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Ultrastructural Variations in Collagen Synthesis Generated by Infrared Laser in Rat Fibroblasts

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ABSTRACT

Introduction: studies on the effect of gallium arsenide infrared laser emissions (IRL) on cell activity indicate this stimulus has a wide range of reactions, manifested by protein synthesis activation, synthesis and activation of numerous enzymes and mitochondrial ATP synthesis, among others. The effects of IRL on tissues are determined by three factors: wavelength radiation; the amount of irradiated energy, and irradiation time. All these factors are manifested in the potential carrying out of biological processes in which presence of morphological or ultrastructural modifications can be observed in the cell.

Previously reported data make it possible to assume that the possible proliferation and development of some of the cell components are the expression of a specific IRL-activated cell function.

To reveal possible changes brought about during the fibrillogenesis process in dense connective tissue, we studied the effect of IRL on the temporomandibular joint of adult Sprague Dawley rats.

The results suggest that after irradiation, the fibroblasts of the temporomandibular joint of adult Sprague Dawley rats undergo modification in the processes of collagen fibril synthesis, causing their diameters to vary among fibrils produced by young cells up to senescent cells fibrils.

Keywords: Fibrillogenesis; Ultrastructure; Infrared Laser.

Introduction

Studies into the effect of gallium arsenide (GaAs) infrared laser (IRL) emissions on cell activity indicate that these stimuli produce a wide range of reactions manifested in protein synthesis activation (Omi *et al*., 2005), the synthesis and activation of numerous enzymes (Geinits *et al*., 2006), and mitochondrial ATP synthesis (Lavi *et al*., 2003), among others.

These changes at cell level translate at tissue level into increased healing and repair speeds, accelerated neovascularization, increased formation of granulation tissue, and a greater number of fibroblasts and collagen fibers (Kitchen and Partridge; 1991; Vecoso,1993).

One explanation for these results is that the initial layers of the target tissue absorb the radiation from the laser, generating a direct action, with local photothermal, photochemical and photoelectric effects, which in turn cause an indirect regional or systemic action (stimulus of microcirculation and increase in trophism) (Martínez and Portero, 1998).

The effects of IRL on tissues are determined by the wavelength of the radiation, the amount of irradiated energy, and the time of irradiation, while its potential expression on biological processes can be observed by the presence of morphological changes (Mester *et al*., 1985; Kulekcioglu *et al*., 2013). Evidence on the matter come from studies conducted by Osawa *et al*. (1998), who demonstrated the formation of nodular bone, increase in alkaline phosphatase activity and osteocalcin gene expression *in vitro* by stimulating cell cultures from *Sprague Dawley* rat skull bone at different stages of development with IRL.

Other reports are provided by Cornejo *et al*., 2009; and Matamala *et al*., 2001, who, working with the ischial nerve in rabbits (*Oryctolagus cuniculus*), demonstrated the existence of a stimulating effect on the fibroblasts, which resulted in an increase in collagen production, and thus an increase in the thickness of the epineurium together with a larger amount of fatty tissue in the epineurium. Additionally, Matamala *et al*. (2009) report macro- and microscopic variations in the temporomandibular joint (TMJ) of rabbit which include variations in the arrangement of the collagen, and alterations in the ordering of the cell layers in both the joint disk and on the surface of the mandibular condyle.

Based on previously reported findings, it may be assumed that the subcellular effects of IRL stimulations generate qualitative, quantitative and/or morphological changes in the cell components, which can be seen by quantitative image analysis.

The TMJ is defined as a bicondylar joint located between the mandibular fossa and the articular eminence or tubercle of the temporal bone and condyle of the mandible, which is stabilized by a joint disk. All these structures are surrounded by a dense fibrous and avascular tissue likely due to the role they fulfill by supporting strong forces and compression during the

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movements of the joint (Griffin and Sharpe, 1960), with collagen being the main executor molecule of these activities (Siadat *et al*, 2021). Collagen fibrils, 20 nm in diameter and larger, are the basic component of the hierarchical structural complex of collagen tissues.

To provide evidence of the possible morphological and morphometric changes that can occur at ultrastructural level during the fibrillogenesis process in the dense connective tissue, we studied the effect of GaAs IRL on the temporomandibular joint (TMJ) of adult *Sprague Dawley* rats by measuring the diameter of the fibrils present in the cells of this joint.

The ability to conduct quantitative studies on the dynamics of the fibrils with optical microscopy is quite limited, which is why normally transmission electron microscopy (TEM) has been used to measure the diameter of the fibrils (Birk and Trelstad, 1986; Flint *et al*., 1984; Hama *et al*., 1976; Starborg *et al*., 2013).

