

Effect of Acupuncture on ST36 Acute Stitches to Accessible Epithelialization Accuracies in Rats of Rats Infected of MRSA Bacteria

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ABSTRACT

Epithelialization is part of skin tissue regeneration to repair the damage. This process will be inhibited by the presence of bacterial infections, especially Methicillin resistant Staphylococcus aureus (MRSA). This study aims to determine the effect of stabbing acupuncture needles on ST36 acupoint on epithelialization on the healing process of MRSA infected wounds in the skin of wistar rats. Thirty six Wistar rats, 3 months old, are divided into 6 groups, 2 groups of negative control, 2 groups of positive control, 2 groups of treatment group (All are sacrificed on the fourth day and sixth day). Injury to the back skin of rats with a knife along the incised 2 cm and depth to subcutaneous. Wound of positive control groups infected by MRSA, wound in the treatment groups also infected MRSA and stabbing at ST36 acupoints. Each group are sacrificed to/for day 4 and 6, the skin tissue is fixed, made histological preparations, stained with HE. The measurement of the epithelial length using Optilab mounted on a light microscope. The data are analyzed by comparing the mean and SD. The epithelial length of the treatment group is higher than the positive control $\{(0,46 \pm 0,19) \text{ vs } (0,21 \pm 0,16); (0,63 \pm 0,76) \text{ vs } (0,42 \pm 0,301)\}$, being compared with the negative control is not much different. Stabbing acupuncture needles on ST36 acupoint accelerates epithelialization in wound healing of the skin MRSA-infected rat.

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I. Introduction

The science of acupuncture has been a medical science in China since 4000-5000 years ago. Many studies show ST36 acupuncture points are able to trigger the body's defense system. The development of acupuncture in Indonesia began in 1963, then on the instruction of the then minister of health (Prof. Dr. Satrio) established the research team of traditional medicine of eastern medicine and since then acupuncture practice was held officially in RS Cipto Mangunkusumo. The basic mechanisms of acupuncture stimulation are local reactions at acupuncture points by acupuncture stimulation (piezoelectric), arising inflammatory reactions and subsequent hypothalamic stimuli (endocrine reactions, neurochemical reactions, and autonomic nervous reactions) (1).

Skin injuries can be caused by various causes such as traumatic injury (mechanical, chemical, thermal, electrical) or may also be caused by blood vessel blockage (such as Buerger disease). The wound causes the loss of the skin structure to be limited to the epidermis or to the dermis even to the point of muscle. If the wound to the structure of the dermis or muscle will be accompanied by bleeding because the blood vessels are also affected. The wound becomes a port d'entry for microorganisms to enter the body, making the wound unsterile. The infection triggers the body's



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immune response to eradicate the germs. The immune process may be non-specific (innate) and may also be specific (acquired) (2).

Infection by Methicillin resistant *Staphylococcus aureus* (MRSA) will inhibit wound healing. Bacteria capable of producing biofilms, toxins and superantigens so as to avoid the immune system and inhibit the epithelial process. These bacteria are also able to protect themselves from destruction by antibiotics by transferring staphylococcal cassette chromosome mec (SSCmec) which produces a protective against antibiotics that have methicillin structures. This type of *staphylococcus aureus* when infected on an incomplete skin (such as a wound) will aggravate the degree of illness (3).

The stabbing of acupuncture needles at the ST36 acuity point, triggering neuron cells around the stabbing occurs the release of P compounds that inhibit cortisol and stimulate the growth factor (epidermal growth factor). This growth factor triggers the formation and proliferation of phagocyte cells (macrophages, neutrophils) that eradicate bacteria. Growth factors also trigger the proliferation of fibroblasts and metalloprotein matrix (MMP) that are important in epithelialization (4)

In this study, we investigated the accelerated epithelialization process in MRSA-infected wounds with acupuncture on ST36 acupuncture points.

The skin is the largest organ of the body, the total weight ranges from 2.7 to 3.6 kg and receives one-third of the body's blood volume, the thickness of the skin varies from 0.5 to 6.0 mm, consisting of cells and extracellular matrix. The skin structure consists of 3 layers, the epidermis is the outermost layer of skin and thin, the dermis is a thick layer and lies within, the layer beneath the dermis there is subcutaneous fat tissue (hypodermis). Hypodermic tissue is a loose connective tissue attached beneath the dermis (5).

