

# Randomized clinical efficacy of superficial peeling with 85% lactic acid versus 70% glycolic acid\*

Estudo randomizado da eficácia clínica dos peelings superficiais de ácido láctico 85% versus ácido glicólico 70%

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**Abstract:** BACKGROUND: *Peeling* is a procedure which aims to accelerate the process of skin exfoliation. OBJECTIVES: Development of formulations containing lactic acid at 85% or glycolic acid at 70% and the evaluation of these formulations on clinical efficacy in reduction of fine wrinkles. METHODS: Preliminary stability tests were carried out and an *in vivo* study was performed with three groups with 9 representatives each. One was the control group, which used only sunscreen; another one used lactic acid+sunscreen, and the last group used acid glycolic+sunscreen. Clinical efficacy was assessed with a CCD color microscope, through the digitization of images before and after treatment. The applications were carried out by a dermatologist, once a month every 30 days, during 3 months. The area with wrinkles was calculated by planimetry point counting, in accordance with Mandarin-de-Lacerda. RESULTS: The formulations were stable in the visual and Ph evaluation. There was no improvement in the control group; for lactic acid, there was significant improvement after the second peeling application on the outer lateral area of the right eye and after the third application on the outer lateral area of the left eye. For the glycolic acid group, there was significant improvement in the outer lateral area of the left eye after the first application, and of the right eye region, after three applications. The formulations used must be kept under refrigeration and should be manipulated every 30 days. CONCLUSIONS: Both peelings were effective in reducing fine wrinkles of the outer lateral eye area after three applications ( $p \leq 0.05\%$ ). It was observed that peeling efficacy in the external-lateral region of one eye might be different compared with that in skin of the external-lateral region of the other eye, relative to the speed of skin improvement.

Keywords: Chemical exfoliation; Lactic acid; Skin aging

**Resumo:** FUNDAMENTOS: *Peeling* visa a acelerar o processo de esfoliação da pele. OBJETIVOS: Desenvolver formulações contendo ácido láctico a 85% ou ácido glicólico a 70% e avaliar sua eficácia clínica na redução de rugas finas. MÉTODOS: Testes preliminares foram efetuados e estudo *in vivo* foi realizado em três grupos com nove representantes cada, separados de forma randomizada. Um grupo foi controle, utilizando apenas fotoprotetor; outro utilizou ácido láctico e fotoprotetor; o último usou ácido glicólico e fotoprotetor. Para eficácia clínica, empregou-se microscópio CCD color, digitalizando-se as imagens do pré e do pós-tratamento. As aplicações foram realizadas por médica dermatologista uma vez por mês, a cada 30 dias, durante três meses. A área com traços de ruga foi calculada pela planimetria por contagem de pontos. RESULTADOS: As formulações foram estáveis na avaliação visual e de pH. Não houve melhora no grupo controle; para o grupo do ácido láctico, houve melhora significativa após a segunda aplicação do peeling na região lateral externa do olho direito e após a terceira aplicação na região lateral externa olho esquerdo. Para o grupo do ácido glicólico, houve melhora significativa na região lateral externa olho esquerdo após a primeira aplicação e, depois de três aplicações, na região lateral externa do olho direito. As formulações magistrais empregadas no estudo devem ser mantidas sob refrigeração e manipuladas a cada 30 dias. CONCLUSÕES: Tanto o *peeling* de ácido láctico quanto o de ácido glicólico foram eficazes na diminuição de rugas finas da região lateral externa dos olhos após três aplicações ( $p \leq 0.05\%$ ). Verificou-se que a eficácia dos peelings na região lateral externa de um olho pode ser diferente da eficácia na pele da região lateral externa do outro olho, em relação à rapidez da melhora da pele.

