Using He-Ne laser irradiation for enhancing cartilage Autografts

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Summary

thirty adult NewZealand rabbits used in this study, they were divided in to two groups (control and treaded with Helium – Neon laser). A square skin flap done on the medial aspect of the auricle of both sides, a square piece of cartilage incised, pealed out from each auricle and fixed in the site of the other, then the flaps sutured .The site of the operation in the rabbits of the treated group were irradiated using a Helium –Neon laser with (5mw) power for (10 days) began after the operation directly, (3 rabbits) from each group used for collection of specimens for histopathological examination at the weeks (1,2,3,4, & 6) weeks post the operation .The results revealed

Early invasion of the matrix with elastic fibers which continue to the surrounding perichondrium, mature lacunae and complete healing occurred later at the 6th week in the treated group.

Conclusion: Helium- Neon laser raised the mitotic activity of the cartilage cells, activated the reproduction processes in addition to the intra and extra regenerative reparation.

Introduction

The cartilage found in the external ear is of fibroelastic origin which consist of typical chondrocytes and yellow elastic fibers pervaded the matrix except around the lacunae, (1). Cartilage autografts widely used but potential autologus cartilage donor sites options expand to include both allogenic and xenogenic cartilages, (2).The therapeutic effect of laser therapy in wound healing had been identified so that a better knowledge about the mechanism of tissue repair sing light energy obtained in the areas of skin, muscle, ligaments, nerves, bones and cartilage which respond

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to doses of light with wavelengths range between 600-1000nm, but the amount of energy absorbed varied from one tissue to another even when the wave length remain constant,(3).Efforts directed to accelerate the new cartilage formation and anchoring the grafts within the articular defects using laser therapy, (4), at the same time combined magneto- laser treatment used experimentally for acceleration and maturation of reparative processes of hyaline cartilage cells in the knee joints at different postoperative periods,(5), while another team of researchers explained the enhancing role of laser radiation of blue and red regions on the early regenerative processes, (6).Many defects may be met in the external ear like imperfect development, preauricular cysts, fistulae and sinuses or even the absence of the meatus,(1), and these defects need cosmetic surgery, which depends. mainly on the cartilage grafting (both allografts and xenografts) and hence this study designed to evaluate the role of Helium-Neon laser on the cartilage grafts, and the mechanism by which it works.

Materials and methods

Thirty adult NewZealand rabbits used in this study, they were divided in to two equal groups (control and treated with Helium-Neon laser). [1]Each rabbit underwent general anesthesia using a mixture of Ketamine Hydrochloride, [2] and Xylazin, [3] administered intramuscularly, (7). The site of the operation was the medial aspect of both auricles where three sided square skin flap done with one and half centimeter for each side, the yellow cartilage exposed nd square incision with one centimeter each side done and pealed out to be kept in a petri dish filled with normal saline and the skin flap sutured with simple interrupted stitches using (4-0 silk). [4]The site of the operation in the treated group irradiated with a Helium-Neon aser (632,8 nm) wavelength, 5mW) power applied directly fter he operation and daily for (10 days) after that with (10 minutes / session), by direct contact of the beam source on the line of the incision.Three animals of each group were anaesthetized and cartilage specimens collected from the edges of the grafts to be consisted of both original and transmitted tissues which then sent for histopathological examination using ordinary Hematoxelin &Eosin stain at the weeks (1,2,3.4 & 6) post the operation.

Results

Samples collected from the control group one weak post the operation showed a line of necrotic tissue between the original and the grafted cartilage, the line was heavily infiltrated with inflammatory cells and clusters of erythrocytes, while samples collected from the treated group for the same period showed inflammatory cells especially macrophages pervaded the matrix, clusters of erythrocytes also seen. Granule's rows seen beneath the perichondrium, but they subsequently connected together as

[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,

[4] Ethicon, Ltd., PO. Box 408,Bankhead

advanced toward the center of the defect where they form elastic fibers which pervaded the matrix to a degree that degenerated cells and newly formed fibroblasts squeezed up in the intervals between them.Samples collected from the control group two weeks post the operation revealed presence of clusters of erythrocytes and large number of macrophages while necrotic tissue still present. The matrix was faint and contained loose network of fibrils and few fibroblasts. Samples collected from the treated group for the same period showed well developed matrix heavily infiltrated with fibroblasts and elastic fibers, the network of the fibers was dense and continue in to the surrounding perichondrium, immature chondrocytes abundant in the matrix, they were spherical shaped surrounded with irregular capsules.After three weeks of the operation, examination of the samples collected from the control group revealed decrease in the density of the inflammatory reaction, while the macrophages decrease in number and the necrotic tissue disappeared. The matrix appeared dense and the network of the fibers seen intense while the fibroblasts increase in number. Examination of the samples collected from the treated group for the same period showed well developed chondrocytes with granular cytoplasm and nuclei, each chondrocyte occupied in a well developed capsule forming a lacunae, the chondrocytes were large and spherical in the center while they were smaller and flat near the periphery, Fig .1. Samples collected from the control group four weeks post the operation showed disappearance of the inflammatory reaction, few numbers of immature chondrocytes could be seen in the treated group for the same period revealed presence of large number of lacunae pervaded the matrix, they were elliptical, semicircular or angular depending upon their position from the perichondrium, isogenous groups also pervaded the matrix, Fig. 2. The stain of the matrix vary depending upon the relation with the lacunae, it stained deep near the capsule (territorial matrix) while it was lighter in the intercellular spaces (interterritorial matrix).Samples collected from the control group after six weeks of the operation showed mature chondrocytes arranged in lacunae scattered in the matrix singly or in isogenous groups which were few in number. Samples collected from the treated group for the same period revealed presence of large number of isogenous cells separated from each other by intense bundles of elastic fibers.

