فاعلية استخدام جرعة عالية من البلازما الفيزيائية الباردة في عملية التئام الجروح الجلدية

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مقدمة:

البلازما الفيزيائية، المعروفة بالحالة الرابعة للمادة. البلازما الفيزيائية الباردة تحديدًا هي غاز متأين جزئيًا يحتوي على أيونات وإلكترونات وفوتونات، بالإضافة إلى جذور حرة وجزيئات، وهو موجود في حالة مثارة. إن الطريقة الأكثر شيوعًا لتوليد بلازما منخفضة الحرارة والحفاظ عليها في التطبيقات التقنية هي تطبيق مجال كهربائي على غاز متعادل أو نبيل، ثم يحدث التأين وتتشكل البلازما عندما تكون طاقة الذرات عالية بما يكفي لتصادمها وإخراج الإلكترونات. وهذه الأشعة الآمنة تستخدم في المجال الطبي في مجالات متعددة مثل علاج الجروح وعلاج الأورام السرطانية و طب الأسنان و غيرها.

مشكلة البحث:

معرفة مدى تأثير زيادة الجرعة العلاجية وارتفاع درجة حرارة أشعة البلازما الفيزيائية الباردة، كظروف تشغيل جهاز نافث البلازما آمنة للتطبيقات الطبية الحيوية.

أسئلة البحث:

- هل زيادة الجرعة العلاجية له تأثير ضار على جسم الحيوان عامة وعلى الجرح بشكل خاص؟
- هل إن ارتفاع درجة حرارة أشعة البلازما الفيزيائية الباردة إلى 40 درجة مئوية له تأثير ضار على عملية شفاء الجروح؟

أهداف البحث:

تقييم فعالية جهاز نافث البلازما الفيزيائي في علاج الجروح الجلدية كاملة السمك في الكلاب، ودراسة تأثير اختلاف الجرعة المستخدمة من أشعة البلازما

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 ISBN:978-9922-9838-7-5

أهمية البحث:

استخدام أحدث الطرق العلمية والصديقة للبيئة وبتأثيرات جانبية تكاد تكون معدومة على الصحة العامة وهي فيزياء البلازما الباردة كونها إحدى حالات المادة وهي تمثل 99٪ من الكون.

منهج البحث:

نهج البحث إلى استحداث علاج للجروح جراحيا ومعالجتها بجرعتين مختلفتين من أشعة البلازما الفيزيائية الباردة لمدة يومين متتاليين وتم اتباع طرق التقييم السريرية والنسيجية المرضية، والكيميائية الحيوية، على التغيرات الحاصلة في الجروح المعالجة استجابةً للبلازما الفيزيائية الباردة مقارنةً بمجموعة السيطرة. في هذا البحث، استُخدم جهاز نافث البلازما الهيليوم في حالتي تشغيل مختلفتين تتضمن (كثافة طاقة البلازما والجرعة) بما يلبي المتطلبات الطبية. وأظهرت النتائج وجود أنواع النيتروجين التفاعلي والأكسجين التفاعلي في طيف خط الانبعاث، وكانت ظروف التشغيل: معدل تدفق (6 أو 10 وحدات بلازما) ودرجة حرارة (32 أو 40 درجة مئوية) على التوالي. كما كشفت الملاحظات السريرية عن التئام مبكر وكامل للجروح المعالجة بالبلازما بجرعات مختلفة مقارنةً بالمجموعة الضابطة. كما أظهرت نتائج التقييم النسيجي المرضي وجود تسلل التهابي بين المجموعات، متمثلاً في زيادة الالتهاب في مجموعتي العلاج (أ، ب) مقارنةً بالمجموعة الضابطة (ج)، مع اختلاف معنوي وبدرجة ($P<0.001$) وكشفت المراقبة الكيميائية الحيوية عن عدم ظهور أي تأثير ضار في عينات الدم المستخدمة في الاختبار خلال فترة التجربة (3، 7، و21) يومًا. وبناءً على ذلك، تُعد ظروف التشغيل المستخدمة آمنة للتطبيقات الطبية الحيوية، وتُسرّع من عملية التئام الجروح الجلدية.

