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# EFFECTS OF MICRO CURRENT, COLLAGEN, AND DMAE ON THE CONNECTIVE TISSUE OF WISTAR RATS EVALUATED BY METHODS HISTOLOGICAL AND GRAVIMETRIC

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## ABSTRACT

**Introduction.** Skin aging is a degenerative process, involving intrinsic and extrinsic factors, being related to photoaging, oxidative stress and inflammatory response, a process that induces the degenerative effect in the collagen network. In order to minimize skin aging, the intake of collagen, micro **current** and DMAE is an alternative in the aesthetic environment, however, the mechanisms of action involved in these phenomena are not fully understood. **Objective.** To evaluate the relationship between CM actions and direct collagen administration such as oral supplementation and DMAE, in the effectiveness or efficiency in the rejuvenation of integrodermal tissues in rats that did not suffer tissue injury, from their observed effects on connective tissue. **Material and Method.** The sample consisted of 12 Wistar rats (*Rattus norvegicus*), subdivided into four groups, respectively, control group (CG) that will not receive application of micro current (CM); treatment-7 group (GT7), which will receive **an** MC applications for one week once a day; collagen treatment group (GTC<sub>o</sub>), in which hydrolyzed collagen will be administered orally for 1 week (1 per day); and dmae treatment group (GTD), in which intradermal microinjection of DMAE will be applied. After treatment, the skin tissues of the animals were analyzed by histological techniques and by gravimetric method. **Results:** The GTC<sub>o</sub> presented an average area in  $\mu\text{m}^2$  and **a** higher percentage when compared to the control by analysis of variance with  $p: 0.044$ . **Conclusion:** The use of oral collagen for seven days in animals was efficient after histological analysis.

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*Keywords: Micro current, elastic fibers, collagen and DMAE.*

## 1. INTRODUCTION

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Wrinkles are natural consequences of the aging process gradually arising due to looseness in the dermo epidermal junctions **and modern** advents focused on research on facial aging, **have** incited studies **and knowledge** about the functional clinical aspects resulting from this process [1].

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**Senescence** and/or cutaneous senility is described as a deteriorating and progressive factor of the collagen network, specifically affecting type I [2].

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As a result of the loss of anchorage and the addition **of** elastic fibers **to** the dermis, there is a decrease in the tension of the collagen fiber network. With this, there is an increase in the stiffness of the tissues, which constitutes obstacles to the processes of transport and diffusion of nutrients from the blood capillaries to the tissue interstice, causing degradation of the cell functions ES [3]. What results from this pathophysiological process is that the skin loses natural elasticity, causing significant changes in collagen and elastic fibers, since the decrease in the activity of fibroblast s, significantly reduces the synthesis and consequent production of collagens [4, 5].

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There are several therapeutic modalities in the area of dermato-functional physiotherapy specific for tissue repair, one of **their** applications of micro currents, a resource used in the treatment of skin aging in aesthetic clinics [6, 7]. **The micro current** seems to induce **the** ATP production phenomenon (adenosine triphosphate), responsible for protein synthesis and

tissue regeneration, since its involvement in all cellular energy processes is proven [8,9], and there is a possible relationship between an increase in the number of fibroblasts and alignment of collagen versus CM fibers, with maximum fibroblast responses observed in the regions close to the cathode stimulation electrode.

In tissue lesions, there are reports that excitation by electrical means promotes an increase in the concentration of growth factor receptors, which can potentiate processes in collagen production [10, 11, 12].

This study aims to investigate the effects of CM in a smaller session of 7 applications in intact dermal tissue in rats that did not suffer tissue damage and histologically quantify possible counts in collagen and elastic fibers as possible formations of neocollagens or greater amounts of elastic fibers and, also, to propose and test an alternative gravimetric method in place of histological techniques frequently used in the approaches of this theme. Therefore, its objective is to aim at a relationship between CM action and direct collagen administration such as oral supplementation and DMAE, in the effectiveness of age or efficiency in the rejuvenation of integrotomic tissues in rats that did not suffer tissue injury, from its observed effects on connective tissue.

## 2. MATERIAL AND METHODS

The sample consisted of 12 Wistar rats (*Rattus norvegicus*), originally from UnirG bioterium, randomly selected with the weight between 250 and 300 g, subdivided into four groups of 4 animals, respectively, control group (CG) that will not receive an application of micro current (MC); treatment-7 group (GT7), which will receive MC applications for one week once a day; collagen treatment group (GTCO), in which hydrolyzed collagen will be administered orally for 1 week (1 per day); and DMAE treatment group (GTD), in which intradermal microinjection of DMAE will be applied.

