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## *Glycolic Acid Modulation of Collagen Production in Human Skin Fibroblast Cultures In Vitro*

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**BACKGROUND.** Glycolic acid has been used extensively for the treatment of photoaging and wrinkles. Suggestions have been made that glycolic acid may have specific dermal effects, although biochemical studies are limited.

**OBJECTIVE.** This study's purpose was to examine the effect of glycolic acid on the radioactively labeled collagen production in human skin fibroblasts in culture.

**METHODS.** Normal dermal fibroblasts were grown to semi-confluence and incubated in the presence of glycolic acid for 24 hours. Radioactive proline was added to the cultures.

*Using a specific amino acid assay, the amount of radioactive hydroxyproline was measured and was used as an accurate index of collagen production.*

**RESULTS.** Results show that glycolic acid caused an elevated collage production in the fibroblasts.

**CONCLUSION.** These results demonstrate a specific stimulatory effect by the glycolic acid and could explain some of the positive benefits from the clinical use of glycolic acid. *Dermatol Surg* 1996;22:439-441.

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**G**lycolic acid is a new superficial chemical peeling agent. Reports suggest that glycolic acid can improve fine wrinkles and photoaging lesions.<sup>1,2</sup> Unlike other chemical peeling agents, glycolic acid may not cause as deep of an injury in order to achieve improvements. Articles have suggested that glycolic acid may have a direct effect on dermal components of the skin, including collagen and ground substances.<sup>3,4</sup> Therefore, a specific action in the skin may be the mechanism for improvement by glycolic acid. The purpose of our study is to biochemically examine the effects of glycolic acid on collagen synthesis by human skin fibroblasts in culture.

### **Materials and Methods**

#### *Cell Cultures*

Fibroblast cultures were initiated from biopsy of normal human skin. All skin samples were obtained with patient consent. Tissue pieces were minced and plated onto 75-cm<sup>2</sup> plastic tissue flasks. Cells were maintained in Dulbecco's modified Eagle's medium, containing glutamine and supplemented with 30 mM Hepes buffer (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (pH 7.6), penicillin (200 U/mL), streptomycin (200 µg/ml), and 20% fetal calf serum. Confluent primary cultures were trypsinized and subcultured in 75-cm<sup>2</sup> flasks at 37°C with 5% CO<sub>2</sub>. The medium was replaced every 2-3 days.

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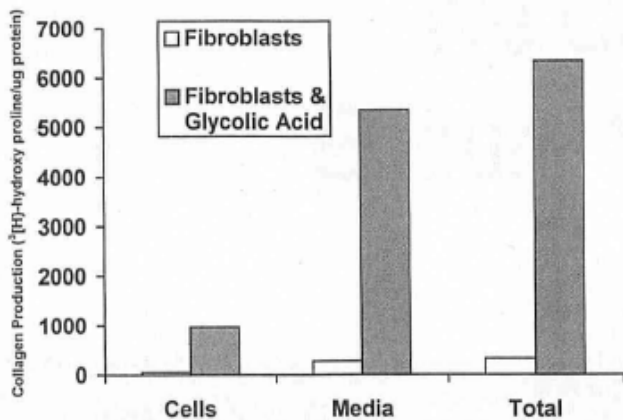
*Collagen Assays*

Fibroblasts in confluent monolayer cultures were preincubated in a medium containing glycolic acid (50 µg/ml) and ascorbic acid (25 µg/ml) for 4 hours and 24 hours, respectively. Control fibroblasts were incubated in medium containing ascorbic acid (25 µg/ml) only. At the end of preincubation, the cells were labeled with [<sup>3</sup>H] proline.<sup>5</sup> Cell and media extracts were collected and then dialyzed to remove unincorporated proline from the samples. Samples were sealed in glass tubes and hydrolyzed at 120°C for 12 hours.