Palavras-chave: Abrasão química; Ácido láctico; Envelhecimento da pele

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## INTRODUCTION

Chemical peeling involves the application of one or more caustic agents to the skin to generate a controlled destruction of the latter. It can cause thickening of the epidermis, collagen deposition, reorganization of structural elements and an increase in dermal volume.<sup>1</sup>

Peeling is classified as: *very superficial*, where destruction occurs from the stratum corneum to the stratum granulosum; *superficial* when destruction occurs in the epidermis; *medium*, reaching the papillary dermis; and *deep*, when it reaches the reticular dermis. Alpha-hydroxy-acids (AHAs) have been the most commonly used agents for superficial peelings.<sup>1,4</sup>

AHAs are organic compounds, which share the same hydroxyl in the alpha position. Important components of this group are found in foods such as fruit, sugar cane and yoghurt, which contain glycolic acid, lactic acid, malic acid, tartaric acid and citric acid.<sup>2,5,6</sup>

The simplest and most-used has been glycolic acid (GA), derived from sugar cane, presenting in its structure only two carbon atoms, allowing for better cutaneous penetration.<sup>5,7,8</sup> Its effect depends on how long it remains on the skin (the longer the period, the stronger the action) and its concentration. To put an end to the peeling effect using glycolic acid, it is necessary to use a formulation that provides acid neutralization, or that can be washed with water.<sup>5,6,9,10</sup>

In previous studies, Okano *et al.* and Hussein *et al.*, described the mechanisms by which the GA could act in the improvement of photoaged skin: GA increases the thickness of the epidermis, activates the fibroblasts and increases the dermal collagen. The increase in density and thickness of the collagen fibers occurs as a result of the ability of the GA to accelerate collagen synthesis by fibroblasts. AHAs can increase the thickness of the skin by increasing the synthesis of glycosaminoglycans and collagen fibers.<sup>11,12</sup>

Lactic acid (LA) comes from sour milk and tomato juice and reduces the thickness of the stratum corneum by decreasing corneocit cohesion.<sup>5</sup> There are few studies on the isolated action of LA peeling.<sup>13</sup>

Thus, the aims of this study were the development of formulations with lactic acid at 85% or glycolic acid at 70%, and the evaluation of their clinical efficacy in reducing fine wrinkles in the external lateral region of the eyes.

## CASUISTRY

### Development of the formulations

Two formulations were developed with hydroxyethylcellulose. LA at 85% was added to the first formulation and GA at 70% to the other (Table 1).

**TABLE 1:** Composition of the formulation developed with lactic acid 85% (F1) and glycolic acid 70% (F2)

Components	F1 (%)	F2 (%)
Hydroxyethylcellulose	0.8	0.8
Methylparaben	0.2	0.2
Glycerin	2.0	2.0
Lactic acid 85%	Qs	-
Glycolic acid 70%	-	Qs

Qs = Sufficient

### Preliminary Stability tests

#### Visual assessment

Visual assessment samples were observed for changes in color, separation of phases and homogeneity at times zero (T0), 7 days (T7), 14 days (T14) and 30 days (T30) after manipulation. They were stored at room temperature ( $25.00 \pm 2.00^\circ\text{C}$ ), in a refrigerator ( $5.00 \pm 2.00^\circ\text{C}$ ) and in hot oven ( $37.00 \pm 2.00^\circ\text{C}$ ) (14).

#### Determination of pH

The pH was analyzed in a peagometer, using samples diluted in distilled water (5%). This test was performed at times T0 and T30, after being stored at room temperature ( $25.00 \pm 2.00^\circ\text{C}$ ), in a refrigerator ( $5.00 \pm 2.00^\circ\text{C}$ ) and hot oven ( $37.00 \pm 2.00^\circ\text{C}$ ).<sup>14,15</sup>

