https://doi.org/10.33472/AFJBS.6.13.2024.4149-4165

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

STUDY THE EFFECT OF ELECTRIC STIMULATION IN WOUND HEALING ACTIVITY USING HYDROGEL CONTAINING BECAPLERMIN AND SERRATIOPEPTIDSE.

A.R. Patil[1], Dr.S.S. Nipate[2]*, Ms.P.K. Hajare[3], R. D. Dighore[4]

[1]Department of Pharmacology, P.E.S. Modern College of Pharmacy, Nigdi, Pune-44 Maharashtra, India.

[2*]Professor, Department of Pharmacology, P.E.S. Modern College of Pharmacy, Nigdi, Pune 44 Maharashtra, India.

[3]Assistant professor,
Department of Pharmacology, P.E.S. Modern College of Pharmacy, Nigdi, Pune-44 Maharashtra, India.

[4]Department of Pharmacology, P.E.S. Modern College of Pharmacy, Nigdi, Pune-44 Maharashtra, India.

Email: animeshp441@gmail.com, pratikshahajare796@gmail.com, rdighore10@gmail.com
Corresponding Email: sonynipate@rediffmail.com

Declaration of interest

Authors declares no conflict of Interest

Article Info

Volume 6, Issue 13, July 2024

Received: 04 June 2024

Accepted: 05 July 2024

Published: 31 July 2024

doi: 10.33472/AFJBS.6.13.2024.4149-4165

ABSTRACT:

A wound is caused by damage to the skin's epithelium, and an infection results from the wound being exposed to microorganism-filled external environment. In recent research, we investigate the effect of low intensity electric stimulation along with the hydrogel of Becaplermin and Serratiopeptidase for the treatment of wound. Electric stimulation accelerates the activation of growth factors like vascular endothelial growth factor, transforming growth factor, fibroblast growth factor, keratinocytes growth factor involved in wound healing. Becaplermin selectively binds to PDGF receptors on fibroblasts and promotes the migration, proliferation, and production of extracellular matrix components like collagen. Protease, also known as Serratiopeptidase, is a proteolytic enzyme. Through the hydrolysis of protein peptide bonds, it breaks down and dissolves inanimate materials that build up at the site of inflammation, blood clots, dead cells, and inflammatory debris. Angiogenesis and anti-inflammatory activity of Becaplermin and Serratiopeptidase protects the wound from microbial infection and oxidative stress. Combine treatment of electric stimulation along with hydrogel of Becaplermin and Serratiopeptidase increases growth factor stimulation, cell migration, fibroblast accumulation, collagen synthesis, angiogenesis which gives excellent wound healing activity. Above therapy shows good percentage of wound contraction and re-epithelization, which can do great revolution in treatment of chronic wounds.

Keywords: Electric stimulation, Becaplermin, Serratiopeptidase, Growth factor, Recombinant human platelet derived growth factor, wound healing

1. BACKGROUND

The largest sense organ in the human body, skin serves as our primary line of defence against a variety of wounds, including chemical and physical ones. An injury to the epidermis, or top layer of skin, is referred to as a wound. Wounds are caused by damage to the epidermis' tissues [1]. The various microorganisms found in the external environment, primarily aerobic bacteria like Streptococcus aureus, E. coli, Pseudomonas, and others, cause infection in wound patients [2]. Chronic wounds arise from long-term wound pain. Body damage from chronic wounds can be quite severe. A number of factors, including diabetes mellitus, inappropriate wound care, and severe microbiological infections, can lead to chronic wounds. Serious sepsis can occasionally result from a severe infection in order to stop the infection from spreading. This body component needs to be ampute [3].

Even though there are many different treatments are present, alternative wound therapy is still an option for improving wound healing. For that, electric therapy might be a better fit. Different types of electric current exist, including AC, DC, pulsed, and others [4]. To treat wounds, we employed low-intensity pulsating direct current in microampere [5]. DC stands for direct current, which is not continuous but rather unidirectional. The growth factor involved in wound healing is activated in part by electric stimulation [6].

Becaplermin is a recombinant platelet-derived growth factor (PDGF)-BB that is made when the Saccharomyces cerevisiae yeast carries the gene. It's been demonstrated that Becaplermin

speeds up wound healing. Becaplermin is the only FDA-authorized growth factor and one of only three biologics that are currently approved by the FDA for the treatment of diabetic foot ulcers. One of the most contentious sophisticated wound treatment products nowadays is Becaplermin [7]. Platelets, macrophages, endothelial cells, fibroblasts, and keratinocytes all generate endogenous PDGF. PDGF stimulates the chemotaxis of various cells, including as collagen and fibroblasts, as well as neutrophils and macrophages at the wound site. Moreover, PDGF promotes collagen crosslinking and remodeling [8]. This overall property of the Becaplermin can play great role in the process of wound healing.

Serratiopeptidase is an essential family of proteins and peptides generated by humans and other living organisms. Proteolytic enzymes are particular enzymes that break down proteins. Serratiopeptidase (SRP) is found in one of the intestinal bacilli of silkworms [9]. It has a number of functions, including strong anti-inflammatory, antiedematic, and bradykinin-decomposing properties. It also enhances the activity of antibiotic at the site of infection [10]. SRP is generally used as an enzyme-based anti-inflammatory medication, either by itself or in conjunction with other medications, to treat arthritis, bronchitis, atherosclerosis, and sinusitis [11]. Proteolytic enzymes kill necrotic and damaged cells by deactivating mediators and harmful substances. They also regulate the discomfort, edema, and promoting more effective wound healing [12]. Combination therapy can yield the excellent result than the single therapy. Therefore, we used hydrogel of Becaplermin and Serratiopeptidase along with electric stimulation to get effective result in wound healing.

2. METHODS

2.1 Equipment

For therapy, a device that outputs low intensity current at 200 µA and 400 µA (microamperes) of pulse DC type is utilized. Together with a display, batteries, control keys, and a pair of electrodes (anode and cathode), it is contained. The gadget has multiple keys that can be used for different functions, such as the ON/OFF key, ENTER key, time adjustment key, and current adjustment knob. The device has lithium batteries within, which allow for recharge. Electrodes for the treatment are positioned in between the wounds.

