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THE EFFECTS OF NEGATIVE PRESSURE WOUND TREATMENT ON BLOOD VESSEL DENSITY AROUND PERCUTANEOUS DEVICES

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I. INTRODUCTION

A growing number of amputees use osseointegrated percutaneous prosthetics [1], which have two components: the endoprosthetic stem implanted directly into the bone and the percutaneous post that protrudes out of the skin [1, 2]. The percutaneous post attaches to the rest of the prosthetic limb [2]. These types of prosthetic devices reduce skin contact and discomfort compared to socket prosthetics that are fitted directly over the residual limb [1, 2]. However, one major limitation of osseointegrated prosthetics is that epithelial downgrowth occurs around the percutaneous post [3]. Downgrowth is when the skin recedes off the device (Fig 1), which is the body's natural way of ridding itself of the foreign object. This can lead to infection and other complications for the patient [1, 3].

To reduce complications for patients, researchers have studied how to reduce downgrowth. In one study, downgrowth was significantly less (50-70%) when Negative Pressure Wound Treatment (NPWT) was applied to animals with percutaneous devices, compared to the animals not treated with NPWT [3]. NPWT mechanically draws wound edges together and removes fluids with vacuum suction [4]. Applying suction significantly reduced downgrowth around the percutaneous posts, although, the morphological mechanism as to why NPWT helped was not clear from this study [3].

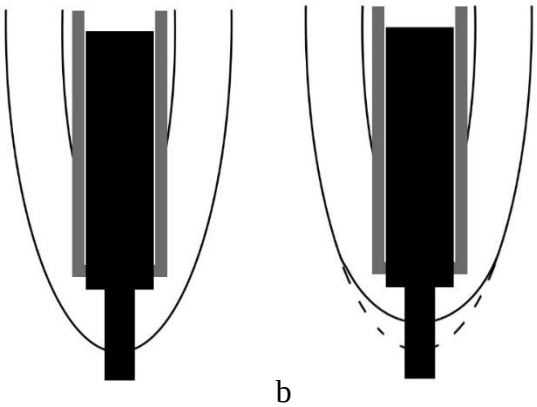

Fig. 1. Parts of an osseointegrated prosthetic without and with downgrowth. White represents the soft tissue, gray represents the bone, and black represents the osseointegrated implant and percutanous post. (a) shows no downgrowth on the percutaneous post. (b) shows downgrowth on the percutaneous post. The dotted line is where the skin was originally and the solid line is where the skin has receded to.

The effects of NPWT on morphological mechanisms surrounding other musculoskeletal and dermis wounds have been studied [5, 6]. Research has demonstrated that blood vessel density and blood flow increase around wounds, which promotes healing by supplying oxygen and nutrients to the wound site and by removing waste from the wound site. Along with increased healing, a study determined that NPWT changed the blood vessel density around wounds [5]. Yet, how the change in density affects downgrowth around a percutaneous device was unknown.

The goal of this research was to determine the effects of NPWT on blood vessel density and the possible interaction between blood vessel density and downgrowth.. It was hypothesized that

the blood vessel density would be greater after NPWT was applied. This was determined by comparing the blood vessel density of tissue samples from 12 rats that had been implanted with percutaneous devices, six of which were treated with NPWT and six of which were not treated. The analyzed tissue samples could help clinicians better understand how NPWT affects blood vessel density and how it plays a role in wound healing around a percutaneous device. A better understanding of NPWT could allow clinicians to improve health and comfort for amputees who use osseointegrated percutaneous prosthetics.

II. METHODS

A. Animal and study design

This research was conducted with an approved animal protocol from the Institutional Animal Care and Use Committee at the University of Utah and Department of Defense. Hairless rats (8 weeks old; Charles River, Raleigh, NC) were implanted with percutaneous devices using an aseptic surgical procedure [3]. The rats were randomly assigned into two groups. NPWT was applied to six rats (Fig 2) and six rats served as untreated controls. All rats remained on the study for four weeks. Continuous NPWT was applied according to an established protocol to the treated rats [3].

