ABSTRACT... Objective: To assess if Topical probiotics and steroids can accelerate the process of angiogenesis in wound repair. Study Design: Longitudinal Experimental study. Setting: Al-Tibri Medical College. Period: Jan 2020 to Dec 2020. Material & Methods: Total 72 male albino rats were acquired from the animal house of the institution, and 18 as a requirement of per day of sampling included 6 animals/gp. An equal size incision of (2x2cm²) was made in all the animals. Group A was given Topical Normal Saline (Control Group), Group B was given the Probiotic (Lactobacillus Acidophilus), and Group C was given Topical Steroid (Dexamethasone). Samples from the wound created surgically were obtained on the 3rd, 7th, 14th, and 21st Day and slides were created to observe the number of blood vessels formed and angiogenesis under a light microscope. Data was analyzed using SPSS version 21.0. To compare the mean between the groups, the ANOVA was applied along with post hoc Tukey’s test. The level of significance was considered P≤0.05. Results: The mean number of blood vessels formed in Group A (Control Group) was 2.75, 4, 5.25, and 5.50, in Group B (Probiotics Group) was 10, 13.50, 2.25, and 1, and in Group C (Steroid Group) was 3.75, 5.50, 3, and 2.25 on the 3rd, 7th, 14th, and 21st Day respectively. Significant difference was seen in the mean number of blood vessels formed when the Probiotic group was compared to Group A and C. Conclusion: Topical Probiotic accelerates the process of angiogenesis in wound repair due to their anti-inflammatory response.

Key words: Albino Rats, Angiogenesis, Steroids, Topical Probiotic, Wound Healing.

INTRODUCTION
One of the many organs in our body, Skin is one of the severing a myriad of functions. These include protection, hydration, thermoregulation, and vitamin D synthesis. The skin consisting of five layers is as important to the body as any other organ. However, the skin is also susceptible to trauma or damage like any other tissue and organ in the body that may lead to the formation of a wound. Damaged to the superficial and deep layers of the skin and the subsequent development of a wound requires a process to heal this defect which is termed as wound healing. Wound repair is a tightly controlled and an intricate process which consists of hemostasis, inflammatory phase, proliferative phase, and a maturation phase occurring in a temporal sequence. The inflammatory phase occurs after the injury to the tissue but is precluded by the formation of clot through the initiation of a coagulation cascade. The inflammatory phase prevents infection and removed dead or non-vital tissues. Following this phase formation of new tissue starts to occur once again through cellular proliferation. During the dermis formation, granulation tissue formation is also seen which consists of an array of different cells. Finally, the maturation stage takes place which strengthens the tissue leaving behind a dense rich region of collagen and extracellular matrix proteins. All of these steps combine together to complete a successful wound healing process in an individual.

Although wound healing is a process well
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Regulated by the body, there are certain factors that can delay or have an antagonizing effect on the process of wound healing. These factors include age, sex, weight, infection, oxygenation, nutritional status, alcohol intake, medications, and diabetes. A sound knowledge of the mechanisms of wound healing, the factors that can impair wound healing, and thorough knowledge of the human skin anatomy is critical for recognizing these factors that may dampen wound healing. The development of chronic wound that are unable to heal is a persistent issue in the medical world, and due to this dilemma, there is a substantial increase in both patient morbidity as well as enormous amount of health care costs.

The goal of enhancing wound healing outcome has been a long time goal of the medical fraternity. As there are factors that can affect wound healing, the world of medicine with its intense research and advancements have also developed therapeutic agents that can facilitate wound repair, thereby aiding doctors and surgeons alike to initiate a strategy that will ultimately speed up the process of wound healing thereby producing a more favorable outcome for the patient. One of these agents is Probiotics, which are living nonpathogenic microorganisms that can provide a beneficial effect to the human body.

Different types and combinations of probiotics are available in the market that are isolated from various resources. Probiotics have the ability to restore the microbial flora of the skin and topical probiotic application has the ability to enhance the barriers of defense of our skin. The most common type of probiotics that the health care sector uses is Lactobacillus, which can facilitate the wound healing process.

To see the effects of probiotics, a study was conducted to assess if topical probiotics and steroids can accelerate the process of angiogenesis in wound repair.

MATERIAL & METHODS
An experimental study was designed and conducted at Al-Tibri Medical College, Isra University Karachi Campus. The duration of the study from Jan-2020 to Dec-2020 (duration 12 months) conducted after approval from ethical committee. Total 72 male albino rats were acquired from the animal house of the institution, and 18 as a requirement of per day of sampling included 6 animals/gp through randomized sampling technique. All of the selected albino rats needed to be healthy, and had to weigh between 150-250 grams which was done through and electronic weighting machine. The health status of the selected albino rats was done by a licensed Doctor of Veterinary Medicine (DVM). All subjects not healthy and not being in the predetermined weight category was excluded from the study.

