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HE-NE LASER IRRADIATION ENCOURAGES REPARATIVE PROCESSES AFTER CARTILAGE LOSS AND ENHANCES THE GROWTH OF AUTOLOGOUS CARTILAGE GRAFTS

*Prof. Dr. Ihsan F.R.Mohammed, **Dr. Ibtisam K. Ali,
***Dr. Noha Saheb AL – Mestowffy

*Dept. of Anatomy, Baghdad University;
**College of Nursing, Baghdad University;
***Ministry of Health of Iraq

The external auricle has a high potential for injury due to its exposed and unprotected position alongside the head. Several of authors have demonstrated the enhancing role of laser radiation of blue and red regions on the early regenerative processes. Many changes occur on the sub cellular levels for example acceleration of collagen and its precursors. This is very important for the healing process especially when known that the collagen form about 50% of the dry weight of the intercellular matrix.

The main purpose of this project was to determine the ability of filling a defect resulting in massive cartilage loss with newly formed tissue which is cartilaginous in origin with just little fibrous tissue . This process depends upon preservation of blood supply to the cartilage which formed the boundaries of the hole left after removing the specimens. The cartilage of the ear has no other blood supply except that supplied by the overlying skin.

Rabbit ears with experimental damage were irradiated with He–Ne laser (632,8 nm wavelength, 5mW power) applied directly after the operation and daily for 7 days after that with 10 minutes / session, by direct contact of the beam source on the line of the incision.

Results showed that low lever laser irradiation enhances microcirculation and seemed to be unique in normalization of the functional features of the area which irradiated with it in addition to rapid increase in the level of Adenosine, Growth hormone, GH and Fibroblast growth factor, FGF. Fiber/capillary, F/C ratio and Capillary's diameter proliferated the aria with marked increase in the diameter of the original blood vessels. Thus the first important factor encourage the healing was provided by the irradiation with the He-Ne laser.

Introduction

The external auricle has a high potential for injury due to its exposed and unprotected position alongside the head. A retrospective study looking at hospital records in auricular injury cases revealed that human bites constitute the most common cause of injury (42%). This was followed by falls (20%), automobile accidents (16%), and dog bites (14%). The most common injury observed was incomplete amputation of the ear, usually helical rim tissue loss. Untreated open auricular injuries invariably result in infection, ensuing deformities, and further tissue loss.

The therapeutic effect of laser therapy in wound healing have been studied in order to achieve better understanding of the mechanism of tissue repair using light energy obtained in the areas of skin, muscle, ligaments, nerves, bones and cartilage which respond to doses of light with wavelengths range between 600-1000 nm. The amount of energy absorbed, vary greatly from one tissue to another, even when the wavelength remains constant. Various authors (1-6) have explained the enhancing role of laser radiation of blue and red regions on the early regenerative processes. Many changes occur on the sub cellular levels for example acceleration of collagen and its precursors. This is very important for the healing process especially when known that the collagen form about 50% of the dry weight of the intracellular matrix.

Materials &Methods

Twenty four rabbits were included in this study. They were distributed into two groups: group A as a control group and group B as a treated one irradiated with He–Ne[(1)] laser.

General anesthesia induced using a mixture of Ketamine Hydrochloride(2) and Xylazin(3) administered intramuscularly. The surgical field was prepared by rubbing the skin of the medial aspect of both auricles with Povidon iodine 0.75% solution. Then three sided square skin flaps were made with 4 mm / axis length on each side. The yellow cartilage was exposed and square incisions with 3 mm / axis was made and pealed out. The skin flap was sutured with simple interrupted stitches using 4-0 silk. Then the animals were injected with systemic antibiotics; penicillin 1000 iu/kg. B.W. and streptomycin 10 mg/kg. B.W. i/m for 3 days after the operation.