Human skin has many important functions, especially as a front line defense, protecting the body from various elements that come from the environment outside the body. In case of skin injury, the integrity of the skin defenses becomes disrupted and becomes the entry point for various microorganisms such as bacteria and viruses. Skin can also be an important factor in mental health and human social conditions (4).

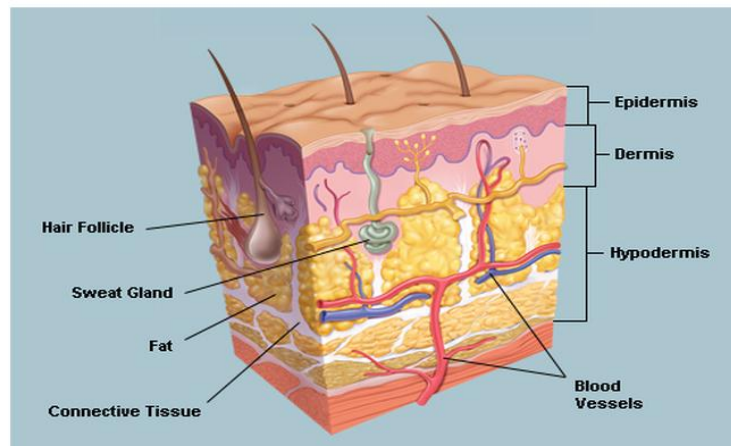


Figure 1. Skin structure

Wound healing process there are 4 phases: phase hemostasis, inflammation phase, proliferation phase, remodeling phase. Hemostasis occurs immediately after trauma, to stop the bleeding by way of open blood vessels experiencing vasoconstriction and activated platelets and then stick together and aggregate in the wound area. Platelets are activated by extracellular collagen (type I). Once platelets interact with collagen, platelets release the mediator (growth factor and cyclic AMP) and glycoproteins, which signal the platelets to become more sticky and accumulate. Platelet alpha granules release glycoproteins of fibrinogen, fibronectin, thrombospondin and von Willebrand factors. When platelet aggregation occurs, the clotting factor of the blood is released causing fibrin to settle in the wound area (6).

This phase occurs 24 hours after trauma to the skin and can last up to 2 weeks depending on whether there is an infection that extends this phase. Mast cells release granules containing enzymes, histamine and other active amines that give rise to signs of inflammation (7).

Fibroblasts migrate to the wound in response to the dissolved mediators released platelets and macrophages. Fibroblasts secrete proteolytic enzymes to facilitate the movement of fibroblasts to the matrix. The secreted enzymes include three types of MMP such as collagenase (MMP-1), gelatinase (MMP-2 and MMP-9) that destroy the gelatin compound, stromelysin (MMP-3) which has several protein compounds in ECM (extracellular matrix)).

Remodeling is the last phase of the wound healing process that occurs after the granulation tissue becomes scar tissue and skin elasticity increases (4).

The epithelization process is a process that includes the attachment of epithelial cells and changes in epithelial structure further migrate, proliferate and differentiate. The stratum basalis epithelial cells proliferate, subsequently attached well to the basement membrane via the aid of the intercellular linkage of desmosomes (for intercellular adhesion) and hemidesmosomes (for epithelial adhesion with basal membranes). Growth factors (EGF, keratinocyte growth factor / KGF, TGF- α) secreted bind to receptors in epithelial cells and lead to migration and proliferation. Bonding growth factor with receptor triggers desmosome and hemidesmosome dissolves so that epithelial cells can migrate (8).

S. aureus bacteria are single-celled organisms called prokaryotes. This bacterium is shaped coccus (round) in groups. The bacterial cell wall is a complex, less elastic structure, and affects the cell shape. The main function of cell walls to protect bacterial cells from greater intracellular pressure differences from extracellular ones at risk of such cells would be rupture. Clinically the cell wall is important because it contributes to the ability of bacteria to cause disease, the part attached to the APC receptor (antigen presenting cell), is also the workplace of antibiotics. *S. aureus* bacterial cell wall consists of peptidoglycan macromolecules, teichoic acid and lipoteichoic acid (9).