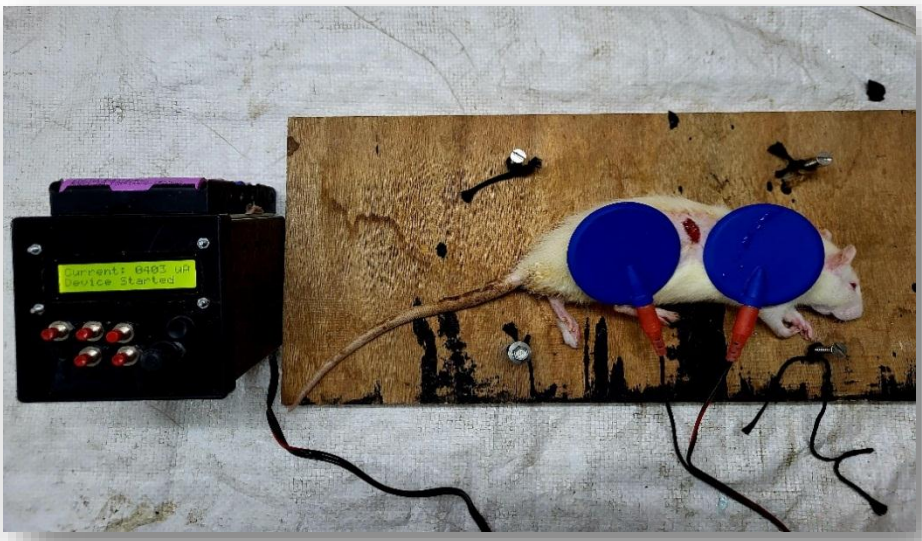


Figure 1- Dosing of animal using equipment.

a. Collection of API

1. Serratiopeptidase powder was received from the advanced enzyme technologies Pvt, Ltd, Thane, Maharashtra, India.
2. Becaplermin was procured from the Dr Reddy's Laboratories, Pvt, Ltd, Delhi, India.

2.3 Preparation of Formulation –

Table 1. Formulation of gel

INGREDIENT	CONCENTRATION
Carbopol 940	1 %
Xanthan gum	1%
Becaplermin	1%
Serratiopeptidase	2%
Dimethyl sulfoxide	0.50%
Benzalkonium chloride	0.25%
Water	QS

Weigh 1 gm of Carbopol and added it in 20ml of water, it kept away for 24 hrs for getting good swelling.1gm of Xanthan gum added was to 20ml of water and mix it properly with the stirrer. Add small amount this dispersion continuously in to the swelled Carbopol and mix it well with the help of mechanical stirrer then added required amount of water in it. Then added Becaplermin and Serratiopeptidase slowly with continuous mixing, using mechanical stirrer. Stirring is continue still getting proper gel formation. Then finally added Dimethyl sulfoxide and Benzalkonium chloride and mix it well [13].

2.4 Evaluation of parameter of gel -

- PH
- Viscosity
- Spreadability
- Diffusion test (Frans diffusion)

2.5 Experimental animal -

Wistar rats (180-200gm) of either sex was purchased from Crystal Biological Solutions, Pune, Maharashtra, India. The animals were kept in polypropylene cages under standard environmental conditions (25 ± 2^0C, 12hr light and dark cycle) with free access to standard pellet feed (VRK Nutritional solutions) and water.

2.6 Ethics Statement -

All experimental procedures carried out in accordance with the guidelines prescribed by committee for the purpose of control and supervision of experiments on animals (CCSEA) and were approved by institutional animal ethics committee (IAEC).

2.7 Animal Grouping -

Total 30 animals are used for the experimental model, there are five groups of animals i.e., are Disease control , Electric stimulation + STD (Hydro heal AM Colloidal silver), Electric stimulation, Hydrogel of Becaplermin and Serratiopeptidase and Electric stimulation + Hydrogel of Becaplermin and Serratiopeptidase. DC group is untreated, ES group is treated with plane electric stimulation (400 µA), ES + STD is treated with electric stimulation(200)

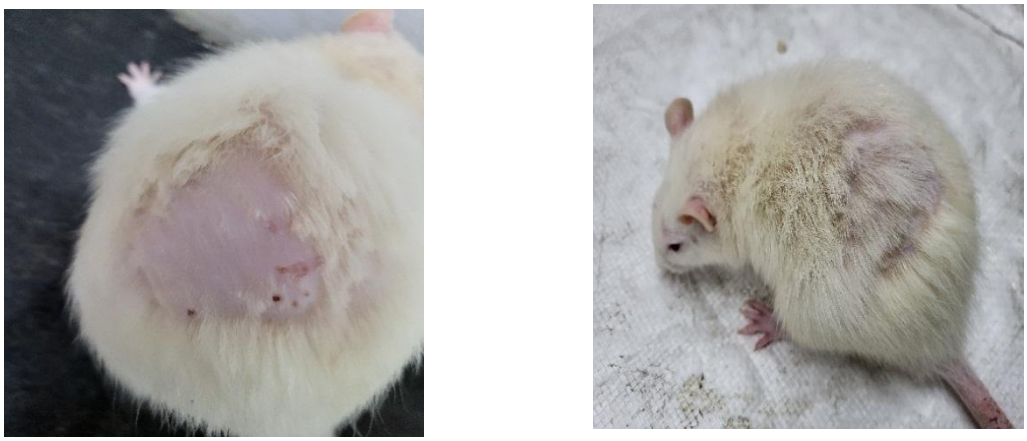
and hydrogel of colloidal silver (Hydro heal AM), HOBS is treated with plane Hydrogel of Becaplermin (1%) and Serratiopeptidase (2%) and ES(200 µA) + HOBS is treated with electric stimulation and Hydrogel of Becaplermin (1%) and Serratiopeptidase (2%).

2.8 Experimental model

All animals were anaesthetized using the ketamine 50 mg/kg (intraperitoneally) [14], Hair of the animal was removed using the electric trimmer. First hairs are trimmed to small size as possible by trimmer, after that remaining hairs were removed using the hair removal cream.

2.8.1 Skin irritation test

Acute dermal irritation test was performed using OECD guideline 404 with modification using two rats . The fur was removed by closely trimming the dorsal area of the trunk on different sites 24hr before the test. About 0.5/ml hydrogel of HOBS was applied to one sites and another site as used as control. Sites were observed critically at 1hr after removal of test substance. The observation was repeated at 24, 48, and 72hr, for days 7 and 14^{th} thereafter.

