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Histological 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 extracellular matrices embedded with autologous platelet rich plasma for cutaneous wound healing in horses by histological 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. Histological evaluation was studied for 0, 5, 15, 30 and 45th day of post treatment period. Microscopically, histological changes were observed as sequential changes in group I when compared to group II animals.

Keywords: Horses, cutaneous wound, collagen, silicone, PRP, pathology, wound healing

1. Introduction

Cutaneous wound healing is a physiologically active process that is triggered after the loss of skin integrity. It consists of four phases; Haemostasis, inflammatory phase, proliferative phase and remodelling phases (Harper *et al.*, 2014) [6]. Wound healing depends on factors like blood supply, tissue tension and mobility of wound margin, size of the wound susceptibility to infection, type and condition of underlying tissue involved (Xu *et al.*, 2020) [12]. Regenerative medicine therapy with extra cellular matrix (ECM) and autologous platelet rich plasma (PRP) provides the adequate microenvironment for quicker healing of wound. PRP stimulates angiogenesis, promotion of growth factor and vascular fibroblast proliferation (Xu *et al.*, 2020) [12]. Collagen acts as a natural substrate for cellular attachment, growth, differentiation and promotes cellular proliferation. Collagen's dressing is commercially available in the form of sheet, powder, gel and sponge which accelerates the epithelialization in the wound (Ruszczak, 2003) [7]. Silicone dressing is synthetic, non-adherent, fully occlusive dressings which promotes wound healing and at the same time restrict the formation of exuberant granulation tissue which acts as limiting factor in wound healing of horses (Desjarlais *et al.*, 2005) [3]. 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 histological 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 treatment

On 0th, 5th, 15th, 30th and 45th day, wound treatment was performed with collagen and

silicone extracellular matrices embedded with autologous platelet rich plasma (PRP).

2.3 Biopsy

Skin biopsies were obtained on the day 0th, 5th, 15th and 30th after treatment. The horses were sedated with 10 per cent xylazine (1mg/kg) and 2 per cent lidocaine spray applied topically. Biopsy samples were collected by using a 6.5 mm-dermatological punch biopsy needle (BISCO, India) under aseptic preparation. Samples were immediately fixed in 10% formalin for 24 hours and then paraffin embedded samples were sectioned to 5 µm thickness and stained by haematoxylin and eosin (H&E) for histopathological examination.

3. Results and Discussion

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

On fifth day, in group I animals, the biopsy samples showed that epidermis had downward hyperplasia of *Stratum basali* with early epithelialization, presence of inflammatory cells with profused granulation tissue characterized by proliferation of endothelial cells and fibroblast cells. In group II animals, epidermis had downward hyperplasia with thickening of epidermis and presence of more inflammatory cells and dermis had profuse fibrous connective tissue proliferation. Fig. 1(a-b), Fig. 3 (a-b)

On fifteen days of treatment, in group I animals, the biopsy samples showed that epidermis had downward hyperplasia of *Stratum basale*, complete epithelialization, profused fibrosis, formation of new blood vessels, presence of collagen fiber, small size hair follicle and sweat gland formation were observed in dermis. In group II animals, the epidermis had epidermal necrosis with partial epithelialization with infiltration of moderate inflammatory cells, fibroblast cell formation, and mild collagen deposition, presence of disorganized flabby connective tissue and irregular arrangement of fibrosis. Fig. 1(c-d), Fig. 3 (c-d)

The collagen with PRP treated biopsy samples collected on 30th day of post wound displayed the organized epithelial cells of epidermis. In dermis there was a regular arrangement of fibrous connective tissue, new numerous blood vessels,

moderate size of hair follicle and sweat gland formation were observed in group I animals. In group II animals, histological section revealed that dermis showed connective tissue with moderate inflammatory cells, sebaceous gland, pilous follicles and angiogenesis of small blood vessels, hair follicle and minimal proud flesh appearance was observed. Fig. 2 (e-f, g-h), Fig. 4 (e-f)

Histological pictures obtained from group I and group II animals on 45th day of post wound treatment revealed formation of mature skin adnexal structure in dermis, hair shaft with primary follicle, sebaceous gland, well developed connective tissue with collagen fiber and scarce amount inflammatory cells. Fig. 2 (i-j), Fig.4 (g-h)