#### Evaluation of the formulations' efficacy

The study was performed according to the Ethics in Research Committee at the Methodist University of Piracicaba. The volunteers taking part in the research were made aware of the *Terms of Free and Informed Consent*. Study inclusion criteria were: healthy women with fine wrinkles on the external lateral region of the eyes, aged 35-60, skin phototypes II to IV according to Fitzpatrick's classification, and signing the informed consent. Exclusion criteria were recent surgical procedures (2 to 6 months) in area of treatment, serious inflammatory injuries, herpes on treated region, pregnant and lactating women due to the risk of appearance of spots; precedents of hipertrophic or queloidic scars because of their reduced capacity to heal and those who had taken systemic retinoids for less than four months.<sup>7,13,16,17</sup>

Three randomized groups were formed, each with 9 representatives. One used a lactic acid plus sunscreen formulation, another used a glycolic acid plus sunscreen formulation, and the last one was the control group, which used only sunscreen. The three groups were homogeneous in terms of age, skin phototype and wrinkle aspect. The sunscreen was standardized for all groups, SPF 30 with physical and chemical broad-spectrum sunscreens (UVA and UVB). The volunteers were advised to apply them every three hours. The use of sunscreen is important since

products containing AHAs may make users more sensitive to ultraviolet radiation, enabling the occurrence of post-inflammatory hyperpigmentation.<sup>2,18,19</sup>

Before the first application of each peeling, the external lateral region of the volunteers' eyes (both the right and the left eye) was photographed with a CCD color skin microscope, model Scope, with a 10x increase. The applications were carried out once a month with an interval of 30 days between them, for 3 months. Each application lasted 3 minutes, which is classified as superficial peelings. The application procedure was standardized, the dermatologist wore gloves on their hands, the peeling was applied with a brush starting on the external lateral region of the eyes and following a clockwise direction. The volunteers put disposable caps on their hair during the application. These were then removed with water, and volunteers dried their faces with paper towels. Next, they applied the sunscreen.<sup>7</sup> The immediate effects were redness and burning (in some cases), no adverse events were observed. At the end of each month, the volunteers' eyes were photographed again.

In order to calculate the percentage of areas with wrinkles, the photographs taken with I Scope equipment were transferred to the Corel Photo-Paint program. The area with wrinkles of each volunteer was calculated by Planimetry point counting, in accordance with Mandarin-de-Lacerda, using a reticle with 266 squares within an area of 1,2 cm<sup>2</sup>.<sup>20</sup> The total number of areas with wrinkles in percentage points was obtained by dividing the number of squares with areas of wrinkles by the total number of photographed squares (266), and then by multiplying this figure by 100 (Figure 1).

The statistical analysis was performed using a statistics software program (GMC) developed by Maya Campos.<sup>21</sup> The preliminary statistical tests indicated a normal sample distribution (parametric) and therefore, an Analysis of Variance was performed followed by the Tukey Test. The probability level for statistical significance on the tests was 5%.

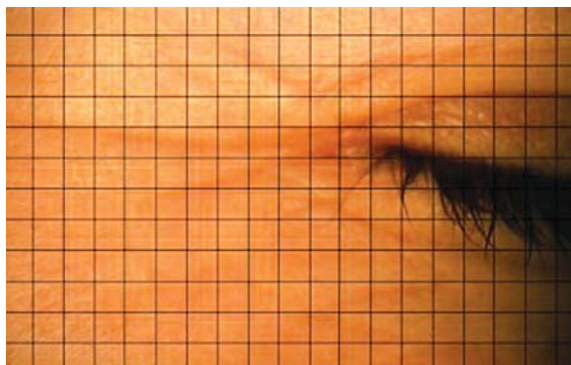


FIGURE 1: External-lateral region of the right eye (RE) of the volunteer, using a reticle with 266 squares within an area of 1.2 cm<sup>2</sup> (10x increase)

## RESULTS

### Development of the formulations

In the development of the formulation, glycerin and methylparaben were firstly mixed, homogenized. Next, the lactic acid or glycolic acid was added to the mixture and hydroxyethylcellulose was then added, heating up, with homogenization, up to 55°C.

