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Original article

Enhancing reparative processes of cartilage defects using He-Ne laser irradiation

*Ihsan, F. Rostum , *Fadhil , A.Hamed & **Nuha, S.
*College of Dentistry – Al- Muthanna University, **Minstry of Health

Abstract

So much remedies and medications were tried to induce healing of the cartilages and increase the rate of their recovery, from that were; hormones, herbals, electromagnetic radiations and also cold lasers. To assess the ability of Helium - Neon (He –Ne) laser on the reparative processes of cartilage defects. The experiment included of using 24 rabbits, 12 of them allocated as a group of standardization while the remaining were considered as a group of treatment with laser radiation. All the animals underwent a surgical operation included making a three sided incision in the skin of the inner side of the auricle, the skin is then folded temporally so that the cartilage below exposed, then a four equal sided incision was made in the cartilage and pealed out the skin incision to appear as a square shaped. The difference between the two groups that the animals of the group which was allocated to be irradiated with the He – Ne laser beam with 5 mW out put postoperatively and continued for seven days. To compile specimens from the site of the operation, the animals of both groups were divided in to 4 subgroups with 3 rabbits each, one subgroup from the both group was specified for collection of specimens at the weeks 1, 2, 4 & 6 post postoperative and sent to the laboratory to be examined histopathologicaaly. Significantly well-developed cartilage growth with the both types of cartilaginous cells (mature - chondroblasts and immature chondrocytes) fill the operation site , there was also a raise in the connective tissue thickness at the same site which composed mainly of elastic fibers and fewer numbers of collagen fibers. Irradiation with cold or biostimulating lasers raised the mitotic activity of the cartilage cells, activated the reproduction processes likewise the intra and extra regenerative repair.

Keywords: Cartilage Lost, He-Ne Laser & Repair.

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Introduction

The cartilage that forms the framework of the auricle is of fibro - elastic origin which consists of typical chondrocytes and yellow elastic fibers embedded in the matrix except the boundaries of the cell nets. Cartilages are known as avascular tissues, because most of their cells are devoid of direct vascularization roots which are located mostly in the perichondrium while the nutrients diffuse to the chondrocytes depending on the variance in osmotic concentrations of the matrix and the perichondrium, (1). For these reasons cartilages have little ability for regeneration and so that their healing carried out by forming fibrous tissue scars, (2). Cosmetic surgery is capable to substitute the defects of the auricles caused by

trauma or congenital malformations like; Microtia (underdeveloped outer ear), Anotia (missing one or both ears), Constricted ears (Flattened or rolled outer ears) and many others, (3). The auricles are vulnerable to high risks of trauma and injuries because they are unveiled and non sheltered structures on either sides of the skull. Surveying studies for the data accumulated from the logs of hospitals contributed that the higher percentage of the injuries of the auricles in man is due to snapping from the human forming about 42% of the cases, followed by falls (20%), automobile accidents (16%), and dog bites (14%), it was found that the prevalent cases noticed included patchy truncation of the auricle, leading frequently to a defect or defects in the edges of the helix. Leaving the causalities of the external ear without treating may conducive to constant inflammations, sepsis, subsequent anomalies in addition to more losses in the adjacent structures, (4). Cold or biostimulating lasers reduce inflammatory reactions, produce analgesia and promote regeneration and healing, how they work, not well known, but it is supposed that there is a generation of non – constrained energy sources which have beneficial effects at low concentrations, and they accelerate the maturation of the main components of the cartilage, and their precursor's, (5). Lasers with 550 – 1000 nm red and near-infrared have low energy which provide no destruction or harms for the organic particles, these lasers direct cold (subthermal) light toward body fine and delicate structural components with no breach or disrupt to them in any way, (6). Group of authors explained the enhancing role of visible lasers on the early regenerative processes contributed to direct effect of these lasers on the prostaglandins and the histamines which act as precursors to attract the components of the vascular system which are responsible for immunity to the area of the trauma, (7), this is very important for recovery steps to continue especially when known that about 50% of the total mass of the inter - cellular matrix is composed of collagen, (1).

Materials &Methods

Twenty four rabbits were used in the current experiment divided equally in to two groups, group 1 considered as standardization while group 2 was considered as a treated group irradiated with He – Ne[(1)] [1] laser. General anesthesia induced using a mixture of Ketamine Hydrochloride[(2)] and Xylazin[(3)] administered intramuscularly, (8). The surgical field which was the inner side of the both auricles prepared by scraping them with Povidon iodine[(4)] 0.75% solution, then three sided square skin flaps done with 4 mm / axis length on each side. The yellow cartilage exposed and square incisions with 3 mm / axis done and pealed out, simple interrupted stitches using 4-0 silk[(5)] used to close the three sided wound which made in the skin , then the animals injected with systemic antibiotics; Penicillin 1000 iu/kg. B.W. and Streptomycin 10mg/kg. B.W. i/m for 3 days after the operation. The area of the operation in the animals of group 2 which was considered as a treated directly postoperative and daily for 7 days after that with 10 minutes / session, (power density ; 0.03333333 Watt/cm^2 and energy density 20 J/cm^2) by direct contact of the beam source on the site of the operation including the rims of the incision. One were irradiated with He - Ne laser 632,8 nm wavelength, 5mW power applied

