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Macroscopical evaluation of cutaneous wound healing in horses treated with collagen and silicone extracellular matrices embedded with autologous platelet rich plasma

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Abstract
The present work was done to study the utility of collagen and silicone extracellur matrices embedded with autologous platelet rich plasma for cutaneous wound healing in horses by Macroscopical assessment. The clinical cases of horses were divided into two groups of six animals each (N=6). Group I animals were treated with collagen extracellular matrices embedded with autologous platelet rich plasma and group II animals were treated with silicone foam dressing embedded with autologous platelet rich plasma. Macroscopical evaluation was studied for 0, 5, 15, 30 and 45th day of post treatment period. Macroscopically, changes were observed as sequential changes in group I when compared to group II animals.

Keywords: Horses, cutaneous wound, collagen, silicone, PRP, Image J, epithelialization wound contraction

1. Introduction
Cutaneous wound and other traumatic injuries of the musculoskeletal system are frequently seen in equine and ponies. Formation of wound occurred by Disruption of the integrity of skin, mucosal surfaces or organ tissue. Typically, this process of wound healing is divided into four distinct phases: haemostasis, inflammation, proliferation and tissue remodelling. Horses suffered with cutaneous wounds on the distal portion of the limb that are difficult to treat and impossible to close and must heal by extra cellular matrices. Wound healing process had a dynamic interaction between extracellular matrix and the cell as a microenvironment, where synthesis, degradation and remodeling of ECM by the cells occurred and the ECM regulation caused tension, polarity, differentiation, migration, proliferation and survival of the cell. (Schultz *et al.*, 2011) [7]. Wound healing depends on factors like The age of the wound, integrity of the local blood supply, degree of contamination, location of the injury, skin loss, tissue tension and mobility of wound margin, local tissue, type and condition of underlying tissue involved (Xu *et al.*, 2020) [8]. Regenerative medicine therapy with extra cellular matrix (ECM) and autologous platelet rich plasma (PRP) provides a platform to create the adequate microenvironment for quicker healing of wound. PRP is a concentrate of growth factors (important for regulating the cellular events involved in wound healing) which stimulates angiogenesis, promotion of growth factor and vascular fibroblast proliferation that ultimately aid to increase the collagen synthesis (Marx, 2004) [5]. The collagen extracellular matrix was bactericidal, anti-inflammatory and promoted wound healing potentially by regulating the fibroblast migration and macrophage activation, making it an ideal dermal substitute for wound regeneration (You *et al.*, 2017) [9]. Silicone dressing prevented the formation of exuberant granulation tissue and improved tissue quality (Desjarlais *et al.*, 2005) [2]. In this relation present study was aimed to investigate and evaluate with comparison the enhancing effect of PRP with collagen and silicone dressing for the management of cutaneous wound in horses by a macrosopical evaluation.

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 selected for this study and detailed clinical examination was performed and the animals which were free from concurrent neurological, metabolic and other diseases were selected for the study.

2.2 Wound Planimetry

Wound planimetry was performed on day 0th, 5th, 15th and 30th day respectively. The wound area was evaluated on each day by using Image J software. The digital photographs were visualized with ImageJ 1.45s software (National Institutes of Health, Rockville, MD; http://imagej. net/ImageJ).

2.3 Image J software protocol

Wound area measurement was carried out as follows:

After opening of Image J software, one file was created (The picture was dragged and dropped into the software).

A segment was drawn along the ruler using "straight line" tool. The software was calculated the distance in pixels of the segment.

Menu> Set scale> Known distance of 10> unit of length (mm) was analyzed. The software automatically recalculated the number of pixels/mm.

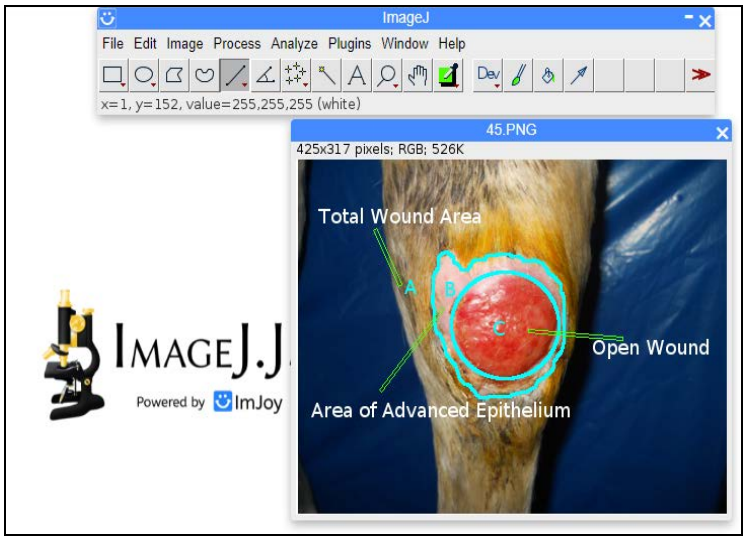
Wound outline was created using "Freehand Selection" tool by tracing wound shape.

Menu> Measure was analysed and the area in cm2 was also calculated.

