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RESEARCH ARTICLE

THE EFFECT OF INTERMITTENT NEGATIVE PRESSURE ON COLLAGEN CONCENTRATION IN MICE SKIN

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ABSTRACT: **Overview:** Physical forces are known to affect biological structure and modulate the extracellular matrix. Collagen is the most abundant protein outside the cell, and it is clearly highlighted through Masson trichrome and van Geison's stain. This research aimed to calculate the specific concentration of collagen within histology slides, after being subjected to sessions of intermittent negative pressure (INP), using Adobe® Photoshop® software and mice models. **Objectives:** To measure the collagen concentration in histology slides through Adobe® Photoshop®, using two types of stains: Masson trichrome, and van Gieson's stain. **Methods:** Sixteen mice were put into sessions of INP ten minutes daily, for three weeks. Skin tissues were extracted from test and control sides. Stained slides were photographed and inserted in the Adobe® Photoshop® program to measure collagen concentration according to the specified calculated areas within the slides. **Results:** Masson's trichrome slides gave a mean Collagen concentration of (58.4%) in the test slides, compared to the control which was (39.9%). In van Gieson stained slides, the collagen gave a mean concentration of (54.93%) in test slides and (41.32%) in the control. The difference was statistically insignificant in both staining. **Conclusion:** Applying INP to mice skin for three weeks did not significantly change the concentration of the collagen in the extracellular matrix.

KEYWORDS: Collagen, Negative pressure, Masson's trichrome, van Gieson stain.

INTRODUCTION:

Collagen is a protein with different subtypes and high abundance in Human and other animals [1,2]. Exactly 28 protein subtypes currently hold the name collagens [2,3,4]. Outside the cell, Collagens are mainly seen as long fibers of ordered or semi-ordered structures, and could vary in length [1]. It gives the extracellular matrix its strength and coherence [3]. Collagen fibers give the tissues its resilient ability, and mechanical forces can affect collagen alignment and expression. Uni-directional stretch in rat mesenchyme promoted the synthesis of collagens I and III which

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commonly found in connective tissue[5,6]. The study results were supported with other similar findings in bladder tissues[7].

Negative pressure is been used in medicine since the invention of traditional cupping therapy. Recently it had been found that applying intermittent negative pressure (INP) enhanced collagen expression within the extra cellular structure. Aiming to assess the potentials of INP in directing the proliferation of mesenchymal stem cells (MSCs), Zhang et al (2010) concluded that collagen-I levels rose, among other findings[8]. The results were confirmed in 2014, in Yang et al reports about apoptosis in MSCs[9].

This current study was performed in mice skin to measure collagen concentration changes due to the INP effect, by the use of Adobe® Photoshop® software as a measurement tool. The concept is this software can outline certain area of the slide according to its color and give a precise percent of the area out of the total slide photographed[10]. Similar study was performed in 2012 by Calvi et al. using four different histology stains and two software's to quantify and assess the structure of collagen in human abdominal wall muscles. Corel Photopaint and UTHSCSA are the two-software used, and they both proved to have high efficacy [11]. The two above mentioned programs are not as commonly found as the Adobe, which is readily downloadable from the internet, and also not as easy to operate.

MATRIALS AND METHOD:

I. Mice preparation and INP application:

This was a case-control experimental study performed in the Histology department at the Sir Lee Stack's referral laboratories, and the faculty of medical laboratories in the East Nile College, Khartoum, Sudan. The practical procedure took seven weeks In the first week the mice were chosen and brought from the center of veterinary research in Soba city, Sudan, to the DANIDA Laboratories in the faculty of medicine University of Khartoum, and in the following three weeks INP sessions were performed, then there was a week of rest period to allow possible trauma-induced changes or inflammatory changes to subside, and in the last two of the seven weeks the samples were extracted, processed, stained, visualized and photographed by microscopes.