Materials and Methods

Fifteen adult *Sprague Dawley* rats were used, all bred and maintained in the vivarium at the Universidad de La Frontera in similar environmental and feeding conditions. Ten of these rats were irradiated on the skin in the TMJ region with GaAs IRL (904 nm, 10 mW, for 10 minutes for 15 consecutive days), with a dose of 8 J/cm^2. Five rats were kept as controls to compare the changes in the macroscopic anatomy of the joint.

Another five of the experimental rats were euthanized the day after the final irradiation (day 16), whereas the five remaining were kept for a period of 30 days post-irradiation and then euthanized (residual effect of the irradiation). The control rats were also euthanized on day 16 of the experiment.

To study the possible ultrastructural changes in the connective tissue, the animals were euthanized by CO^2 saturation and the TMJ surgically dissected with the help of a stereoscopic microscope. Then, the joint disk was separated from the joint complex to then extract small pieces of connective tissue that were fixed in a solution of glutaraldehyde 2% in phosphate buffer 0.15 M, pH 7.2 and at room temperature for 2 hours. Later, the samples were washed in a solution of 6 g NaCl and 73 g saccharose, dissolved in 1 liter of distilled water. Post-fixation was done with osmium tetroxide 1% dissolved in the previously described buffer solution for one hour at room temperature.

Next, the samples were washed and immediately dehydrated in increasing acetone concentrations (30 to 100%) to ultimately be embedded in Araldite 6005. Ultra-thin sections approximately 70 nm thick were obtained in an ultramicrotome (MT1, Sorval, , Dupont Instrument), which were stained with uranyl acetate 2% for 40 minutes and lead citrate 0.5% for 10 minutes. Finally, the samples were studied and microphotographed using a Phillips EM 300 electron microscope.

From the photos obtained at magnifications of 11,300X and 17,000X, an image analysis was performed using the SigmaScan-Pro SigmaScan-Pro5 software (SPSS).

For greater precision in the estimation of the average diameter of the fibrils, in the image analysis only those fibrils that appeared cut cross-sectionally or longitudinally were measured, whereas fibrils that appear diagonally were not included for not corresponding to the real diameter.

To determine if there are statistically relevant differences between the group control and the irradiated and residual groups, a Kruskal-Wallis H test was performed.

Results

The control tissue exhibited fibroblasts with vesicular, spherical and central nuclei in which an abundant amount of euchromatin in addition to some areas with a small amount of heterochromatin were observed. In the cytoplasm next to the nucleus, there were some very distended cisternae of the rough endoplasmic reticulum that exhibit a central electro-lucid content enclosed by a membrane surrounded by electrodense granules.

The pericellular matrix of the fibroblasts shows very fine collagen fibrils forming a network that binds collagen fibers that exhibit different diameters (Fig.1).

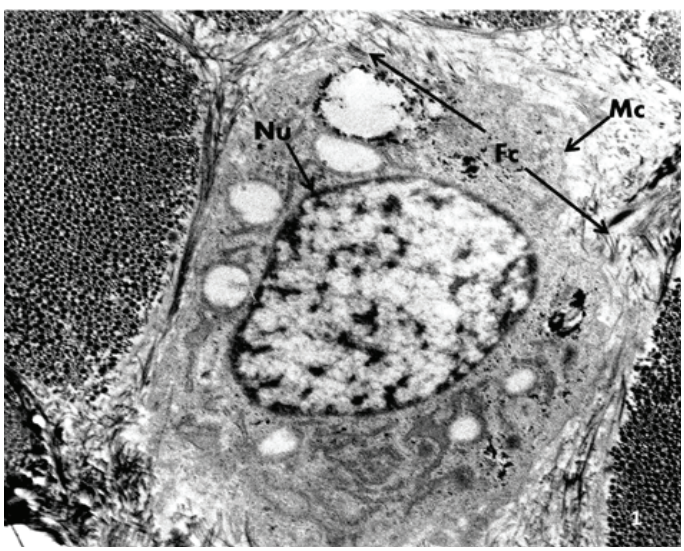

Figure 1. Microphotograph of a control fibroblast from the rat temporomandibular joint obtained by transmission electron microscopy. 11,300X. Mc. cellular membrane; Nu: nucleus; FC. Collagen fibers.