الكلمات المفتاحية: البلازما الفيزيائية، جرعة البلازما الفيزيائية، والتئام الجروح.

The Efficacy of High Dose of Cold Physical Plasma on Healing Process of Skin Wounds

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Abstract:

The goal of the research is to evaluate the effectiveness of a physical plasma device in treating skin wounds in dogs, and to study the differences in the effects of plasma radiation. Also, the significance of the research is the use of the latest scientific methods and cast iron, and the impact of cold plasma physics on virtually no public health outcomes, is used to cover special cases, representing 99% of the universe. The study has adopted the descriptive analytical approach to study the research problem to reach results and solutions.

As well as, evaluations for clinical, histopathological and biochemical test evaluations were based on the changes of treated wounds in response to cold physical plasma treated compared to control group. Helium plasma jet in this research, was used two different operating condition (plasma power density and dose) that meets medical requirements. The results showed the presence of reactive nitrogen and reactive oxygen species in the emission line spectrum as well the operating conditions were: a flow rate of (6 slm or 10 slm) with a temperature of (32 °C or 40 °C) respectively. In addition, Clinical observations revealed early and complete closure of plasma-treated wounds of different doses in comparison with control group. Also, the results of the histopathological scoring were in inflammatory infiltration between groups represented by increased inflammation in treatment groups (A& B) than control group (C) with significant difference ($P<0.001$), and biochemical observation revealed no toxic effect appear in blood samples used in test during experiment period (3,7 and 21) days. As, conclusions, the operating conditions used were regarded as safe for biomedical applications and accelerating the healing process in skin wounds.

Keywords: Physical plasma, physical plasma dose, wound healing.,

Introduction:

Open wounds healing had been considered as the treatment of choice for centuries (Demaria, et al, 2011), severe skin injury, can be a-life-threatening. While, the repair process contains the interaction of cells, growth factors, and cytokines, included in wound closure (Tottoli, et al, 2020). As well, several Factors that interfere with wound healing may be divided by source into physical, endogenous, and exogenous categories. Diabetes has been considered as one of the most emerging disease condition in both human and companion animals, and is one of the common endocrinopathy of dog, it can cause impairment of healing process (Winkler, 2024).

Plasma is one of the four fundamental states of matter, first systematically studied by Irving Langmuir in the 1920s, that, it consists of a gas of ions – atoms or molecules which have one or more orbital electrons stripped, and free electrons (Heinlin, et al, 2011). So, Non-thermal plasma jet device is a newly multi-displimentary strategy that benefits from cold physical plasma's chemical and physical properties. It has medical value, like disinfection, sterilization, decontamination, acceleration of wound healing, and tissue regeneration, in addition to the low cost and safety of non-thermal plasma therapy. CAP is used another area of interest, the surface modification ability of cold physical plasma to revealing a potential improvement in the osseointegration of dental implants. Aa well as, there are other areas within dentistry used to study CAP such as surface bleaching, disinfection, and cavity preparation (Silva, et al., 2023).

Aims of the study are: Evaluation of non-thermal plasma jet role in acute open wound healing experimentally induced in dogs. Creation of treatment conditions and cold physical plasma dose suitable for healing process, and, studied the effect of usage of high dose of cold physical plasma on healing process.