The animals were kept during the experimental protocols in the same environment of the bioterium, at an average temperature of 25 °C and controlled lighting with a photoperiod of 12 light hours/12 hours-dark and minimum noise, confined in cages lined with shavings and fed with commercial ration ad libitum, and the operational procedures were performed in the venous period. All rats were positioned in a polypropylene container to minimize handling and promote animal stress.

The animals CG and GT7 were anesthetized with sodium thiopental at a dose of 50 mg/kg intraperitoneally, and in the rats of the CG only simulations of the current applications were made with the device switched off. The rats of GT-Co (administered 0,5 mL of oral collagen daily for 7 days) and GTD (intradermal microinjection of 0,2% of DMAE (Dimethylaminoethanol) daily for 7 days) were not anesthetized.

In the MC group, the Ibramed ® (Brazil) type device was used, modulated to (parameters: 700 µA, 500 Hz and 30 minutes/day), in which two electrodes with 4 cm<sup>2</sup> of the area were coupled to the trichotomized region using electrolytic gel, maintaining a distance of 5 cm, in sliding movements on the surface.

At the end of the experimental protocols, all 12 animals were sacrificed by thiopental overdose, diaphragmatic perforation and injection of 0,1 mL of KCl 3 M into the heart, then skin samples with about 1 cm<sup>2</sup> of the trichotomized surfaces were removed for histological analysis.

The tissue samples were placed in identified vials containing 20 mL of 10 % buffered formaldehyde solution. After the fixation process, the second routine protocol for the preparation of histological slides was processed, using hematoxylin-eosin (HE) stains to verify differences in collagen tissue in relation to the control.

Photographs were taken with the zensys trinocular microscope, and 10 photographs of histological slides of each rat were obtained, obtaining 30 images of each group. Subsequently, the images were analyzed by the J Image program to detect the percentage of collagen in each image. An average of 30 images were made to obtain the percentage of collagen per group.

## 3. RESULTS

After the time of the experiment was completed, the sacrifice of the experiment was performed, and the tissue was removed, processed and coradofor histological analyses. A histological cut of each rat was obtained, and 10 photos of each blade were made per mouse with a 4x lens. As there were three rats per experiment, 30 photos were obtained per experiment.

The images were analyzed by the ImageJ software used for image processing and analysis, developed by Wayne Rasband at the National Institute of Mental Health, USA, in Java language. With this software you can view, edit, analyze,

80 process, save and print images. In ImageJ, the calculation of the areas is done by counting pixels of the regions selected  
 81 by the user or by a specific algorithm [13]. ImageJ acts on the image by the intensity, or gray level of the pixels [14].

82 In the ImageJ software, an adjustment was made to allow the quantification of collagen in the histological slides.  
 83 Measurements were made in  $\mu\text{m}^2$  and in percentage throughout the visual field of the image. The results are expressed  
 84 in table 1 and figure 1.

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88 **Table 1. Description of areas in  $\mu\text{m}^2$  and percentages of collagen in histological sections of connective tissue**  
 89 **of Winstar rats submitted to treatment with micro current, collagen and DMAE. Gurupi, Tocantins, Brazil 2022.**

