JOURNAL OF VETERINARY AND APPLIED SCIENCES
2014 VOL. 4(1): 9 - 16
*Manuscript No. JVAS/2014/029; Received: 01/04/2014; Accepted: 07/07/2014*
*Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria*

# COMPARATIVE HISTOPATHOLOGICAL ASSESSMENT OF WOUND HEALING MARKERS ON SURGICALLY INDUCED SKIN WOUNDS OF RABBITS (*Oryctolagus cunnicullus*) SUTURED WITH COTTON HAIR THREAD AND SILK SUTURE MATERIALS

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***ABSTRACT***

***Histopathological assessment of tissue reactions in surgically induced skin wounds of rabbits sutured with cotton hair thread (CHT) and silk suture was investigated. Twenty four (24) clinically healthy male rabbits of New Zealand breed; aged 6 – 8 months with a weight range of 1.5 - 1.8 kg were used for the study. Following acclimatization for a period of two weeks, they were randomly assigned into two groups A and B of twelve (12) rabbits each. A 6 cm paralumber skin incision was aseptically performed on rabbits in both groups using xylazine (0.5 mg/kg body weight) and ketamine (22 mg/kg body weight) as pre-medicant and anesthetic respectively. The incised skin in group A was apposed with conventional silk suture while the incised skins in group B were approximated with CHT. Surgical sites in both groups were excised from two rabbits each for histopathological evaluation at post-operative days (pds) 1, 7, 10, 14, and 21. The histopathological wound healing markers such as re-epithelialization, polymorphonuclear leucocytes (PMNLs) and tissue macrophages infiltrations, fibroblasts, collagenization and neovascularization were evaluated and scored semi-quantitatively thus, none = 0, few = 0.5, moderate = 1, many = 2 and marked = 3. The semi-quantitative data of the wound healing markers when tested statistically, showed no significant difference (p > 0.05) in both groups A and B throughout the study. The results showed that cotton hair thread elicited similar histopathological reactions with the conventional silk suture. Therefore CHT is biocompatible after sterilization and could be used as skin suture in rabbits.***

***Keywords*:** Cotton hair thread, silk, suture, skin wound, rabbits.

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## INTRODUCTION

Wound healing is an intricate process in which tissues are repaired after injury [1]. Normal skin consists of the epidermis (outermost layer) and dermis (inner or deeper layer) which form a protective barrier against the external environment. Once the protective barrier is broken, the normal (physiologic) process



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**ISSN: 2315 - 6856**

of wound healing is immediately set in motion [2]. The initial inflammatory responses to injury provide the necessary framework to the subsequent production of a new functional barrier. In this phase of healing, cellular activity predominates. The major events during this phase are the creation of a permeability barrier (i.e., re-epithelialization), the establishment of appropriate blood supply (ie, angiogenesis), and reinforcement of the injured dermal tissue - fibroplasia [3].

Re-epithelialization is the process of restoring an intact epidermis after cutaneous injury. It generally involves several processes, including the migration of adjacent epidermal keratinocytes into the wound, the proliferation of keratinocytes used for the supplemention of the advancing and migrating epithelial tongue, the differentiation of the neo-epithelium into a stratified epidermis, and the restoration of an intact basement membrane zone (BMZ) that connects the epidermis and the underlying dermis [3].

Fibroplasia describes a process of fibroblast proliferation, migration into wound fibrin clot, and production of new collagen and other matrix proteins, which contribute to the formation of granulation tissue. As an early response to injury, fibroblasts in the wound edges begin to proliferate and by approximately day 4 start to migrate into the provisional matrix of the wound clot, where they lay down a collagen-rich matrix, including collagens, proteoglycans, and elastin [4,5]. Once the fibroblasts have migrated into the wound, they gradually change to profibrotic phenotypes and switch their major function to protein synthesis. Fibroblasts are also modulated into phenotypes of myofibroblasts and participate in wound contraction [6].

Angiogenesis refers to new vessel growth by the sprouting of preexisting vessels adjacent to the wound. As in most normal adult tissues, dermal blood vasculatures remain quiescent. In response to the injury, microvascular endothelial cells initiate an angiogenic process consisting of activation of endothelial cells, local degradation of their basement membrane, sprouting into the wound clot, cell proliferation, and tubule structure formation, reconstruction of the basement membrane, and, eventually, regression and involution of the newly formed vasculature as tissue remodeling [7].