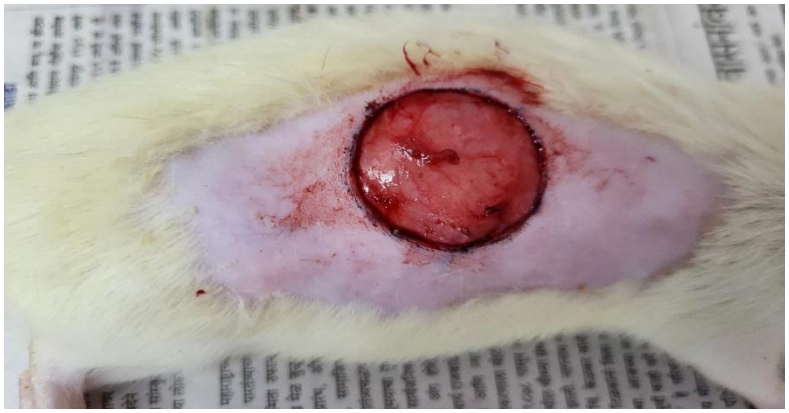


(Fig 2. Skin irritation test OECD -404)

2.8.2 Circular Excision Model

In this model circular wound of 500 mm^2 was made using the surgical scissor and forceps. Wound is formed from 1 cm away from the vertebral column. Firstly, the skin is sterilized with the help of 90% alcohol solution. After that circle of 500 mm^2 is drawn, then hold the skin with the help of forceps and was cut down. Excess of blood is cleaned with surgical cotton [15].

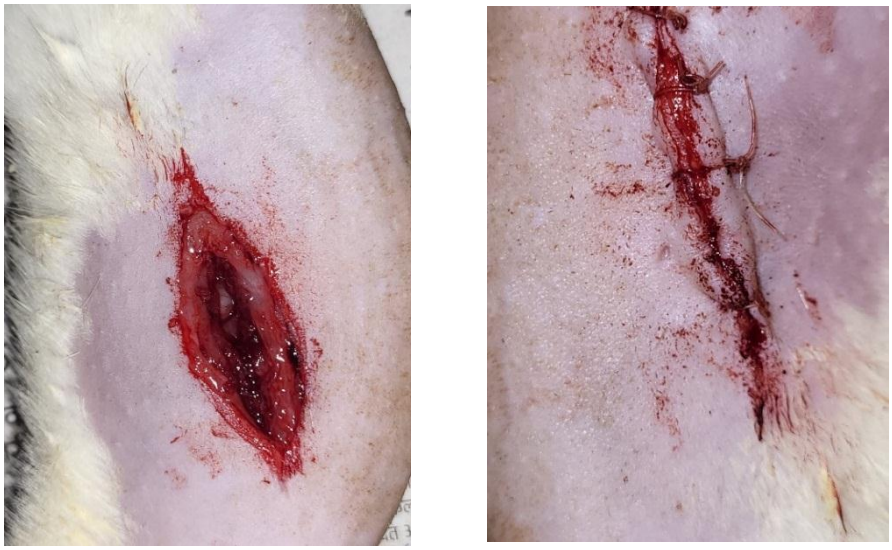
$$\% \text{ Wound contraction} = \frac{\text{Initial wound size - Specific day wound size}}{\text{Initial wound size}} \times 100$$ [16].



(Fig 3. Circular excision wound model).

2.8.3 Linear Incision Model

In this model linear incision of 5 cm length was made using the surgical blade. Wound is formed 2 cm away from the vertebral column. At First, skin was sterilized with the help of 90 % alcohol. Then skin was stretched so as to get perfect linear incision cut. Using the surgical blade and forceps deep cut was made up to the visceral layer of the skin. Excess of blood was cleaned with surgical cotton [17].



(Figure 4. Linear incision wound model)

2.9 Wound size measurement

2.9.1 Wound tracing

Daily wound was traced using tracing paper and randomly three diameters were selected and taking the average were taken. Using the formula of the circular area of wound contraction were calculated [18].

2.9.2 Photographs -

Daily wound photographs were taken for wound using a digital camera and wound contraction was observed [19].

2.10 Histology -

In excision wound models, a sample of skin tissues from the control, standard, and treatment groups was removed from the animals for histological analysis. The thin slices were cut and preserved in 10% formalin, and histological alterations such fibroblast proliferation, collagen deposition, tissue granulation, and neovascularization were examined under a microscope [20].

2.11 Statical Evaluation -

The results were expressed as mean ± SEM. Statistical analysis was performed using GraphPad Instat software, version 3.00 for Windows 95, San Diego, California USA. The values were analyzed by a one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test at a significance level of $p < 0.05$.

3. RESULTS

3.1 Evaluation parameters of hydrogel –
3.1.1.PH

Table 2. PH of Hydrogel -

Sr.no	Standard PH range	PH
1	4 to 6	5.1

3.1.2. Viscosity
Table 3. Viscosity of Hydrogel -

Sr.no	Standard viscosity range (cps)	Viscosity (cps)
1	2000-4000	3570

3.1.3. Spreadability
Table 4. Spreadability of Hydrogel -

Sr.no	Standard Spredability range (cm)	Spredability (cm)
1	5 - 7	5.8 / 15sec

3.1.4. Diffusion Test

Table 5. Diffusion test of the Hydrogel (PB - 6.8) -

Sr no	Time (min)	Absorbance
1	15	0.302
2	30	0.398
3	45	0.446
4	60	0.512
5	75	0.578

We applied 200 µA and 400 µA of pulse direct current (PDC) to the wound twice a day for five minutes each to get effective result in wound healing.

3.2 Circular Excision Wound Model
3.2.1 Percentage of area of wound contraction -
Circular wound of the 500 mm^2 area were made regular tracing of wound is done with help of tracing paper and taking the photographs of the wound. From the 4^{th} day wound started to contracts. The results of the percentage of wound contraction by circular excision model are presented in below table. The values presented in the table represents percentage of area of contraction at 4,7,11,15,19 and 21^{th} day for DC, ES + STD, ES, HOBS + ES and HOBS group. It was observed that wound contraction ability of animals healed with ES + STD, ES, HOBS + ES and HOBS group was found to be significantly higher than that of disease control group. However, ES(400µA) treated group shows higher percentage of wound contraction among all other group.

Table 6. Percentage of area of wound contraction

DAYS	DC	STD+ ES	ES	HOBS	HOBS + ES
4	1.69±0.30	31.46±1.76	34.60±0.79**	37.20±1.13**	38.02±2.51**
7	2.46±0.99	47.09±0.89	47.29±1.48**	51.31±2.01**	53.17±1.32**
10	4.03±0.01	70.12±3.11	71.96±2.57**	73.38±3.64**	75.43±2.63**
13	14.38±0.81	81.30±1.65	83.50±3.30**	87.80±2.10**	90.05±0.98**
16	28.59±1.72	91.61±3.25	93.54±0.98**	95.01±0.81**	97.21±0.65**
19	42.15±3.69	94.96±0.76	96.21±0.70**	97.10±0.37**	98.03±0.25**
21	72.39±7.01	97.20±0.64	97.93±0.51**	98.01±0.25**	99.67±0.79**

Values are expressed as mean± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple tests for comparison. #p<0.05, as compared to C; *p<0.05, **p<0.01.
DC- Disease control group, STD+ES – Standard+ Electric stimulation, ES – Electric stimulation, HOBS – Hydrogel of Becaplermin and Serratiopeptidase, ES + HOBS – Electric stimulation + Hydrogel of Becaplermin and Serratiopeptidase.