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	<i>Control</i>		<i>Collagen</i>		<i>DMAE</i>		<i>Micro current</i>	
	<i>Area</i> <i>(<math>\mu\text{m}^2</math>)</i>	<i>%</i>	<i>Area</i> <i>(<math>\mu\text{m}^2</math>)</i>	<i>%</i>	<i>Area</i> <i>(<math>\mu\text{m}^2</math>)</i>	<i>%</i>	<i>Area</i> <i>(<math>\mu\text{m}^2</math>)</i>	<i>%</i>
<i>Image 1</i>	590,085	54,851	666,084	61,916	560,787	52,128	857,465	79,705
<i>Image 2</i>	364,067	33,842	316,455	29,416	390,876	36,334	438,955	40,803
<i>Image 3</i>	142,041	13,203	316,455	29,416	489,082	45,463	377,653	35,105
<i>Image 4</i>	309,181	28,740	548,410	50,977	340,158	31,619	473,007	43,968
<i>Image 5</i>	465,729	43,292	310,619	28,874	258,570	24,035	306,535	28,494
<i>Image 6</i>	234,634	21,810	303,537	28,215	439,562	40,859	506,436	47,076
<i>Image 7</i>	530,681	49,329	610,514	56,750	634,577	58,987	308,019	28,632
<i>Image 8</i>	372,894	34,662	654,300	60,820	120,973	11,245	229,659	21,348
<i>Image 9</i>	508,335	47,252	676,310	62,866	153,313	14,251	764,907	71,102
<i>Image 10</i>	286,492	26,631	330,551	30,726	128,716	11,965	343,597	31,939
<i>Image 1</i>	286,019	26,587	251,094	23,340	139,857	13,000	258,940	24,070
<i>Image 2</i>	476,142	44,260	455,458	42,337	363,149	33,756	653,715	60,766
<i>Image 3</i>	349,382	32,477	255,377	23,739	517,003	48,058	174,672	16,237
<i>Image 4</i>	335,151	31,154	627,965	58,372	758,865	70,540	174,672	16,237
<i>Image 5</i>	516,840	48,043	700,333	65,099	276,614	25,713	210,345	19,553
<i>Image 6</i>	645,405	59,993	706,777	65,698	275,490	25,608	162,003	15,059
<i>Image 7</i>	652,277	60,632	420,049	39,046	665,124	61,826	548,059	50,945
<i>Image 8</i>	236,181	21,954	258,359	24,016	291,649	27,110	525,446	48,843
<i>Image 9</i>	522,940	48,610	330,503	30,722	800,528	74,413	540,222	50,216
<i>Image 10</i>	599,100	55,689	248,837	23,131	622,652	57,878	671,964	62,462
<i>Image 1</i>	383,018	35,603	451,144	41,936	154,031	14,318	364,821	33,912
<i>Image 2</i>	509,539	47,364	390,352	36,285	314,655	29,249	271,387	25,227
<i>Image 3</i>	186,950	17,378	297,163	27,623	267,520	24,867	265,538	24,683
<i>Image 4</i>	589,892	54,833	212,007	19,707	316,989	29,466	188,744	17,545
<i>Image 5</i>	349,805	32,516	386,429	35,920	559,039	51,965	621,479	57,769
<i>Image 6</i>	218,691	20,328	550,538	51,175	405,768	37,718	155,869	14,489
<i>Image 7</i>	589,054	54,755	264,019	24,542	568,045	52,802	132,717	12,337
<i>Image 8</i>	149,173	13,866	249,260	23,170	561,544	52,198	538,047	50,014

Image 9	167,511	15,571	541,203	50,307	545,981	50,751	293,787	27,309
Image 10	153,924	14,308	142,317	13,229	327,503	30,443	251,471	23,375

91 %: Percentage

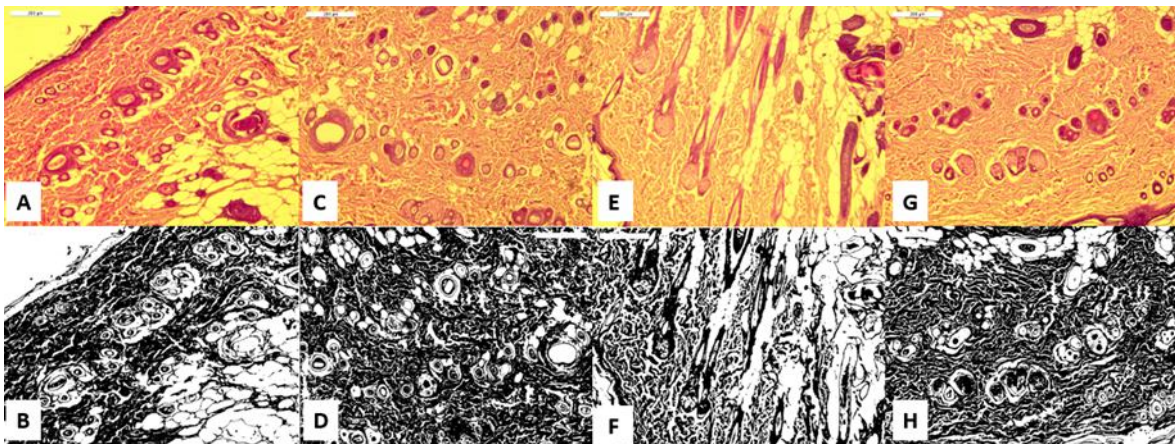
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93 **Table 2. Analysis of descriptive and statistical variables, by ANAVA test, of area measurements in  $\mu\text{m}^2$  and**  
94 **percentage of collagen in histological sections of connective tissue of Winstar rats submitted to treatment with**  
95 **micro current, collagen and DMAE. Gurupi, Tocantins, Brazil 2022.**  
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	Control		Collagen		DMAE		Micro current	
	Area ( $\mu\text{m}^2$ )	%	Area ( $\mu\text{m}^2$ )	%	Area ( $\mu\text{m}^2$ )	%	Area ( $\mu\text{m}^2$ )	%
<b>Average</b>	390,266	35,76	415,366	38,13	407,766	37,43	386,466	35,50
<b>Standard deviation</b>	163,044	15,12	169,783	15,71	190,954	17,66	197,249	18,26
<b>CI 95%</b>	144,5 a 285,5	24,57 a 46,96	252,64 a 323,85	26,80 a 49,31	236,96 a 236,96	25,90 a 48,82	152,40 a 274,32	23,77 a 46,82
<b>P</b>	-		0,0441		0,250		0,200	