Three animals of both groups underwent anesthesia to collect small pieces of cartilages from the rim of the holes left after pealing of the cartilage and the specimens submitted to the laboratory to be examined

1 Mellis griot, gas laser He-Ne, U.S.PAT, 4311,1986.
2 Ketallar, 50 mg/ml, Park Devise Co. Gwent, U.K.
3 Rompun, 20mg/ml, Partex, Holland, B.V.DE Hoeve 28.
4 Al Ansari for Antiseptic ,ALEPPO-SYRIA.
5 Ethicon, Ltd., PO. Box 408, Bankhead Avenue, Edinburgh, & Scotland.

histopathologicaaly using ordinary Hematoxelin & Eosin stain at the weeks 1, 2, 4 & 6 post the operation. Precise analogy carried out for the dimensions of the square holes left after pealing the cartilage by a very small metal ruler at the ends of the same periods to asses the progress of the healing processes. Measurements achieved from analogy of the holes were estimated statistically using t test.

Results

Specimens collected from the standardization group one week postoperative showed the rims of the defect covered with perichondrium from both the upper and lower surfaces, the perichondrium is formed of two layers; the external which is fibrous and the internal which is chondrogenic which contains just few chondroblasts and some chondrocytes, the fibrous layer is thick and contains few blood vessels and corpuscles due to hemorrhage which accompany the surgical operation, their were also neutrophils.

For the same time; the specimens collected from the animals which irradiated with laser shown significant variations, the perichondrium was thin and it`s outer layer seen with numerous blood vessels which invade the area to accommodate with the need of proliferation while the inner chondrogenic layer contained large number of chondroblasts which were active and in different stages of mitosis and located beneath the perichondrium directly. Specimens collected from the animals of standardization group two weeks after the operation showed a significant difference in the structure of the rims of the site of the operation, there was a clear decline in the perichondrium which was composed of the outer layer which is mainly consisted of fibrous tissue and the inner layer which contains chondrocytes, the number of the blood vessels in the outer layer was nearly the same without significant increase, while the chondrocytes are limited and disseminated. For the same time; specimens obtained from the treated group showed increase in the thickness of the outer layer with a large number of blood vessels newly formed needed to accommodate the proliferative processes. The inner layer which was the chondrogenic showed large number of chondroblasts at different stages of mitosis, the matrix was thick and contained many lacunae is with two chondrocytes and some which contains four cells. After four weeks post the operation, the specimens collected from the animals of the standardization group were not so different , that the perichondrium was in the same state as the two weeks before but there were a very important change that chondroblasts moved toward the outer layer leaving the matrix depth and show no mitotic or proliferative activities , also the elastic fibers in the matrix were not intensive but hazy and appeared as single strands, the chondrocytes were almost sphere , there were no flattened cells. Specimens collected from the animals of the treated group for the same time showed a very active inner layer which contained chondroblasts disseminated a long the whole thickness of the layer, and all the stages of the mitosis and proliferation were seen , tend to be mature as it passed toward the depth of the layer, the matrix contained intensive elastic fibers and less numbers of collagen fibers , also the chondrocytes tend to differentiate as the move toward the surface to be flattened , while the oval one remain in the depth of the cartilage, Figure; 1 and Table; 1.