Sixteen albino mice, with ages range from six to nine weeks were chosen, from which, ten were used to extract the Masson slides, and six to extract the van Gieson slides. Thirty two histology slides were prepared from the test side, and the same number from a control side. Extra tissue samples were preserved in 10% formaldehyde solution. Mice were put through sessions of Intermittent Negative Pressure (INP), on selected areas in their abdominal wall skin, using a manual vacuum suction. INP was delivered in intervals alternating between suction pressures and resting periods, usually each took between five and eight seconds. The cycle extended to ten minutes for each mouse, and repeated two times daily for three weeks. Skin tissues were excised and preserved in 10% Formaldehyde.

II. Slides preparation:

The skins were processed and fixed in Paraffin blocks, then were cut by Leica® microtome. Slices were de-paraffinised and then fixed in microscopic slides with addition of the specific stain. The histological stains used in this experiment are Masson's Trichrome, and van Gieson Stain; both are excellent for collagen demonstration. The slides were photographed through a microscope by a histopahology specialist, who commented on the findings.

III. Collagen concentration measurement:

Further analysis of slides was performed using Adobe Photoshop program; the colors were separated to measure the concentration of the desired element as follows[10]: Right clicking the area of interest and shadowing through an Adope® Photoshop® CS version 8.0 tool, which is called the magic wand, the area of similar adjacent color will be automatically selected.

Other options for highly irregular spots are available in new versions of Adobe Photoshop. On the right of the screen, the values of width and height of the selected part of the image are recorded. By multiplying W×H, we can obtain the shadowed part of the image. The whole selected areas are then divided by 100 to obtain the percent of the color, hence Collagen, within the slide. Arithmetic mean of the test and controls will be calculated for comparison. Figure (1) demonstrates the steps of calculation of the color concentration by the Adope® Photoshop®. Percent summation was performed by two calculators simultaneously, and Statistics analysis was done by Microsoft® 2007 Excel, and Adope® Photoshop CS version 8.0.

All practical procedures on mice were performed under the regulations of the (2002) Institutional Animal Care and Use Committees (IACUCs)[12], and animal use protocol (AUP).

RESULTS:

I. **Massons trichrome stain:** The collagen concentration measured was a mean of 58.4%, compared to the control which was 39.9% of the total slide. The 18.42% difference in collagen concentrations between the test and control slides was statistically insignificant; revise table (1), and figure (2).

II. **Van Gieson's stain:** The collagen gave a mean concentration of 54.93% in the test tissues and 41.32% in the control. The difference between the two means was 13.61%, and it was statistically insignificant. Table (2) shows the important statistics values in relation to Van Gieson stain. Figure (3) shows diagrammatic comparison between the test and the control on selected slides.

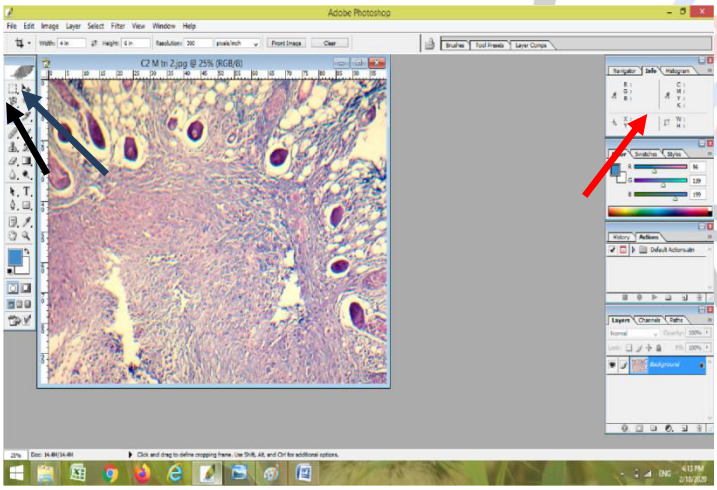

Figure 1: Description of the steps of measuring color concentration; after the file is chosen, the magic wand tool (blue arrow), or the magnetic lasso tool (black arrow), is used to highlight the area desired to be measured. The width- W, and height- H percent will appear on the right side of the screen (red arrow). The value obtained by multiplication of the two numbers will represent the percent of pixels from the total area of the slide.

Fig 2: Masson trichrome ascending Distribution; Red line refers to the test slides distribution, blue line refers to the control distribution.