The site of the operation in the treated group was irradiated with a He–Ne laser 632,8 nm wavelength, 5mW power applied directly after the operation and daily for 7 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 specimens collected from the edges of the holes left after pealing of the cartilage to be consisted of both original and transmitted tissues.

The specimens were sent for histopathological examination using ordinary Hematoxelin & Eosin stain at the weeks 1, 2, 4 & 6 post the operation. The dimensions of the square holes left after pealing the cartilage were measured using a very small metal ruler at the ends of the same periods to assess the advance of the healing processes. The values obtained from measuring of the holes were estimated statistically using T test.

Results

Samples collected from control group one week post-operation showed high infiltration of inflammatory cells (neutrophils) in the area of the operation with damaged blood vessels, thrombi and punctuate hemorrhage. While samples collected at the same period from the animals of the group treated with laser therapy showed few damaged blood vessels, thrombi and punctuate hemorrhage with infiltration of neutrophils in the area of operation. A thin layer of connective tissue could be seen in the same area consisting mainly of collagen fibers with small amount of elastic fiber. Samples collected from the animals of the control group two weeks post-operation revealed infiltration of inflammatory cells (neutrophils), damaged blood vessels and thrombi appeared lesser in numbers.

Part two- autologous cartilage growth

In the 19th century, various alloplastic materials were used as reconstructive material, the autologous bone was the most common one. In 1941, Peer described the resistance to resorption of autologous septal and auricular cartilage. Autologous cartilage grafts can be used to provide a structural framework for tissue that has been modified by trauma, or congenital malformation. One method to reshape cartilage for use in reconstructive procedures involves laser irradiation.

The aim of this part of the study was to assess autologous cartilage grafts irradiated with diode laser to determine whether there is an increase in growth and a greater preservation of architectural features compared with non-irradiated cartilage grafts.

24 New Zealand white rabbits were divided in to two equal groups (control and treated with diode laser). Sites chosen for the operation were the medial aspect of the right auricle as a donor and the upper part of the right flank as a recipient site.

Surgical operations were carried out under general anesthesia, the donor site was prepared surgically and a three-sided square skin flap was made with one and half centimeter each side. The skin was elevated to expose the yellow cartilage beneath it, then a square incision was made in the cartilage with one centimeter each side and pealed out to be kept in a petri dish filled with normal saline, the skin was resutured with simple interrupted stitches using 4-0 silk.

The upper part of the right flank was prepared surgically and a three sided skin flap made to expose the subcutaneous region where each harvested cartilage segment was transmitted under sterile conditions serving as recipient site, the cartilage segments were placed in contact with the subcutaneous tissue, the flap closed and sutured with interrupted stitches using 3-0 silk.

The site of the operation in the treated group was irradiated using a diode laser with wavelength 904nm, output 10mW for 10 minutes/session and total 10 sessions started after the operation directly, the laser used was window type and it`s energy carried to the site of operation by direct contact of the window to the flank.

Three rabbits of each group were anaesthetized and the implanted cartilages were pealed out for deremination. Gross analysis included a measurement of the area (area [cm^2] = length [cm] x width [cm]) of each harvested cartilage graft. Results were analyzed using paired samples t- tests to determine differences in mean area and resorption rates.

Subjective assessments on the gross specimens included determination of the pliability of each segment of cartilage and inspection for the presence of an inflammatory exudate surrounding the cartilage graft or within the recipient bed. Sections were prepared from the specimens of each time stained with hematoxylin-eosin for histopathological examination.

Results

Palpation of the skin overlying the implanted cartilage revealed a subjective difference in the ability

for manual displacement of grafts within the subcutaneous pocket from postoperative day 1 until the third postoperative week, when all grafts were subjectively immobile. On harvesting the cartilage grafts, there was no gross, subjective evidence of an inflammatory exudate, either within the recipient bed or surrounding the cartilage grafts. All grafts were noted to have a thin, fibrous capsule surrounding them.