According to the protocol, NPWT at -70mmHg was applied to the treated rats for thirty-six hours, then not applied for twelve in a repeating cycle during the four-week period. The untreated rats received dressing changes in the same 36-12 cycle as the treated rats. However, a vacuum tube was not bandaged to the untreated rats. All the dressing changes were performed while the rats were under general anesthesia of isoflurane (2%).

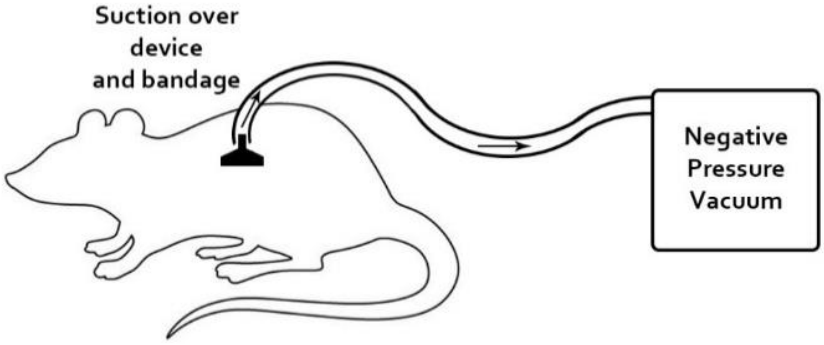

Fig. 2. NPWT applied to device implanted in rat. A tube, which applies negative pressure from a small clinical vacuum machine, is bandaged over the percutaneous device.

B. Downgrowth Measurements

During each dressing change, pictures were taken of each percutaneous post to recorded any downgrowth. At the end of the four-week period, the rats were sacrificed using 5% isoflurane. At this time, the final downgrowth of each rat was measured by using a caliper to measure the distance between the percutaneous post and the surrounding skin in the medial, lateral, cranial, and caudal directions. Any measurement other than 0 mm recorded was considered downgrowth.

C. Tissue Staining

Tissue samples were collected from three locations per rat. First tissue was taken from the three-point junction, which is the point at which the air, the epithelial, and the device first make contact. Next a tissue sample was taken from along the top of the implanted device, called the over-device tissue. Finally, tissue was collected from another region of the skin that was not affected by the device, as control tissue (Fig 3). After the tissue samples were harvested, they were fixed in formalin for paraffin embedment. Using a microtome, a small slicing tool, 3-4 micrometers thick tissue sections were obtained and stained with CD31 antibody (Abcam, Cambridge, USA) which stains for blood vessels. A liver from one of the rats was used as an IgG control for the CD31 staining.

D. Cell Quantification

After staining, the blood vessels in the tissue samples were quantified. The vessels were counted under a high magnification microscope (Nikon Instruments INC, Melville, NY) to observe the number of blood vessels in the tissue that was surrounding the implant. The vessel density was calculated by counting the total number of CD31 positive structures, with visible lumen, per area. An area with radius of 500 µm was drawn on microscope image of the NPWT and untreated tissue, with the center of the circle being the three-point junction. The blood vessel density for each group was expressed as the mean number of CD31 positive structures per mm^2 ± SD. The area within which blood vessels were quantified was averaged. Based on that average, blood vessels were counted within similar areas in the control tissue and the over-device tissue (Fig 3).

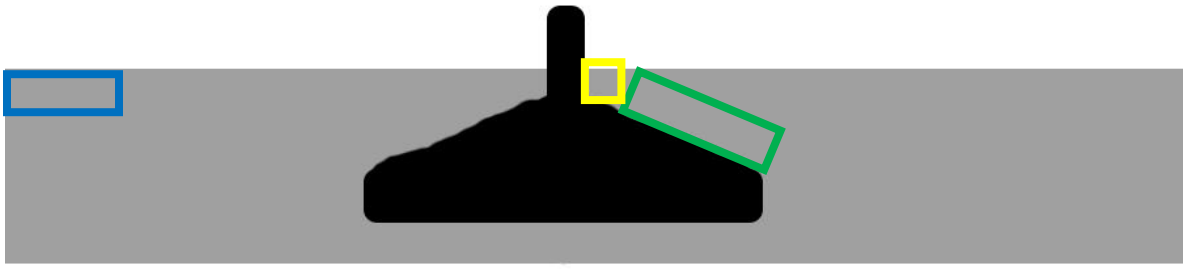

Fig. 3. Locations from which tissue samples were taken. Blood vessel density was quantified in three tissue samples per rat: control tissue (blue rectangle), over-device tissue (green rectangle), and three-point junction tissue (yellow rectangle).