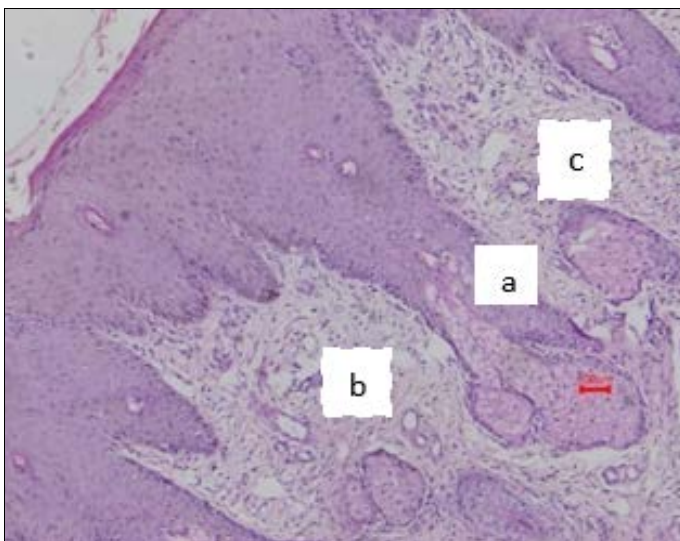
Collagen dressing embedded with autologous platelet rich plasma was found to promote early epithelialization of wound contraction and improved the total percentage of wound healing in equine when compared to silicone foam embedded with autologous PRP.

In the present study, inflammatory phase was seen in 5th day of post treatment exhibiting neutrophils and inflammatory cells in both the groups. These findings were in accordance to the inflammation phase of equine wound which was reported by Silver (1982) [8]; Harper *et al.* (2014) [6], Theoret, (2016) [10] and Guo and Dipietro (2010) [4]. Collagen deposition with signs of healing were observed on 15th day of treatment in the both groups which showing the signs of healing and it agreed with the findings of Stashak (2009) [9] and which was differed from the conclusion of Guo and Dipietro (2010) [4] who reported that the collagen deposition at 1-7 days post injury

At 30th day, both the group I and group II animals showed signs of epithelialization and at 45th day showed maturation and contraction. These findings concurred with the reports of Bertone (1989) [1]; Stashak (2009) [9]; Deodhar and Rana (1997) [2]; Theoret, (2005) [11] and Harman *et al.* (2018) [5].

4. Conclusions

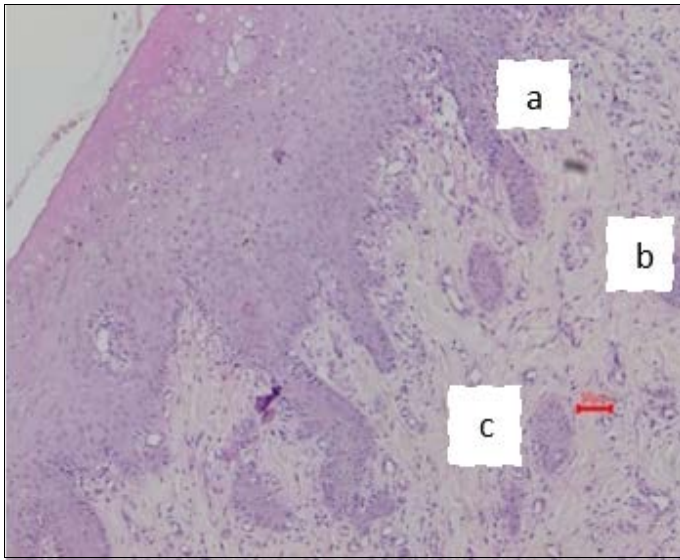
Based on the above histological studies, Group I animals had better healing when compared with Group II animals because on 15th day treatment in group I animals' biopsy samples revealed early epithelialisation, formation of new blood vessels, and presence of collagen fibers and small size hair follicle, sweat gland formation observed in dermis when compared to Group II animals.



a). GI- a. Downward hyperplasia of stratum basali. b. Mild inflammatory cells. c. Profused granulation tissue characterized by endothelial cells and fibroblast cells.