The principal role of suture is to approximate wound edges so as to enhance wound healing. Chemical and physical configuration of sutures affects the rate of wound healing [8]. The search for more appropriate suture material has resulted in a variety of natural and synthetic suture materials (absorbable and non-absorbable). These materials influence the biological reaction to the suture permitting a great diversity of clinical application [9,10].

The main objective of this study was to histologically compare the variant behavior of wound healing markers; reepithelialization, polymorphonuclear leucocytes (PMNLs) and tissue macrophages infiltration, fibroblasts proliferation, collagenization and vascularization following the use of CHT and silk suture on surgically induced skin wounds of rabbits.

## MATERIALS AND METHODS

Twenty four (24) clinically healthy male New Zealand breed of rabbits aged 6 – 8 months and purchased from Ibagwa market, Nsukka were used for the study. They were housed in the Experimental Animals House of the Department of Veterinary Surgery, University of Nigeria, Nsukka. They were acclimatized for two weeks and randomly assigned into two groups, A and B of twelve (12) rabbits each. They were kept one per cage in the fly-proof house. The animals were fed with sweet potato leaves supplemented with commercial grower feed (Vital® Feed, Jos, Nigeria). Water was provided *ad-libitum* throughout the study period.

### Pre-operative preparation

The non-conventional cotton hair thread (CHT) along side with the complete surgical pack was sterilized by autoclaving prior to surgery. The paralumber region of each rabbits was carefully shaved of hairs using

powered surgical clipper. The shaved area was aseptically scrubbed using chlorhexidine hydrochloride antiseptic. Xylazine (0.5 mg/kg body weight, Indian Immunologicals, Arendont-Belgium ) and Ketamine (22 mg/kg body weight, Rotexmedica, Trittau, Germany) were administered intramuscularly as pre-medicant and anesthetic respectively and the area draped accordingly.

**Surgery**

A 6 cm paralumber skin incision was asepetically created in all the experimental animals. The incised skin in group A was apposed with silk suture using simple interrupted suture pattern while the incision in animals in group B was approximated with cotton hair thread (CHT) using the same suture pattern.

**Histopathological evaluation**

The surgical sites from two animals in each of the two groups (A and B) were excised for histological evaluation of wound healing markers at post surgical days (psd) 1, 7, 10, 14 and 2. The excised tissues were preserved in 10% formalin. The specimens were labeled and routinely processed for histological examination. Briefly, the tissues were passed through graded concentrations of ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Sections of 5µm thickness were obtained with Leitz microtome model 1512. They were stained with haematoxylin and eosin for light microscopy examination [11].

The prepared tissue sections were evaluated histologically using the semi-quantitative method to grade the wound healing markers; re-epithelialization, polymorphonuclear leucocytes (PMNLs), tissue macrophages, fibroblast, collagenisation and vascularization. The semi-quantitative evaluation and comparison of the wound healing indices were based on a five point score as follows: none = 0, few =0.5, moderate =1, many = 2 and marked =3 [12]. The microscopic examination and grading were done in a blinded fashion and the mean values from at least three different fields of each specimen were used for statistical comparison.

**Statistical analysis**

The data obtained were summarized and presented as means ± S.E.M and analyzed using the Student's t-test. Significance was accepted at 5% probability level ($p < 0.05$).

**RESULTS**

At day 1 post-surgery (Figures 1A and 1B), the wounds in both groups A and B were characterized by little re-epithelialization while inflammatory cells (PMNLs) were considerably prominent especially under the necrotic points at the line of incision where fibrin was also noted. Fibroblasts and tissue macrophages were sparsely seen at this stage while revascularization and collagen formation were absent in both groups A and B.

At psd 7 (Figures 2A and 2B), re-epithelialization was slightly more in the group B (CHT) as seen by thick and greater number of epithelial layers and the differentiation of keratinocytes to keratinized cells. Fibroblasts, new blood vessels and non-organized collagen were also prominent at this stage in both groups. However, PMNLs and tissues macrophage were sparsely distributed in the two groups.

At psd 10 (Figures 3A and 3B) the re-epithelialization process was stable. PMNLs decreased greatly while fibroblast, proliferation of new blood vessels and collagenization were similar in both groups. At psd 14 (Figures 4A and 4B), the process of re-epithelialization has drastically reduced just like PMNLs and tissues macrophages. Re-vascularization and collagen formation were markedly high in groups A and B.