The irradiated tissue showed fibroblasts with elongated, vesicular nuclei with abundant euchromatin and little heterochromatin. Unlike what was observed in the normal nuclei, the perinuclear cytoplasm showed little presence of cisternae of the rough endoplasmic reticulum in whereas in the cell cytoplasm little fibrillogenetic activity was observed. The pericellular matrix is lacking collagenous fibrils, whereas the

intercellular matrix exhibits collagenous fibers with fibrils of uniform diameter (Fig.2).

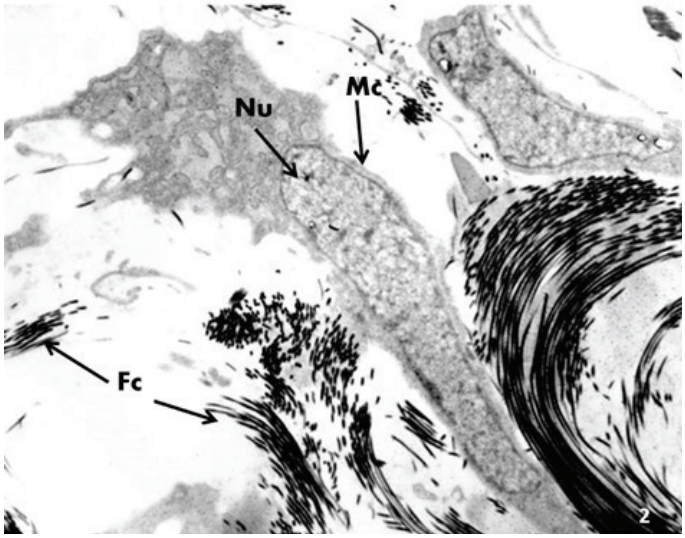

Figure 2. Microphotograph of an irradiated fibroblast from the rat temporomandibular joint by transmission electron microscopy. 17,000X. Mc. Cellular membrane; Nu: nucleus; Fc. Collagen fibers.

The tissue kept to evaluate the residual effect of the treatment presented fibroblasts with an irregularly shaped nucleus and abundant heterochromatin (Fig.3). The cell cytoplasm presented a small number of vesicles of the rough endoplasmic reticulum, which is consistent with the scarce fibrillogenetic activity observed. The plasma membrane also showed a series of folds with jagged edges and in some cases the fibrillogenesis process was noted due to the presence of collagen fibrils.

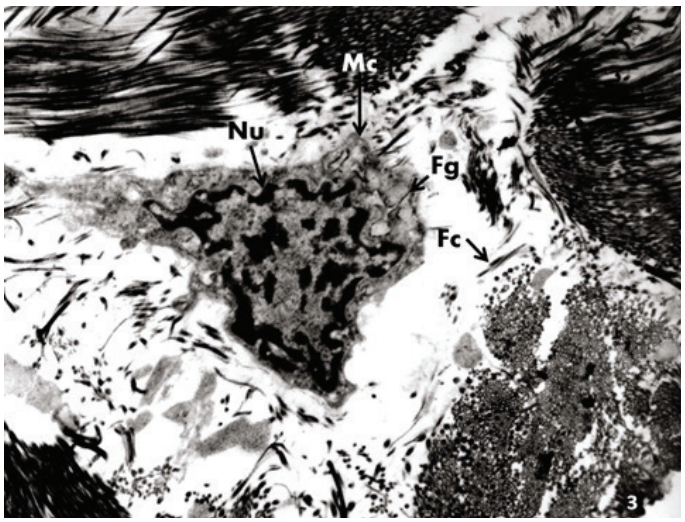

Figure 3. Microphotograph of a residual fibroblast from the rat temporomandibular joint by transmission electron microscopy. 17,000X. Mc. Cellular membrane; Nu: nucleus; Fc. collagen fibers.

The image analysis made it possible to estimate the diameter of the collagenous fibers. In the case of the control tissue, it varied between 10 and 70 nm in diameter with a predominance of fibers of 40 nm (Table 1, Fig 4). In the case of the irradiated cells, the fiber diameter reduced in range to values between 20 and 40 nm with a predominance of fibers 30 nm in diameter (Table 1). The residual cell samples showed that the diameter of the collagenous fibers varied between 10 nm and 70 nm; however, the most abundant fibers were smaller (10 nm) (Table 1).