Methods: Nine dogs were devided to three equal groups (A) used He flow rate (4 slm), and (B) used He flow rate (6 slm), as treated groups, while

(C) as control group. Surgical wounds were made under the effect of general anesthesia to create 3x3cm full-thickness wounds in all groups, each dog subjected to 4 skin wounds. The wound healing process was evaluated clinically and wound contraction measurements with their ratio by Scientific Image J software analysis, also, Histopathologically in consequence periods (3 ,7 and 21 days) post-operation. Also, Explanation of used scale in the semi-quantitative evaluation of histological sections (sultana et al, 2009). Also, the histopathological samples were stained by H&E; Masson's Trichrome stains. As well, statistical analysis was used to analyzed the data by the following software, Microsoft excel, Minitab v17, and IBM SPSS V26. The results reported in this study were expressed as mean +_ SE. Student Independent t-test to test the means between two groups. One-way analysis of variance (ANOVA) were used to test between groups or days after treatment.

The plasma treatment plan: All wounds twice directly exposed to the atmospheric pressure plasma jet (N-APPJ) each $1cm^2$ for 60 sec. at zero and first day post wounding. The operating conditions Cold physical plasma jet (CPP J) or N-APPJ operating Conditions and cold physical plasma dose was used in the experimental study for dog model were in table 1:

Distance between the nozzle of plasma jet and the skin wound	15 mm	
Gas flow rate	6 slm	slm 4
Helium (He) plasma temperature	(32)◦C	(41)◦C
The power density	(44)mWatt/cm^2	(50)mWatt/cm
Plasma radiation energy dose	(76) J/cm^2	(95) J/cm^2

Results: The results of Clinical observation of all groups: All animals showed equitable viability, good appetite. no marked differences in body temperature, no mortality, and no signs of infection. While, clinical observation of control group (C): Shows signs of restlessness reflected by rubbing their back with the ground, its severity gradually decreased as the wound continued to heal. The wounds appeared more blood oozing, complete closure had been found in (30-35) days post wounding. In treatment groups (A& B) the dogs were appeared with Less blood oozing wounds, minimal signs of pain, and complete closure was found in plasma treatment group (NT) (25-30) days post wounding. The study, investigated the role of cold atmospheric plasma (CAP) in reducing itching and pain, atopic eczema, scars, and epidermal barrier defects (ichthyosis), in addition to an essential role of CAP in accelerating the healing process. but the mechanism of the plasma jet effect in reducing itching or pain was not studied even it was proven clinically (Breathnach, et al, 2018). Furthermore, Clinical observations revealed early and complete closure of plasma-treated wounds in dogs compared with control wounds. There were significant changes between treatment groups (A&B) and (C)in ($p< 0.05$) at 21 days post wounding, in comparison with 30 days, the difference was significant ($P< 0.001$) in treatment groups (A&B) in comparison with (C), that appeared in table 2.

Table2: shows wound area measurement in all groups during days 3,7, 21and 30 days by medical imaging (image J):

TIME-days	Groups\ A	B	C	P-value*
0	972.4_+ 68	966.8_+ 54	961.2_+ 51	0.858
3	1178.2_+94.6	1133.4_+69.7	1283.5_+66.2	0.375
7	892.0_+137	959.3_+78.1	993.0_+141	0.329
21	84.1_+5.59	43.1_+26.4	202.0_+104.0	0.039*
30	7.8_+13.9	2.4_+17.1	72.0_+37.5	0.001***

Data presented as mean± SD, One-way anova were used to test between groups, *, **, *** significant ($P<0.05$), highly significant ($P<0.01$), very high significant ($P< 0.001$) respectively.

The wounds of all groups (A, B and C), macroscopically shows full thickness the 3x 3cm wounds. In period 0,3,7,21,30 days P.O. At **3 days** (C) group shows redness of the wound edges**,** exudation at wound bed**.** At (A): group wound, with lesser signs of inflammation (pale wound edges), area of coagulated blood in the wound bed, while, at (B) group wounds, shows redness of the wound edges and area of coagulated blood in the wound bed scab formation. At **7 days**, in (C) beginning of granulation tissue formation. In (A) the wound is filled with granulation tissue**,** and in (B) the wound is filled with granulation tissue**.** At **21 days** (C), area of scar tissue formation surrounding the wound area, (A), scar formation occupying wound area, and the wound bed is smaller in size. (B), scar formation occupying wound area, and the wound bed is smaller in size. NOTE: the scar tissue area in (A) was smaller than (B). At **30 days**, (C) scar tissue formation, the wound not completely closed. (A), complete closure with scar tissue formation**,** and (B) complete closure with scar tissue formation.

