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Low-intensity current (LIC) stimulation of subcutaneous adipose derived stem cells (ADSCs) – A missing link in the course of LIC based wound healing

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ABSTRACT

Millions of people die as a result of fatal injuries accounting for 9% of the total global annual deaths. Non fatal injuries generally result in variety of wounds. The normal wound healing process is slow and takes weeks to months, depending on the type of wound. In last two decades, electrotherapy called low-intensity currents (LIC) for the treatment became popular for faster wound healing, as well as in management of nonresponding and ulcerative wounds. It was reported that LIC mimics 'the current of injury' which is generated by body on wounding and helps in faster wound healing. Researchers have also studied the migration of localized cell and other bio-molecules under the influence of LIC helping the wound to heal faster. Literature review has also suggested that, electrical stimulation of isolated adipose tissue derived stem cells (ADSCs) releases growth factors and differentiates in to specialized cells like fibroblasts and keratinocytes in laboratory conditions. These research areas are well explored and emerged as independent state-of-the-arts therapies and technologies.

Considering the fact, that adipose tissue (along with ADSCs) is present subcutaneously, a new hypothesis is proposed which states that 'low intensity current (LIC) stimulation of wound stimulates subcutaneous adipose tissue containing ADSCs which releases different growth factors and also differentiates into certain cells like fibroblasts, neurons and keratinocytes. These cells easily migrate to wound site due to lipolysis and loosening of fat tissue, resulting in faster wound healing'. Thus this hypothesis provides a missing link between two state of the art technologies; first one is 'LIC based electrotherapy' and second one is '*in-vitro* LIC stimulation of ADCSs' where role and significance of *in-situ* ADCSs were never studied.

Introduction

An injury that causes a wound is either in the form of a cut, a blow or other impact to living tissue, typically to the skin. According to WHO, Injuries are a global public health problem. More than 5 million people die each year globally accounting for 9% of the world's deaths as a result of injuries. These deaths represent only a small fraction of those injured. Whereas, billions of people suffer from injuries that lead to hospitalization, accident and emergency department, general practitioner treatment or treatment at home [1]. Typically there are five types of wounds abrasion, laceration, puncture, avulsion and burn. There are typically six stages of wound healing haemostasis, inflammation, proliferation, angiogenesis, re-epithelialisation and maturation/remodelling [2]. The normal healing process is slow and takes weeks to months, depending on type of wound. Nonresponding wounds and ulcers are the most challenging wounds in the wound healing therapy.

In this article, new hypothesis for LIC based wound healing is proposed which is based on the understanding that LIC stimulates ADSCs present naturally in subcutaneous adipose tissue, releasing different GFs and differentiating into different cell types which migrate to wound site and help in faster wound healing.

Discussion

Existing State-of-the-art technologies

Electrotherapy based Wound healing

In last two decades, many researchers have studied the healing effect of low-intensity currents (LIC) in the treatment of acute, chronic or nonresponding standard wounds, arterial ulcers, diabetic ulcers, stasis ulcers etc. The results obtained from these studies were very encouraging and thus LIC has gained popularity in the last few years [3–11].

Abbreviations: LIC, low intensity current; ADSCs, adipose derived stem cells; GF, growth factors; VEGF, vascular endothelial growth factors

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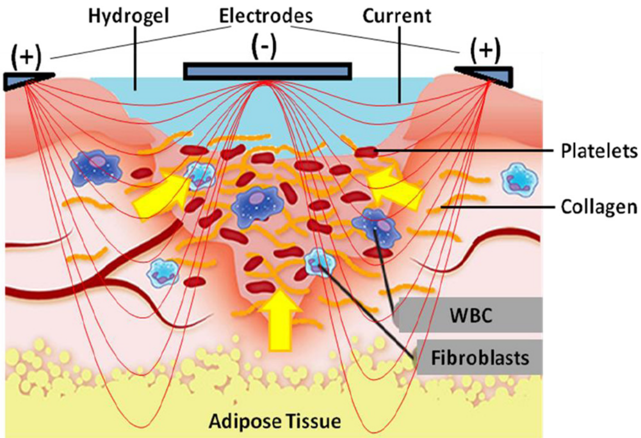

Fig. 1. Known electrotherapy based wound healing mechanism.

LIC mimics 'the current of injury' which is generated by body on wounding. Researchers have proposed that 'the current of injury' is mediated through cell membrane potential and generated by transportation of Na^+ into the cell creating negative pole outside the cell, this system is called as 'Skin battery' [12]. The current of injury ranges from 200 to 800 µA and has been observed to be decreasing in non-responding wounds. This observation triggered the thought of applying the LIC equivalent to 'current of injury' externally, to improve wound healing (Fig. 1) [13].