Table 1: The statistic results for Masson trichrome stain.

Masson trichrome gategory	Mean	Median	Standard deviation	95% Confidence interval	P value of the difference
Control	39.98 %	41.41 %	21.47	-2.96% - 82.92%	0.9976
Test	58.40 %	59.77 %	19.00	20.40% – 96.40%	

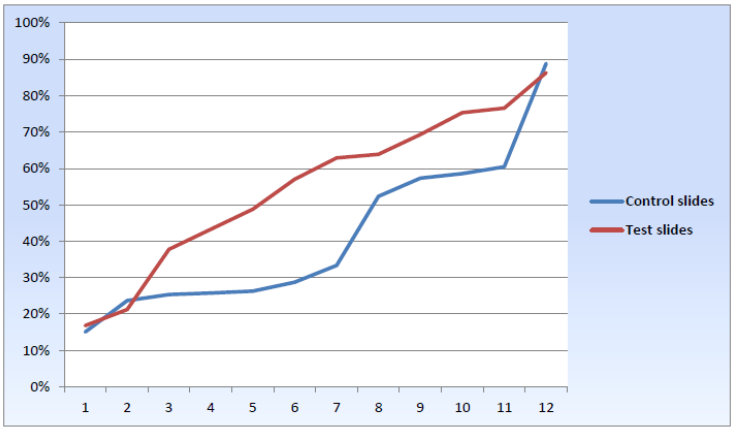

Fig 3: van Geison stain ascending distribution; red line refers to the test slides distribution, blue line refers to the control distribution.

Table 2: The statistic results for Van Gieson's stain.

van Gieson category	Mean	Median	Standard deviation	95% Confidence interval	P value of the difference
Control	41.32%	31.04%	21.83	-2.34% - 84.98%	0.9291
Test	54.93%	60.44%	21.90	11.13% - 98.73%	

DISCUSSION:

This study aimed to measure collagen difference in histology slides of mice skin after exposure to sessions of intermittent negative pressure. The Collagen concentration increased in the test slides by a difference of 18.42% for Masson's trichrome, and 13.61%, for the van Gieson stain. Both differences were statistically insignificant, but the fact that the raise in collagen level was on test slide for both stains, as seen from the diagrams in figures (2) and (3), is suggestive of the potential effect of the negative pressure on collagen concentration. When INP was applied to the skin of albino mice for 14 days, it caused decrease in the number of sebaceous glands, accumulation of fluid, and increased local concentration of leucocytes, and collagen appeared thicker and well organized in the test slides, but these observations are dependent on the pathologist experience [13].

This negative result may be attributed to the ineffectiveness of the method, the short period of the experiment, or the low magnitude of negative pressure delivered; a pressure level that could be re-tested in humans without inflicting pain or harm. The mice might have particular resistance to localized negative pressure. Previous experiments on the plants-Wheat, Lettuce, and Radish, had shown their capabilities to withstand a short period of decompression [14]. After restoring the normal pressure and gas concentration, these plants resumed growth without an apparent damage [14]. Tardigrades also have remarkable tolerance to the low-pressure state. These eight-legged micro-invertebrates had shown their resistance to the low-pressure environment in experiments [15,16]. Adobe® photoshop® usage in the measurement of molecule concentration is supported by a single study [10], and the overall literature concerning software use in histology is scarce. The advances in Immunohistochemistry made the above-mentioned method obsolete and non-beneficial, but it could be handy when a specific concentration is needed. The 2010 Zhang and

2014 yang study both used the molecular technique to measure the expression of collagen, but they didn't measure the specific concentration, in addition, they used prolonged intervals of negative pressure in cell cultures[9,10].

For further researches in this area, it is recommended to re-assess the methodology of software-operated color analysis of histological slides, and estimate its accuracy. Once the concept of Software dependent slide analysis is accredited, the computer analytical software should be enhanced to give more sensitive and specific results. Better research preparations are needed, particularly the negative pressure administration and measurement tools.

