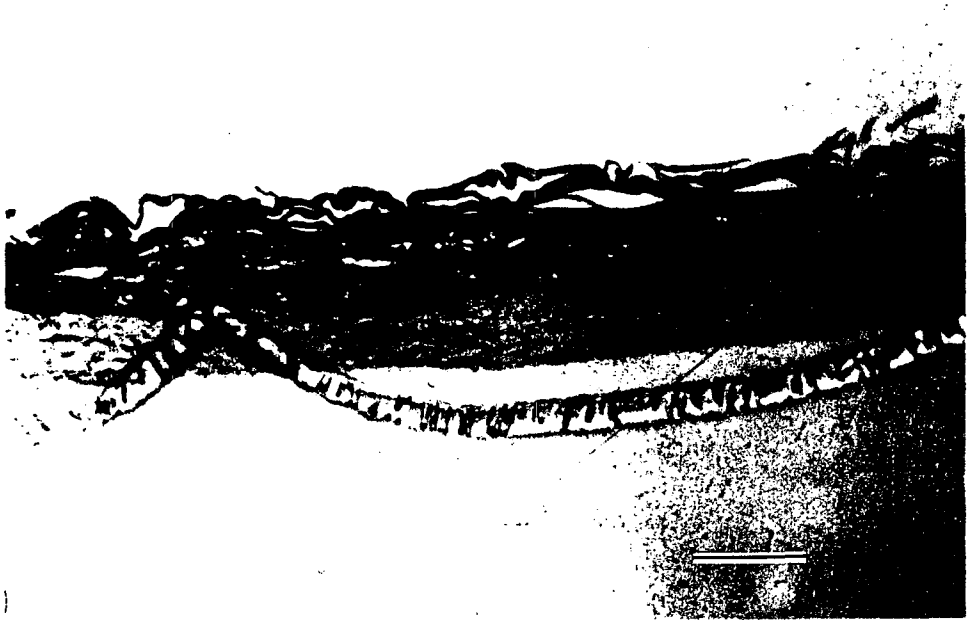
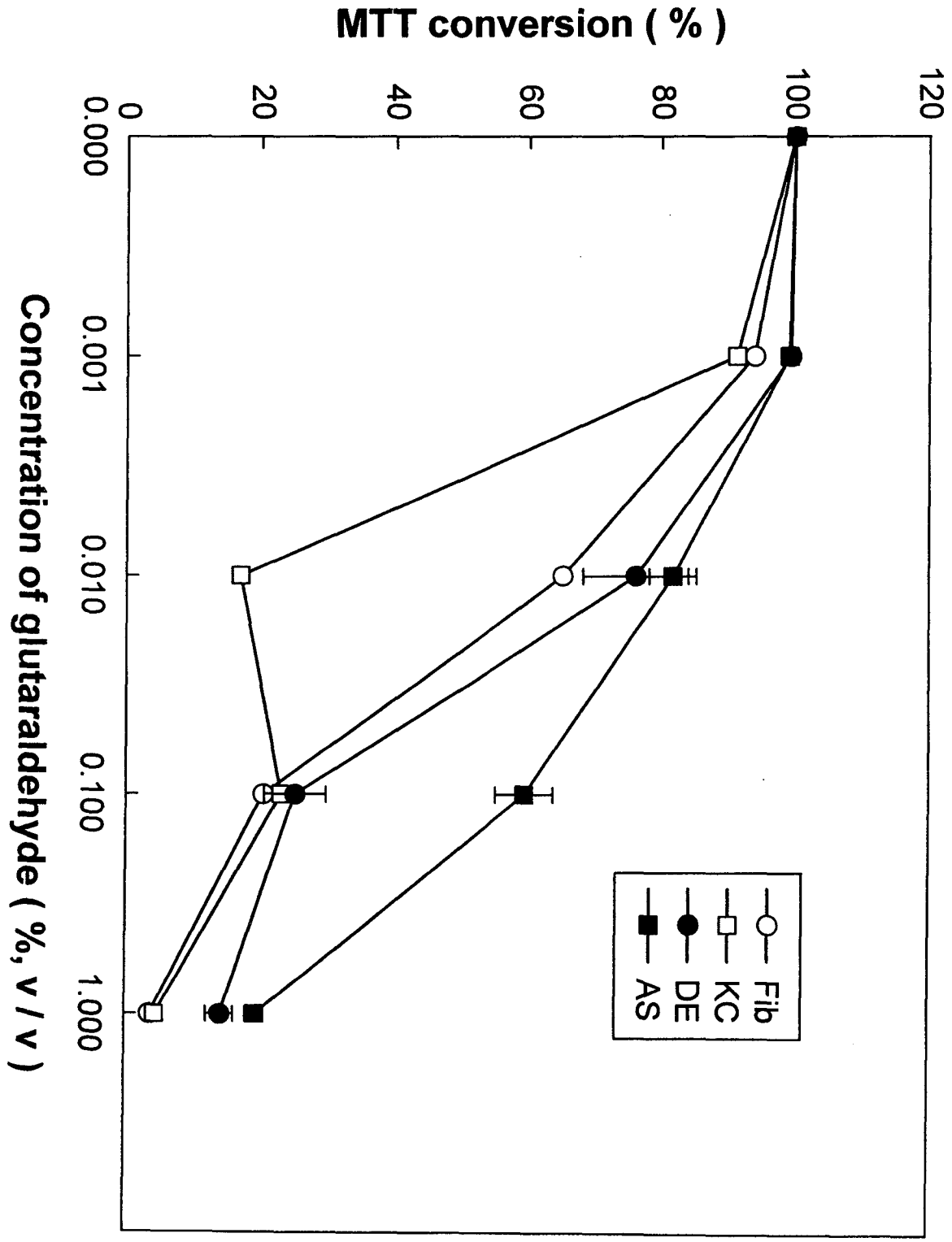
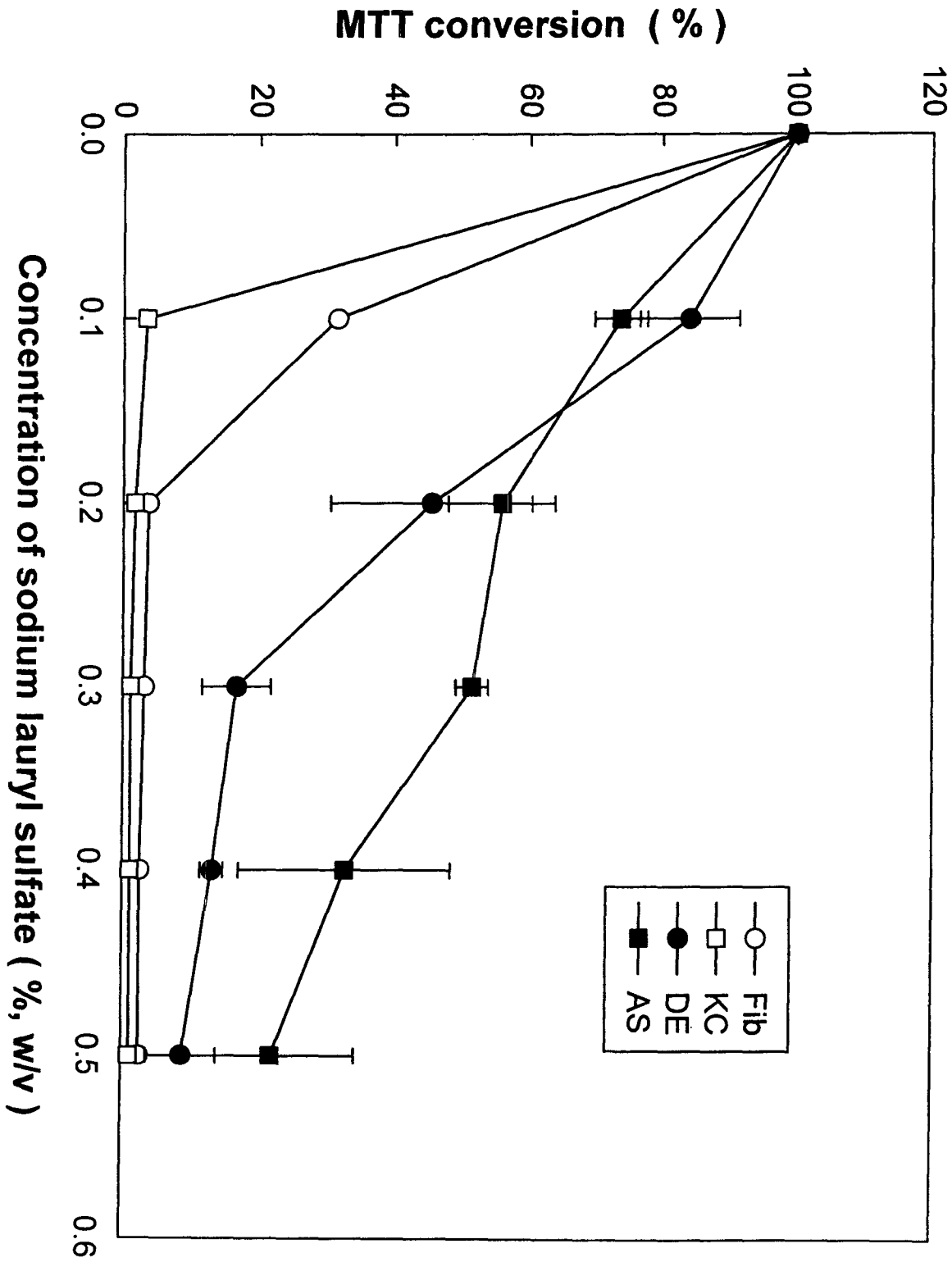
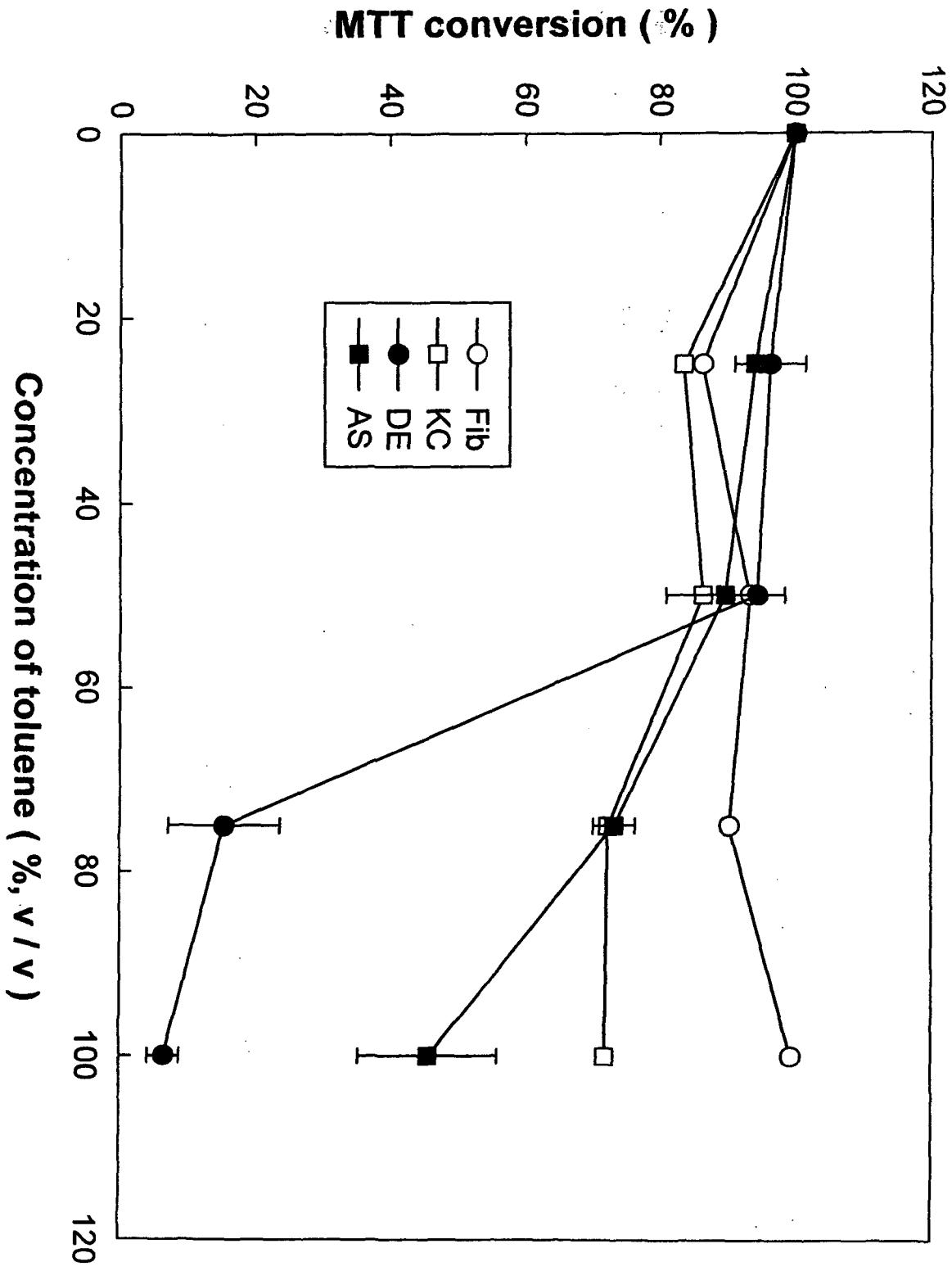


Fig. 2. Histological feature of artificial skin 14 days after air/liquid interface culture. The keratinocytes-containing epidermal layer was cultured on a type I collagen-containing dermal layer contracted by fibroblasts. SC = stratum corneum; EP = epidermal layer; DE = dermal layer. Haematoxylin and eosin staining,× 200, bar = 50 μm.