The mechanism proposed for faster wound healing in this state-of-the-art states that LIC stimulates the migration of the localized cells (WBC, endothelial cells and fibroblasts) in the wound due to membrane potential difference generated by electrodes of LIC device (from + to − terminal). LIC application to these cells also stimulates release of GFs and collagen at the wound site helping the wound to heal faster.

In-vitro LIC stimulation of ADSCs

In human body, adipose tissue is mainly located beneath the skin [subcutaneous fat/white adipose tissue (WAT)]. It is also present around internal organs (visceral fat), intermuscular (Muscular system), in bone marrow (yellow bone marrow) and in the breast tissue. It was reported that adipose tissue derived stem cells (ADSCs) obtained from adult adipose tissue provide pool of multi-potent adult stem cells that are noncontroversial in nature, relatively abundant, easy to isolate and are expandable [14]. There are recent reports stating electrical stimulations of adipose-derived stem cells (ADSCs) generating specialized differentiated cells like nerve cells, cardiomyocytes, fibroblast, etc. and also stimulating secretion of FGs like VEGF-A, NGF etc. (Fig. 2) [15–17].

Thus, this state-of-the-art work proved that LIC stimulation of ADSCs can differentiate them into various functionalised cells and also improves release of GFs. Interestingly; all the studies reported for this type of work are *in-vitro* studies with isolated ADSCs.

Neurogenesis and Angiogenesis go hand in hand

Neurogenesis and Angiogenesis are critical aspects of wound healing. Insufficient angiogenesis results in impaired wound healing and formation of chronic wound. Recent reports suggest that neurogenesis and angiogenesis share some molecular signals that act synergistically [18–20]. The expression of vascular endothelial growth factor (VEGF-A) with LIC stimulation of endothelial cells was reported along with the role of other growth factors like Nerve Growth Factor (NGF) in neurogenesis and angiogenesis [21–23]. It was reported that wounded neurons in wound show nerve regeneration in the direction of LIC stimulation (+ to −). This regeneration takes place mainly due to release of VEGF. This GF is released due to LIC stimulation of Shwann cells present on the wounded neurons (Fig. 3). This increased release of VEGF additionally helps in sprouting of new blood vessels. Thus, LIC induces neurogenesis and angiogenesis, helping wound to heal faster [24–26].

The literature survey suggests that, LIC positively plays a role in conditioning of isolated ADSCs, differentiating them into specific cell types and secretion of certain growth factors. Improved wound healing due to LIC was also well established. However, there are no studies reported on the effect of LIC stimulation of ADSCs present *in-situ* in adipose tissue. Thus, there is still a 'gap' between these two State-of-the-art research areas. This gap has provided the unique opportunity to explore the effect of LIC stimulation of subcutaneous adipose tissue containing ADSCs specifically from the wound healing prospective.

Hypothesis

By understanding the gap between the present studies and established technologies and taking advantage of the opportunity to explore new research area, a novel hypothesis is proposed here stating 'low intensity current (LIC) stimulation of wound stimulates subcutaneous adipose tissue containing ADSCs which releases different growth factors and also differentiates into certain cells like fibroblasts, neurons and keratinocytes. These cells easily migrate to wound site due to lipolysis and loosening of fat tissue, resulting in faster wound healing'.

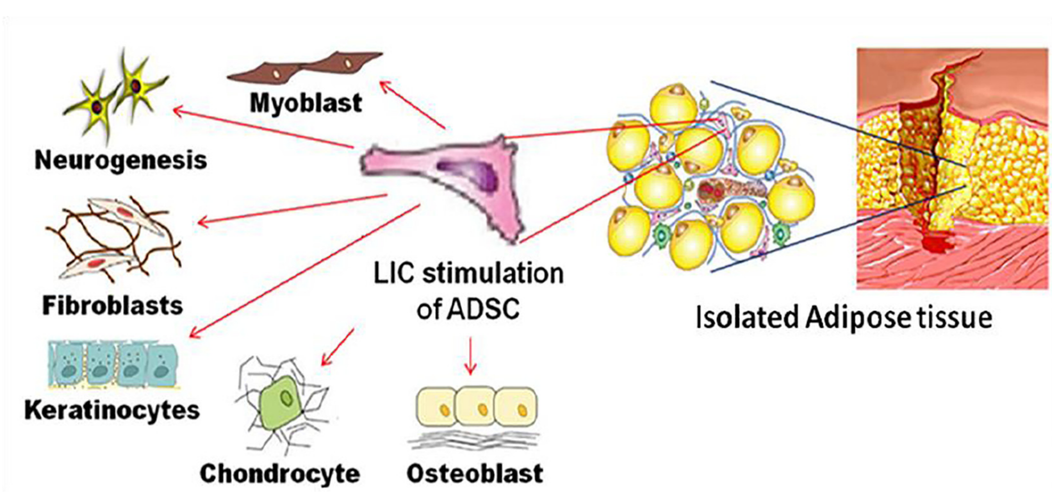

Fig. 2. Differentiation of adipose derived stem cells (ADSCs) in the presence of LIC.