The results of the histopathological scoring were in table (3), that, the inflammatory infiltration between groups represented by increased inflammation in treatment groups (A&B) than control group, with non-significant difference at 3 days post wounding, while, at 7 days the inflammation decreased in (A&B) than control group non-significance, Whereas, at (21) days the inflammation was reduced in all groups with non-significant difference. Therefore, the amount of granulation tissue had increased all C and (A&B) groups, at 3 days PO., but there was a significant increase between groups at ($p< 0.05$), while, at 7 days the granulation tissue was increased in all groups with non-significant difference. So, in 21 days, high significant changes were found between groups (at $p<0.01$). while, the amount of early collagen fibers at 3 days PO was increased in treated groups while in C was minimal, there was a significant difference between C & (A) at ($p< 0.05$). So, at 7 days, it was increased in all groups with significant difference at ($p< 0.05$). As well as, 21 days, it was decreased in all groups with significant changes at (p<

0.05). where, the amount of mature collagen fibers had minimal increased in treated groups at 3 days PO., there was significant difference between C&A groups at ($p< 0.01$). At 7 days there were significant changes between all groups at ($p< 0.05$), As well, it increased at 21 days, there were significant changes between all groups at ($p< 0.05$).

Table 3 : Shows results of scoring evaluation of histological sections, between groups during wound healing stages , using one way ANOVA

days	Histological parameters	C \mean_+ SD	A \mean_+ SD	B \mean_+ SD	P value*
3	Inflammatory infiltration	1.50_+0.50	1.00_+0.00	1.25_+0.50	0.134
	P value*	0.543	0.430		
	Amount of granulation tissue	2.25_+0.50	1.50_+0.58	1.75_+0.50	0.024*
	P value*	0.024*	0.430		
	Amount of early collagen	2.00_+0.00	1.25_+0.50	1.25_+0.50	0.442
	P value*	0.024*	1.00		
	Amount of mature collagen	----	3.00_+0.00	3.00_+0.00	1.50
	P value*	a	1.00		
7	Inflammatory infiltration	1.75_+0.50	2.00_+0.00	2.00_+0.50	0.422
	P value*	0.365	1.00		
	Amount of granulation tissue	1.50_+0.58	2.00_+0.00	1.75_+0.50	0.154
	P value*	0.134	0.442		
	Amount of early collagen	1.25_+0.50	2.00_+0.00	2.25_+0.00	0.036*
	P value*	0.024*	0.365		
	Amount of mature collagen	3.00_+0.00	2.00_+0.00	2.00_+0.00	0.024*
	P value*	0.050*	1.00		
days	Histological parameters	C \mean_+ SD	A \mean_+ SD	B \mean_+ SD	P value*
21	Inflammatory infiltration	2.75_+0.50	3.00_+0.00	3.00 _+0.58	0.134
	P value*	0.375			
	Amount of granulation tissue	2.75_+0.50	3.75_+ 0.50	3..75_+0.00	0.024*
	P value*	0.002**			
	Amount of early collagen	2.75_+0.50	3.75_+ 0.50	3.75_+ 0.00	0.024*
	P value*	0.030*			
	Amount of mature collagen	2.00_+0.50	1.25_+0.50	1.00_+0.50	0.30*
	P value*	0.024*	0.430		

Data presented as mean± SD: One-way **ANOVA** were used to test between groups, *, **, *** significan
(b) is highly significant ($P<0.01$), (a) is equal to very high significant ($P< 0.001$) respectively.