E. Statistical Analysis

After all the blood vessels in the tissue samples were quantified, a Student t-test was used to determine any statistical significance between the densities of the NPWT treated group and the untreated group, from tissue at the three-point junction, over-device, and control tissues. The alpha level was set at 0.05.

III. RESULTS

At the time of tissue harvesting the untreated rats showed downgrowth, while none of the treated rats showed downgrowth. None of the rats had signs of infection around their devices. With no signs of infection around the implants, the blood vessel counts were unaltered. After harvesting, the tissues were fixed to prevent tissue degradation and allow for more accurate study of the blood vessels. However, during fixation, one of the untreated (control rats) tissue samples was destroyed, so only blood vessels in tissue samples from five untreated rats were able to be quantified.

The blood vessel densities for each tissue type were quantified using a Nikon microscope software. There was not a significant difference found between the untreated control tissue (43.736 ± 7.330 blood vessels per square millimeter (BV/mm^2), mean ± std. dev) and the NPWT treated control tissue (37.054 ± 6.277 BV/mm^2). Furthermore, in the over-device tissue there was a 91.3% increase in blood vessels density in the treated tissue (79.105 ± 21.533 BV/mm^2) compared to the untreated tissue (41.342 ± 12.746 BV/mm^2). There was also a 96.6% increase in blood vessels from the untreated three-point junction tissue (139.664 ± 42.141 BV/mm^2) to the treated three-point junction tissue (274.574 ± 80.494 BV/mm^2) (Fig 4). Because there was not a significant different in the control tissue blood vessel densities, but there was in the over-device and three-point junction tissue densities, we can confidently determine that NPWT does make a statistically difference in the blood vessel density in tissue around a percutaneous device.

From this study we were able to observe that NPWT does increase blood vessel density around percutaneous devices.