Two determinants of suspected virulence factor *S. aureus* are *ica* genes encoding poly-N-acetylglucosamine / polysaccharide intercellular adhesion (PNAG / PIA) and insertion of IS256 gene. IS256 plays a role in genetic adaptation at the time of infection, by insertion of the locus *ica* or on *agr*. Insertion in *ica* will increase the formation of PNAG / PIA, while insertion in *agr* will inhibit the regulatory function of biofilm formation so that biofilm becomes thicker. Both of these virulence factors help *S. aureus* to colonize both the commensal and the infectious. The role of PNAG / PIA, PGA, and protease ScpA protects bacteria against the antibody protein produced by non-specific (innate) body defenses (3).

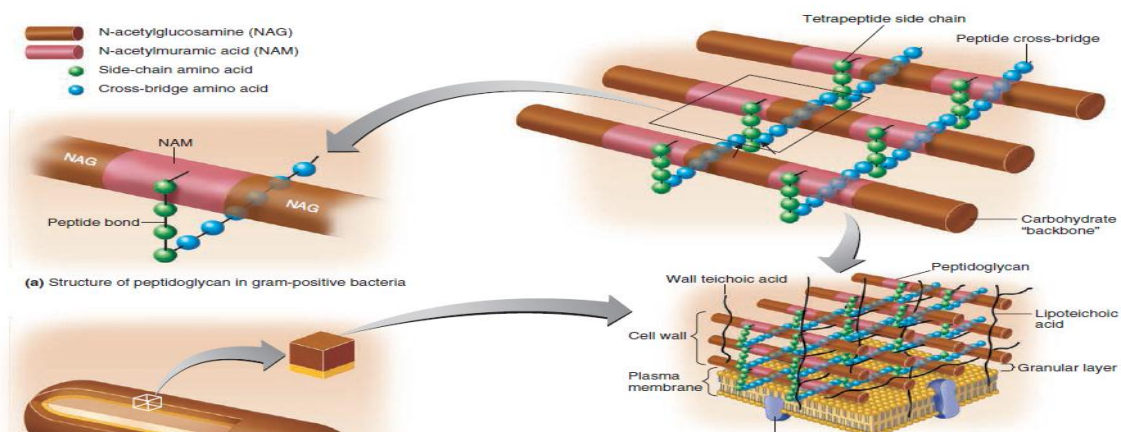


Figure 2. *S. aureus* cell wall structure

S. aureus produces several toxins: exotoxins (5 cytolytic toxins, 2 exfoliative toxins, enterotoxins and toxin shock syndrome toxin-1 / TSST-1) and endotoxins. Exfoliative toxins A, enterotoxins and TSST-1 include a class of polypeptides called superantigens (9).

The infection begins when the pathogen breaks through the anatomical barrier of the host. Some mechanisms of a non-specific immune system are activated to include several groups of well-dissolved molecules present in extracellular fluid, blood, and secreted epithelial cells. Phagocytosis in non-specific immune systems by cells such as neutrophils and macrophages (10).

