REVIEW ARTICLE

Stimulus-triggered Fate Conversion of Somatic Cells into Pluripotency in Chronic Wounds in Human Beings?

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Abstract:

Bone-marrow derived stem cells are multi potential or totipotent and are able to differentiate into numerous cell types. Their application is indicated in various reconstructive and restorative surgeries for rapid healing. A technique for creating cells that have the embryonic ability to turn into almost any cell type in the mammalian body has been reported. Recently, an unexpected phenomenon of somatic cell reprogramming into pluripotent cells by exposing to sublethal stimuli such as citrate based acidic medium has been reported. With the concept of creating acidic environment in chronic infected wounds to make a condition unsuitable for growth and multiplication of bacteria using 3% citric acid has been reported. It would be interesting to study whether the phenomenon of pluripotency takes place in chronic infected wounds in human beings following the application of 3% citric acid and plays an important role in formation of healthy granulation tissue.

Keywords: STAP, Chronic Wounds, Citric Acid

Introduction

Wound healing is a complex and well-orchestrated process involving multiple biological pathways and signaling various kinds of intra- and intercellular mechanisms encompassing the immune system, blood coagulation cascade and proinflammatory signaling. These are intricately associated with the healing and repair process [1, 2].

The process of wound healing progresses through the normal healing process consisting of haemostasis, inflammation, formation of healthy granulation tissue and reepithelization. Any obstruction in this normal process of wound healing results into nonhealing of wound [3].

The routine ways of treatment of wounds include: (i) Irrigation to decontaminate and eliminate bacterial population from wound thereby preventing the subsequent liberation of toxic products, which may initiate inflammatory response. (ii) Debridement of wound to remove necrotic tissue and foreign body, if any, to expose the underlying viable tissue, and to reduce the bacterial burden with an objective to promote and expedite wound healing [4, 5]. (iii) Use of antiseptics and antimicrobials to control infections. (iv) Adherent and nonadherent dressings to cover the wounds with an intension to avoid gross microbial contamination, to prevent the spread of infection to other patients and to absorb the wound exudates [6], and (v) Miscellaneous topical applications [7, 8].

Somatic Cells and Pluripotency

Bone-marrow derived stem cells are multi potential or totipotent and are able to differentiate into numerous cell types. Because of this ability, their application is indicated in various reconstructive and restorative surgeries for rapid healing. Stem cells possess distinctive ability to

renew themselves by mitotic division as well as they are able to differentiate into wide spectrum of cells [9]. In adults, stem cells are involved in different repair processes and also maintain normal turnover of regenerative organs like skin. Because of their plasticity to differentiate, they can be adjusted when transplanted on foreign tissues. The stem cells are also capable of producing various cytokines and growth factors, which act as triggering factors to accentuate the healing process [10] and may participate in active inflammation for the repair and healing of chronic nonhealing wounds [11]. Thus, stem cells have been reported to augment faster healing of wound.

A technique for creating cells that have the embryonic ability to turn into almost any cell type in the mammalian body has been reported in 2006 by Japanese researchers. The differentiated cells can be reprogrammed to an embryonic - like state by transfer of nuclear contents to oocytes or by fusion with embryonic stem cells. These cells, which are designated as induced Pluripotent Stem (iPS) cells from mouse embryonic or adult fibroblasts, exhibit the morphology and growth properties of embryonic stem cells and express embryonic stem cell marker genes. These results demonstrate that pluripotent stem cells can be generated directly from fibroblast cultures. This study further suggests that it may be possible to create pluripotent cells directly from somatic cells of patients [12].

Recently in 2014, another Japanese team discovered an unexpected phenomenon of somatic cell reprogramming into pluripotent cells by exposing to sublethal stimuli [13-16]. Two recent studies show that cells isolated from newborn mice lose their identity on exposure to citrate based acidic medium (mild acidic conditions) and get converted into pluripotent cells [13, 14]. Instead of triggering cell death or tumor growth, as might be expected, a new cell state emerges that exhibits an unprecedented potential for differentiation into every possible cell type [16]. This unexpected phenomenon of somatic cell reprogramming into pluripotent cells by exposure to sublethal stimuli such as low pH exposure is known as stimulus triggered acquisition of pluripotency (STAP) [13, 14]. This phenomenon of reprogramming does not require nuclear transfer [17, 18] or genetic manipulation [12].