Table 1. Summary table showing the diameter of the collagen fibers and their frequency according to the treatment analyzed

Fiber Quantification			
Diameter (nm)	**Control**	**Irradiated**	**Residual**
10	8	0	168
20	60	11	100
30	122	144	45
40	151	18	36
50	80	0	45
60	23	0	13
70	1	0	3
80	0	0	0

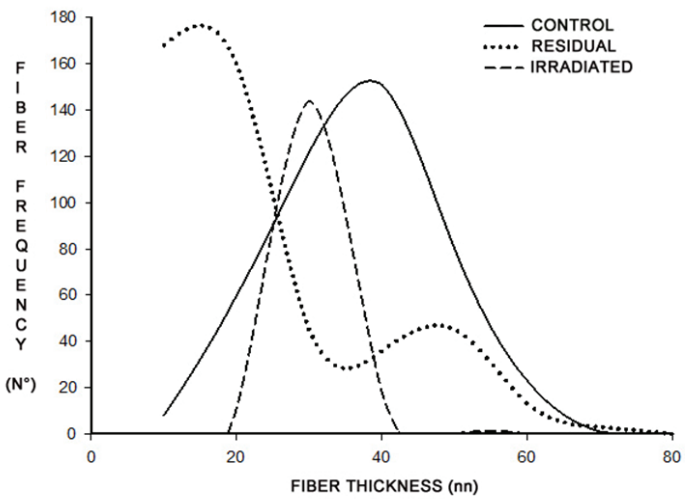

Figure 4. Graph showing the sample frequency versus thickness of collagen fibers according to the treatment analyzed.

The result of the Kruskal-Wallis H test indicates that the H value is 22.9981 (2, N = 900) with the p value being 00001 and statistically significant at a value of $p < .05$. Consequently, the results suggest there are statistically significant differences between the control group and the irradiated and residual groups.

Discussion

Information reported in the literature indicates that the effect of IRL-induced stimulations causes a wide range of reactions; the data contributed by Zhang *et al.* (2003) in experiments with human fibroblasts showed that inductions can activate more than a hundred genes, many of which are associated with functions related to cell proliferation processes. Other data were

contributed by Omi *et al.* (2005), who reported that irradiations produce a noticeable reaction in protein synthesis as well as in collagen synthesis-secretion (Abergel *et al*., 1987).

Data have been compiled and analyzed related to the synthesis and distribution of collagen fibril diameters in different connective tissues, and it has been established that the distribution of the diameter of collagen fibrils at birth and in the early stages of development is unimodal and does not exceed 20 nm, whereas in the case of mature or differentiated tissues, the mean diameter of the collagen fibrils is generally greater than at birth and the size distribution of the fibrils can be unimodal or bimodal depending on the tissue type and can vary between 10 and 300 nm in diameter. With respect to senescence, few data are available, but in most cases the diameter of the collagen fibrils is smaller than those at maturity and the distributions of the fibrils are mainly bimodal (Parry *et al*., 1978; Ross and Pawlina, 2011).

The results reported here, both from the ultrastructural perspective as well as those obtained from the image analysis of cross-sectional sections of rat temporomandibular joint, show that normal fibroblasts synthesize collagenous fibers that vary between 10 nm and 70 nm in diameter, with a maximum frequency around 40 nm, thus acquiring a unimodal distribution (Parry *et al*., 1978). This suggests that this joint is in a stage of tissue maturity since the diameters clearly exceed the 20 nm described for embryonic tissues (Parry *et al*., 1978; Ross and Pawlina, 2011).

In the case of the irradiated fibroblasts, the variation range of the diameter of collagenous fibers was considerably reduced, between 20 and 40 nm, with the greatest frequency of fibrils being focused around 30 nm in diameter. In this case, the unimodal distribution of the previously described fibrils is repeated, although the greatest frequency clearly shows a predominance of 30 nm filaments, suggesting that the laser stimulus acts mainly by inducing the production of fibers with a diameter characteristic of younger cells in agreement with what was reported by (Parry *et al*., 1978; Ross and Pawlina, 2011).

In the case of the sections from rats in the residual group, the presence of collagenous fibers was also observed with a wide distribution range. In this case, the diameters fluctuated between 10 nm and 70 nm in diameter; nevertheless, the greatest fiber frequency was around 10 nm with a second increase, although smaller, in the fiber frequency of 50 nm of diameter. This may suggest that this animal group exhibits a bimodal distribution in the diameter of the fibers, which may possibly correspond to an activity characteristic of senescent cells (Parry *et al*., 1978).

It has generally been established that low-power gallium arsenide infrared laser stimulation is photochemical, stimulating normal cell functions (Ebensperger *et al*., 2008). Ultrastructurally, this is reflected in the appearance of abundant rough endoplasmic reticulum vesicles in the cell cytoplasm, which is an indicator of the formation of collagen fibrils (among other products), since the procollagen chains form in these vesicles, whereas their processing occurs in the cisternae (Ross and Pawlina, 2011).