Fig. 3. Dose-response comparison of MTT conversion in cell systems exposed to four test chemicals, glutaraldehyde, SLS, toluene, and polyethylene glycol. The dermal equivalent (● , DE), artificial skin (■ , AS), monolayer cultures of human dermal fibroblasts (0 , Fib), and human keratinocytes (□ , KC) were exposed to the indicated concentrations of the four chemicals (percentage weight). The cell systems were exposed to the chemicals for 24 h at 37℃ in the presence of 5% CO_2 and then assayed for their capacity to convert MTT. The data are expressed as the mean percentage absorption ± standard deviation (SD) for 2-3 replicates per concentration. The results have been normalized to the appropriate negative control representing a 100% MTT conversion for each of the cell systems, which were subjected to all the experimental conditions except for exposure to the test sample. For vehicle controls, the DE and AS were exposed to 0.08 ml water. The MTT conversion in these vehicle controls was essentially identical to that seen in the negative controls to which no additions were made.


MTT conversion (%)
0
20
40
60
80
100
120
0.000
0.001
0.010
0.100
1.000
Concentration of glutaraldehyde (%, v / v)
Fib
KC
DE
AS


MTT conversion (%)
0
20
40
60
80
100
120
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration of sodium lauryl sulfate (%, w/v)
Fib
KC
DE
AS


MTT conversion (%)
0
20
40
60
80
100
120
Concentration of toluene (%, v / v)
AS
DE
KC
Fib