Then a screenshot of the picture including the measurement window was taken.

Measurements were plotted in a Microsoft Excel sheet (Microsoft Corporation, Bellevue) for wound area measurement.

The wound margin (At the border between the normal skin and the wound) and the outlined area were considered as the total wound area. The area between these two margins was considered as the area of epithelialization. Area within the margin of advancing epithelium was defined as unhealed wound area or open wound (Fig1).



2.4 Percentage of epithelialization

$$\text{Percentage of epithelialization on day n} = \frac{100 \text{ x Area of epithelium on the day n}}{\text{Total wound wound area day n}}$$

2.5 Percentage of wound contraction

$$\text{Step 1: Total wound on dayn as \% of original} = \frac{100 \text{ x Total wound area day n}}{\text{Original wound area day 0}}$$

Step 2: Per cent wound contraction day n = 100- Total wound on day n as percent of original

2.6 Percentage of wound healing

$$\text{Step 1: Open wound dayn as \% of original} = \frac{100 \text{ x open wound area at day n}}{\text{Original wound area day 0}}$$

Step 2: Per cent total wound healing dayn = 100 - open wound dayn as % of Original

3. Results and Discussions

Wound planimetry were taken in both group I and II on day 5, 15, 30, 45 interval periods to evaluate the macroscopical changes on wound healing during post treatment.

The Image J software was found to be a very useful tool for performing wound planimetry studies in equines. It was able to assess percent epithelialization, per cent wound contration and per cent wound healing. The illustration of wound planimetry procedure was displayed in Fig 1.

3.1 Percentage of epithelialisation

The Mean ± SE of per cent epithelialization in group I animals were 13.63±6.12, 30.01± 8.71, 50.48±8.92 and 66.63. ±8.62 at 5th day, 15th day, 30thday, 45th day respectively. The Mean ±SE of per cent epithelialization in group II animals were 1.97±0.28, 11.86±3.11, 27.28±5.18 and 31.31±10.01 at 5th day, 15th day, 30th day and 45th day respectively. Statistical analysis revealed a significant difference observed between group I and group II animals ($p \leq 0.05$) at 30th day and highly significant difference ($p \leq 0.01$) observed at 45th day respectively.

The Mean ± SE of percentage of epithelialization values are represented in Table 1 and Fig. 2.

Table 1: Percentage of epithelialisation (Mean ±S.E) of cutaneous wound in group I and group II animals

Treatment	5 Day Epithelialisation	15 Day Epithelialisation	30 Day Epithelialisation	45 Day Epithelialisation
Group I	13.63±6.12	30.01±8.71	50.48±8.92	66.63±8.62
Group II	1.97±0.28	11.86±3.11	27.28±5.18	31.31±10.01
't' value	1.90^{NS}	1.96 ^{NS}	2.24*	3.12 **

NS – Non-Significant (P≥0.05)
* – Significant at 5% Level of significant (p≤0.05)
** - Highly Significant at 1% Level (P≤0.01)

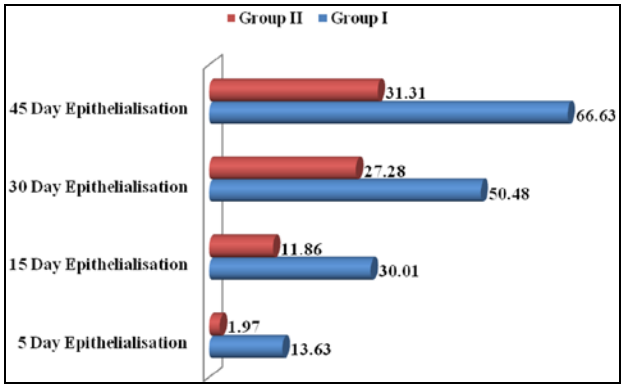


Fig 1: Percentage of epithelialization

3.2 Percentage of wound contraction

The Mean ± SE of per cent wound contraction in group I animals were 16.70±4.62, 29.35±7.37, 43.51±6.88 and 59.15±5.31 at 5th day, 15th day, 30th day, 45th day respectively. The Mean ±SE of per cent wound contraction in group II animals were 13.43±0.88, 25.88±2.88, 29.38±5.40 and 35.08±6.68 at 5th day, 15th day, 30th day and 45th day respectively. Statistical analysis revealed a significant difference observed between group I and group II animals (p≤0.05) at 30th day and highly significant difference (p≤0.01) observed at 45th day respectively.

The Mean ± SE of percentage of wound contraction values are represented in Table 2 and Fig. 3.