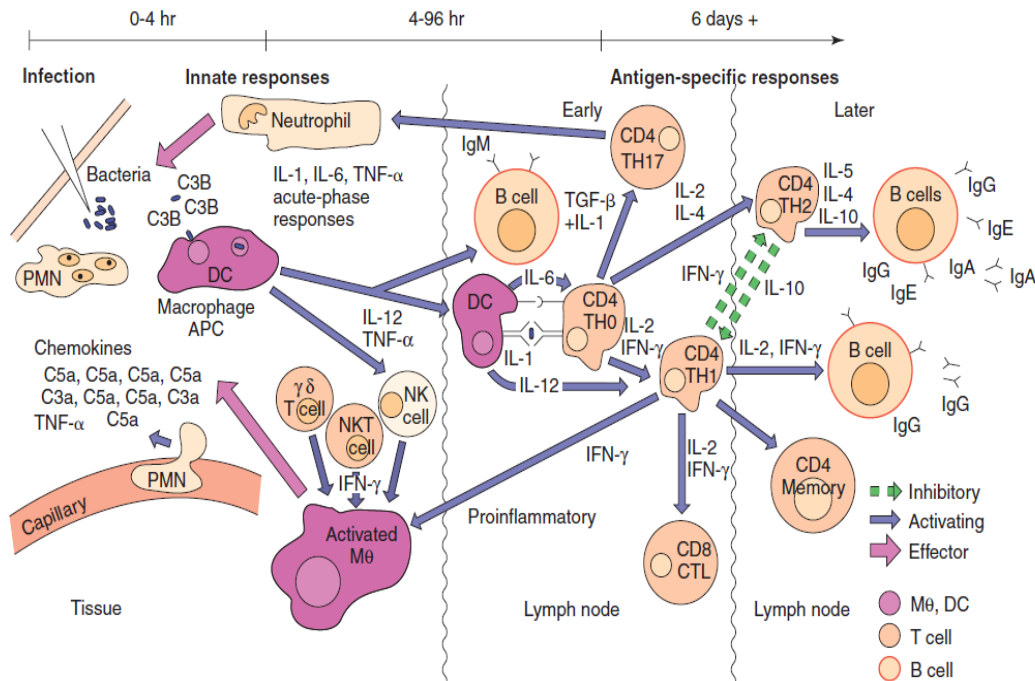


Figure 3. Immune response to MRSA infections in skin lesions

The Zusanli acupuncture point (足三里) is a gastric meridian known as the Leg Three Li, the He point group or the personal point (tonification) or the point of the vitamin or the distant point of the foot and is coded ST36. The stabbing at the ST36 acupuncture points causes the release of substance P by the edge of the peripheral nerve. further activates an inflammatory signal pathway that converts polarization of macrophages into M1 and triggers the formation of macrophages in the bone marrow through the formation of proinflammatory cytokines (11).

Substance P is a protein encoded by the TAC1 gene (the location on chromosome 7 in humans) and is part of the tachykinin hormone consisting of three neuropeptides namely neurokinin A, neuropeptide K and neuropeptide Y. Substance P binds to the receptors NK1R and CCR2 (chemokine β receptor) will activate second messenger (Ca²⁺, Diacyl Glycerol, Inositol Triphosphate, cAMP). Second messenger passes the signal to mitogen-activated protein kinase (MAPKK), then activates extracellular signal-related kinases 1/2 (ERK 1/2), enters the cell nucleus and mediates expression of cytokines IFN- γ , IL-2, mTOR, AP1 and NF- κ B. This formation of proinflammatory cytokines suppresses the glucocorticoid hormone produced by the adrenal cortex in the hypothalamic axis - the pituitary - the adrenal gland. The inhibition of the glucocorticoid hormone triggers the release of growth factors that play a role in shaping the necessary microenvironment in epithelialization (12,13).

II. Method

Before you begin to format your paper, first write and save the content as a separate text file. Keep your text and graphic files separate until after the text has been formatted and styled. Do not use hard tabs, and limit use of hard returns to only one return at the end of a paragraph. Do not add

any kind of pagination anywhere in the paper. Do not number text heads-the template will do that for you.

This type of research is a pure experimental research in the laboratory (True Experimental) because this research is given intervention with all external variables that influence controlled, this research using Post Control Only Control Group Design research design. The object of the study was a 3-month-old *Rattus norvegicus* strain of Wistar weighing 200-250 grams. There were 6 groups of study group O1 (negative control observed until day 4), O2 (negative control observed until day 6), OK1 (positive control was observed until day 4), OK2 (positive control was observed until day 6), OP1 (treatment group observed until day 4), OP2 (treatment group observed until day 6). Negative control was rat wound without MRSA infected, positive control was MRSA infected mice wound, treatment group was wound rats infected with MRSA and performed stabbing at ST36 acupuncture point. Each group consisted of 6 rats (a total of 36 rats), each 6 rats were numbered 1 to 6 with paper attached to the tail, then each random mouse was inserted into 6 groups until each group had 6 rats.