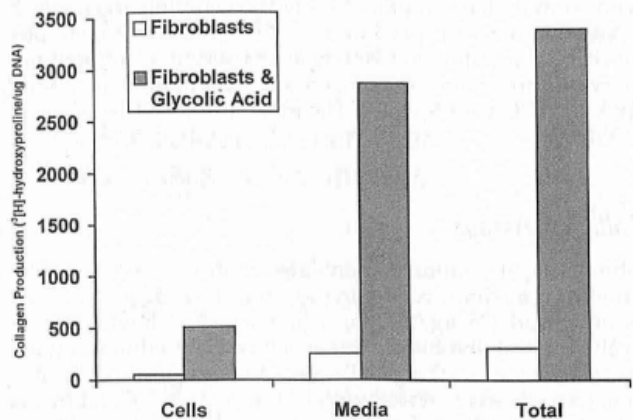
Cell and media sample fractions were tested with a specific radiochemical assay for nondialyzable hydroxyproline. Sample fractions were buffered with 8 mg sodium pyrophosphate using 0.2 mg chloramine-T, and the samples were oxidized to remove free proline. Samples were then heated to 100°C to extract radioactive hydroxyproline. A colorimetric test and radioactive scintillation count were made on the hydroxyproline samples, since the amount of radioactive hydroxyproline is an accurate marker of procollagen production by the fibroblasts.<sup>6,7</sup>

**Results**

Our experiment was designed to study the effects of glycolic acid on collagen production. Cells were preincubated with 50 µg/ml glycolic acid for 4 or 24 hours, respectively. Cells were then labeled with radioactive proline. The synthesis of radioactive hydroxyproline in nondialyzable fraction was taken as an index of procollagen production. Incubation of glycolic acid with fibroblasts for 4 hours failed to affect the synthesis. At 24 hours of preincubation with glycolic acid, procollagen production was increased dramatically compared with control fibroblasts without glycolic acid [Figures 1 and 2]. Control fibroblasts produced 308.1 + 209.6 radiation units (RU)/cell DNA, while fibroblasts preincubated with glycolic acid produced 3393.2 + 1453.5 RU/cell DNA [Table 2]. Thus, glycolic acid caused an approximate 10-fold upgrade in the production of procollagen. With respect to the cell protein, the control fibroblasts produced 333.9 + 78.3 RU/cell protein while fibroblasts preincubated with glycolic acid produced 6308.6 + 2606.3 RU/cell protein [Table 1].



**Figure 1.** Collagen production stimulation by glycolic acid. Cultures of normal human skin fibroblasts were grown in tissue flasks. Glycolic acid (50 µg/ml) was added to half the cultures. Hydroxyproline was used as an index of collagen production per microgram of cellular protein. The fibroblasts with the glycolic acid had a greatly increased level of collagen production compared with the fibroblasts without the glycolic acid.



**Figure 2.** Collagen production stimulation by glycolic acid. Cultures of normal human skin fibroblasts were grown in tissue flasks. Glycolic acid (50 µg/ml) was added to half the cultures. Hydroxyproline was used as an index of collagen production per microgram of cellular DNA. The fibroblasts with the glycolic acid had a greatly increased level of collagen production compared with the fibroblasts without the glycolic acid.

Table 1. Collagen Production per Cell Protein

	Cell	Media	Total
Control	53.6 + 48.9	280.3 + 48.9	333.9 + 78.3
Glycolic acid	967.4 + 306.2	5341.2 + 2300.1	6308.6 + 2606.3

*Fibroblast culture production of collagen expressed as an index of [<sup>3</sup>H]Hydroxyproline per cellular protein.*

Table 2. Collagen Production per Cell DNA

	Cells	Media	Total
Control	52.4 + 30.8	255.7 + 178.8	308.1 + 209.6
Glycolic acid	510.5 + 168.4	2882.7 + 1285.1	3393.2 + 1453.5

*Fibroblast culture production of collagen expressed as an index of [<sup>3</sup>H]Hydroxyproline per cellular DNA.*

## Discussion

In our study, we examined the effects of glycolic acid on cultured human skin fibroblasts. We wanted to determine the biochemical basis of glycolic acid's effects on connective tissue metabolism. At physiologic levels, glycolic acid was incubated for 24 hours with the cultured fibroblasts. The amino acid assay specified for the amount of hydroxyproline was an index of total collagen produced.