The selected 18 animals were then randomly divided into 3 groups, each group consisting of 6 rats. Group A was given Topical Saline Solution (Control Group), Group B was given Topical Probiotic (Lactobacillus Acidophilus), and Group C was given Topical Steroid (Hydrocortisone 1%, Zafa Pharmaceuticals). A wound of equal size (2x2cm2) was created on all the animals, through a sterilized scalpel blade (Blade No.11, Scalpel Handle No.15) under topical anesthesia (Lidocaine).

Once the therapeutics were applied to the animals of their respective groups, they were all kept in separate cages and all the cages were labeled to avoid any confusion. 6 Samples from each group (totaling 18 samples) were collected from the animals on the 3rd, 7th, 14th, and 21st day after topical therapeutic application. The sample of the skin was acquired from the wound site. Samples were taken from the artificially created wound to observe the healing process in all of the relevant intervention groups. Slides were prepared accordingly to be placed under a light microscope for observing under a magnification of 400x. Trichome stain was used to observe the blood vessels. Under the light microscope, we observed the number of blood vessels and the process of angiogenesis. The probiotic was isolated from fresh yogurt, that was purchased from a nearby local dairy shop. The organisms of interest, which in this case was Lactobacillus Acidophilus was then isolated. The number of the organisms was increased by growing it in MRS agar plates and the presence of Lactobacilli was
confirmed by performing the catalase test which was negative. The data that was collected and analyzed was done through Statistical Package of Social Sciences (SPSS) Version 21.0. To compare the mean between the groups, the ANOVA was applied along with post hoc Tukey’s test. The level of significance was considered $P \leq 0.05$. A wound of equal size (2x2cm2) was created on all the animals, through a sterilized scalpel blade (Blade No.11, Scalpel Handle No.15) under topical anesthesia (Lidocaine).

RESULTS
Figure-1 Shows the Mean numbers of blood vessels among different therapeutic groups (x400) on the 3rd, 7th, 14th, and 21st Day. There was significant increased number of blood vessels found among probiotic group on day 7 as compare to others.

Photomicrograph-1 shows the Epidermal tissue showing numbers of blood vessels per field area among different therapeutic groups. The increase numbers of vessel were observed in probiotic given therapeutic group as compare to others.

DISCUSSION
Wound healing has both considerable economic and humanistic burdens, both for the individual suffering from the chronic wound and at a health care level due to the ever rising costs. Treatment costs for wounds are considered to be significant and are estimated to account for 1-3% of the total health care expenditure in developed countries, but this is stated to be an underestimation. Probiotics are microorganism that don’t elicit a pathological response and are administered to maintain the balance in the microbial system, especially in the gastrointestinal tract. They consist of a variety of bacteria and are regulated through dietary supplements and foods. Various mechanisms are known through which probiotics help to exert their favorable effects, which include lowering the pH of the intestinal tract, reduce colonization and invasion of pathogenic organisms, and modify the immune response of the host. It should be noted however that a benefit associated with one strain or species of probiotic doesn’t necessarily associate it with others.

Steroids were first introduced in the 1960s for treating brain edema. Since their advent, the use of steroids has been found in almost all branches of medicine and by all routes.
They are synthetic analogs of the natural steroid hormone made by the human body in the adrenal cortex and consist of two types’ mineralocorticoids and glucocorticoids. Steroids are the most commonly prescribed drug class in the world, having a market share of more than 10 billion dollars per annum.\textsuperscript{11}

The purpose of this study was to see if probiotics can provide a beneficial effect in the process of wound healing by promoting neovascularization/angiogenesis in surgically created wounds in albino rats. Probiotics are essential in restoration of the flora of the skin and have critical action in forming a barrier of protection.\textsuperscript{12} Mentioned previously, Lactobacillus is the most commonly employed Probiotic stain, and its used in clinical medicine is steadily on the rise because of its myriad of benefits.\textsuperscript{13} Prevention and amelioration has been shown by the action of probiotics in the course of treating disorders of the digestive system which include acute, nosocomial, and antibiotic-associated diarrhea; allergic diseases such as atopic dermatitis and rhinitis in children; and diarrhea caused by the super infection of Clostridium difficile-associated diarrhea and lastly inflammatory bowel disorders in children.\textsuperscript{14}

In the wound healing process, during the proliferative phase the formation of new blood vessels occurs, this is known as angiogenesis or neovascularization. The process of angiogenesis involves the migration of endothelial cells migrating, proliferating, and branching to form new blood vessels. Angiogenesis is vital for wound healing to take place effectively. The presence of angiogenesis helps to supply nutrition and oxygen so that ongoing cellular proliferation as well as regeneration takes place without any impedance. Pro-angiogenic signals such as VEGF, help in promoting endothelial motility, sprouts are then converted into endothelial tubules and create connections with other vessels that require localized suppression of motility and the creation of new cell-cell junctions.\textsuperscript{15} Among the major reasons which cause persistent delay of wound healing, disrupted angiogenesis is one of them along with other reasons such as chronic inflammation, disturbance in growth factors, and recurring microbial infections. This unfortunately leads to repeated hospitalizations, greater healthcare cost for the individual and the system, reduced quality of life, and in worse case scenarios amputation of the affected limb or organ.\textsuperscript{16}