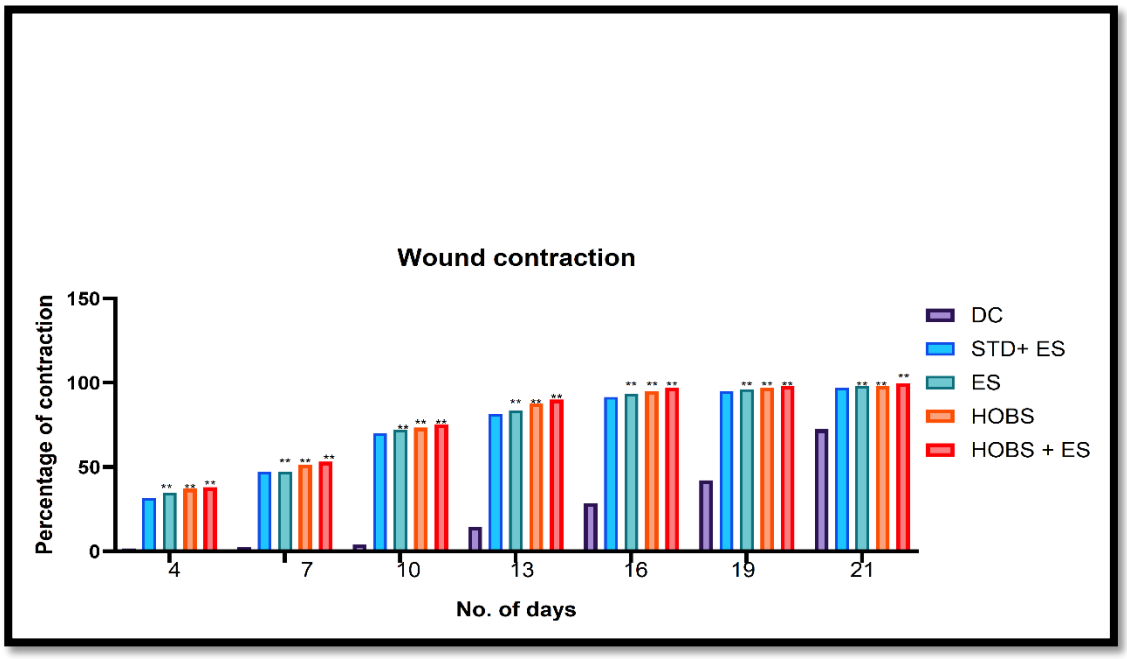


Figure 6 - Wound contraction of circular excision model.

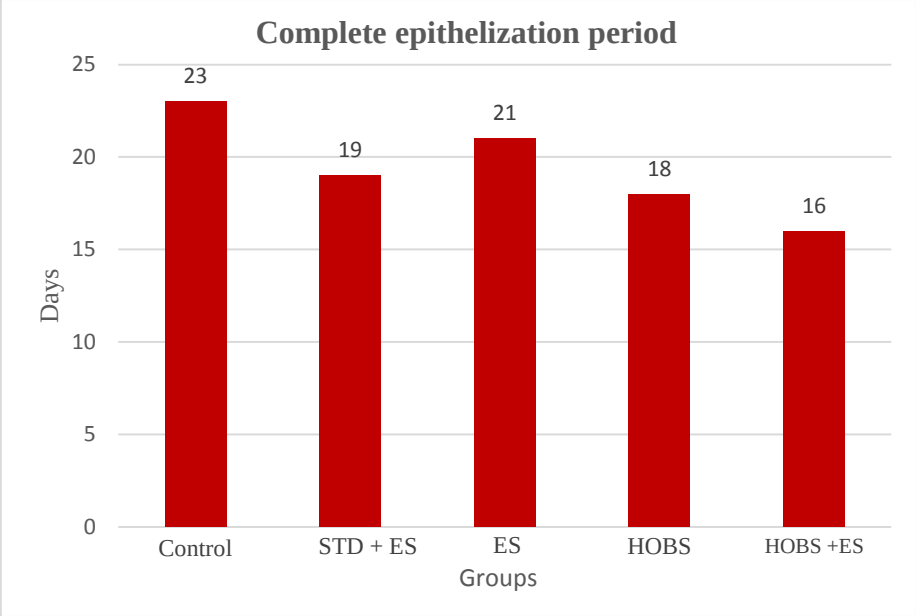


Figure 5. Percentage area of wound contraction.

Values are expressed as mean± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple tests for comparison. #p<0.05, as compared to C; *p<0.05, **p<0.01. DC- Disease control group, STD+ES – Standard+ Electric stimulation, ES – Electric stimulation, HOBS – Hydrogel of Becaplermin and Serratiopeptidase, ES + HOBS – Electric stimulation + Hydrogel of Becaplerin and Serratiopeptidase.

3.2.2 Complete epithelisation period

Complete epithelization is the time required for wound to heal completely. The result of complete epithelization period of circular excision wound model were presented in below table. It was observed that complete epithelization period of animals treated with ES + STD, ES, ES +HOBS and HOBS groups less than that of DC groups. DC group completely heal in 23 days, ES+ STD heal in 19 days, HOBS heal in 17 days, HOBS + ES heal in 15 days. However, plain ES treated group showed less epithelization period among all other groups.