Previous works with chemical peels discuss the postpeel development of a zone of collagen. The zone of collagen is a deposit of a new collagen that is laid down in the upper dermal layers after a chemical peel. Both phenol and trichloroacetic acids (TCAs) have been histologically studied to compare the amount of new deposition in the zone of collagen. The deeper depth of necrosis caused by the chemical peeling agent resulted in a deeper zone of collagen. Thus, more damaging chemical peels can smooth deeper layers of the skin. For instance, higher concentrations of TCA, at 50-70%, can penetrate to layers of the reticular dermis and also cause a zone of new collagen to that same depth. However, higher concentrations of trichloroacetic acid can lead to more scars and risks than TCA applied at lower concentrations.

By working with glycolic acid, we are finding a way to stimulate fibroblasts without relying on peels that cause significant damage to cells and connective tissue. Glycolic acid may enhance the deposition of collagen in the upper dermis, which may eventually lead to a new zone of collagen. Thus, the glycolic acid does not have toxic effects on fibroblasts at the glycolic acid concentrations used. The effect on collagen production has a direct stimulatory effect and not a nonspecific or damaging effect on fibroblasts. Tretinoin has also been shown to act on directly on fibroblasts by increasing fibroblast activity and increasing collagen deposition on electron microscopy.<sup>8</sup> In addition, tretinoin stimulates an increased production of procollagen Type I in photodamaged skin.<sup>9</sup>

Fibroblasts synthesize a number of connective tissue components, including collagen, elastin, and glycosaminoglycans.<sup>10,11</sup> Fibroblast cell modulation is still being researched. Cytokines, such as granulocyte-macrophage colony-stimulating factor<sup>12</sup> and sclerosing basal cell factor,<sup>13</sup> have been shown to directly stimulate collagen production.

The results demonstrate that glycolic acid has a precise stimulatory effect on the collagen production in fibroblasts. This positive outcome may be a mechanism of action through a cytokine-line effect by the glycolic acid. Direct glycolic acid effects on collagen metabolism have been proposed from clinical experience accumulated about glycolic acid. The amino acid assay specified for the amount of hydroxyproline was an index of total collagen produced.

Previous work with chemical peels has established that a zone of collagen develops post-peel.<sup>14,15</sup> This zone of collagen is proportional to the strength and penetration of the chemical peeling agent, usually TCA or phenol. Elastotic deposits from photodamage are replaced by the zone of collagen. The increased collagen stimulation in normal dermal fibroblasts by glycolic acid may account for a comparable zone of collagen after glycolic acid chemical peels.

The deeper depth of necrosis caused by the TCA or phenol chemical peeling agent resulted in a deeper zone of collagen. Thus, more damaging chemical peels can smooth deeper layers of the skin. For instance, higher concentrations of TCA, at 50-70%, can penetrate to layers of the reticular dermis and also cause a zone of new collagen to that same depth.<sup>16</sup> However, higher concentrations of trichloroacetic acid can lead to more scars and risks than TCA applied at lower concentrations.<sup>17</sup>

By working with glycolic acid, we are finding a way to stimulate fibroblasts without relying on peels that cause significant damage to cells and connective tissue. Glycolic acid may enhance the deposition of collagen in the upper dermis, which may eventually lead to a new zone of collagen. As shown, higher concentrations of glycolic acid caused increased stimulation of collagen production. Thus, the



glycolic acid does not have toxic effects on fibroblasts at the glycolic acid concentrations used. The effect on collagen production has a direct stimulatory effect and not a nonspecific or damaging effect on fibroblasts.

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