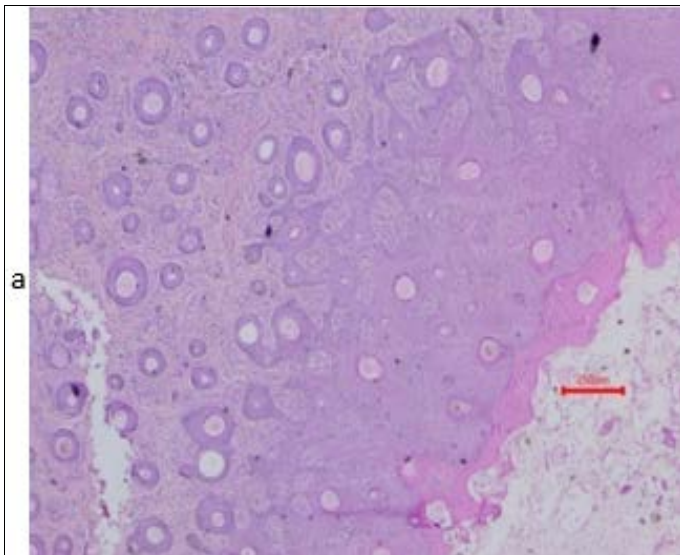
b) 5th day post treated wound by collagen and PRP ECM



c) GI – a. Downward hyperplasia of stratum basali. b. Early epithelialization. c. Profused fibrosis and formation of new blood vessels, presence of collagen fiber, small size hair follicle and sweat gland formation

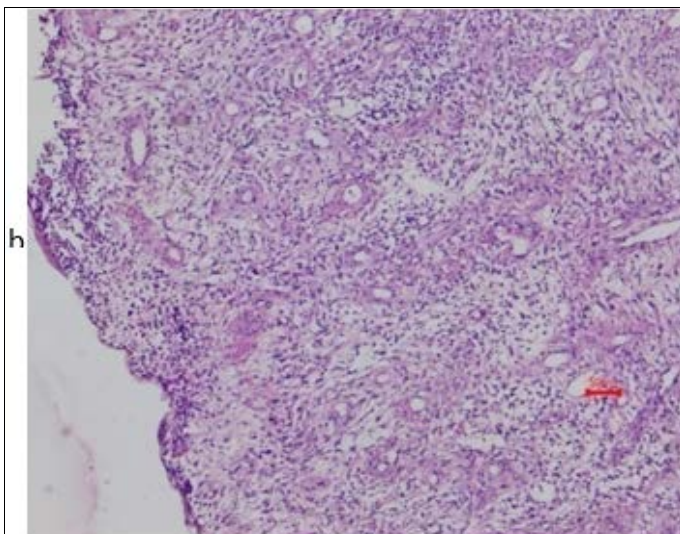
d) 15th days post treated wound by collagen and PRP ECM

Fig 1: Histopathological examination for group I animals



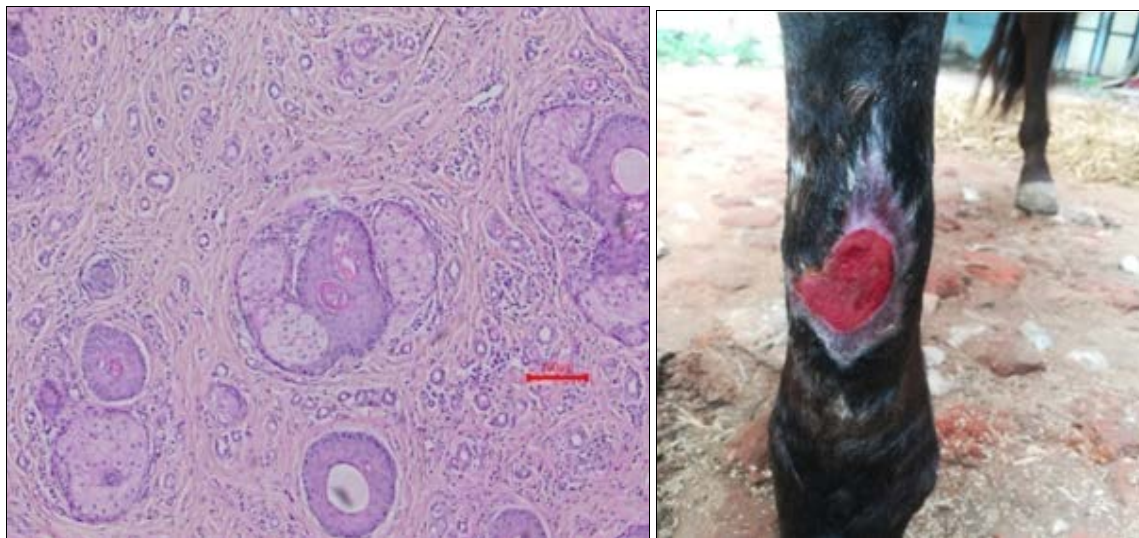
e) GI- a. Hyperplasia of epithelial cells of epidermis, dermis. b. Regular arrangement of fibrous connective tissue, new numerous blood vessels, moderate size of hair follicle, sweat gland