It has been observed that exposure of $CD45^+$ splenic lymphocytes from mouse to citrate based acidic pH of 5.7 for 30 minutes and their subsequent culture in presence of Leukemia Inhibitory Factor (LIF) has been found to induce pluripotency related marker proteins and marker genes. The STAP stem cells generated so were found to express protein and RNA markers for pluripotent cells, showed a substantial decrease in DNA methylation in the regulatory regions of pluripotency marker genes and had a nuclear fine structure similar to embryonic stem cells. In differentiation cultures they were found to generate ectodermal, mesodermal and endodermal derivatives *in vitro* and formed teratomas *in vivo*. After blastocyst injection, STAP stem cells efficiently contributed to chimeric mice in which germline transmission was seen. In tetraploid complementation assay they were able to generate mice capable of growing adults and producing offspring [13, 14].

These results show that citrate based acidic pH has potential to induce pluripotency that has many functional potentials and can play key role in the treatment of many diseases for which no suitable alternative treatment approaches are available.

Acidic Environment and Wound Healing

With the concept of creating acidic environment in chronic infected wounds to make conditions unsuitable for growth and multiplication of bacteria using 3% citric acid is being practiced at Maharashtra Institute of Medical Sciences and Research (MIMSR) center for more than 19 years. Application of citric acid as a sole antimicrobial agent to treat chronic infected wounds not responding to conventional treatment modalities in human beings has been proved to be very effective approach for the treatment of a variety of chronic infected wounds. Findings of earlier studies at MIMSR center has shown that the chronic infected wounds in patients of diabetes [19], burns [20], leprosy [21], HIV/AIDS [22] or necrotizing fasciitis[23], sarcoma [24] and chronic wound infections caused by multiple antibiotic resistant bacteria pathogens [25], respond very well to local application of 3% citric acid ointment prepared using petroleum jelly (prepared by mechanical mixing of 3gm citric acid with 97 gm of 100% pure petroleum jelly, a hydrocarbon base not absorbed by the skin, was used as an inert vehicle for citric acid). This treatment modality is very effective in the management of a variety of wounds. Three percent citric acid has antiseptic property, which may be due to lowering of pH that makes an environment unsuitable for the growth and multiplication of bacteria causing infection. In four to five applications, 3% citric acid ointment accelerates wound healing process by controlling infection, promoting formation of healthy granulation tissue and renewal of epithelium by enhancing epitheliazation, which is a major factor in wound healing. In addition to treat a variety of chronic infected wounds, the use of citric acid has also been reported in the control of biofilm formation by pathogens like *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* because of the broad bactericidal activity of citric acid against these pathogens. Hence, it can also be used in controlling the biofilm formation on a variety of cutaneous traumatic and surgical wounds [26, 27]. Recently its use has also been reported for preparation of wound bed for skin grafting in wounds with large raw areas [28]. The experience at MIMSR center shows that citric acid is active against almost all bacterial pathogens including multiple antibiotic resistant strains.

A histological study has revealed that use of citric acid enhances epitheliazation and found to actuate the wound healing process by boosting fibroblastic growth and neo-vascularization, which in turn increases microcirculation of wounds that enables the formation of healthy granulation tissue thereby leading to faster healing of wound [29]. These exciting and fascinating results of the earlier studies in different patients, including immunodeficient ones, give reason to believe that citric acid is a powerful tissue sensitizer and has potential to direct the different cell types involved in wound healing process.

It has been reported in earlier studies that the acidic environment promotes healing of wounds by controlling infections caused by a variety of bacterial pathogens including multiple antibiotic resistant strains, increasing antimicrobial activity of topical antimicrobial agents, altering protease activity, releasing oxygen, reducing toxicity of bacterial end products, enhancing epitheliazation and angiogenesis, etc. The results of these studies show that acidic environment in a wound bed positively influences wound healing process.

It has been found that the proteases are more

active in alkaline conditions and their end products are toxic to wound tissues. In acidic environment, the activity of these enzymes is reduced because of lowering of pH thereby reducing the formation of their end products and the toxicity of end products [30]. The release of oxygen enhances epitheliazation, which occurs better in well-oxygenated tissues. This also explains the importance of hyperbaric oxygen therapy. Increased oxygenation of local tissue because of acidic environment is one of the important reasons for promotion of epitheliazation [31]. The acidic environment also promotes epitheliazation and angiogenesis. In addition to these activities, the acidic environment has been shown to enhance the destruction of abnormal collagen in the wound bed, increase macrophage and fibroblast activity, and control activities of various enzymes participating in wound healing process [31-33].

So far in the studies on citric acid treatment of chronic infected wounds, the molecular mechanism of effect of citric acid and low pH has not been studied. What exactly happens at molecular and cellular levels following the application of 3% citric acid is not fully known. Whether the phenomenon of pluripotency takes place in chronic infected wounds in human beings following the application of 3% citric acid and plays an important role in formation of healthy granulation tissue may be an interesting field of research that would be helpful to achieve more useful and concrete conclusions.

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