Palpation of all grafts revealed a subjective difference in the structural integrity between grafts treated with laser irradiation and grafts of control group. Grafts of control group were softer, more pliable, and more likely to be curled up on themselves compared with grafts treated with laser irradiation.

Samples collected from the animals of control group (3 weeks post-operation) showed bleeding and damaged blood vessels with large number of inflammatory cells (neutrophils and macrophages).

Samples collected from the treated group for the same period showed lesser amounts of erythrocytes and damaged blood vessels in the area of the operation, macrophages and neutrophils seen in the area. The cartilage seen surrounded with perichondrium, chondroblasts, the matrix seen containing collagen and elastic fibers , Fig. 1.

Samples collected from the animals of control group 6 weeks post operation, showed slight decrease in the number of inflammatory cells. While the samples collected from the animals of the treated group at the same period showed the cartilage surrounded by the perichondrium, chondroblasts and chondrocytes seen numerous in number proliferating by the deep perichondrial cells differentiating by mitosis embedding in the matrix that contain collagen and elastic fibers. The area of operation showed

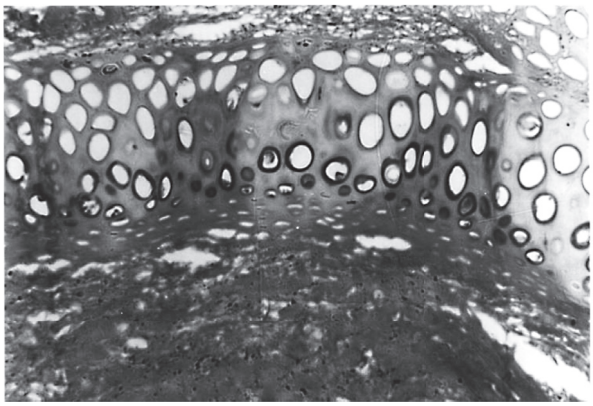

Fig. 1: Samples collected from the treated group (3 weeks post operative) showed decrease in the number of the inflammatory cells, formation of connective tissue while the chondrocytes and chondroblasts seen large in number, (H&E X1000)

connective tissue formation consist of collagen and elastic fibers with early formation of blood vessels.

Samples collected from the control group (9 week) post operation showed disapperance of inflammatory reactions.

Samples collected from the treated group for the same period revealed that the perichondrium showed thick layer of connective tissue consist of collagen and elastic fibers. The tissue mass enlarged as the cartilage cells surrounded themselves with the substantial matrix the generate, Fig. 2.

Table 1

Graft area (cm²) in both groups (control and laser treated)

No.	C3	L3	C6	L6	C9	L9	C12	L12
1-	1.151	1.577	1.050	1.857	1.155	1.949	1.254	2.102
2-	1.030	1.355	1.060	1.768	1.166	1.850	1.265	2.120
3-	0.720	1.488	0.824	1.880	1.134	1.887	1.299	2.110
4-	1.054	1.377	1.065	1.804	1.210	1.987	1.276	2.103
5-	0.931	1.610	1.255	1.752	1.175	1.964	1.322	2.185
6-	1.032	1.761	0.960	1.910	1.188	1.891	1.334	2.194

Table 2

Mean, Standard Deviation SD, Standard Error SE, Value of Probability and Degree of Significance of both groups.

Readings	Groups							
	C3	L3	C6	L6	C9	L9	C12	L12
Mean	0.986	1.528	1.036	1.8285	1.1713	1.9213	1.2917	2.1357
SD	0.148	0.153	0.142	0.0636	0.0264	0.0531	0.0321	0.0423
SE	0.060	0.063	0.058	0.026	0.011	0.022	0.013	0.017
P- Value	0.000		0.000		0.000		0.000	
Degree of Significance	*		*		*		*	

*Very Highly Significant: P< 0.001

Samples collected 12 weeks after the operation showed increase in number of chondroblasts and