Each mouse was weighed and put into a cage measuring 20 x 15 x 15 cm for adaptation. Mice in anesthesia use a ketamine solution. The preparation of a ketamine solution of 3 ml ketamine plus 1 ml of xylazine was diluted with aquabidest for 6 ml injection, then injected in rats (0.1 ml per rat) until the mice was already in a state of calm and only abdominal breathing was observed. After the rat in anesthetized condition was shaved with the area of 3 cm x 3 cm, disinfected with betadin then performed an incision on the skin of the back of the mouse with a knife skapel with wound length \pm 2 cm with depth until subcutaneous layer (14).

In the positive control group and the treatment group, skin lesions were infected with MRSA bacteria with 50 micro liter of suspension 0.5 mcFarland by using micropipet on the wound. Provision of bacteria is done after the wound stops the bleeding. Acupuncture needle stabbing done 2 days after infection. On day 4 and 6 after treatment, each group including a negative control group was sacrificed by using ketamine as anesthesia. Then the skin was cut with a normal skin coverage of 0.5-1 cm from the wound edge then inserted into 10% formalin buffer fixation solution for 15-24 hours and then dead rats were buried (15).

The pieces of skin tissue are then made preparations and HE is painted. Afterwards it was observed and measured the length of the epithelium with Optilab mounted on an ocular lens microscope with 40x magnification.

III. Results and Discussion

The mean and standard epithelium (SD) epithelial lengths in the negative control group, positive control and treatment are shown in Table 1, showing an increase in epithelial length in the treatment group both on day 4 and day 6 than in the positive control group.

Treatment group compared to negative control group showed not much difference of epithelial length either on day 4 or day 6.

Table 1 Mean and standard deviation of epithelial length (conversion from μ m to mm) in skin of injured Wistar rat in negative control group, positive control and treatment

Group		4 th Day	6 th Day
Control negative	Mean	0,59	0,73
	SD	0,711505	0,0083666
Control positive	Mean	0,21333	0,42
	SD	0,162682	0,301993