97 IC: Confidence Interval; p: Significance level; %: Percentage

98 There was no difference between the DMAE and Micro Current group with the.

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101 **Fig. 1. Images of histological sections of connective tissue, cordoned with HE (A: Control, C: Collagen, E: DMAE**  
102 **and G: Micro Current) and treated by ImageJ (Images B: Control, D: Collagen, F: DMAE and H: Micro Current).**  
103 **Gurupi, Tocantins, Brazil 2022.**  
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#### 105 4. DISCUSSIONS

106 During the aging process the amount of acetylcholine, elastic fibers and collagen in the skin tissue gradually decreases.  
107 The most efficient way to obtain firmer skin and stronger muscles is by introducing and biostimulating rejuvenation  
108 precursors through aesthetic procedures [15].  
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110 However, the need to evaluate the effects of DMAE, Microcurrent and oral collagen intake under the cutaneous tissue  
111 through histological blades effectively certifies the amount of collagen after the treatments proposed in this research.  
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113 In [16] conducted a study to evaluate the effects of hydrolyzed collagen on skin photoaging in rats. The authors observed  
114 through histological analysis that in the treatment group there was the repair of elastic fibers, and collagenous fibers, in  
115 addition to maintaining the proportion of collagen type II.  
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118 A recent study conducted by [17] sought to evaluate through a cutometer the effects of hydrolyzed collagen intake for four  
119 weeks on skin elasticity in postmenopausal women. The results found showed positive statistical significance compared to

120 the placebo group. Although this study was conducted with humans, it was possible to observe through physical  
121 evaluation the effects of collagen on skin tissue.

122 Conducted a study [18] evaluating the effect of micro current on skin tissue repair in rats. Twenty-six animals were then  
123 used: 13 in a control group and 13 in intervention groups (manual passage of the current, lasting two minutes, for three  
124 weeks). In the histopathological analysis there was no significant effect in relation to fibroblast proliferation, corroborating  
125 the results of this study compared with the other techniques studied.

126 Another study evaluated the effects of micro current in Wistas rats in which 12 animals were submitted to daily treatment  
127 for 10 days. After histological analysis, the authors observed an increase in angiogenesis, collagensynthesis and number  
128 and quality of fibroblasts presenting synergistic action on skin tissue compared to the control group [19]. This contradicts  
129 the results of this study, since there was convergence with the proposed treatment days.

130 Studied [20] the effects of DMAE on skin aging. The intervention consisted of the topical use of DMAE for 30 days in  
131 women, resulting in improved hydration, firmness, elasticity and skin viscosity. Although, this study presents as an  
132 intervention the use of injectable DMAE in rats, it was possible to notice agreement in the biomechanical effects of the  
133 skin, since the collagen network positively induces these characteristics.

#### 134 **4. CONCLUSION**

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136 Currently, several resources are available for the prevention of skin aging and to induce collagen stimulation is to delay  
137 this event. In this study, the use of oral collagen for seven days in animals proved to be efficient after histological analysis,  
138 which is in contact with the bibliography presented. Therefore, this stimulus, associated with the other interventions of this  
139 study may be predictors for a more efficient result, since all interventions were effective in the collagen network. However,  
140 further studies with this methodology should be carried out in order to observe this effect on human subcutaneous tissue.

#### 141 **Ethical Approval :**

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144 This experimental study using laboratory animals, was approved by the Ethics Committee on the Use of Animals (CEUA)  
145 of Gurupi University - UnirG under opinion number 004/2021.

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