f) 30th day post treated wound by collagen and PRP ECM



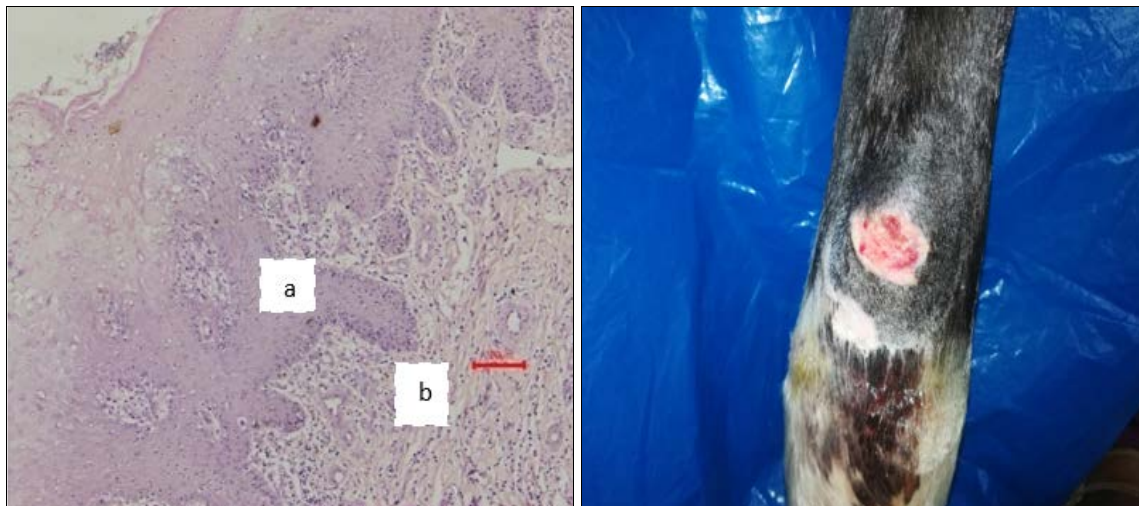
g) GI – Proud flesh – a. Hyperplastic epidermis with inflamed granulation tissue. b. proliferating solid nest of endothelial cell nest

h) 30th day post treated wound by collagen and PRP ECM with proud flesh wound

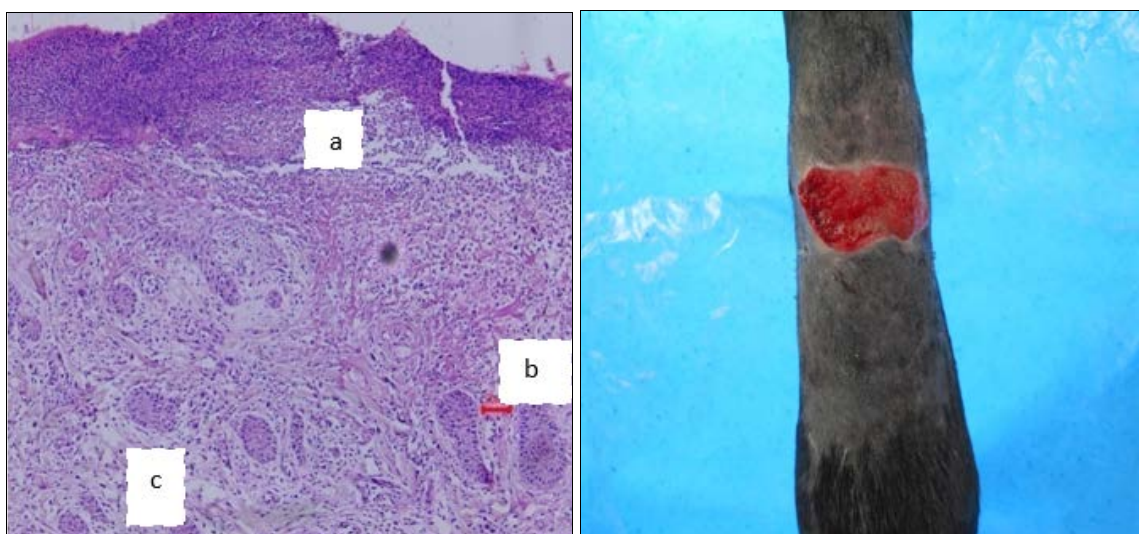


i) GI – a. Formation of mature skin adnexal structure in dermis. b. Hair shaft with primary follicle and sebaceous gland well developed collagen fiber and absence of inflammatory cells
 j) 45th day post treated wound by collagen and PRP