The choice of hydroxyethylcellulose as a thickening agent was due to its high capacity to bear the wide range of pH, remaining stable after the addition of acid.

### Stability tests

#### Visual assessment

The visual evaluation results for the lactic acid formulation are shown in table 2, and those for glycolic acid, in table 3.

#### Determination of pH

The results for the determination of pH on the studied formulae, lactic acid and glycolic acid, are displayed in table 4.

#### Evaluation of the formulations' efficacy

The results for areas occupied by wrinkles in the external-lateral region of the eyes, expressed in percentages, on the right eyes (RE) and left eyes (LE) of the volunteers, at moments zero (T0), 30 days (T30), 60 days (T60) and 90 days (T90) in the control groups, lactic acid and glycolic acid, are displayed in table 5.

## DISCUSSION

Drawing on the visual evaluation results, it was noticed that both the lactic acid and glycolic acid formulations remained stable until T14 in the analyses of color, odor, separation of phases and homogeneity (Tables 2 and 3). However, at T30, there was a change in the color parameters of both formulations when stored at room temperature and in a hot oven. In accordance with this data, it was found that it is important to manipulate a new product every 30 days and keep it under refrigeration, as the high temperature influences stability.

In respect to homogeneity, the two formulations were liquefied when stored in a hot oven at 37.00 ± 2.00°C for 30 days. However, this result was expected, since Mambro *et al.* showed that formulations added with hydroxyethylcellulose may lose viscosity when stored at high temperatures for over 21 days.<sup>22</sup>

The study published by Bennett & Henderson affirms that the pH of the glycolic acid formulations may vary from 0.08 to 2.75.<sup>5</sup> The pH results found in this experiment varied from 2.05 to 2.50, and the formulations were not neutralized (Table 4).

Clark & Scerri assert that the formulae which were not previously neutralized are more efficient, as they are found in their free acid form.<sup>2</sup> Smith and Van Scott & Yu also observed that the lower the pH of the

**TABLE 2:** Visual analysis of the lactic acid formulation, taking into account color, odor, separation of phases (SP) and homogeneity at times T0, T7, T14 and T30, when stored at room temperature ( $25.00 \pm 2.00^\circ\text{C}$ ), in a refrigerator ( $5.00 \pm 2.00^\circ\text{C}$ ), and in a hot oven ( $37.00 \pm 2.00^\circ\text{C}$ )

	<b>Color</b>	<b>Odor</b>	<b>SP</b>	<b>Homogeneity</b>
<b>T0</b>				
Room Temperature	colorless	characteristic	No	homogeneous
<b>T7</b>				
Room Temperature	colorless	characteristic	no	homogeneous
Refrigerator	colorless	characteristic	no	homogeneous
Hot oven	colorless	characteristic	no	homogeneous
<b>T14</b>				
Room Temperature	colorless	characteristic	no	homogeneous
Refrigerator	colorless	characteristic	no	homogeneous
Hot oven	colorless	characteristic	no	homogeneous
<b>T30</b>				
Room Temperature	Slightly Brown	characteristic	no	homogeneous
Refrigerator	colorless	characteristic	no	homogeneous
Hot oven	Slightly brown	characteristic	no	fluid

**TABLE 3:** Visual analysis of the glycolic acid formulation, taking into account color, odor, separation of phases (SP) and homogeneity at times T0, T7, T14 and T30 when stored at room temperature ( $25.00 \pm 2.00^\circ\text{C}$ ), in a refrigerator ( $5.00 \pm 2.00^\circ\text{C}$ ) and in a hot oven ( $37.00 \pm 2.00^\circ\text{C}$ )