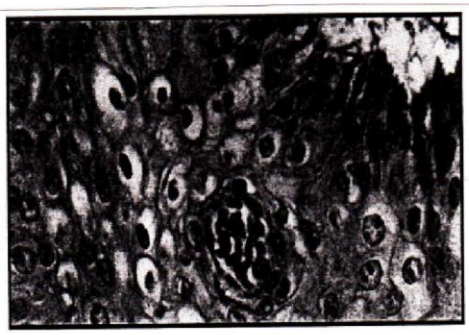

Fig. 1; presence of well developed chondrocytes located in lacunae , they were large and spherical in the center. (H&E X 400)

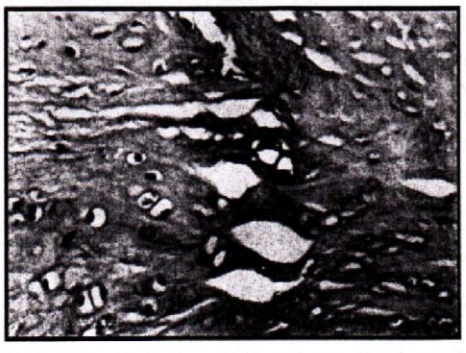

Fig. 2; large number of semicircular and elliptical lacunae. (H & E X 100)

Discussion

Cartilage's are known as avascular tissues, because most of their cells are far from exchange vessels which are located mostly in the perichondrium while the nutrients diffuse to the chondrocytes depending on the difference between the concentrations, also the cartilages consist an anti-angiogenic factor which inhibits vascular invasion in addition to that they consist inhibitors for chondrolytic proteases,(1) , for these reasons cartilages have little ability for regeneration and it is well known that they heal by forming fibrous tissue scars,(2).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 team of workers found that the laser's energy absorbed by the endogenous chromphores located in the mitochondria and cell membrane, furthermore, they proved that this energy potentiate the synthesis of RNA, DNA, Proteins and various enzymes resulting in cell proliferation and earlier tissue regeneration, (3). 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 effects take place due to transmission of the laser energy to an energy of chemical bonds in addition to nonlinear optic effects like, acoustic, ultrasound, standing waves and mild ultra- violet radiation which emerge in to the body tissues, (8), thus many changes occur on the subcellular levels far example acceleration of collagen and its precursors, (9), and this is very important for the healing process especially when known that the collagen form about 50% of the dry weight of the inter cellular matrix ,(1).On the cellular level, stimulation of the nuclear functions lead to many changes, like raising of mitotic activity of the cell, activating reproduction processes as well as intra and extra cellular physiological and reparative regeneration, (9), while increasing morphofunctional activity and substantial expansion of the anti inflammatory effect and stimulating of phagocytosis and leukocyte migration taking place at the tissue level, (8&9). The forward facts explain how the mitotic processes stimulated in the area of incision at the early reparative stages and the increase in the number of the immature young chondrocytes which then replaced with mature chondrocytes in addition to the active interstitial cartilage growth in the treated group and these results agreed with those obtained by serduchenko and his colleagues, (6).

Conclusions

Regardless the morphofunctional nature of the cartilage which make them of just little ability of healing, their response to the laser energy resemble those of all the body tissues, the irradiation with the laser raised the mitotic activity, activated the reproduction processes in addition to intra and extra regenerative reparation.

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التشعيع بليزر الهليوم-النيون لتشجيع الطعم الغضروفية الذاتية

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الخلاصة

استخدمت في هذه الدراسة ثلاثين أرنبا نيوزلنديا بالغا تم تقسيمها إلى مجموعتين متساويتين(السيطرة والمعالجة بليزر الهليوم – نيون).تم استحداث قطع لساني مربع في الجلد على الجانب الأنسي لكلا الأذنين وصولا إلى الغضروف حيث تم استحداث قطع مربع في كل جانب وتم سلخه لتثبيته في مكان الغضروف في الجانب الثاني تبعه خياطة اللسان الجلدي.تم تشعيع موقع العملية في حيوانات مجموعة المعالجة بوساطة ليزر الهليوم – نيون قوته (٥ ملي واط) لمدة(١٠ أيام) بدأت بعد العملية مباشرة،تم تخصيص(٣ أرانب) من كل مجموعة لغرض جمع النماذج الخاصة بالفحوصات النسجية المرضية للأسابيع(١،٢،٣،٤ و٦) بعد إجراء العملية.أظهرت النتائج غزو مبكر للنسيج البيخلوي بالألياف المرنة والتي تمتد إلى الغلافي الغضروفي المحيط ، ظهور فجوات ناضجة مع اكتمال الألتآم في مجموعة المعالجة بعد مرور (٦ أسابيع).

الاستنتاجات: رفع ليزر الهليوم – نيون القابلية الانقسامية غير المباشرة للخلايا الغضروفية ، العمليات التكاثرية علاوة على عمليات إعادة النمو الإصلاحية داخل وخارج الخلايا.