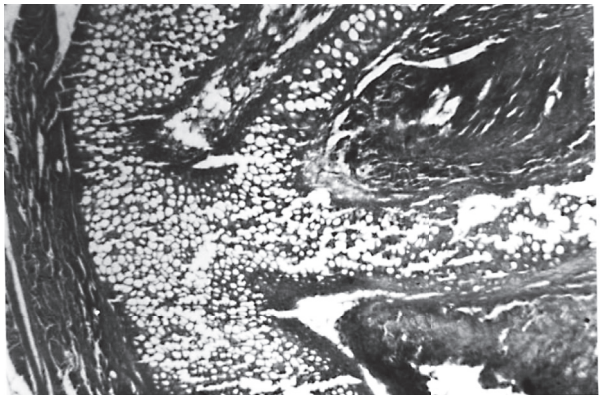

Fig 2: Samples collected from the treated group (9 week post operative showed proliferation of well developed cartilage, thick matrix and high increase in the number of the chondrocytes and chondroblasts, (H & E X 100)

chondrocytes. The matrix appeared thick layer of connective tissue surrounding the cartilaginous cells. Few numbers of macrophages and lymphocytes could be seen in the area of operation while samples collected from the treaded group for the same period revealed high developing in the cartilage proliferation in the area of operation. Thick connective tissue matrix with high increase in numbers of chondroblasts and chondrocytes that make cartilage grow in size and show penetration into the surrounding tissue.

Discussion

The main purpose of this project was to determine the ability of filling a defect resulting in massive cartilage loss with newly formed tissue which is cartilaginous in origin with just little fibrous tissue.

The process depends upon preservation of blood supply to the cartilage which formed the boundaries of the hole left after removing the specimens. The cartilage of the ear has no other blood supply except that supplied by the overlying skin. Many authors (1-6) have experimented with increasing the vascularization of the sites irradiated with low level lasers and this has been showed to be a specific reaction.

Conclusions

There have been no previous studies using laser diode to regulate the growth and quality of autologous cartilage graft. The current study revealed significant improvements in both the gross and histological features of implanted cartilage using laser irradiation. The results may illustrate how cartilage grafts can be prepared and treated by laser in order to increase growth and quality. These results can be considered to be applicable in both nasal and auricular augmentation to reduce the resorption rate and in preserving the structural integrity of the cartilage. The results of this study also agree with those, which

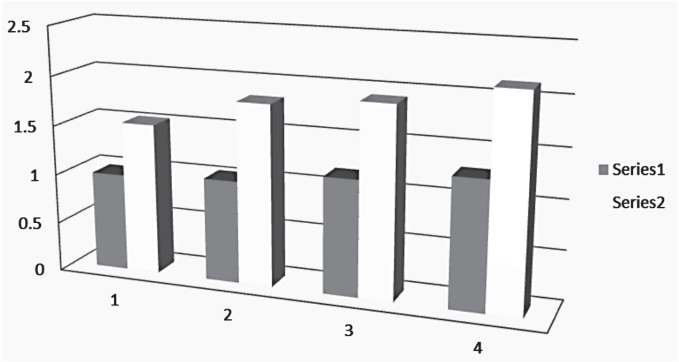

Figure 3: Comparison of the means in different groups regarding the follow up period

demonstrated the benefits of enhancing cartilage autografts with either fibrin sealant and aFGF or bFGF or simply fibrin sealant alone.