	<b>Color</b>	<b>Odor</b>	<b>SP</b>	<b>Homogeneity</b>
<b>T0</b>				
Room Temperature	colorless	odorless	no	homogeneous
<b>T7</b>				
Room Temperature	colorless	odorless	no	homogeneous
Refrigerator	colorless	odorless	no	homogeneous
Hot oven	colorless	odorless	no	homogeneous
<b>T14</b>				
Room Temperature	colorless	odorless	no	homogeneous
Refrigerator	colorless	odorless	no	homogeneous
Hot oven	colorless	odorless	no	homogeneous
<b>T30</b>				
Room Temperature	Slightly yellow	odorless	no	homogeneous
Refrigerator	colorless	odorless	no	homogeneous
Hot oven	Slightly yellow	odorless	no	fluid

**TABLE 4:** Result of the average (n=2) and standard deviation of the pH of the formulae of lactic acid and glycolic acid, performed at T0 at room temperature ( $25.00 \pm 2.00^\circ\text{C}$ ) and at T30 when stored at room temperature ( $25.00 \pm 2.00^\circ\text{C}$ ), in a refrigerator ( $5.00 \pm 2.00^\circ\text{C}$ ) and in a hot oven ( $37.00 \pm 2.00^\circ\text{C}$ )

	<b>Lactic Acid</b>	<b>Glycolic Acid</b>
<b>T0</b>		
Room temperature	$2.10 \pm 0.007$	$2.05 \pm 0.007$
<b>T30</b>		
Room temperature	$2.50 \pm 0.000$	$2.41 \pm 0.007$
Refrigerator	$2.45 \pm 0.007$	$2.43 \pm 0.007$
Hot oven	$2.44 \pm 0.007$	$2.44 \pm 0.000$

**TABLE 5:** Percentage of areas with wrinkles of the right eye (RE) and left eye (LE) of the control, lactic acid and glycolic acid groups, at T0, T30, T60 and T90. Different letters represent statistically different results according to the Tukey's test ( $p \leq 0.05\%$ )

	Right eye (RE)				Left eye (LE)			
	T0	T 30	T60	T90	T0	T30	T60	T90
Glycolic acid	17.66	17.29	12.78	15.03	13.9	10.52	12.4	6.01
	8.27	7.89	7.89	8.64	8.27	9.39	6.76	13.15
	18.79	11.65	13.15	11.65	12.03	8.64	8.27	9.39
	5.63	7.51	7.14	6.76	10.15	10.15	11.27	6.01
	4.88	3	3.38	3.38	3.38	3	3	9.39
	2.25	1.5	1.5	0	0	0	0	3
	18.42	16.91	17.66	12.03	13.53	12.4	13.15	0
	9.39	5.63	9.02	4.88	9.77	8.64	9.77	10.52
	4.51	4.51	4.51	2.63	4.13	4.13	4.13	9.02
	<b>Average</b>	9.98a	8.43a	8.56a	7.22b	8.35a	7.43b	7.64b
<b>Standard deviation</b>	6.57	5.73	5.21	4.99	4.86	4.09	4.53	4.21
Lactic acid	10.9	11.27	7.89	10.9	15.03	15.03	13.53	10.9
	10.15	6.01	5.26	6.76	7.51	6.39	4.88	4.88
	5.63	3.38	3.75	5.63	4.51	5.26	5.63	3
	12.4	6.01	6.01	4.88	8.64	7.51	4.88	6.76
	11.27	9.77	7.51	8.64	13.9	10.9	9.02	6.01
	23.3	15.41	12.78	12.78	19.54	16.16	19.92	15.78
	18.04	13.9	13.9	12.4	16.54	15.41	17.66	14.66
	5.26	0.75	0.37	0	4.13	1.5	3	1.5
	16.54	10.15	11.65	9.39	15.78	6.01	5.63	7.14
	<b>Average</b>	12.61a	8.52a	7.68b	7.93b	11.73a	9.35a	9.35a
<b>Standard deviation</b>	5.84	4.84	4.44	4.09	5.63	5.24	6.19	4.95
Control	12.4	12.03	10.15	12.78	9.02	9.77	10.15	10.52
	0.75	3	2.25	1.5	8.27	7.89	11.27	7.51
	6.39	5.26	7.14	4.88	7.89	6.01	6.01	5.63
	13.53	13.53	13.9	10.9	10.52	11.27	7.51	10.52
	2.63	1.87	3.38	2,6	4,2	4.51	4.88	4,50
	12.4	10.15	8.27	9.02	10.52	7.51	8.64	8.64
	10.52	5.63	10.9	11.27	12,15	15.03	11.65	14,1
	8.64	10.15	9.77	9.77	4.13	4.88	4.51	4.88
	13.53	14.28	15.41	12.4	9.39	9.39	10.52	9.77
	<b>Average</b>	8.97a	8.43a	9.02a	8.35a	8.45a	8.47a	8.35a
<b>Standard deviation</b>	4.77	4.6	4.35	4.26	2.75	3.34	2.75	3.15