stain). In addition, in group (B), thin tongue of epithelial cells under inflammatory cells, moderate vascular granulation tissue by (H & E stain). At 7 days PO. histological sections showed in group C, inflammatory cells, immature granulation tissue, highly vascularization, collagen fibers deposition (M.T. stain). In group (A), shows granulation tissue, dense blue color collagen fibers at the deep part by (M.T. stain). So, in group (B), shows vascular granulation tissue, profound early collagen fiber, epithelial layer by (M.T. stain). Therefore, at 21 days PO. In group C, shows complete epidermal layer with small rete ridge, mature granulation tissue by (H & E stain). So, in group (A) shows, full thickness epidermal layer, well-formed rete ridge, and mature granulation tissue by (H & E stain). As well, in group (B), full thickness epidermal layer with reteridge over mature granulation tissue, formation hair follicle and sweet glands by (H&E stain).

Discussion: The results of previous study (Al-Qaseer et al, 2021) showed the presence of reactive nitrogen and reactive oxygen species in the emission line spectrum as well the operating conditions were: a flow rate of (4 slm or 6 slm), this results match the results of this study that, revealed the efficiency of helium plasma jet as generating cold physical plasma, also the effectiveness of treatment conditions of plasma jet used in the study.

In this study the clinical observations found, the same with study (Busco et al, 2020) they assumed that, CAP is a-dose-dependent therapy for endothelial cell-mediated angiogenesis, and used to increase proliferation of endothelial cells, fibroblasts and keratinocyte cells wounding, also, they mentioned that, the no beneficial effects in wound healing that had been attributed to its functional action on angiogenesis, inflammation, cell proliferation, matrix deposition, and remodeling. Another study (Martinez, et al, 2019) discovered that helium CAP treatment decreased the pH in the aqueous medium, which may lead to wound acidification and, as a result, promotes the healing process.

In histopathological results demonstrated the modulation effect of NAPPJ in inflammatory stage of healing process in treatment groups in compression control group and there is minimal difference between treatment group A&B because the plasma dose is different and radiation intensity also helium gas temperature, that inflammation become same at 7 days PO. Between A&B group. Research study the effect of NTPJ on the macrophage, which was done by (Kubinova, et al, 2017) who suggested that Macrophage infiltration was not affected by CAP treatment into the wound and in gene expression analysis on day 3 post skin excision, while they found that inflammatory marker has transient up-regulation on day 7 after NTP treatment, also they found that CAP treatment improved the healing efficacy of acute skin wounds without causing side effects or activating pro-inflammatory signaling. As well, the study by (Dang, et al, 2021) who study non-thermal argon-based plasma flux effect on macrophage and in mouse models of burn wounds with or without Staphylococcus aureus infection, they were found that plasma flux enhanced ROS, and induced anti-inflammatory macrophages also, promoted the burn-wound healing process partly through the decrease in macrophage NF-κB. also, NTP improved the migratory function of fibroblasts, instead of, fibroblast proliferation. Therefore, this finding match the finding of this research, that, there were early fibroblast proliferation and collagen deposition and faster maturation of collagen in wound bed which demonstrated macroscopically and microscopically in current study. Also the clinical observation suggests the reduce the area of scar in treatment groups A than B.

In this study the histopathological sections shows, full thickness epidermal layer, well-formed rete ridge, and mature granulation tissue, and formation hair follicle and sweet glands these data were supported the previous findings by (Vandersee et al, 2014), who stated that plasma-treated wounds had an earlier onset of the proliferative phase, resulting in accelerated wound healing. Also, (Breathnach, et al, 2018) demonstrated that, the giant cell infiltrates were thought to be caused by endogenous

foreign material at the biopsy site (e.g., hair fragments),The plasma-treated group had more biopsies with moderate giant cell infiltrates than the control group. Furthermore, (Fathollah, et al, 2016) revealed that in the histological image at (7) days after NTP treatment, there was the formation of the epidermis after plasma treatment at ($P<0.01$) from the control group, neo-vascularization largely in the dermis layer, and granulation tissue filled the incision space with collagen production. In addition, (Wang, et al, 2020) the study by (Al-OKLA et al, 2021), who mentioned that the CAP stimulates keratinocytes and fibroblasts, resulting in faster cell proliferation and migration, which can shorten the wound healing process, through activation of cytokines and growth factors synthesis like transforming growth factors (TGF-ß1/2) and alpha-smooth muscle act.