Table 7. Complete epithelization period

Groups	Complete epithelization period (days)
Control	23
Standard + E. S	19
E. S	21
HOBS +	18
HOBS + E.S	16

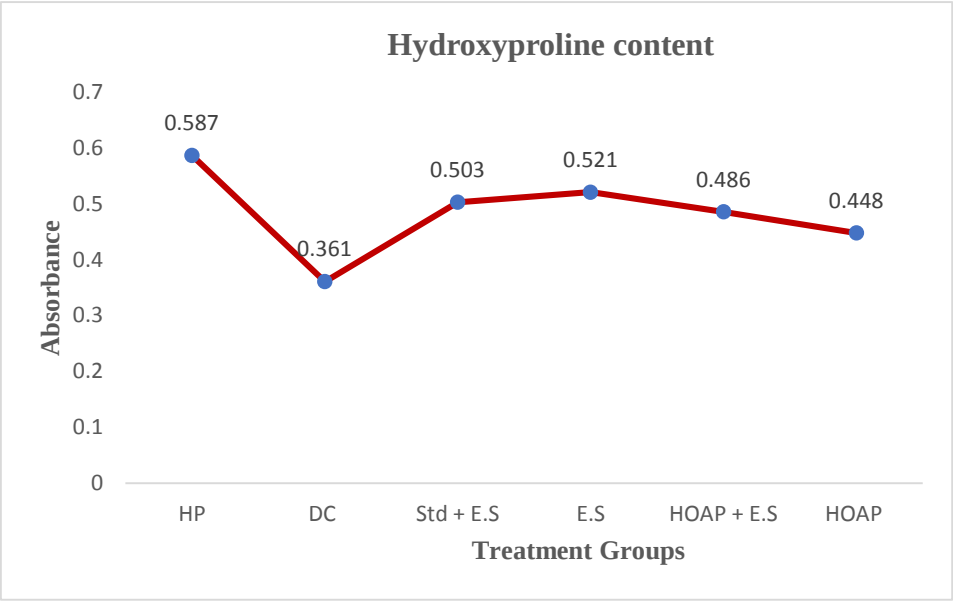


Figure 7. Complete epithelization period.

3.2.3 Hydroxyproline content

Hydroxyproline is the amino acid involve in collagen synthesis more amount of the HP increases the wound healing. In present study hydroxyproline content in treated group was found to be significantly increased as compare to the Disease control group. However, group treated with HOBS +ES shows increased Hydroxyproline content among all other groups.

Table 8. Hydroxyproline content

Sr no.	Groups	Absorbance
1	HPC	0.586
2	DC	0.360
3	Std + ES	0.448
4	ES	0.398
5	HOBS	0.503
6	HOBS+ ES	0.584

Figure 8. Hydroxyproline content

3.1.4 Histopathology -

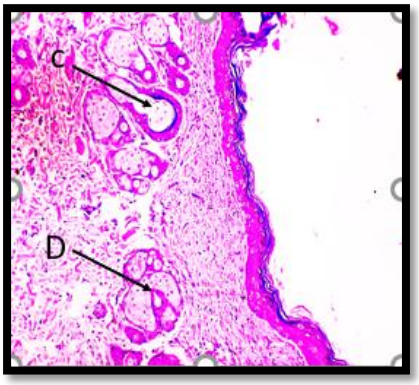


Fig 9 a- (NDC)

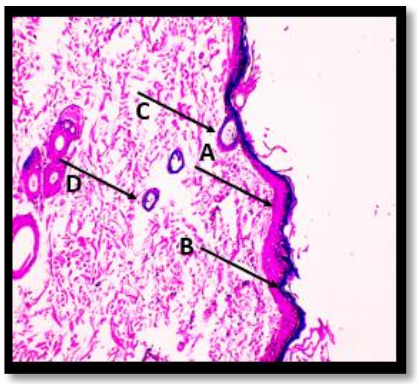


Fig 9 b- (E.S)

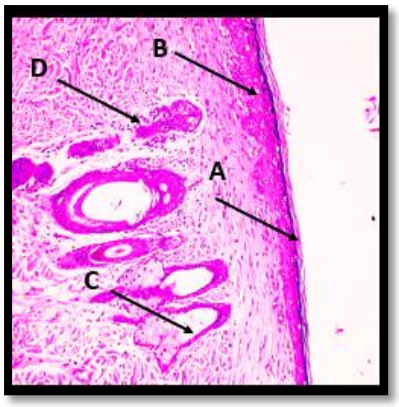


Fig 9 c- (STD + E.S)

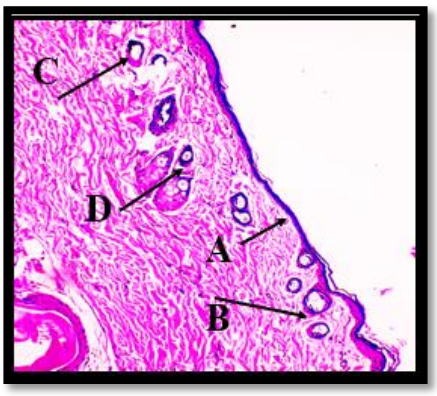


Fig 9 d- (Hydrogel of HOBS)

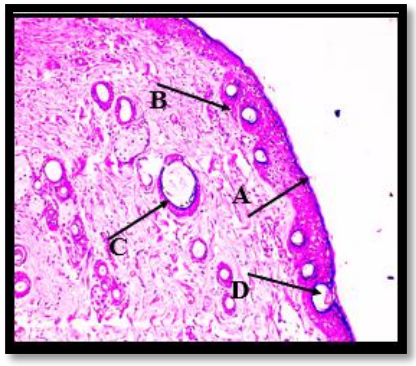


Fig 9 e- (HOBS +E.S)

Figure 9: Histopathological investigation of DC- Disease control group, ES- Electric stimulation, STD+ES – Standard+ Electric stimulation, HOBS – Hydrogel of Becaplermin and Serratiopeptidase, ES + HOBS – Electric stimulation + Hydrogel of Becaplerin and Serratiopeptidase.

Histopathological reports of the untreated group were characterized by the less collagen deposition and fibroblast proliferation. Reduced collagen deposition and fibroblast proliferation denotes poor wound healing. ES + STD, ES, ES +HOAP and HOAP treated groups shows significant tissue granulation **(a)**, neovascularization **(b)**, fibroblast proliferation **(c)** and collagen deposition **(d)** these leads to fast re-epithelization of tissue, which is the signal of marked wound healing.