Table 2: Percentage of wound contraction (Mean± S.E) of cutaneous wounds in group I and group II animals

Treatment	5 Day Wound Contraction	15 Day Wound Contraction	30 Day Wound Contraction	45 Day Wound Contraction
Group I	16.70±4.62	29.35±7.37	43.51±6.88	59.15±5.31
Group II	13.43±0.88	25.883±2.8852	29.38±5.40	35.08±6.68
't' value	0.91^{NS}	1.10^{NS}	2.21*	3.78**

NS – Non-Significant (P≥0.05)
* – Significant at 5% Level of significant (p≤0.05)
** - Highly Significant at 1% Level (p≤0.01)

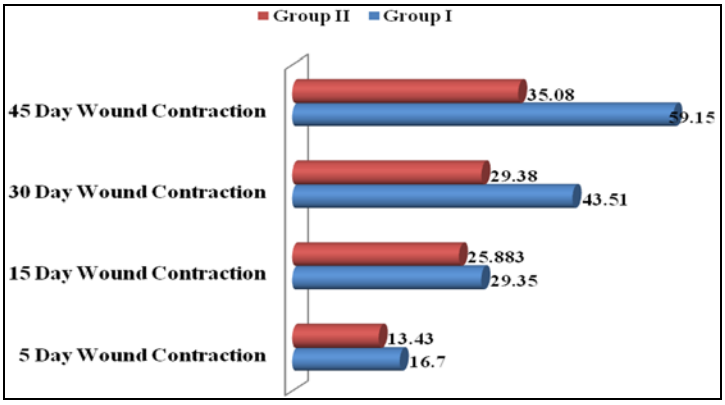


Fig 2: Percentage of wound contraction

3.3 Percentage of wound healing

The Mean ± SE of per cent total wound healing in group I animals were 27.58±7.38, 46.41±9.76, 70.21±8.17 and 82.78±5.63 at 5th day, 15th day, 30th day, 45th day respectively. The Mean ±SE of per cent total healing in group II animals were 25.36±1.05, 27.91±5.16, 36.53±6.93 and 45.76±7.55 at 5th day, 15th day, 30th day and 45th day respectively. Statistical analysis revealed a significant difference between Group I and Group II animals (p≤0.05) at 15th day and a highly significant difference (p≤0.01) was observed at 30th and 45thday respectively.

The Mean ± SE of percentage of total healing values are represented in Table 3 and Fig. 4.

Table 3: Percentage of wound healing (Mean ±S.E) of cutaneous wounds in group I and group II animals

Treatment	5 Day Wound Healing	15 Day Wound Healing	30 Day Wound Healing	45 Day Wound Healing
Group I	27.58±7.38	46.41±9.76	70.21±8.17	82.78±5.63
Group II	25.36±1.05	27.91±5.16	36.53±6.93	45.76±7.55
't' value	0.85 NS	2.156*	2.923**	3.545**

NS – Non-Significant (P≥0.05)
* – Significant at 5% Level of significant (p≤0.05)

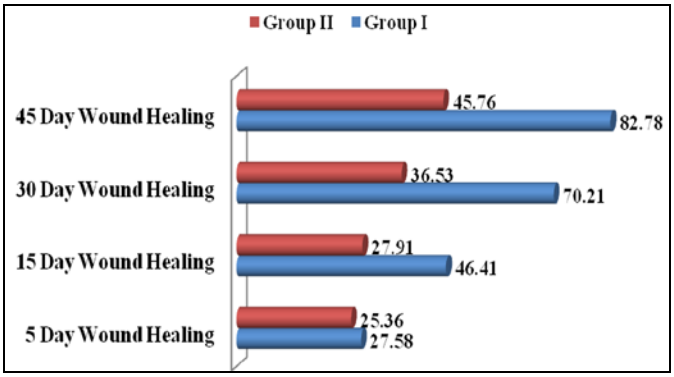


Fig 3: Percentage of wound healing

In the present study, wound Planimetry was performed using Image J software to assess the percent epithelialization, percent wound contraction and per cent total wound healing. In group I animals, the per cent epithelialization at 30th day was 50.48±8.92 when compare to group II animals which had epithelialization of 27.28±5.18 and at 45thday the percent epithelialization in group I animals was 66.63±8.62 and 31.31±10.01 in group II animals and a statistically significant similar pattern was observed in percent wound contraction and percent total healing between group I and group II animals. The percent assessment of wound healing by planimetry were concurrence with the findings of Plassmann (1995) [6], Foltynski *et al*., (2015) [3] and Jorgensen *et al.*, (2016) [4] for application of digital planimetry in wound healing assessment. However, Chang *et al.*, (2011) [1] described a quicker inexpensive method using square or rectangle for wound healing assessment but added in his findings that digital palnimetry had several advantages over manual method. Yvorchuk-St Jean *et al.*, (1995) [10] found that there were no significant differences in the total wound epithelialized, or contraction areas were detected between the porous bovine collagen membranes - treated and control (nonadherent-treated) wounds and this differed from the present study findings. The author further studied the rates of wound healing were not statistically different between the two treatment groups and were significantly slower in the hind limbs compared with the forelimbs.

4. Conclusions

The Image J software was found to be a very useful tool for performing wound planimetry studies in equines. It was able to assess per cent epithelialization, per cent wound contrition and per cent wound healing. Early epithelialization was observed in group I animals on day 5 when compared to group II which occurred on day 15. A statistically significant difference was observed in percent epithelialization between group I and Group II animals at ($p \leq 0.05$) 30th day and ($p \leq 0.01$) at 45th day respectively. Similar changes were observed for per cent wound contraction and per cent total wound healing.

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