formulation, the greater their bioavailability and effectiveness, that is to say, the greater the capacity for stimulation of cellular renewal.<sup>23,24</sup>

Based on the average age of the volunteers and the skin characteristics observed by the dermatologist involved in the study, the investigation was tried in homogenous groups.

In accordance with table 5, it was noted throughout the whole experiment that the results found for the control group did not present significant differences ( $p \leq 0.05\%$ ). It was observed that the use of sunscreens alone is not sufficient to reduce fine wrinkles. Also, there was significant improvement in the

reduction of fine wrinkles after the second peeling application of LA on the RE, and after the third application on the LE. For the group treated with GA, there was a significant improvement in the reduction of fine wrinkles on the LE after the first peeling application, though on the RE, this result appeared only after the third application.

Given that the analyses were performed on both the RE and LE, the differences found on the eyes from the same group can be explained by the type of wrinkles presented by the volunteer, which can vary in depth and size. Since the superficial peeling basically reaches the epidermis, it is more effective against fine

wrinkles.<sup>2,5,16,17</sup> As the action of AHA is time-dependent, an exposure of 3 minutes is classified as superficial.<sup>25</sup> Thus, it a reduction of fine wrinkles was observed, though not of the deep ones, which justifies the reduction of wrinkles on only one side of the face in the same group. The skin around one of the eyes may present differences concerning the depth of wrinkles when compared to the other, probably due to daily habits, for instance having one side that is more exposed to solar radiation, sleeping positions, among others.

According to Clark & Scerri (2), if the GA peeling with 70% free acid remains in contact with the skin for 15 minutes, it has a similar effect to a medium peeling of trichloroacetic acid (ATA) 40%, enabling action on deeper wrinkles. These same authors explain that there is a risk of hyperpigmentation, as glycolic acid increases the dispersion of melanine granules through the epidermis.

The experiment was conducted in women with a skin phototype II to IV, according to the classification of Fitzpatrick. The methodology used was safe, as no cases of hypopigmentation or hyperpigmentation occurred during the study.

It has been suggested that skin could be pre-treated for 14 to 21 days before peeling, allowing for a more uniform penetration of the peeling agent, redu-

cing the risk of hyperpigmentation by using substances that decrease melanin production.<sup>5,6,25</sup> This procedure was not performed in order to analyze its effectiveness on skins not subjected to this pretreatment, since this practice is commonplace in medical offices. Thus, it can be verified that even in the absence of pretreatment, the results obtained were satisfactory, highlighting the relevance of the study.

## CONCLUSION

The magistral formulations used in the peelings with lactic acid or glycolic acid should be kept in the refrigerator and should not be stored for long periods. It is suggested that they be remade every 30 days.

Both the lactic and glycolic acid peelings were effective in reducing fine wrinkles on the external-lateral region of the eyes, after three applications ( $p \leq 0.05\%$ ). It was observed that peeling efficacy in the external-lateral region of one eye might be different compared with that in skin of the external-lateral region of the other eye, relative to the speed of skin improvement. □

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