Table 9. Evaluation parameters of histopathology

Sr.no	Histopath evaluation parameter	NDC	E.S	STD +ES	HOBS	HOBS+ES
1	Tissue granulation (a)	–	+	+	++	+++
2	Neovascularization (b)	–	+	+	++	+++
3	Fibroblast Proliferation (c)	+	+	++	+++	+++

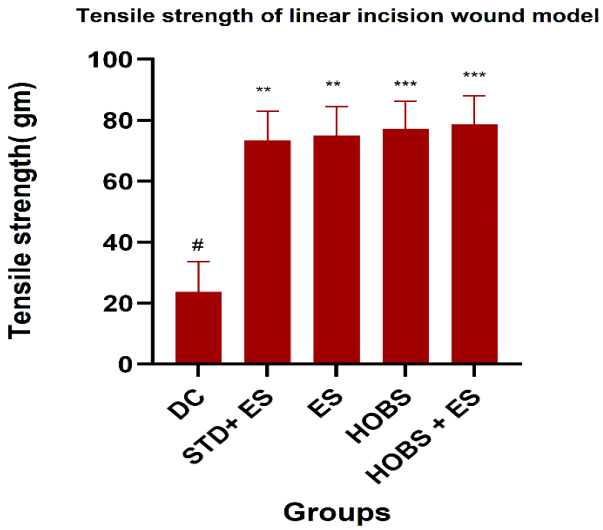
4	Collagen Deposition (d)	+	+	++	+++	+++

3.3 Linear Incision Wound Model

On 10th days stiches were removed with the scissor and tensile strength i.e., wound breaking strength was measured using the tensile strength. The apparatus result of tensile strength of linear incision wound model are presented below. It was observed that groups treated with ES+ STD, ES, HOAP +ES, HOAP showed more tensile strength than that of DC group. However, HOBS + ES healed group showed highest tensile strength among all other groups.

Table 10. Tensile strength of linear incision wound model

GROUPS	TENSILE STRENGTH
DC	231± 0.38
STD+ ES	398±0.69***
ES	399±1.40***
HOBS	445± 1.46***
HOBS +E.S	499±2.38***



(Figure 10. Tensile strength of linear incision wound model)

Values are expressed as mean± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Tukey's multiple tests for comparison. $p<0.05$, as compared to C; *$p<0.05$, **$p<0.01$, ***$p<0.001$.

DC- Disease control group, STD+ES – Standard+ Electric stimulation, ES – Electric stimulation, HOBS – Hydrogel of Becaplermin and Serratiopeptidase, ES + HOBS – Electric stimulation + Hydrogel of Becaplerin and Serratiopeptidase.

4. DISCUSSION

Prolonged wounds have the potential to cause significant harm to the body. Chronic wounds are mostly caused by diabetes mellitus, severe microbial infections, inadequate wound management, and other factors. These could result in sepsis, a severe infection that could cause the body to lose an organ [21]. Numerous therapies, including dressings, antibiotics, and antiseptics, are available for the treatment of wounds [22], [23]. Nevertheless, with the emergence of new obstacles to wound healing, such as medication resistance, there is still a great need for alternative therapies for wound infection. Different models exist for assessing

the rate of wound healing[24]. In written study the linear incision model and the circular excision model were applied. The stimulation of growth factors involved in wound healing, such as transforming growth factor (TGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and keratinocyte growth factor (KGF), were accelerated by low-intensity electric current, according to a review of the literature [25].

The rate of cell migration, proliferation, angiogenesis, collagen synthesis, and other processes are critical and process of wound healing accelerated when these growth factors are activated [26]. The human body naturally produces an electric field [27], but growth factors are greatly activated by mild external currents. There are various types of current, such as AC, DC, and pulsed. In this study we employed a low-intensity pulsed DC current, of 200 µA and 400 µA, which is a unidirectional and noncontinuous current applied for five minutes. For the treatment we selected the voltage of 9V as same voltage is used in the physiotherapy previously. As current flow from one electrode to another caused cell migration which helps in wound contraction [28]. It is found that group applied with current shows contraction in direction of flow of the current.

Enzymatic therapy can contribute to the body's natural inflammatory process, and in doing so, it may aid in and accelerate healing process [29]. Proteolytic enzymes are widely used as anti-inflammatory pharmaceuticals. Within this class, SRP, a metalloprotease generated by Serratiamarcescens, is widely used and offers superior pain and inflammatory situational control for wound healing [30].

Becaplermin has been shown to dramatically speed up wound closure and encourage the growth of granulation tissue throughout the healing process. Fibroblasts and endothelial cells, which are essential for wound healing activities including collagen production and angiogenesis, have been demonstrated to proliferate and migrate in response to it.

.

We have created a hydrogel with Becaplermin and Serratiopeptidase as a therapeutic agent, xanthan gum as a jellying agent, and carbopol as a polymer or crosslinker in the current study. Studies in the literature have shown that xanthan gum-containing gels readily conduct current [31] and have higher concentrations of water, which also conducts electricity [32]. Therefore, hydrogel that has been developed can form the bridge between the two electrodes which increases the rate of conduction of current through the wound area. As mentioned above proliferation, anti-inflammatory property of both the drugs protects the wound away from secondary infections. Therefore, we can propose that electric stimulation along with hydrogel of Becaplermin and Serratiopeptidase can lead to better wound contraction and re-epithelization in circular excision model and linear incision wound model.

In the circular excision model, the parameters used to evaluate the wound healing activity are wound contraction, tissue re-epithelization time, and hydroxyproline content [33]. The wound area will ultimately shrink in size, as shown in the percentage of wound contraction. On fourth day of therapy is there is an increase in wound contraction Observed. Re-epithelialization of the tissue is the time needed for the tissue to restore its original structure [34]. When collagen fibers are formed, hydroxyproline plays a crucial part. This amino acid, hydroxyproline, is necessary for the formation of collagen [35]. The study revealed that the treatment groups exhibited greater significance in terms of wound contraction, tissue re-epithelization time, and hydroxyproline content when compared to the control group. Additionally, rats treated with ES exhibited exceptional hair growth in incision forms. The considerable increase in hair growth is also proof that growth factors involved in wound healing are being stimulated. Since hair development is influenced by several of the growth factors involved in wound healing [36]. In linear excision model is done. Tensile strength of wound is the breaking strength of re-epithelized tissue [37]. Groups treated with electric stimulation and hydrogel of Becaplermin and Serratiopeptidase showed significant tensile strength which represents better wound

healing activity compared to control group. Neovascularization is the development of new blood vessels that trigger angiogenesis, which boosts blood flow to the wound site and quickens healing. Fibroblasts create collagen and other proteins that promote wound healing and regeneration. One of the key components of skin is collagen.
The study of histology revealed that the treatment groups exhibited notable neovascularization (angiogenesis), collagen deposition, fibroblast proliferation, and tissue granulation [38]. As the use of Electric Stimulation with electroconductive gel [39] reduced the period of wound contraction, causes, angiogenesis and collagen deposition leading to fast wound healing. Hence form the study, we can state that ES along with the gel containing Becaplermin and Serratiopeptidase containing growth factors possess wound healing activity.