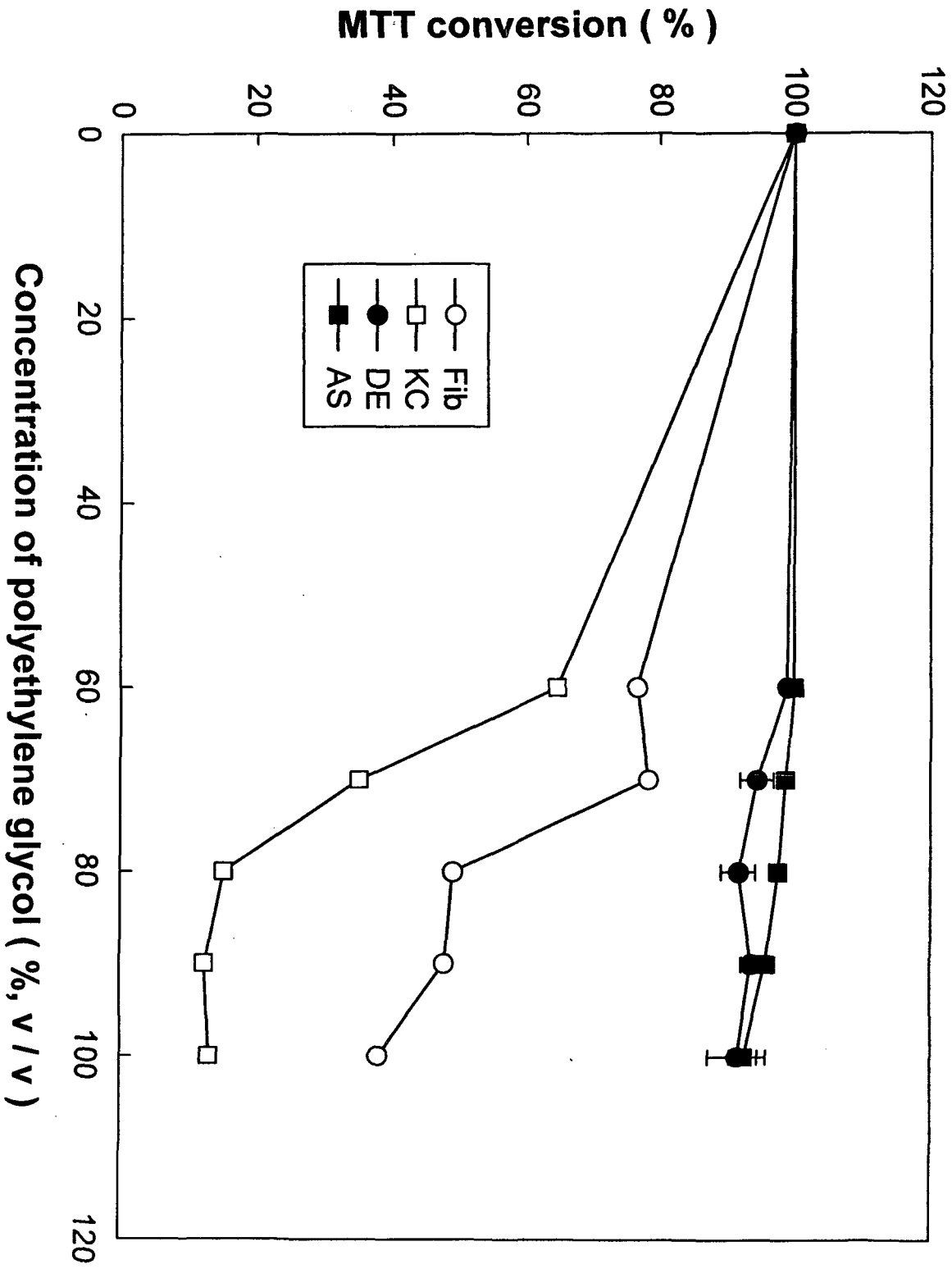
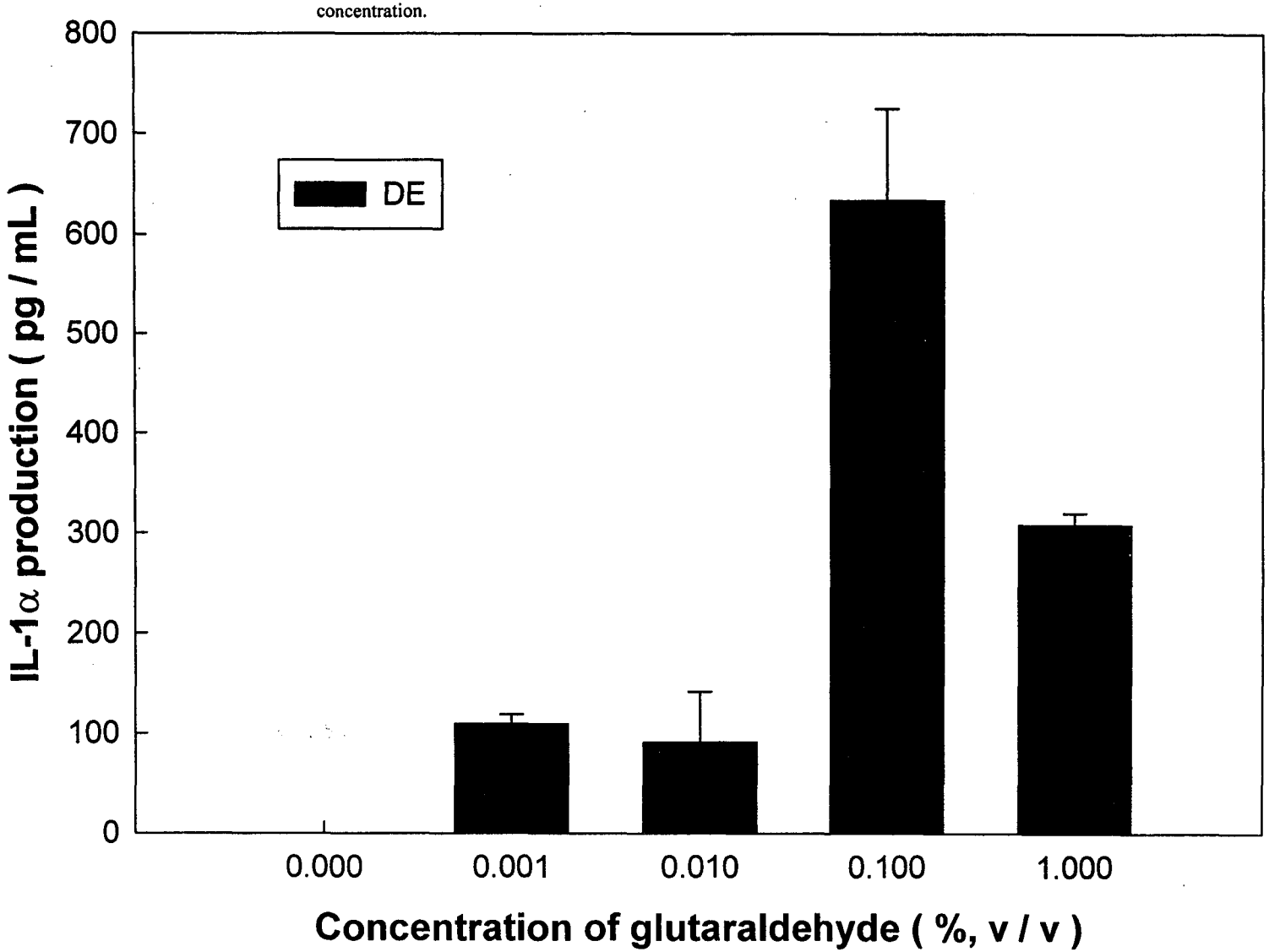
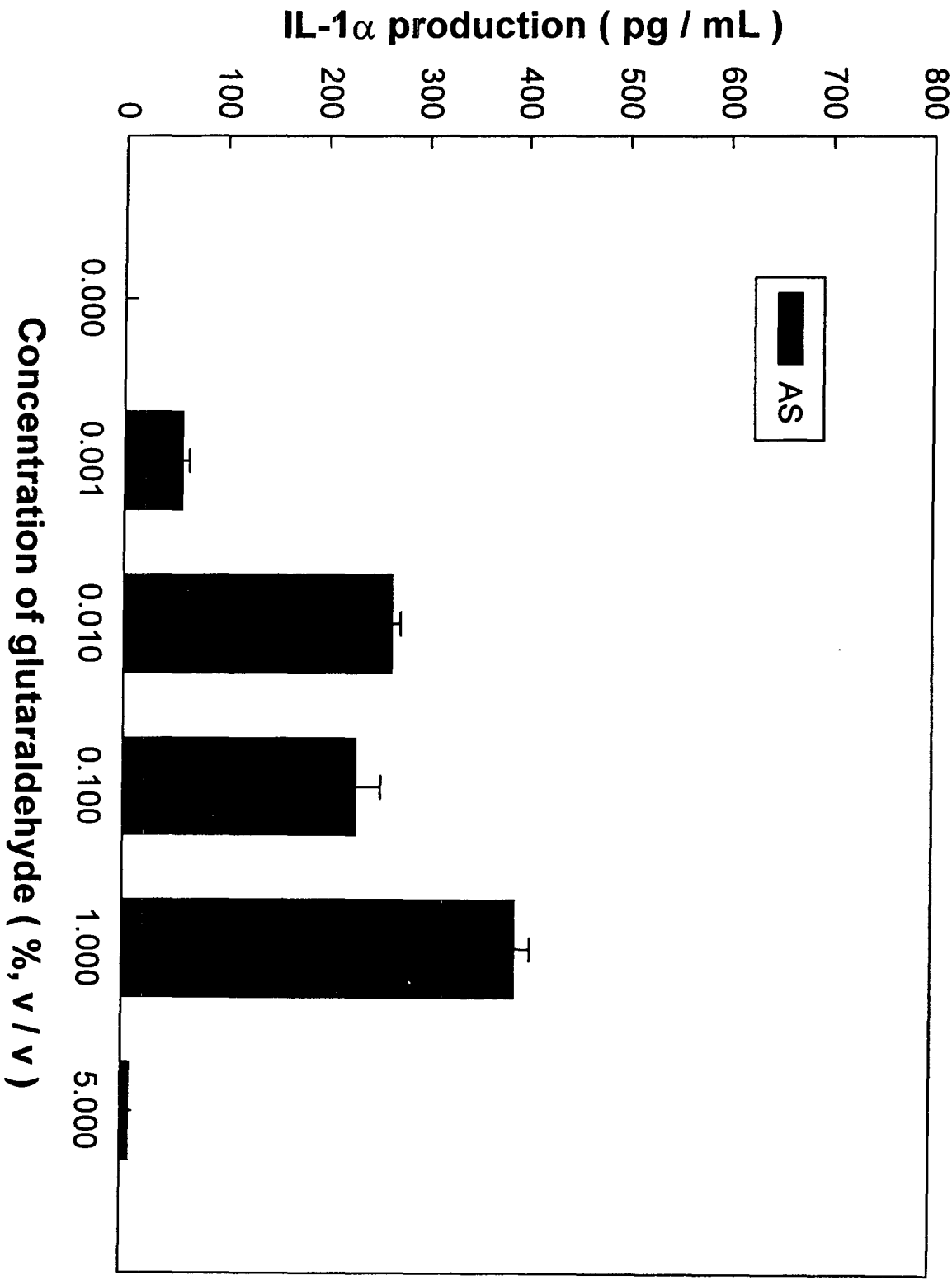
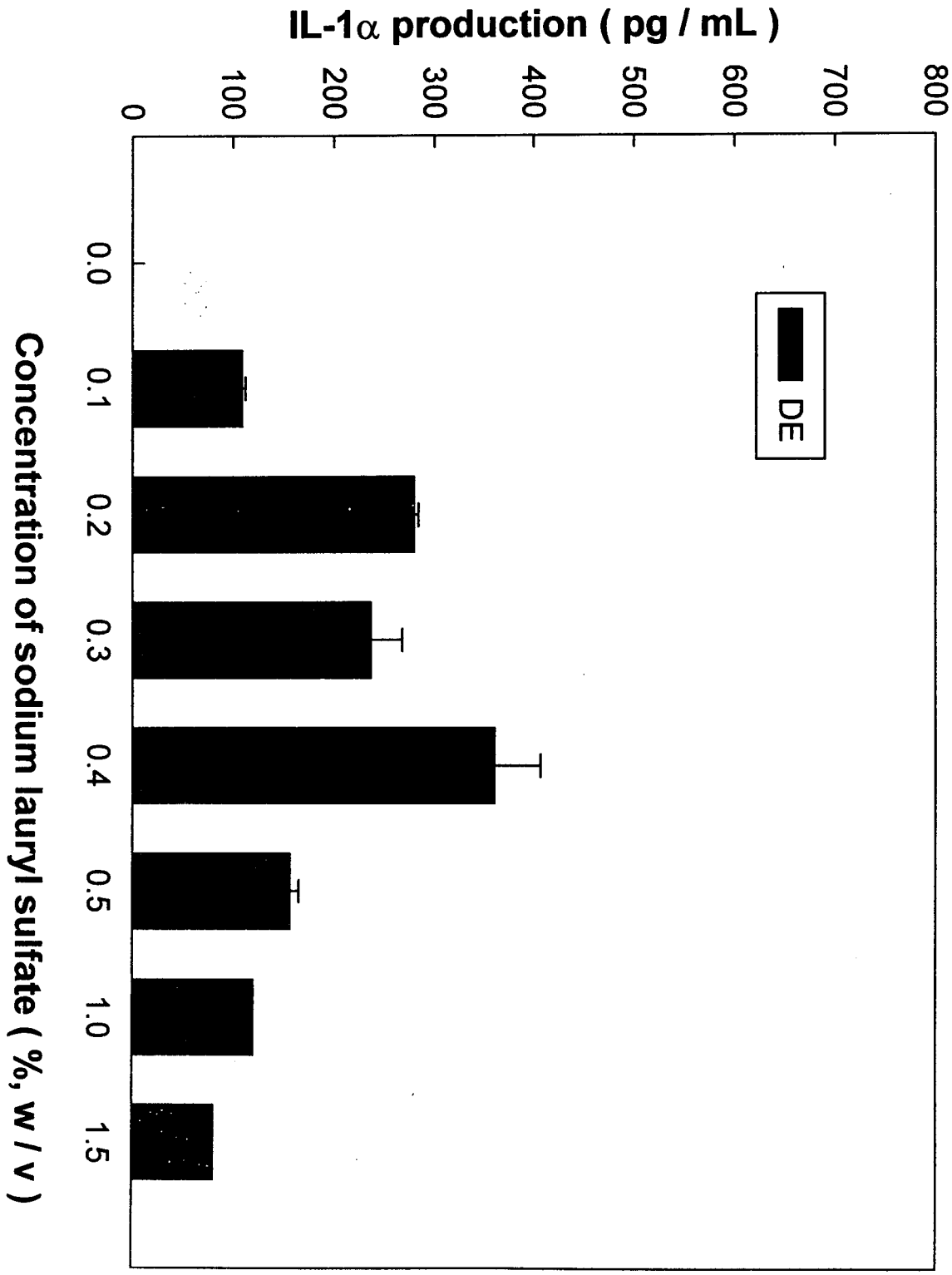

MTT conversion (%)
0
20
40
60
80
100
120
Concentration of polyethylene glycol (%, v / v)
0
20
40
60
80
100
120
Fib
KC
DE
AS

Fig. 4. Release of interleukin-1α (IL-1α) into culture medium of human skin cell systems such as dermal equivalents (DE) and artificial skins (AS) after topical application of various doses of test chemicals, glutaraldehyde (a, b) and SLS (c, d). The cell systems were exposed to the chemicals for 24 h at 37℃ in the presence of 5% CO_2 and then assayed for their IL-1α productions. The data are expressed as the mean percentage absorption ± standard deviation (SD) for 2-3 replicates per concentration.




IL-1α production (pg / mL)
0
100
200
300
400
500
600
700
800
AS
0.000
0.001
0.010
0.100
1.000
5.000
Concentration of glutaraldehyde (%, v / v)


IL-1α production (pg / mL)
0
100
200
300
400
500
600
700
800
DE
0.0
0.1
0.2
0.3
0.4
0.5
1.0
1.5
Concentration of sodium lauryl sulfate (%, w / v)

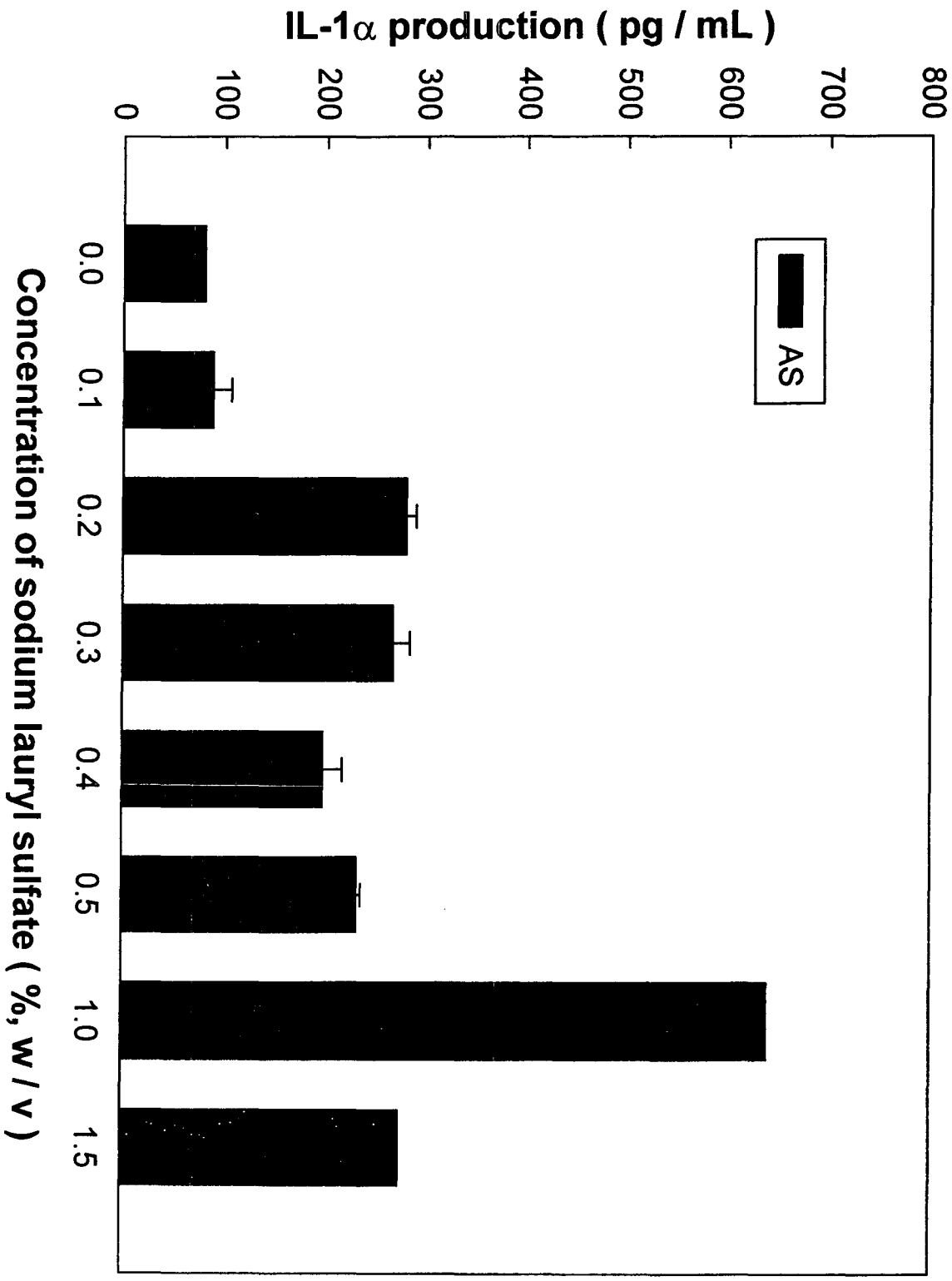
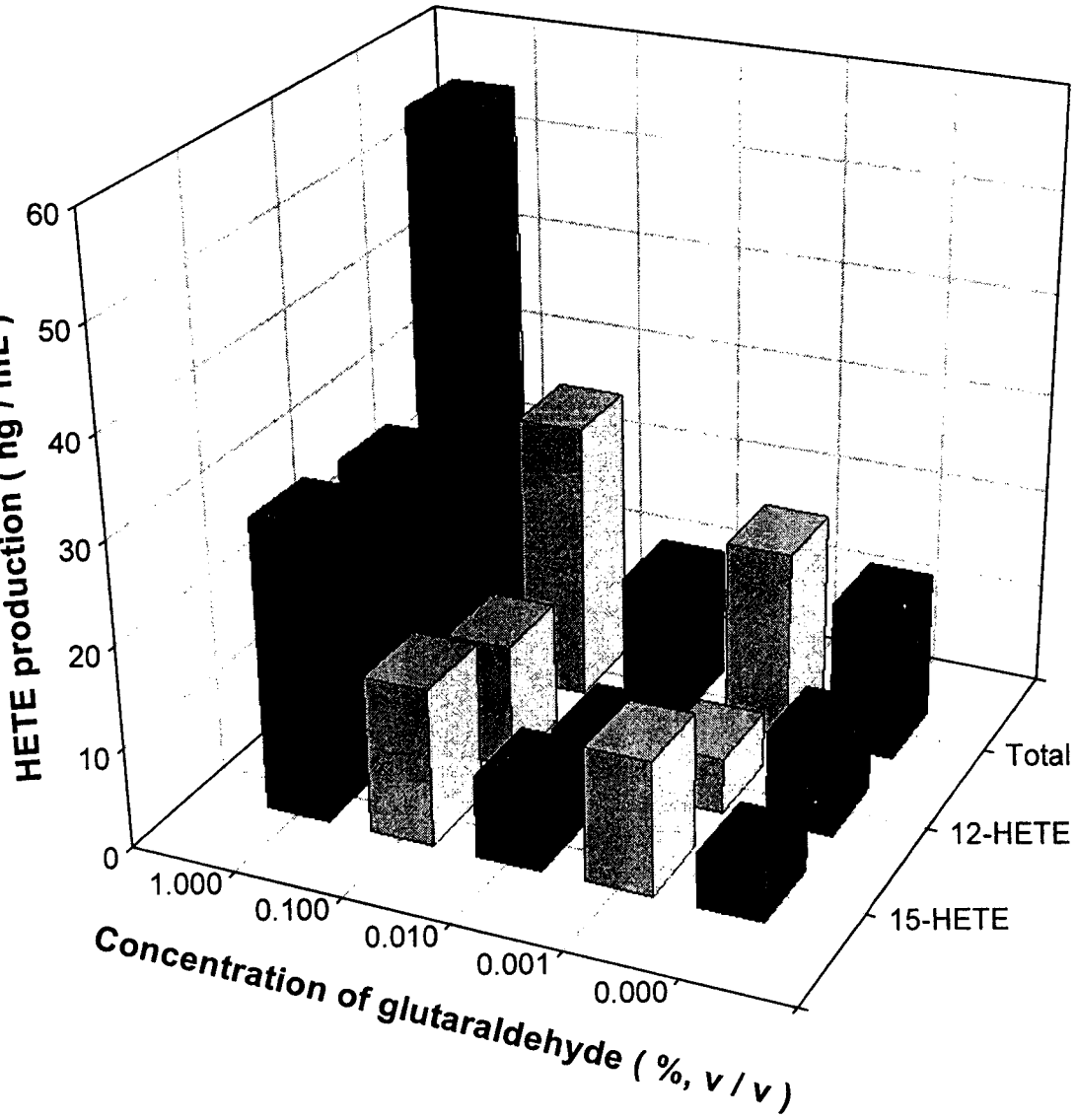
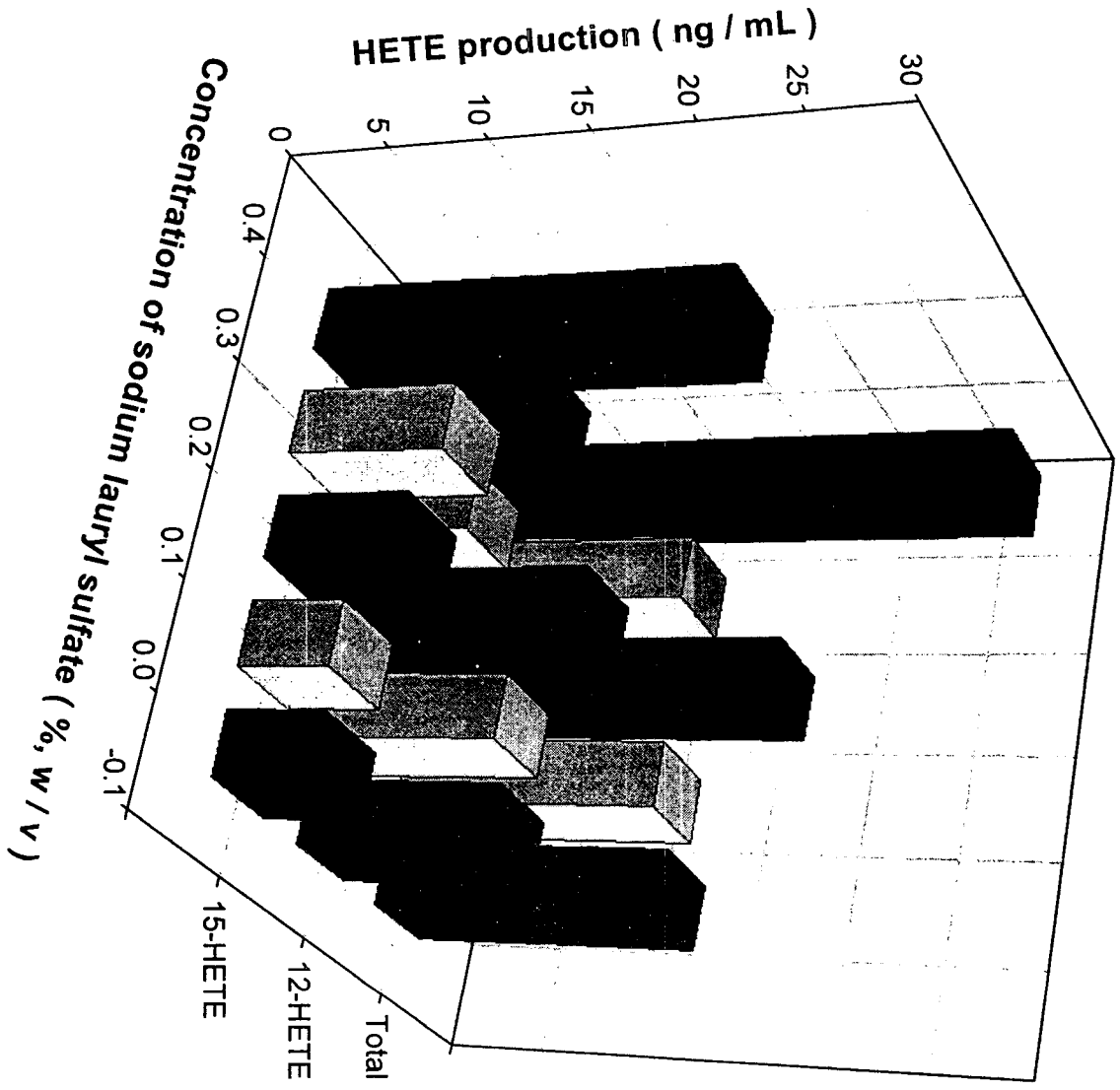

IL-1α production (pg / mL)
0
100
200
300
400
500
600
700
800
AS
0.0
0.1
0.2
0.3
0.4
0.5
1.0
1.5
Concentration of sodium lauryl sulfate (%, w / v)

Fig. 5. Production of 12- and 15-HETE into culture medium of human artificial skins after topical application of various doses of test chemicals, glutaraldehyde (a) and SLS (b). Total HETEs indicates the sum of 12-HETE and 15-HETE. The artificial skins were exposed to the chemicals for 24 h at 37°C in the presence of 5% CO_2 and then assayed for their IL-1α productions. The data are expressed as the mean percentage absorption ± standard deviation (SD) for 2-3 replicates per concentration.




HETE production (ng / mL)
0
5
10
15
20
25
30
Concentration of sodium lauryl sulfate (%, w / v)
0.4
0.3
0.2
0.1
0.0
-0.1
15-HETE
12-HETE
Total