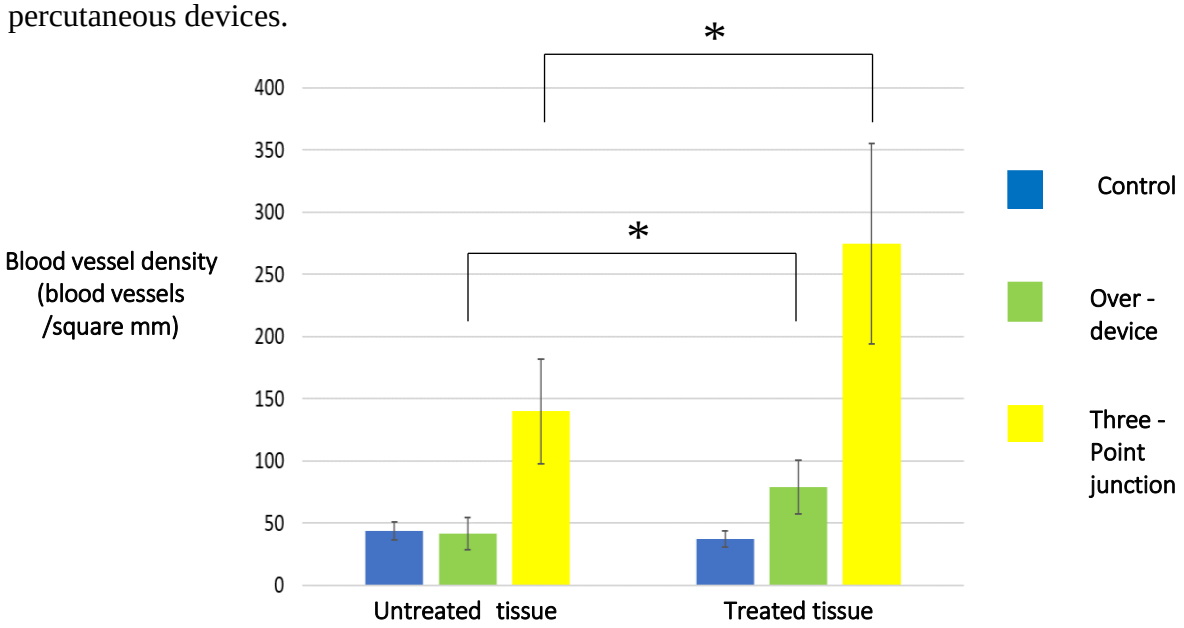

*Fig. 4. Blood vessel density in untreated verses NPWT treated rat tissue. There was not a significant difference between the untreated and treated control tissue densities. However, there was a significant increase in blood vessel density over-device and at the three-point junction when NPWT was applied. The significance denoted by * was set at p = 0.05.*

IV. DISCUSSION

In this study, it was hypothesized that blood vessel density would be greater around percutaneous devices after Negative Pressure Wound Treatment (NPWT) was applied. It was found that blood vessel density did indeed increase around percutaneous devices when NPWT was applied.

Previous studies in this field have determined that NPWT promotes wound healing [5] and reduces downgrowth [3]. Other studies have also been done to determine the mechanisms of downgrowth around percutaneous implants [3]. In addition, NPWT has been used to close diabetic ulcer wounds [4]. Moreover, researchers have shown that the blood flow changes around wounds when NPWT is applied [5, 6]. However, before this study, the combined relationship between NPWT, downgrowth around percutaneous devices, and blood vessel density had not been researched.

This research showed that the mechanism of increasing blood vessel density correlates with the reduction of downgrowth around a percutaneous device. This was shown in three different types of tissue samples (Fig. 3). The blood vessel density in the control tissue (tissue not affect by the presence of the device) of both the untreated and NPWT treated rats remained constant (Fig 4.), while the blood vessel density significantly increased in the three-point junction tissue and the over-device tissue in the treated rats. This may be because the negative pressure applied to the skin increases the blood flow in that area [5] which results in a greater need for blood vessels. This may lead to greater oxygenation, nutrient supply and waste removal, promoting healing of downgrowth. Further studies need to be performed to solidify a relationship of correlation or causation between blood vessel density, NPWT, and downgrowth. If blood vessel density is determined to be the factor that causes reduced downgrowth, other methods of increasing blood vessel density around percutaneous prosthetics could be studied which would further reduce the discomfort and inconvenience for the amputee.

Although our results confirmed our hypothesis, there were several limitations of this study. First, wound healing occurs in overlapping stages [3] and tissues samples were only taken at a single timepoint, 4 weeks after implantation. Because this was an explorative mechanistic study, this was an appropriate time point because acute wound healing usually occurs within 30 days of initial wound [8]. The study did show reduced downgrowth in the NPWT treated rats, so we can still state that increased blood vessel density correlates with reduced downgrowth. In the future, research can be done in which tissue samples are taken at multiple time points throughout the study, to look at blood vessel density throughout the stages of healing. This study also did not explore how the skin around the percutaneous device would behave after NPWT termination or long-term use. Blood vessel quantification can be done in longevity and termination studies in the future. An addition limitation is that rat tissue heals differently than human tissue [7]. Therefore, humans may have differing results from NPWT than the rats in the study. However, this kind of research must be proven safe in animal populations before it can be put into clinical trials for humans.

This research increases the understand of how NPWT interacts with skin around a percutaneous device. This understanding can be used to improve current prosthetics and to design new, better prosthetics. It can also be used to improve other percutaneous medical devices.

Blood vessel density may not be the only factor that reduces downgrowth around a percutaneous device. In the future, other factors that are impacted by NPWT, such as macrophage and other cell densities, and their role in reducing downgrowth can be studied. To do this, the current study could be repeated but instead of staining for blood vessels, tissues could be stained for other cell types.

Because we better understand that blood vessel density may be involved in NPWT limiting downgrowth, clinicians can better utilize NPWT to improve prosthetic health and comfort for amputees. This understanding could improve the lives of many patients.

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