Fig 2: Histopathological examination for group I animals

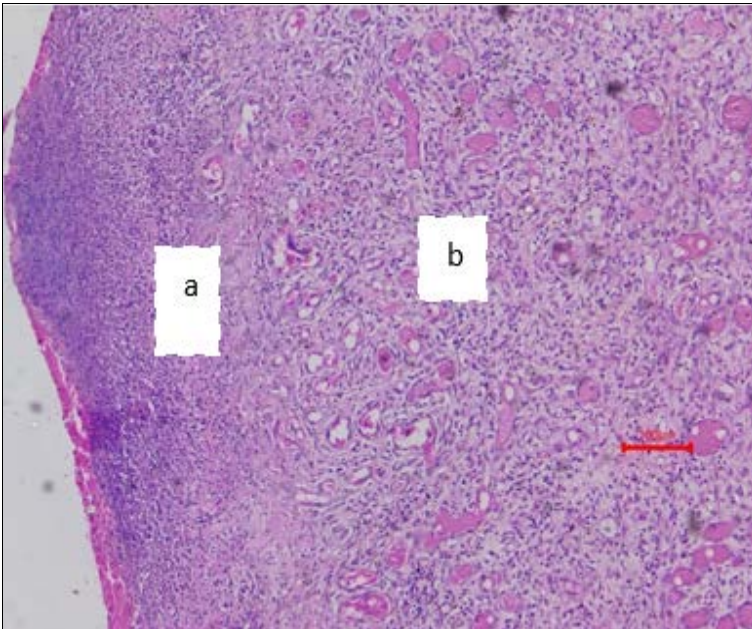


a) GII- a. Hyperplasia with thickening of epidermis. b. profuse fibrous connective tissue
 b) 5th day post treated wound by Silicone and PRP ECM



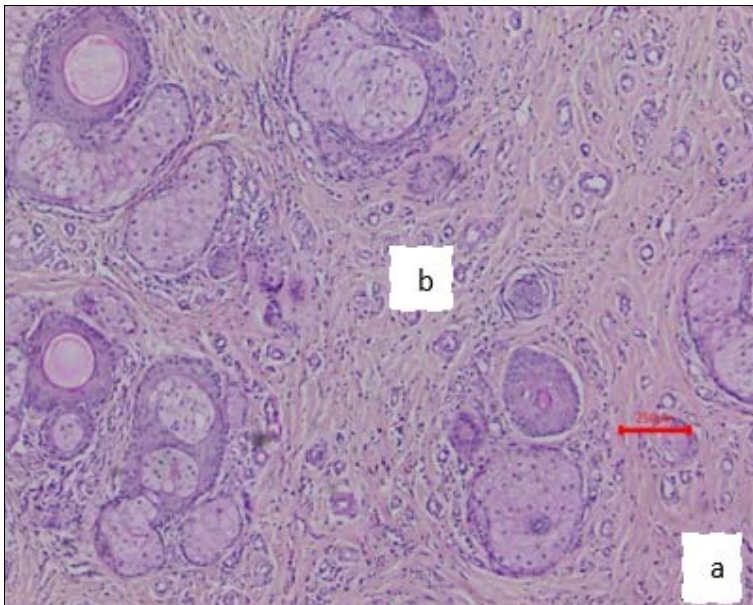
c) GII- a. Epidermal necrosis with fibroblast cell formation and mild collagen deposition. b. Presence of flabby connective disorganized, irregular arrangement of fibrosis. c. Infiltration of moderate inflammatory cells
 d) 15th day post treated wound by Silicone and PRP ECM

Fig 3: Histopathological examination for group II animals



e) GII- a. Dermis shows connective tissue with sebaceous gland and pilous follicles. b. Small blood vessels and hair follicle. Absence of proud flesh

f) 30 days post treated wound by silicone and PRP



g) GII – a. Formation of mature skin adnexal structure in dermis hair shaft with primary follicle and sebaceous gland. b. Well-developed collagen fiber and absence of inflammatory cells

h) 45 day post treated wound by silicone and PRP

Fig 4: Histopathological examination for group II animals

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