In the histological section in the skin of at 21 days post wounding, revealed that, the regenerative effect of CAP had appeared by the formation of hair follicles. Studies had reached the same results (Chatraie et al, 2018) suggested that at day 21, a new epidermis layer had constructed over all wounds, but the epidermis in the treated wounds was noticeably thicker than in the control wounds. They also discovered that in plasma-treated animals, new hair follicles and sebaceous glands formed in the epidermis and dermis layer, whereas these structures were missing in control animals. However, (Lou, et al, 2020) who was investigated recently, The molecular mechanism by which the He-Ar-CAPJ induces keratinocyte migration, which is a key step in cutaneous wound healing, indicated that the He-Ar-CAPJ generates high levels of ROS and RNS for reducing E-cadherin and activating p-ERK, cyclin-D1, and Cdk-2 expression, all of which are important factors for EMT and cell proliferation, and provokes keratinocyte migration, resulting. Moreover, (Wang, et al, 2020) revealed that, when to compare between all the wounds of both NTP treated and control groups on day 21, the scars were totally and utterly epithelialized, but the scar diameter in the plasma group was not only smaller ($p<0.05$), but the scars also had better re-epithelialization than the control group. Also, another study used CAP for wound

treatment, they found healthy epidermis and skin accessory structures, and collagen fibers (Tan and Zuoren, 2020).

CONCLUSIONS:

- The necessity of using artificial intelligence (AI) in simulating surgical operations and the importance of using it as a program to measure the area of wounds as fast and accurate tool. AI used as program to determine the shape of cells in pathohistological examination for more accuracy.
- The results demonstrated the efficiency of cold physical plasma with highly reactive oxygen and nitrogen species that has potent multifunctional agents during tissue regeneration.
- The effectiveness of treatment conditions and plasma doses (for acceleration of wound healing, wound contraction, and tissue regeneration, as well as complete wound closure in all treated wounds.
- The results revealed that the N-AP-He-PJ could improve the quality and the timing of healing stages of acute wounds. In addition to its simple composition, fast, low cost device.
- 4. The clinical and wound contraction measurement results detected the effectiveness of physical plasma therapy in reducing wound closure time, and postoperative complications such as itching. In addition, the pro-coagulant effect of plasma jet was clear in treatment wounds.
- The histological analysis suggested that an a-physical plasma jet has supported the regenerative effect of plasma treatment through modulating the inflammation process, increasing angiogenesis, re-epithelization, new hair follicles, fibroblast proliferation and collagen fiber deposition as well as its maturation.
- The results revealed the safe usage of high dose of cold physical on general health and positive effect of plasma on healing process.

Refrences:

1. (AL-OKLA, S., Souad, M.,Al Nazwani, S., Al-Mudarris, F. (2021). Review Article; Overview of Cold Atmospheric Plasma in Wounds Treatment. Med. Clin. Res. J. V; 5,I: 10.,280.)
2. (Al-Qaseer, Sh.M, Khalaf, M.K., Salih, S.I. (2021-A) Optimal Power of Atmospheric Pressure Plasma Jet with a Simple DBD Configuration for Biological Application. J. Phys.: Conf. Ser. 1999- 012058.)