The results presented here suggest that after the irradiation process on the fibroblastic cells of the temporomandibular joint of adult Sprague Dawley rats, a change takes place in the collagen fibril synthesis processes, causing them to vary in their diameters among fibrils which are normally produced by young cells up to fibrils characteristic of senescent cells.

References

1. Abergel, R. P, Lyons R F, Castel J C , Dwyer R, Uitt Biostimulation of wound healing by lasers: experimental approaches in animal models and in fibroblast cultures. J. Dermatol. Surg. Oncol. 1987.
2. Birk DE, Trelstad RL Extracellular compartments in tendon morphogenesis: collagen fibril, bundle, and macroaggregate formation. J Cell Biol.1986
3. Cornejo R.; Matamala F; Silva H, Garrido O. Morphometric and ultrastructural studies of effect infarred laser on rabbit temporo-mandibular joint fibroblasts. Bras. Arc of Biol and Tech. 2009.
4. Ebensperger A, Vargas R, Romero S, Romero M, Matamala F Efecto microscópico y macroscópico del láser infrarrojo sobre ligamentos de la rodilla en conejos: ¿extrapolable a humanos? Rev Estud. Med Sur. 2008
5. Flint MH, Craig AS, Reilly HC, Gillard GC, Parry DA, Collagen fibril diameters and glycosaminoglycan content of skins--indices of tissue maturity and function. Connect Tissue Res. 1984
6. Geinits A., Moskvin S, Asisov G. Intravenous laser blood irradiation. Moscow, Triada Publishing House. 2006.
7. Griffin C, Sharpe ,The structure of the adult temporomandibular meniscus. Aust. Dent J. 1960.
8. Kitchen SS, Partridge CJ, A review of low level laser therapy, part I: background, physiological effects and hazards. Physiotherapy. 1991
9. Kulekcioglu S, Sivrioglu K, Ozcan O, Parla R Effectiveness of low-level laser therapy in temporomandibular disorder. Scand. J. Rheumatol. 2013.
10. Lavi R, Shainberg A, Friedman H, Shneyvays, Rickove O, Eichler M, Kaplan D Lubart R Low energy visible light induces reactive oxygen species generation and stimulates an increase of intracellular calcium concentration in cardiac cells. J. Biol. Chem. 2003.
11. Matamala F, Veuthey C, Molina B Efecto del láser de uso clínico sobre el perineuro del nervio isquiático de conejo. Arch Med Vet. 2001
12. Matamala F, Cornejo R , Vasconcellos C, Silva Efecto del láser AsGa sobre la Anatomía Normal de la Articulación Témporo Mandibular (ATM) de conejos. Rev. Científica FCV-LUZ. 2009.
13. Martínez M, Portero S, Láser. en: Martínez, M, Pastor J.M. (Eds.), Manual de Medicina Física. Editorial Harcourt, 1998.
14. Mester E, Mester A, The biomedical effects of laser application. Lasers Surg. Med. 1985.
15. Omi T, Kawana S, Sato S, Takezaki S, Honda M, Igarashi T, Hankins R. W, Bjerring P,. Thestrup-Pedersen K, Cutaneous immunological activation elicited by a low fluence pulsed dye

laser. Br. J. Dermatol. 2005.
16. Ozawa Y, Shimizu N, Kariya G, Abiko Y, Low-energy laser irradiation stimulates bone nodule formation at early stages of cell culture in rat calvaria cells. Bone Apr. 1998.
17. Parry DA; Barnes D, Craig AS, A comparison of the size distribution of collagen fibrils in tissues as a function of age and a possible relation between fibril size distribution and mechanical properties. Proc. R. Soc. Lond. B. 1978
18. Ross M.H, Pawlina W, Histología, Texto y Atlas color con Biología Celular y Molecular. 6 edición. Editorial Panamericana, 2011.
19. Siadat SM, Silverman AA, DiMarzio CA, Ruberti J W. Measuring Collagen Fibril Diameter with Differential Interference Contrast Microscopy. J. Struct. Biol. 2021.
20. Starborg T, Kalson NS, Lu Y, Mironov A, Cootes TF, Holmes DF, Kadler KE, Using transmission electron microscopy and 3View to determine collagen fibril size and three-dimensional organization. Nat Protoc. 2013
21. Vecoso Me.. Láser em Fisioterapia. 18 ed. Sao Paulo, Ed. Lovise Científica. 258 p.1993
22. Zhan, Y, Song, Fong C C, Tsan C H, Yang Z., Yang T, cDNA microarray analysis of gene expression profiles in human fibroblast cells irradiated with red light. J. Invest. Dermatol, 2003.

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