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## Ultra sonographic assessment of wound healing in horses

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### Abstract

The present study was aimed to investigate the utility of collagen and silicone extracellular matrices embedded with autologous platelet rich plasma for cutaneous wound healing in horses by analysing the vascularity with the use of ultrasound imaging. The horses with cutaneous wound were randomly divided into two groups with six animals each. Assessment of vascularity by ultrasonography was studied for 0, 5, 15, 30 and 45<sup>th</sup> day of post treatment period. Vascularity assessment by power doppler ultrasound examination revealed median score value which was statistically significant on 30<sup>th</sup> and 45<sup>th</sup> day of treatment between group I and group II animals which exhibited proper and complete healing.

**Keywords:** Horses, wound healing, PRP, collagen, silicone, ultrasound, vascularity

### 1. Introduction

Cutaneous wound healing is a physiologically active process which consists of four phases: Haemostasis, inflammation and proliferation which included angiogenesis, epithelialisation and remodelling phase<sup>[1]</sup>. In horses, distal limb wounds are commonly encountered in clinical practice and are a challenge to treat. The standard management of wound requires an adequate debridement and application of a proper bandage for several weeks to even months. In some cases, the recovery time is pivotal for owners, and veterinarians are enforced to give additional wound care to hasten wound healing<sup>[2, 3, 4, 5]</sup>. Wound healing depends on some factors like blood supply, tissue tension, size of wound etc. The choice of wound management depends on the experience of the Veterinarian and as well as, the budget of the owner. Platelet rich plasma (PRP) is a concentrate of growth factors which modulating inflammation and increasing angiogenesis and re-epithelialisation<sup>[6]</sup>. PRP is commonly applied by mixing it with other extracellular materials. Collagen acts as a natural substrate and available in the form of sheet, powder, gel and sponge which accelerates the epithelialization in the wound<sup>[7]</sup>. Silicone dressing is synthetic, non-adherent, fully occlusive dressing which promotes wound healing<sup>[8]</sup>. With this background, the present study was designed to analyze the treatment efficacy of PRP with collagen and silicone in cutaneous wound healing in horses through vascularity assessment by ultrasonography.

### 2. Materials and Methods

#### 2.1 Animals

The study was conducted in horses with cutaneous wound reported to the Large Animal Surgical Outpatient Unit, Madras Veterinary College Teaching Hospital, Chennai-600 007, for the period from 2017 to 2021. Twelve horses with open cutaneous wound irrespective of breed, age, sex and location of wound were recorded. Detailed clinical examination was performed and the animals which were free from concurrent neurological, metabolic and other diseases were selected for the study and all horses were received Tetanus Toxoid (TT) injection at the dose rate of 5000 IU intramuscularly. The horses with cutaneous wound were randomly divided into two groups with six animals each.

#### 2.2 Wound treatment

On 0<sup>th</sup>, 5<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day, wound treatment was performed with collagen and silicone extracellular matrices embedded with autologous platelet rich plasma (PRP). Photographs were captured of each wound during bandage changes which was displayed in Fig. 1(a-e).

### 2.3 Ultrasonographic assessment of wound healing for vascularity

Wound healing was assessed through colour flow doppler ultrasonography on 0<sup>th</sup>, 5<sup>th</sup>, 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day respectively, a standard ultrasound unit via 10 to 12 MHz linear transducer with harmonic ultrasound capabilities (upto 14 MHz) were used. The Sonoscope S6 system and 15L8 linear transducer (Probe frequency 7 – 13 MHz) was used for ultrasound imaging. Wound was scanned in the centre and on either side of the wound using adjacent tissue to identify the position of the wound surface and base, in each group of treatment. Scan viability and neovascularization of blood vessels were checked for healing assessment. Ultrasound examination was done as the method described by Rippon *et al.* [9] and displayed in Fig. 1(f).

### 2.4 Statistical analysis

The data obtained were statistically analyzed using Mann-Whitney ‘U’ Test and the values were considered significant when ( $p < 0.05$ ).

### 3. Results and Discussion

The evaluation of vascular status in an animal’s wound; clinically guide the veterinarians on decision making. For that purpose, in the current study, power doppler method was employed for assessment of wound healing and vascularity in group I and group II animals. This is also accordance with the report of Reetz *et al.* [10] who stated that the colour-flow doppler ultrasonography was important for vessel identification.

In the present study, a scoring method for subjective assessment of presence or absence of blood vessels signals was employed in various stages of wound healing. Vascularity assessment by power doppler ultrasound examination in group I and group II animals are displayed in Fig.1 (g-j) and the median values of ultrasound examination were represented in Table 1. The power doppler score was tabulated and the median value of group I was 3, 2, 2, 1, 0 and group II was 3, 3, 2, 2, 1 respectively and there was no significant different between groups on 0<sup>th</sup>, 5<sup>th</sup> and 15<sup>th</sup> day respectively. A significant difference between the groups ( $p \leq 0.05$ ) in their power doppler score was found at 30<sup>th</sup> and 45<sup>th</sup> day which showed the healing stages of the wound. Lactingola *et al.* [11] described that power doppler as a potent source for blood vessels presence or absence in healing in equine tendons and the findings in present study concurred with it and also explained about the power Doppler US grade scoring system for neovascularization of wound and tendonopathy healing (Grade 0 -No flow: grade 1- Single vessels: grade 2-  $\leq 50$  per cent vessels: grade 3-  $\geq$  More than 50 per cent lesion filled with vessels) and stated that power Doppler scoring system could be used for assessment of tendon healing in horses.