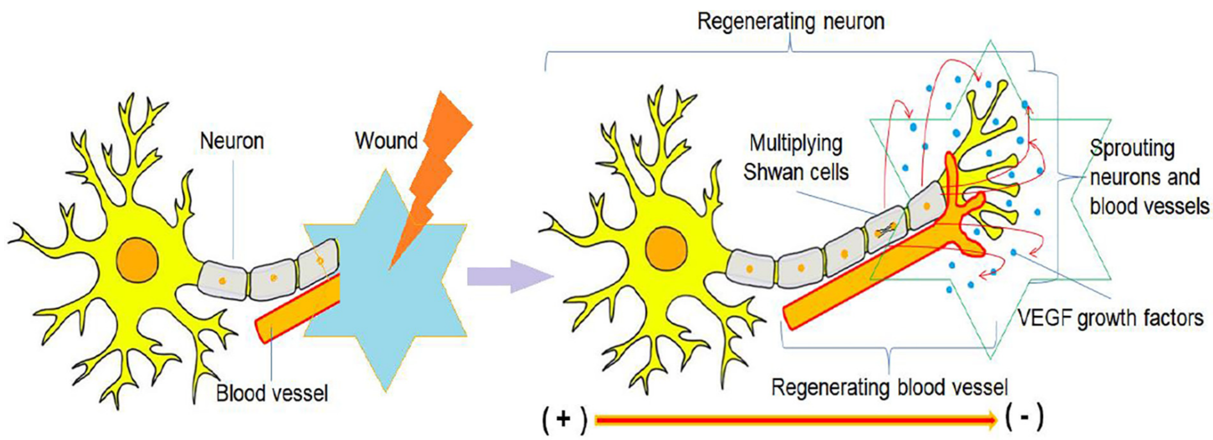

Fig. 3. LIC stimulation of Shwann cells on wounded neurons help in regeneration of neurons and blood vessels.

Probable wound healing mechanism of proposed hypothesis

The LIC wound healing therapy is based on two key components one is hydrogel and other is electrotherapy device. Hydrogel when applied on the wound fills the wound completely and uniformly. It provides the moist environment for wound healing and distributes LIC uniformly throughout the wound. The proposed hypothesis predict that the applied electric current passes through hydrogel and wound surface stimulating WBC, endothelial cells, fibroblasts and Shwann cells present on the damaged neurons. Stimulation of endothelial cells and fibroblast release VEGF and collagen respectively followed by migration and accumulation of collagen and fibroblast cells at wound site. Stimulation of Shwann cells on neuron release VEGF helping in sprouting and regenerating neurons and blood vessels. Further, when electric current reaches the adipose tissue, two phenomenon take place. First the LIC will lyses (lipolysis) some adipocyte cells opening the tight junction between the adipocyte cells and loosening fat tissue. This will help in faster migration of growth factors and differentiated cells generated by LIC stimulation of ADSCs to the wound site, which is the second phenomenon. Thus, if we combine all the effects of LIC stimulation of the wound, overall we get faster wound healing which is the likely mechanism for proposed hypothesis (Fig. 4) [27]. The novelty of the hypothesis lies in bridging the gap between electrotherapy based wound healing studies and isolated ADSCs based wound healing studies.

Upshot of the hypothesis – advancement and innovations in science

Understanding the role of swelled or inflamed subcutaneous fate cells on regulation of ADSCs and wound healing'

The hypothesis will supports the claim of faster wound healing owing to the LIC stimulation of subcutaneous ADSCs leading to differentiation of the stem cells and growth factor release. All the normal healthy individuals have some amount of adipose tissue preset subcutaneously, thus we expect improved wound healing with LIC stimulation in these individuals.

It is worth to study the effect of LIC stimulation on wound healing in obese individuals. This study will be very interesting as there are two contradictory conditions. Obese individual will have excess amount of adipose tissue developed due to adipose homeostasis associated with blocked insulin signals with inflammatory cytokines released by inflamed adipose tissue [28]. Thus, these individuals will have comparatively more number of ADSCs and will help in faster wound healing. On the other hand, the swelled or inflamed adipose tissue will tightly enclose ADSCs in the excess of adipocytes cells which are full of fat. The presence of excess of tightly arranged [very less water filled extracellular matrix (ECM)] swelled adipose cells (full of fat which is poor conductor of electricity) might increase the resistance to the LIC and further hamper wound healing [29].