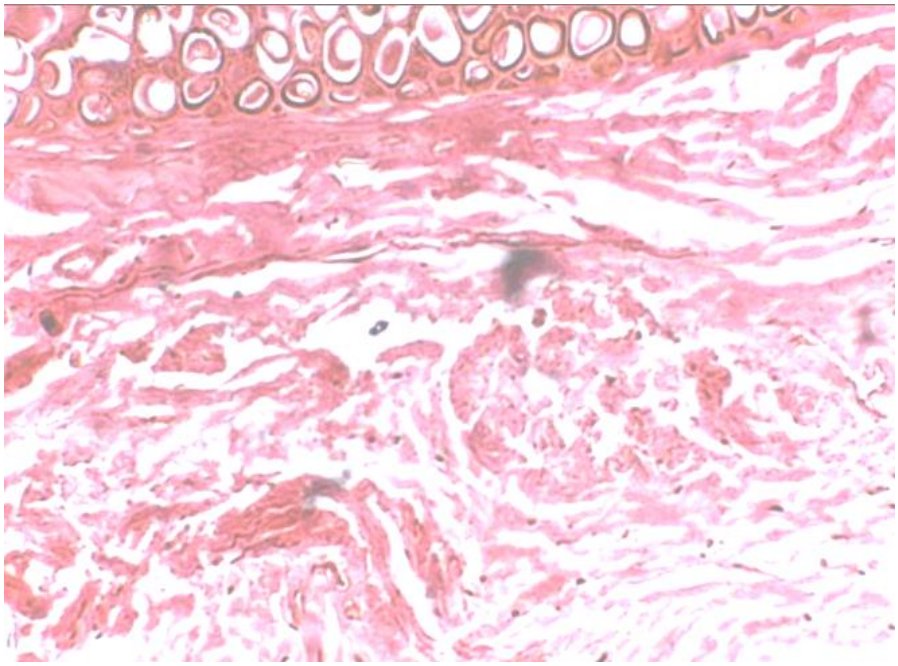

Fig. 1: Specimen collected from the treated group four weeks post operation showed active inner layer of the perichondrium which contained chondroblasts at all the stages of the mitosis and proliferation, the matrix contained intensive elastic fibers and less numbers of collagen fibers, the chondrocytes tend to differentiate as they move toward the surface to be flattened, while the oval ones remain in the depth of the cartilage.

Table 1: Length of axis (mm) / week for both groups showing the rapid filling of the defects done in the treated group animals, which ended with sealing at the sixth week after the operation.

Group	Length of axis (mm) / Week			
	1st. week	2nd. week	4th. week	6th.week
Control A	2.8 ± 0.089	2.3 ± 0.44	1.6 ± 0.34	1.1 ± 0.85
Treated B with He – Ne Laser	2.2 ± 0.58	1.6 ± 0.23	0.9 ± 0.34	Sealed
Level of Significance	*	*	*	**

*Significant
** Highly Significant

At the sixth week post the operation; the specimens collected from the animals of the standardization group showed no mitotic or proliferative activities at the site of the operation and the matrix was filled with intensive strands of elastic fibers and just little collagen fibers, it showed the elastic connective tissue is the main component of the matrix which fill the defect. While the mitotic and proliferative activities were obvious in the specimens collected from the treated group animals, the chondroblasts which were

present on the surface tend to be mature as the pass toward the center of the tissue and changed to chondrocytes flattened as they became near the center and located in lacunae which contained two or four of these cells, lacunae with four cells were dominant, numerous elastic fiber tissue and collagen fibers surrounded the lacunae, Figure; 2 & 3 and Table; 1 .

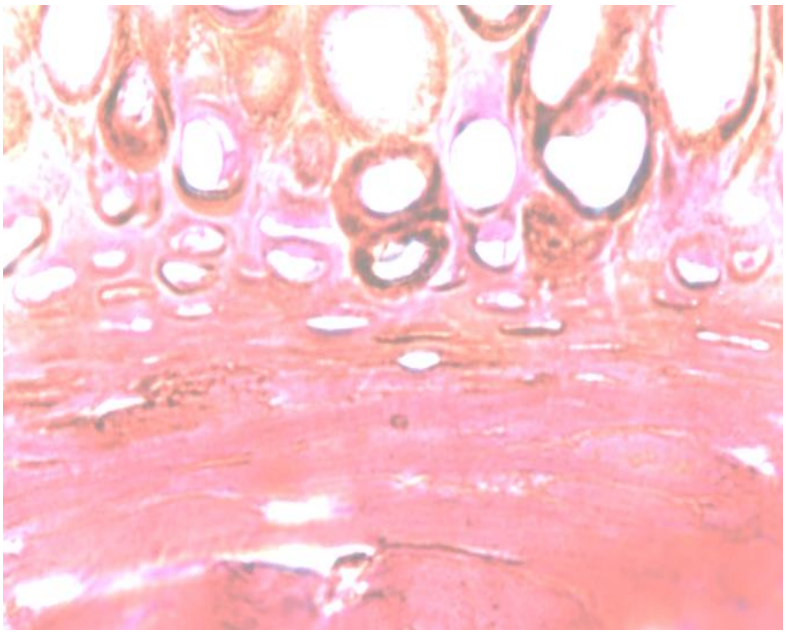

Fig. 2: Specimens collected from the treated group six weeks post operation showed well developed cartilage growth in the area of the operation, chondroblasts and chondrocytes invade the area, numerous elastic fiber tissue and collagen fibers surrounded the lacunae which contained the chondrocytes.