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ИЗЛУЧЕНИЕ ГЕЛИЙ-НЕОНОВОГО ЛАЗЕРА СТИМУЛИРУЕТ РЕПАРАТИВНЫЕ ПРОЦЕССЫ ПОСЛЕ ПОТЕРИ ХРЯЩА И УСИЛИВАЕТ РОСТ АУТОЛОГИЧЕСКИХ ТРАНСПЛАНТАТОВ ХРЯЩА

Ихсан Ф.Р. Мохаммед, **Ибтисам К. Али,* Ноа Саеб Аль-Местоффи*
**Кафедра анатомии Багдадского университета,*
***Колледж медсестер Багдадского университета,*
****Министерство здравоохранения Ирака*

Внешние ушные раковины имеют высокий потенциал травматизации из-за открытого и незащищенного расположения на голове. В ряде работ показана высокая эффективность использования лазерного излучения красной и синей областей спектра на ранних этапах регенеративного процесса. Многие процессы при этом происходят на субклеточном уровне, например, ускорение образования коллагена и его предшественников. Это очень важно для процесса заживления, так как известно, что коллаген составляет почти 50% сухой массы межклеточного матрикса.

Целью работы было определение возможности заполнения дефекта хряща новообразованной тканью, являющейся хрящевой по происхождению с малым количеством фиброзной ткани. Этот процесс зависит от сохранения кровоснабжения на границе раны. Хрящ ушной раковины не имеет иного кровоснабжения, кроме обеспечиваемого вышерасположенной кожей.

Уши кроликов с экспериментальными ранами облучали излучением гелий-неонового лазера с длиной волны 632,8 нм и мощностью 5 мВт сразу после операции, а затем ежедневно в течение 7 дней с экспозицией 10 минут на линию разреза (контактно).

Результаты показали, что низкоинтенсивное лазерное излучение усиливает микроциркуляцию крови, нормализует функциональное состояние зоны облучения, а кроме того, быстро увеличивает уровень аденозина, гормона роста (GF) и фактора роста фибробластов (FGF).

Данные исследования показали, что облучение гелий-неоновым лазером раны ушного хряща приводит к активации процесса восстановления хрящевой ткани.

ВИПРОМІНЮВАННЯ ГЕЛІЙ-НЕОНОВОГО ЛАЗЕРА СТИМУЛЮЄ РЕПАРАТИВНІ ПРОЦЕСИ ПІСЛЯ ВТРАТИ ХРЯЩА І ПОСИЛЮЄ РОСТ АУТОЛОГИЧНИХ ТРАНСПЛАНТАТІВ ХРЯЩА

** Іхсан Ф.Р. Мохаммед, ** Ібтісам К. Алі, *** Ноа Саєб Аль-Местоффі*
** Кафедра анатомії Багдадського університету,*
*** Коледж медсестер Багдадського університету,*
**** Міністерство охорони здоров'я Іраку*

Зовнішні вушні раковини мають високий потенціал травматизації внаслідок відкритого і незахищеного розташування на голові. У ряді робіт показана висока ефективність використання лазерного випромінювання червоної та синьої областей спектра на ранніх етапах регенеративного процесу. Багато процесів при цьому відбуваються на субклітинному рівні, наприклад, прискорення утворення колагену і його попередників. Це дуже важливо для процесу загоєння, так як відомо, що колаген складає майже 50% сухої маси міжклітинного матриксу.

Метою роботи було визначення можливості заповнення дефекту хряща новоствореною тканиною, яка є хрящовою за походженням з малою кількістю фіброзної тканини. Цей процес залежить від збереження кровопостачання на кордоні рани. Хрящ вушної раковини не має іншого кровопостачання, крім забезпечуваного вишеразташованою шкірою.

Вуха кроликів з експериментальними ранами опромінювали випромінюванням гелій-неонового лазера з довжиною хвилі 632,8 нм і потужністю 5 мВт відразу після операції, а потім щодня протягом 7 днів з експозицією 10 хвилин на лінію розрізу (контактно).

Результати показали, що низькоінтенсивне лазерне випромінювання підсилює мікроциркуляцію крові, нормалізує функціональний стан зони опромінення, а крім того, швидко збільшує рівень аденозину, гормону росту (GF) і фактора росту фібробластів (FGF).

Дані дослідження показали, що опромінення гелій-неоновим лазером рани вушного хряща призводить до активації процесу відновлення хрящової тканини.