Treatment	Mean	0,46	0,638333
	SD	0,194422	0,761326

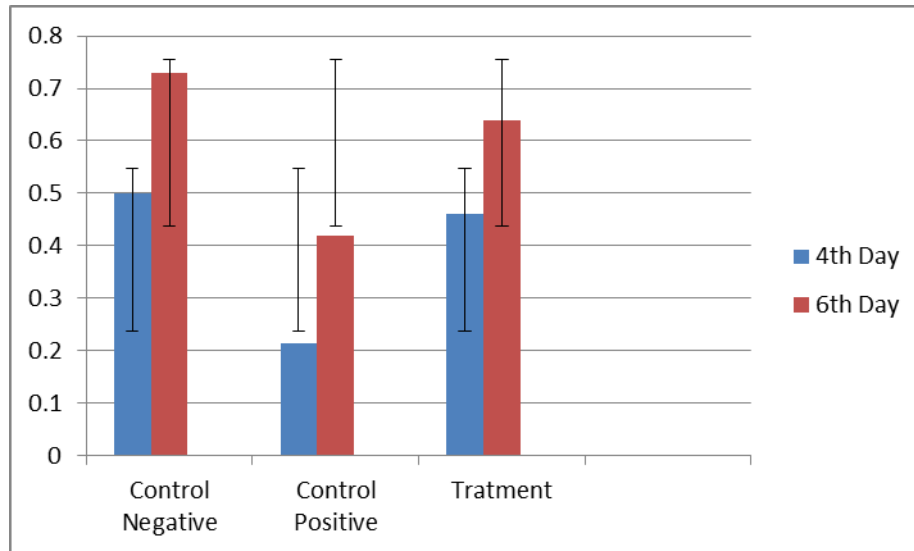


Figure 4 Distribution and mean length of each group epithelial

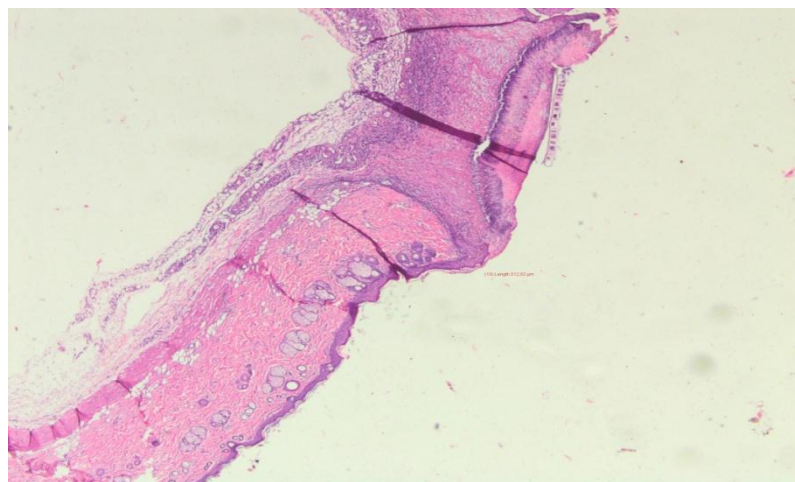


Figure 5. Microscopic picture of epithelial tissue on day 4 after injury in the negative control group (40x magnification)

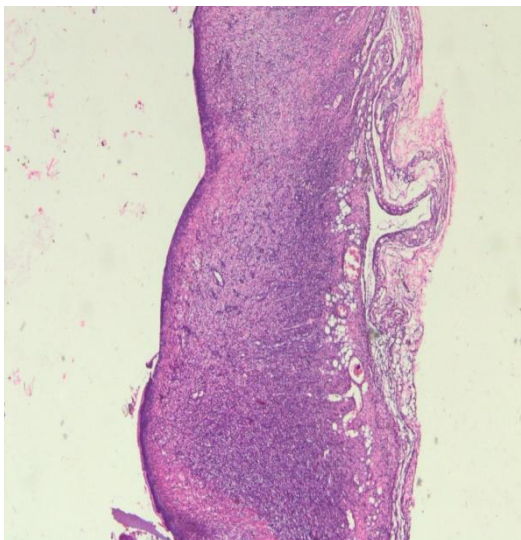


Figure 6. Microscopic picture shows no process

epithelial formation on day 6 after injury in positive control group (40x enlargement)

In this study, long-term outcomes of Wistar mouse skin epidermis on negative control, positive control, treatment showed improvement from day four and sixth. This indicates that epithelization has occurred in all three groups. This condition is in accordance with the theory of wound healing process, namely epitelisasi process begins on day 3, the epithelial layer cover the base of the wound. In the negative control group and treatment there was keratin, whereas in the positive control group was not seen. At 24 hours after the injury, keratinocytes migrate laterally and regenerate basement membranes. After the new basal membrane is formed, the keratinocytes stop migrating then breed to its peak in the 4th heri.

The epithelial layer continues to elongate and thicken, then the newly formed epithelial tissue undergoes ripening and a new corneum lining appears. With regeneration of the basement membrane, keratinocytes return to their original form and attach the hemidesmosome back to the basal lamina. The presence of retepres suggests that epithelialization is underway to form normal epithelial tissue. Migratory epithelial cells will be interconnected and cover the surface of the wound. After achieving normal epithelial thickness, epithelial cell migration stops.

In the positive control group showed the lowest average epithelial length. This is due to an extension of the inflammatory process due to MRSA infection. Fibroblasts still actively form an inflammatory complex that produces pro-inflammatory cytokines IL-1 β . Fibroblasts also multiply and differentiate to synthesize granulation tissue components (collagen, elastin, and proteoglycans).

In the treatment group, the mean length of the epithelium was almost the same as that of the negative control group. This shows the stabbing at ST36 acupuncture points that are capable of triggering epithelization so that the wound healing time equals the physiological wound healing time.

IV. Conclusion

Based on the results of this study, the conclusion of needle stabbing at the acupuncture point ST36 influential in accelerating epithelialization in the wound healing process on the skin of Wistar mice infected with MRSA bacteria.

This research is expected to be the basis for subsequent research. Further research with ST36 acupuncture point stabling is required to look at the substance P levels and the number of phagocyte cells.

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