5. CONCLUSION

The research study shed light on the hydrogel of Becaplermin and Serratiopeptidase and electric stimulation's amazing potential for wound healing. For the first time, this type of research was conducted employing hydrogel combinations containing Becaplermin and Serratiopeptidase, along with electric stimulation. The study's other key discovery is the effectiveness of Becaplermin and Serratiopeptidase together as an natural growth factor and enzyme combination in wound healing. Therefore, this research has the potential to significantly transform the field of wound healing treatment by establishing an alternative therapy for the activity of wound healing.

ABBREVATIONS
AC - Alternating Current
DC - Disease Control
DC - Direct Current
ES - Electric Stimulation
FGF - Fibroblast Growth Factor
rhPDGF – Recombinant human platelet derived growth factor B
HOBS - Hydrogel of Becaplermin and Serratiopeptidase
KGF - Keratinocytes Growth Factor
Min - Minute
PDC - Pulsed Direct Current
ROS - Reactive Oxygen Species
STD - Standard
TGF - Transforming Growth Factor
VEGF - Vascular Endothelial Growth Factor
SYMBOLS
µA – microampere
hrs - hours

6. REFERENCE

1. Abdo, J.M., Sopko, N.A. and Milner, S.M. (2020). The applied anatomy of human skin: A model for regeneration. *Wound Medicine*, 28, p.100179. doi: https://doi.org/10.1016/j.wndm.2020.100179.
2. Bowler, P.G., Duerden, B.I. and Armstrong, D.G. (2001). Wound Microbiology and Associated Approaches to Wound Management. *Clinical Microbiology Reviews*, [online] 14(2), pp.244–269. doi: https://doi.org/10.1128/cmr.14.2.244-269.2001.

3. Falanga, V., Isseroff, R.R., Soulika, A.M., Romanelli, M., Margolis, D., Kapp, S., Granick, M. and Harding, K. (2022). Chronic wounds. *Nature Reviews Disease Primers*, [online] 8(1), pp.1–21. doi:https://doi.org/10.1038/s41572-022-00377-3.
4. Houghton, P.E. (2014). Clinical Trials Involving Biphasic Pulsed Current, MicroCurrent, and/or Low-Intensity Direct Current. *Advances in Wound Care*, 3(2), pp.166–0446.183. doi:https://doi.org/10.1089/wound.2013.
5. Mehmandoust, F.G., Torkaman, G., Firoozabadi, M. and Talebi, G. (2007). Anodal and cathodal pulsed electrical stimulation on skin wound healing in guinea pigs. *The Journal of Rehabilitation Research and Development*, 44(4), p.611. doi:https://doi.org/10.16 82/jrrd.2007.01.0007.
6. Urabe, H., Akimoto, R., Kamiya, S., Hosoki, K., Ichikawa, H. and Nishiyama, T. (2021). Effects of pulsed electrical stimulation on growth factor gene expression and proliferation in human dermal fibroblasts. *Molecular and Cellular Biochemistry*, [online] 476(1), pp.361–368. doi:https://doi.org/10.1007/s11010-020-03912-6.
7. Papanas N, Maltezos E. Benefit-risk assessment of Becaplermin in the treatment of diabetic foot ulcers. *Drug Saf*. 2010;33(6):455–461.
8. Nagai MK, Embil JM. Becaplermin: recombinant platelet derived growth factor, a new treatment for healing diabetic foot ulcers. *Expert Opin Biol Ther*. 2002;2(2):211–218.
9. Vandana Gupte, Umesh Luthra (2017) Analytical techniques for Serratiopeptidase: A review -. J. Pharm. Anal., 7(4): 203-207.
10. Al-Khateeb TH, Nusair Y (2008) Effect of proteolytic enzyme serrapeptase on
a. Swelling pain and trisums after surgical extraction of mandibular third molars.
b. Int. J. Oral. Maxillofac. Surg., 37: 264-68.
11. Manju Tiwari (2017) The role of Serratiopeptidase in the resolution of inflammation. Asian Journal of Pharmaceutical Science, 12 (3): 209-215.
12. Chandanwale A, Langade D, Sonawane D and Gavai P (2017) A Randomized, Clinical Trial to Evaluate Efficacy and Tolerability of Trypsin: Chymotrypsin as Compared to Serratiopeptidase and Trypsin: Bromelain: Rutoside in Wound Management. Adv. Ther., 34(1):180-198.
13. Singh, H., Sarkar, B.K., Arya, J.C., Pal, S., Kumar, R., Gupta, V., Singh, R. and Verma, S.C. (2019). Phytochemical and Anti-Inflammatory Evaluation of Herbal Gel Prepared from Bark Extract of Mesua Ferrea Linn. *Journal of Drug Delivery and Therapeutics*, 9(5-s), pp.53–56. doi:https://doi.org/10.22270/jddt.v9i5-s.3638.
14. Linsenmeier, R.A., Beckmann, L. and Dmitriev, A.V. (2020). Intravenous ketamine for long term anesthesia in rats. *Heliyon*, 6(12), p.e05686. doi:https://doi.org/10.101 6/j.heliyon.2020.e05686.
15. Amritkar, A., Gaikwad, V., Pore, G., Kotkar, A., Nipate, S., Akanksha, P., Amritkar, Ashwini, C., Kotkar, Sonali, S. and Nipate (n.d.). EVALUATION OF GEL OF CARICA PAPAYA LATEX AND BOERHEVIA DIFFUSA LINN. LEAVES FOR WOUND HEALING ACTIVITY IN EXPERIMENTAL ANIMALS Section A-Research paper EVALUATION OF GEL OF CARICA PAPAYA LATEX AND BOERHEVIA DIFFUSA LINN. LEAVES FOR WOUND HEALING ACTIVITY IN EXPERIMENTAL ANIMALS. *Eur. Chem. Bull*, [online] 2023(12), pp.100–105. Available at: https://www.eurchembull.com/uploads/paper/0f2be9cb36fa497940215 49dd9 50aa46.pdf [Accessed 13 Sep. 2023].
16. Chen, L., Mirza, R., Kwon, Y., DiPietro, L.A. and Koh, T.J. (2015). The murine excisional wound model: Contraction revisited. *Wound Repair and Regeneration*, 23(6), pp.874–877. doi:https://doi.org/10.1111/wrr.12338.
17. Gamelli, R.L. and He, L.-K. (2003). Incisional Wound Healing: Model and Analysis of Wound Breaking Strength. pp.037–054. doi:https://doi.org/10.1385/1-59259-332-1:037.