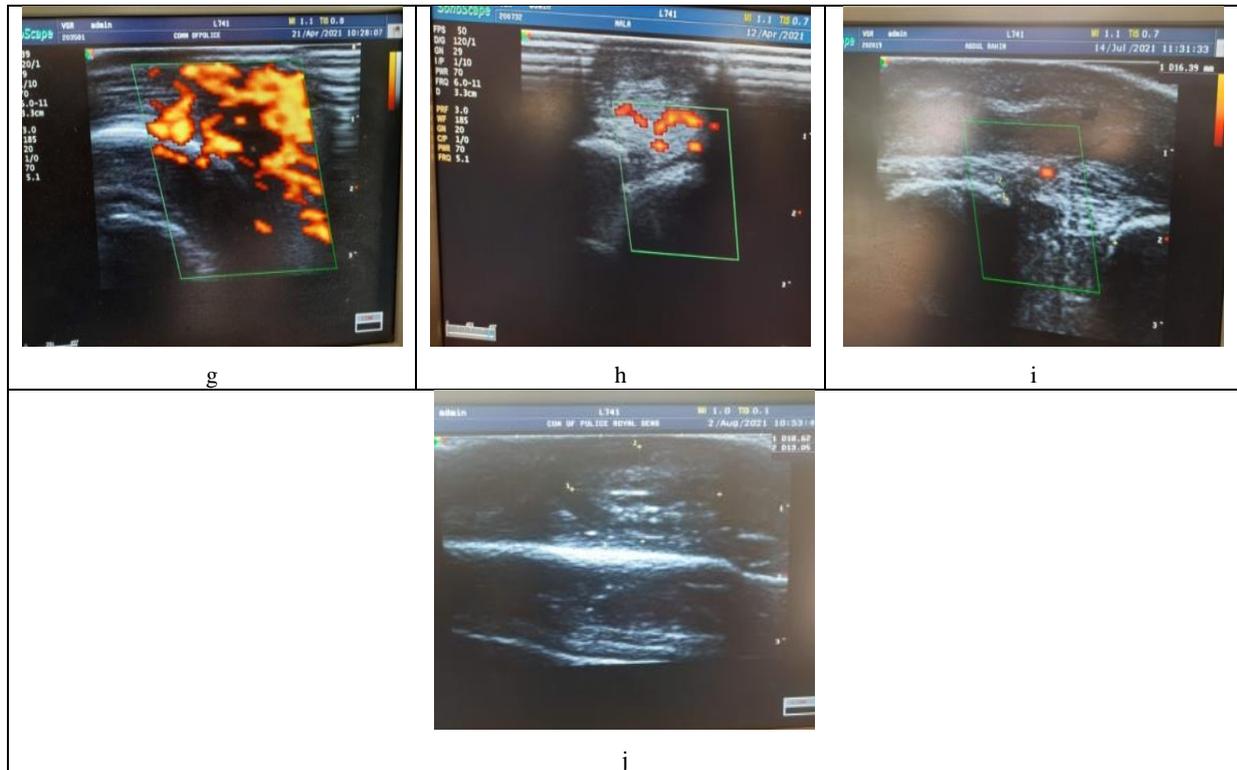
In addition, use of a linear high frequency probe was very useful for assessment of cutaneous structure and was in agreement with the findings of Rippon *et al.* [12] and Dyson *et al.* [13]. However, the time of presentation and different location of wound in the present study had a limitation in identifying wound aetiology with ultrasound and which was differed from the finding of Wendelken *et al.* [14].

**Table 1:** Vascularity assessment by colour flow doppler ultrasound examination with median score value

Treatment	0 Day	5 <sup>th</sup> Day	15 <sup>th</sup> Day	30 <sup>th</sup> Day	45 <sup>th</sup> Day
Group I	3	2	2	1	0
Group II	3	3	2	2	1
Mann-Whitney‘U’ Test	18.00 <sup>NS</sup>	24.00 <sup>NS</sup>	18.00 <sup>NS</sup>	30.00*	31.00*

NS – Non Significant ( $P \geq 0.05$ ): \* – Significant at 5% Level of significant ( $p \leq 0.05$ )





**Fig 1:** Cutaneous wound – Post treatment (a-e): a-0<sup>th</sup> day; b-5<sup>th</sup> day; c-15<sup>th</sup> day; d-30<sup>th</sup> day; e-45<sup>th</sup> day; f- Ultrasound machine with linear (High Frequency Probe) and ultrasound examination of wound margin for healing assessment; (g-j- Power doppler ultrasound examination) g- Power doppler signal for wound grade 3 score signal occupying greater than 50 per cent of the field; h- Power doppler signal for wound grade 2 score signal occupying less than 50 per cent of the field; i- Power doppler signal for wound grade 1 score signal present in single vessel; j- Power doppler signal for wound grade 0 score signal present representing healing.

#### 4. Conclusion

Based on the above ultrasound studies, it may be suggested that in both the groups showed statistically gradual healing at 30<sup>th</sup> and 45<sup>th</sup> day of treatment. From these results, it is concluded that power doppler ultrasonographic method was useful for assessment of wound healing based on vascular status in the wound.

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