3. (Breathnach, R., McDonnell, K.A., Chebbi, A., Callanan, J.J. and Dowling, D.P. (2018) Evaluation of the effectiveness of kINPen Med plasma jet and bioactive agent therapy in a rat model of wound healing. J Bio.Inter.phases Vol(13), iss(5)1.5046489.)
4. (Busco,G., Eric Robert, E. and Chettouh-Hammas, N. et al., (2020). The emerging potential of cold atmospheric plasma in skin biology. Free Radical Biology and Medicine J. Vol. 161, 290-304.)
5. (Chatraie, M., Torkaman, G., Khani, M., Salehi, H., Shokri, B. (2018). In vivo study of non-invasive effects of non-thermal plasma in pressure ulcer treatment. J. of Sci. Rep. 4;8(1):5621.)
6. (Dang, C.P., Weawseetong, S., Charoensappakit, A., Sae-Khow, K.,Thong-Aram, D.and Leelahavanichkul, A. (2021). Non-Thermal Atmospheric Pressure Argon-Sourced Plasma Flux Promotes Wound Healing of Burn Wounds and Burn Wounds with Infection in Mice through t).
7. (Demaria, M. and Stanley, B. J. (2011). Effects of Negative Pressure Wound Therapy on Healing of Open Wounds in Dogs. Vet. Clin.s of Nor. Amer. Small Anim. Prac.J. 658-669.)
8. (Fathollah S, mirpour, S., mansouri, P., dehpour, A.R.,et al. (2016). Investigation on the effects of the atmospheric pressure plasma on wound healing in diabetic rats. Sci. Rep.;6:19144.)
9. (Heinlin, J., Isbary, G., Stolz, W., and et al., (2011).Plasma applications in medicine with a special focus on Dermatology. J. Eur. Acad. Dermatol. Venereol. 25(1), 1-11.)
10. (Kubinova, S., Zaviskova, K., Uherkova, L., and et al., (2017). Non-thermal air plasma promotes the healing of acute skin wounds in rats, Nature Research. J. of Sci. Rep. V.(7), no: 45183.)
11. (Lou, B-Sh., Hsieh, J-H., Chen, C-M., and et al., (2020). Helium/Argon-Generated Cold Atmospheric Plasma Facilitates Cutaneous Wound Healing. Front. Bioeng. Biotechnol. J. 8: 683.)
12. (Martinez, L.G., Dhruv, A., Lin, L., Balaras, E. (2019). Interaction between a helium atmospheric plasma jet and targets and dynamics of the interface. J. of Plasm Sources Sci. and Technol. 28 (11), 2411-2502.)

13. (Silva, N., Marques, J. and Brito da cruz, M., (2023). The applications of cold atmospheric Plasma in Dentistry. Plasma Processes and Polymers. 20(12).)
14. (Sultana, J., Molla, M.R., Kamal, M., Shahidullah, M., Begum, F., Bashar, Md., (2009). Histological differences in wound healing in Maxillofacial region in patients with or without risk factors. Bangladesh J. Pathol. 24 (1): 3).
15. (Tan, F. and Zuoren, Y.Z., (2020). Synergy between Cold Atmospheric Plasma and Acidic Fibroblast Growth Factor during Wound Healing, Angiogenesis, Neurogenesis, and Osteogenesis. Authorea J. (10).41.1-28.)
16. (Tottoli, E.M., Dorati, R., Genta, I., Chiesa, E., Pisani, S. and Conti, B. (2020). Skin Wound Healing Process and New Emerging Technologies for Skin Wound Care and Regeneration. Pharmaceut. J. 5;12(8): 735.)
17. **(Vandersee, S., Richter, H., Lademann, J., and Lange-Asschenfeldt, B. (2014). Laser scanning microscopy as a means to assess the augmentation of tissue repair by exposition of wounds to tissue tolerable plasma. Vol 11(11), ID: 115701.)**
18. (Wang, X-F., Fang, Q-Q., Jia, B., Hu, Y-Y., and Tan, W-Q. (2020). Potential effect of non-thermal plasma for the inhibition of scar formation: a preliminary report. Sci. Rep. 10: (1)1064.)

(Winkler, K.P., (2024). Factors that Interfere with Wound Healing in Small Animals. MSD veterinary manual book, chapter; wound manegment of small animals.)