It is therefore prudent that there must be a reduction in the microbial load at the site of the wound, as well as a subsequent reduction in the inflammatory phase of wound healing which may then lead to early onset of angiogenesis thereby making wound healing more favorable and faster which will ultimately improve the quality of life, reduce health cost, and hospitalizations. This is exactly what is being illustrated in our study as Probiotic composed of Lactobacillus created the most amount of blood vessels on the 3rd and 7th day after its topical application. Significant difference was seen when the Probiotic group was compared to both Group A and Group C. This finding of accelerated wound healing and a reduction of inflammation was also seen in another study, which also demonstrated an increase in amount of collagen deposition, an increase in re-epithelialization, and epidermis production, while the inflammation rate was significantly less than what was compared to the control group of that study.\textsuperscript{17}

Furthermore, a reduction in the activity of myeloperoxidase (MPO) enzyme was also detected in the intervention group of the study mentioned. Mohtashami et al (2020) also in his study that was carried out on Wistar rats and diabetic wound healing using Lactobacillus bulgaricus and Lactobacillus plantarum through histopathology and gene expression analyses on the 3rd, 4th, and 14th day post-wound period showed that the use of Probiotics helped in speeding up the wound healing process and modulated the inflammatory cells in the wound site in the 14 day period, alteration of the mRNA levels of the inflammatory cytokines was observed at the wound sites after the treatment with probiotics.\textsuperscript{18} A similar effect can be observed in our study in which the topical probiotics enhanced the wound healing process by increasing angiogenesis, however the current
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study was only based on histopathology, and our study wasn’t based on gene expression analyses. In another study, researchers used a different species of Lactobacillus (Lactobacillus brevis and Lactobacillus plantarum), in which a 1.5x1.5cm full thickness wound was created on 45 rats divided into 4 groups with samples being collected on the 1st, 3rd, 7th, 14th, and 21st day for histology and statistical study, but the conclusion was the same with significant difference compared with the control and negative control group (p<0.05) showing a reduction of neutrophils and acceleration of wound healing as compared to the control and negative control groups of the study. A reduction in the inflammatory phase leads to quicker onset of angiogenesis, which will ultimately lead to granulation tissue being formed much earlier as well. This is exactly what was also present in our study. Probiotics should be strongly considered as a valid contender for being used as an agent for optimizing wound healing as it is readily available and cost-effective in terms from a consumer point of view and health care cost.

Our previous study also related to Probiotics shows how effective it is in initiating angiogenesis and reducing the level of inflammation in rats when it was compared with antibiotics. Steroids aren’t recommended for being used to promote wound healing, as the data from our studies also prove that they showed no significant benefit in optimizing angiogenesis. In fact studies indicate that use of systemic steroids has no clinically significant effects on wound healing, furthermore long-term chronic use of steroids can cause an array of complications and can actually prove to impair the wound healing process rather than improve it. More therapeutic agents can be studied and compared with Probiotics to see which of the following is superior to the other in regards to reducing the inflammatory levels, and promoting angiogenesis. This includes Low level laser therapy, which has shown promising results when it comes to promoting wound healing. The literature has yet to produce many studies on postbiotics, however a recent study has been conducted on this very subject using heat-killed Lactococcus chungangensis CAU 1447 on type I diabetic mice and demonstrated significantly decreased skin wound size indicating that they can also be used in the future for wound healing treatment. Although our study showed that Probiotics is a tremendous affect for increasing neovascularization the study didn’t have a greater number of subjects (due to a small sample size used), to study the effects of probiotics more accurately.

Furthermore, other parameters were not assessed in this study which such as the neutrophil count, Myeloperoxidase levels, and gene expression. Other effective agents such as low level laser therapy should be compared with topical probiotics along with including a greater sample size to assess which is more superior to the other which will eventually help clinicians in making certain decisions of what to use to enhance wound healing in patients. It is also prudent that in future studies diabetic subjects be incorporated as diabetes is a well-known factor for impeding wound healing, therefore a comprehensive study is required to establish the safety measures in immunocompromised patients especially those that have diabetes mellitus.

CONCLUSION
Topical Probiotic helps to accelerate the process of angiogenesis in wound repair due to their anti-inflammatory response, and most primitive availability faster the healing process as compare to other topical agents. Corticosteroids however don’t demonstrate the same desirable effects that are seen with topical probiotics and therefore should not be used for wound healing acceleration.

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REFERENCES


**AUTHORSHIP AND CONTRIBUTION DECLARATION**

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