CONCLUSION:

This was an experimental case-control study performed in albino mice to determine the effect of applying intermittent negative pressure in their skin collagen concentration. The practical procedure took three weeks. Collagen concentration was measured by the Adobe® Photoshop® program. The end result was: the concentration of collagen slightly rose by 18.42% in Massons trichrome slides, and by 13.61% in the van Geison stain slides. The increase in concentration, however, was not of statistic significance.

REFERENCES:

[1]. Richard-Blum S. The collagen family. Cold Spring Harb. Perspect Biol. Jan. 2011; 3(1): a004978.
a. DOI: 10.1101/cshperspect.a004978.
[2]. Dutov P, Antipova O, Varma S, Orgel J, Schieber JD. Measurement of Elastic Modulus of Collagen Type I Single Fiber. PLoS One. 2016: 11(1): e0145711. DOI: 10.1371/journal.pone.0145711.
[3]. Kadler KE, Baldock C, Bella J, Boot-Handford RP. Collagens at a glance. J Cell Sci. 2007;120(12):1955-1962.
[4]. Mienaltowski MJ, Birk DE. Structure, physiology, and biochemistry of collagens.Adv Exp Med Biol. 2014;(802):5-29.
[5]. Zhang L, Kahn CJ, Chen HQ, Tran N, Wang X. Effect of uniaxial stretching on rat bone mesenchymal stem cell: orientation and expressions of collagen types I and III and tenascin-C. Cell Biol Int. 2008;32(3):344-52.
[6]. Chiquet-Ehrismann R. Tenascins. Int J Biochem Cell Biol. 2004; 36(6): 986-90.
[7]. Chen S, Peng C, Wei X, Luo D, Lin Y, Yang T, Jin X, Gong L, Li H, Wang K.. Simulated physiological stretch increases expression of extracellular matrix proteins in human bladder smooth muscle cells via integrin α4/αv-FAK-ERK1/2 signaling pathway. World J Urol. 2017; 35(8):1247-1254.
[8]. Zhang YG, Yang Z, Zhang H, Wang C, Liu M, Guo X, Xu P. Effect of negative pressure on human bone marrow mesenchymal stem cells in vitro. Connect Tissue Res. 2010;51(1): 14-21. DOI: 10.3109/03008200902855891.
[9]. Yang Z, Yao JF, Xu P, Zhang JB, Zhang YM, Zhu YJ, Qin SQ, Liu L, Liu H, Hou WK, Xu K.Functions and mechanisms of intermittent negative pressure for osteogenesis in human bone marrow mesenchymal stem cells. Mol Med Rep. 2014;9(4):1331-6. DOI: 10.3892/mmr.2014. 1968.
[10]. Abbadi OS. Measuring Collagen Concentration in Histology Slides of Mice Skin Using the Adobe® Photoshop® Software. CPQ Medicine. 2019; 6(5): 01-08.
[11]. Calvi EN, Nahas FX, Barbosa MV, Calil JA, Ihara SS, Silva Mde S, Franco MF, Ferreira LM. An experimental model for the study of collagen fibers in skeletal muscle. Acta Cir Bras. 2012;27(10):681-6.
[12]. "Office of Laboratory animal welfare. Institutional Animal Care and Use Committee Guidebook," 2nd ed. Bethesda (MD): National Institutes of Health; c 2002.

[13]. Abbadi OS, Idris DS, Nafie KM. The histological changes attributed to intermittent negative pressure on mice skin. Chinese J Med Res. 2019; 2(4): 66-69.

[14]. Wheeler RM, Wehkamp CA, Stasiak MA, Dixon MA, Rygalov VY. Plants survive rapid decompression: Implications for bioregenerative life support. AdSpR. 2011;47(9):1600-1607. DOI:10.1016/j.asr.2010.12.017

[15]. Jönsson KI, Rabbow E, Schill RO, Harms-Ringdahl M, Rettberg P. Tardigrades survive exposure to space in low Earth orbit. Curr Biol. 2008;18(17):R729-R731.

[16]. Jönsson KI. Tardigrades as a potential model organism in space research. Astrobiology. 2007;7(5):757-66. DOI: 10.1089/ast.2006.0088.

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