Hence it will be interesting to understand the effect of LIC on modulation of up-regulation and down-regulation of ADSCs in normal

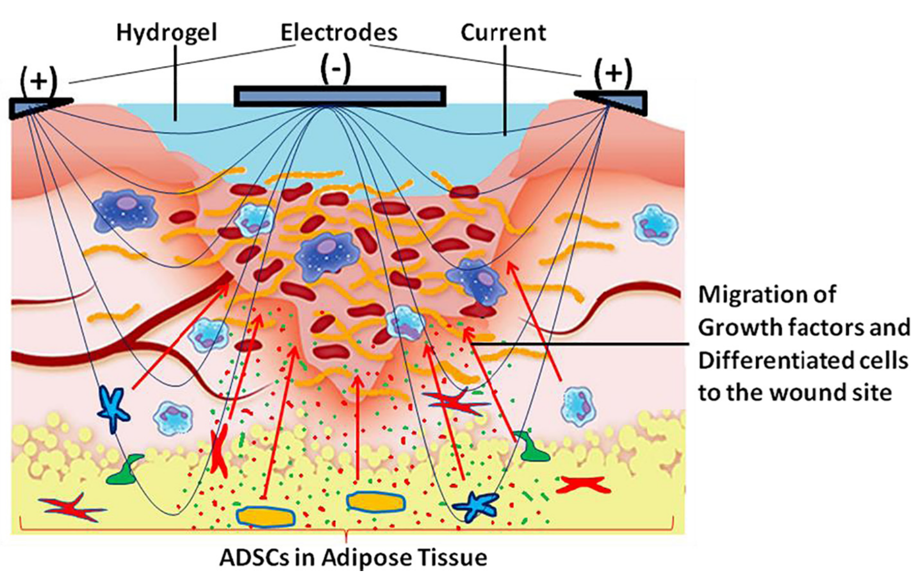

Fig. 4. Mechanism of proposed hypothesis.

and obese individuals. It will also shade a light on effect of LIC on modulation of adipose homeostasis and insulin resistance. Thus both of these areas can become innovations in science.

Developing advanced wound healing therapies

As mentioned earlier, LIC based wound healing therapy is based on two components, hydrogel and electrotherapy device. Hydrogel when applied on the wound fills the wound completely and uniformly, helping to distribute the LIC equally through the wound. The formulation of hydrogel itself is an established technology and developing hydrogel for LIC based wound healing will take it to the next level. The advancement is possible by formulating medicated hydrogel. Here, hydrogel containing pain relieving medicament, antimicrobial agents, nutrients and growth factors/promoters can be formulated to improve wound healing [30–36]. One can improve the electrical conducting property of the hydrogel using metallic nanoparticles and conducting polymers [37–40] or a medicated conducting hydrogel combining both the properties can also be formulated and used to improve LIC based wound healing. The electrotherapy device can also be advanced by developing handheld unit with varying current (from 10 to 800 μA) and flexible moving electrodes. These advancements in present LIC based wound healing therapies in the light of proposed hypothesis might enhance wound healing therapy significantly.

Conclusion

In conclusion, the thorough understanding of present state of LIC based wound healing therapies, LIC stimulation experiments on isolated ADSCs and LIC based neurogenesis and angiogenesis experiments suggested faster wound healing with LIC but these studies evolved separately. The proposed hypothesis in this article suggest the cohere effect of all the above mentioned studies systematically playing individual roles for rapid wound healing.

The hypothesis states that 'low intensity current (LIC) stimulation of wound stimulates subcutaneous adipose tissue containing ADSCs which releases different growth factors, and also differentiates into certain cells like fibroblasts, neurons and keratinocytes, which migrate easily to wound site due to lipolysis and loosening of fat tissue, resulting in faster wound healing'. The probable mechanism showing the cohere action of individual studies reported as 'the applied electric current passes through wound stimulating WBC, endothelial cells, fibroblasts and Shwann cells present on the damaged neuron. Stimulation of endothelial cells and fibroblast release VEGFs and collagen respectively followed by deposition of the same on wound site, stimulation of Shwann cells on neuron also release VEGF helping in sprouting and regenerating neurons and blood vessels. As the electric current reaches the adipose tissue, two phenomenon occur. First the LIC will lyses (lipolysis) some adipocyte cells opening the tight junction between the adipocyte cells and leads to loosening of fat tissue. This will help in faster migration of growth factors and differentiated cells generated by LIC stimulation of ADSCs (which is the second phenomenon which occur simultaneously) at the wound site helping in faster wound healing.

The proposed hypothesis can advance the present LIC based wound healing therapy to next level using medicated, conductive (improved) hydrogel and handheld electrotherapy device. It will also provide the knowledge about the role of swelled or inflamed subcutaneous fat cells on regulation of ADSCs and ultimately in wound healing of obese/diabetic obese people.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.02.039.

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