18. Nagar, H.K., Srivastava, A.K., Srivastava, R., Kurmi, M.L., Chandel, H.S. and Ranawat, M.S. (2016). Pharmacological Investigation of the Wound Healing Activity of Cestrum nocturnum (L.) Ointment in Wistar Albino Rats. *Journal of Pharmaceutics*, 2016, pp.1–8. doi:https://doi.org/10.1155/2016/9249040.
19. Wendelken, M.E., Berg, W.T., Lichtenstein, P., Markowitz, L., Comfort, C. and Alvarez, O. (2011). Wounds measured from digital photographs using photodigital planimetry software: validation and rater reliability. 23(9), pp.267–75.
20. Gupta, A. and Kumar, P. (2015). Assessment of the histological state of the healing wound. *Plastic and Aesthetic Research*, [online] 2, pp.239–242. doi:https://doi.org/10.4103/2347-9264.158862.
21. Han, G. and Ceilley, R. (2017). Chronic Wound Healing: A Review of Current Management and Treatments. *Advances in Therapy*, [online] 34(3), pp.599–610. doi:https://doi.org/10.1007/s12325-017-0478-y.
22. Barrigah-Benissan, K., Ory, J., Sotto, A., Salipante, F., Lavigne, J.-P. and Loubet, P. (2022). Antiseptic Agents for Chronic Wounds: A Systematic Review. *Antibiotics*, 11(3), p.350. doi:https://doi.org/10.3390/antibiotics11030350.
23. Norman, G., Dumville, J.C., Mohapatra, D.P., Owens, G.L. and Crosbie, E.J. (2016). Antibiotics and Antiseptics for Surgical Wounds Healing by Secondary Intention. *Cochrane Database of Systematic Reviews*, (3). doi:https://doi.org/10.1002/14651858.cd011712.pub2.
24. Borda, L.J., Macquhae, F.E. and Kirsner, R.S. (2016). Wound Dressings: A Comprehensive Review. *Current Dermatology Reports*, 5(4), pp.287–297. doi:https://doi.org/10.1007/s13671-016-0162-5.
25. Kloth, L.C. (2005). Electrical Stimulation for Wound Healing: A Review of Evidence From In Vitro Studies, Animal Experiments, and Clinical Trials. *The International Journal of Lower Extremity Wounds*, 4(1), pp.23–44. doi:https://doi.org/10.1177/1534734605275733.
26. Goudarzi, I., Hajizadeh, S., Salmani, M.E. and Abrari, K. (2010). Pulsed electromagnetic fields accelerate wound healing in the skin of diabetic rats. *Bioelectromagnetics*, p.n/a-n/a. doi:https://doi.org/10.1002/bem.20567.
27. Zhao, M. (2009). Electrical fields in wound healing—An overriding signal that directs cell migration. *Seminars in Cell & Developmental Biology*, 20(6), pp.674–682. doi:https://doi.org/10.1016/j.semcdb.2008.12.009
28. Snyder, S., DeJulius, C. and Willits, R.K. (2017). Electrical Stimulation Increases Random Migration of Human Dermal Fibroblasts. *Annals of Biomedical Engineering*, 45(9), pp.2049–2060. doi:https://doi.org/10.1007/s10439-017-1849-x.
29. Chandanwale A, Langade D, Sonawane D and Gavai P (2017) A Randomized, Clinical Trial to Evaluate Efficacy and Tolerability of Trypsin: Chymotrypsin as Compared to Serratiopeptidase and Trypsin: Bromelain: Rutoside in Wound Management. Adv. Ther., 34(1):180-198.
30. Vélez-Gómez JM, Melchor-Moncada JJ, Veloza LA, Sepúlveda-Arias JC (2019) Purification and characterization of a metalloprotease produced by the C8 isolate of Serratiamarcescens using silkworm pupae orcasein as a protein source. Int. J. Biol. Macromol., 15(135): 97-105.
31. Kaith, B.S., Sharma, R. and Kalia, S. (2015). Guar gum based biodegradable, antibacterial and electrically conductive hydrogels. International Journal of Biological Macromolecules, 75, pp.266–275. doi:https://doi.org/10.1016/j.ijbiomac.2015.01.046.
32. Ageev, I.M. and Rybin, Yu.M. (2020). Features of Measuring the Electrical Conductivity of Distilled Water in Contact with Air. Measurement Techniques, 62(10), pp.923–927. doi:https://doi.org/10.1007/s11018-020-01714-2.

33. Nagar, H.K., Srivastava, A.K., Srivastava, R., Kurmi, M.L., Chandel, H.S. and Ranawat, M.S. (2016). Pharmacological Investigation of the Wound Healing Activity of Cestrum nocturnum (L.) Ointment in Wistar Albino Rats. Journal of Pharmaceutics, 2016, pp.1–8. doi:https://doi.org/10.1155/2016/9249040.
34. Rousselle, P., Braye, F. and Dayan, G. (2019). Re-epithelialization of adult skin wounds: Cellular mechanisms and therapeutic strategies. Advanced Drug Delivery Reviews, 146, pp.344–365. doi:https://doi.org/10.1016/j.addr.2018.06.019.
35. Albaugh, V.L., Mukherjee, K. and Barbul, A. (2017). Proline Precursors and Collagen Synthesis: Biochemical Challenges of Nutrient Supplementation and Wound Healing. The Journal of nutrition, [online] 147(11), pp.2011–2017. doi:https://doi.org/10.3945/jn.117.256404.
36. Bernard, B.A. (2016). Advances in Understanding Hair Growth. F1000Research, 5, p.147. doi:https://doi.org/10.12688/f1000research.7520.1.
37. Widodo, A., Rahajoe, P.S. and Astuti, R.T. (2020). TGF-β expression and wound tensile strength after simple interrupted suturing and zip surgical skin closure (IN VIVO study). Annals of Medicine and Surgery, [online] 58, pp.187–193. doi:https://doi.org/10.1016/j.amsu.2020.08.009.
38. Thawer, H.A. and Houghton, P.E. (2001). EFFECTS OF ELECTRICAL STIMULATION ON THE HISTOLOGICAL PROPERTIES OF WOUNDS IN DIABETIC MICE. *Wound Repair and Regeneration*, 9(2), pp.107–115. doi:https://doi.org/10.1046/j.1524-475x.2001.00107.x.
39. Badhe, R.V. and Nipate, S.S. (2019). Low-intensity current (LIC) stimulation of subcutaneous adipose derived stem cells (ADSCs) – A missing link in the course of LIC based wound healing. *Medical Hypotheses*, 125, pp.79–83. doi:https://doi.org/10.1016/j.mehy.2019.02.039.