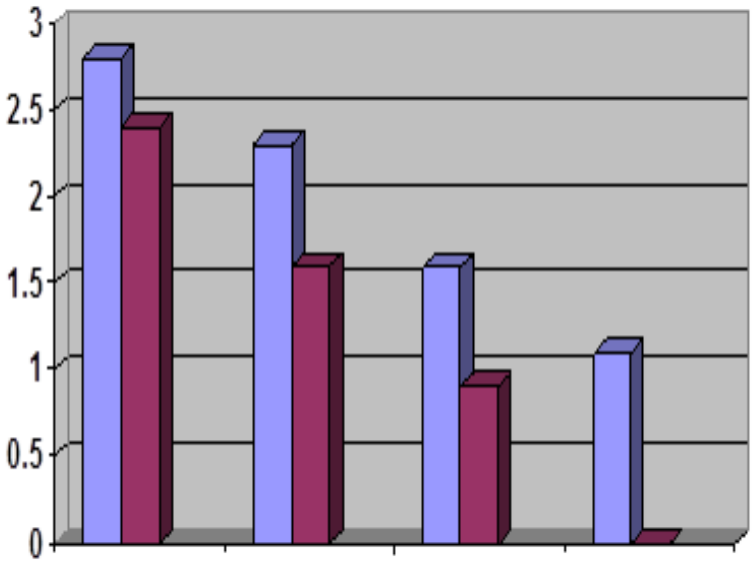

Fig. 3: Length of axis (mm) / week for both groups; standardization and treated with He – Ne laser, showing the rapid filling and sealing of the defects in the animals of the treated group. Blue color: Standardization &Violet color: Treated

Comments

The main aim of the current study was to detect the ability of filling a defect due to automobile accidents , avulsion due to human bites , burns and frostbites resulting in massive soft tissue loss (cartilage) with tissues lately originated which is cartilaginous in origin with just little fibrous tissue. The clinical feasibility of this process depends upon preservation of the vascularization to the cartilage which formed the boundaries of the hole left after removing the specimens. The surrounding skin is the only source of vascularization to the underlying cartilages of the auricle, (9). Many authors proved increasing in the vascularization of the sites treated with cold or biostimulating lasers and this has been considered as one of the laser specific reactions, (10). L.L.L.T. enhances fine circulation looked as fred settlement for the functions carried out in the site of the operation which irradiated with it , moreover the rapid raise in the teir of Adenosine, Growth hormone, GH and Fibroblast growth factor, FGF, Fiber/capillary, F/C ratio and Capillary's diameter infiltrate the site of the operation with significant raise in the native blood vessels diameter, (11). Thus the first important factor encourage the healing was provided by the irradiation with the He - Ne laser, applying biostimulating lasers to experimental defects shows accelerated healing by affecting various aspects of reparative process including defect contraction and healing by new tissue formation and probably it has a major role in dealing with protracted, and stagnant wound which resist healing, (12). Many researches designed to evaluate different therapeutic methods which accelerate the healing of grafted cartilages without or with just little amount of fibrous tissues, one of these therapeutics are the low level lasers, a group of workers found that the laser's power accommodated by the endogenous chromphores located in the mitochondria and cell membrane, furthermore, they proved that this energy potentiate production of RNA, DNA, proteins and various enzymes resulting in cell proliferation and earlier tissue regeneration, (5) . When the laser interacts with a biological tissues, a series of changes carried out, the therapeutic role of the laser depends on many multifactor effects on the body tissues as a whole, some of these implications executed due to transmission of the laser energy to an energy of chemical bonds moreover the non-linear optic effects like, acoustic, ultrasound, standing waves and mild ultra - violet radiation which emerge in to the tissues of the body, (13). Fibroblast growth factor FGF-2 implemented in an experimental work to chondrocytes in to hues (single layer media and triple proportional circumstances), they founded that FGF-2 is a unique gene mitotic for chondrocytes in single layer media and in vivo .However; reports about the utilization of FGF-2 to culture cartilage tissue in vitro in a triple proportional circumstances have been contradictory. It was indicated that FGF-2 contribute to decline of the slandered mature virtual properties of cartilage explants and raise the level of multiplication without influencing virtual properties of chondrocytes - seeded implants in the existence of FGF-2, (13). Laser irradiation act as trigger for the releasing of Prostaglandins, (14), which in turn enhances the incentive of Fibroblast growth factor, FGF. Experimental works indicated increase production of prostaglandins E&F following L.L.L.T., (15) .The forward facts explain how the mitotic processes stimulated at the site of the incision at the early reparative stages and the immature young chondrocytes numeral raised which then replaced with mature chondrocytes and active interstitial cartilage growth in the animals which were specified to be irradiated with laser irradiation. Irradiation with biostimulating lasers indicated to reduce inflammatory reactions, produce analgesia and promote regeneration and healing. Its root of work is unknown, but an assumption referred to ability of the light to generate freebies radicals which have beneficial effects at low concentrations, (16), L.L.L.T. accelerates the collagen and it`s precursor`s maturations, promote the improvement of microcirculation, they also activate the mast cells, (17).

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