At day 21 post-surgery (Figure 5A and 5B), the whole process of wound healing was complete in both groups as seen by a regression in some of the wound healing features. Fibroblast recruitment was

enhanced in both groups while angiogenesis remained almost the same for all the two groups. Thick collagen was observed. The semi-quantitative evaluation of the wound healing markers showed no significant difference ($p > 0.05$) between the two groups throughout the study (Table 1).

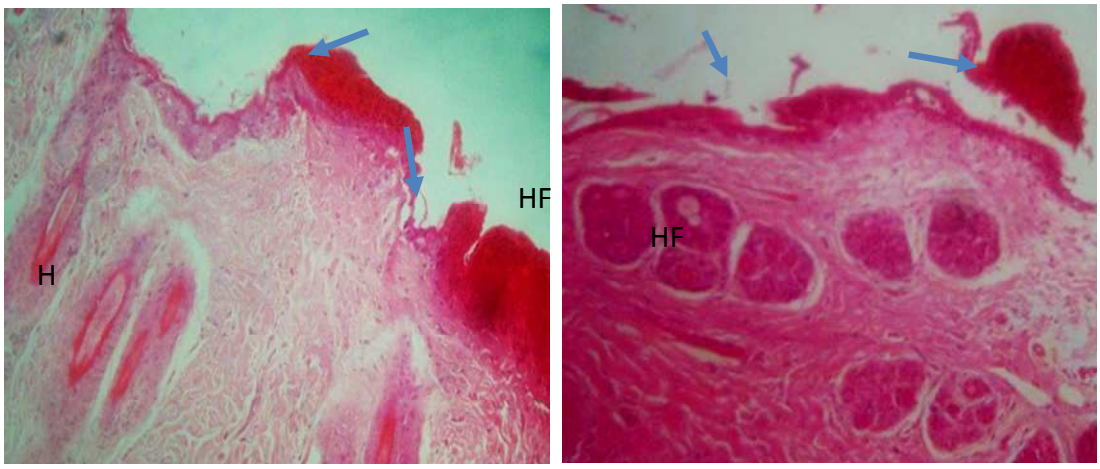


Figure 1: A section of skin wound sutured with Silk (Grp A = left) and CHT (Grp B = right) at day 1 post-surgery showing the hair follicles (HF) and blood clot (arrow) (H&E x100)

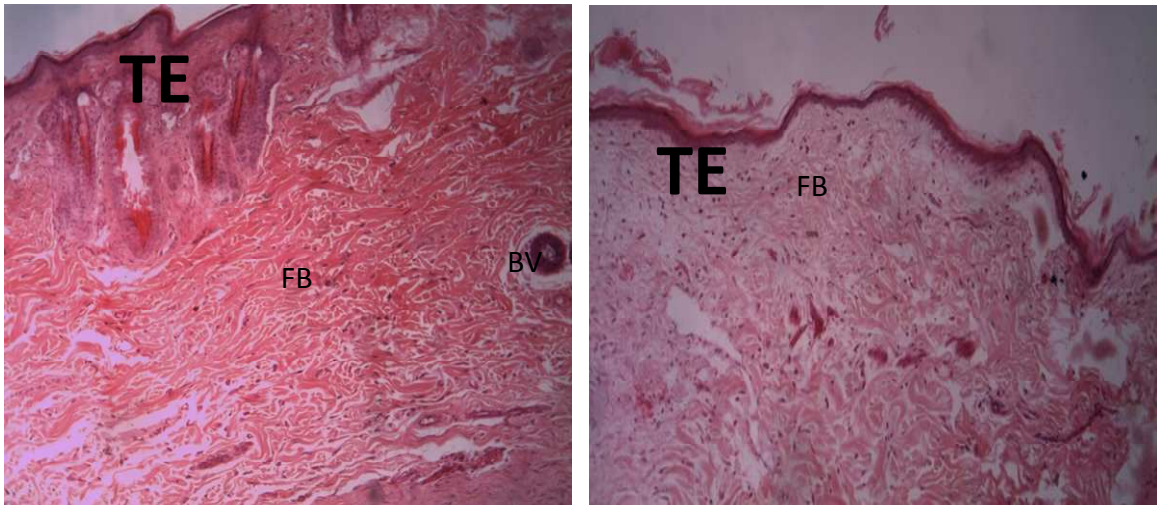


Figure 2: A section of skin wound sutured with silk (Grp A = left) and CHT (Grp B = right) at day 7 post-surgery showing blood vessels (BV), fibroblasts (FB) and thick epithelium (TE) (H&E x100).

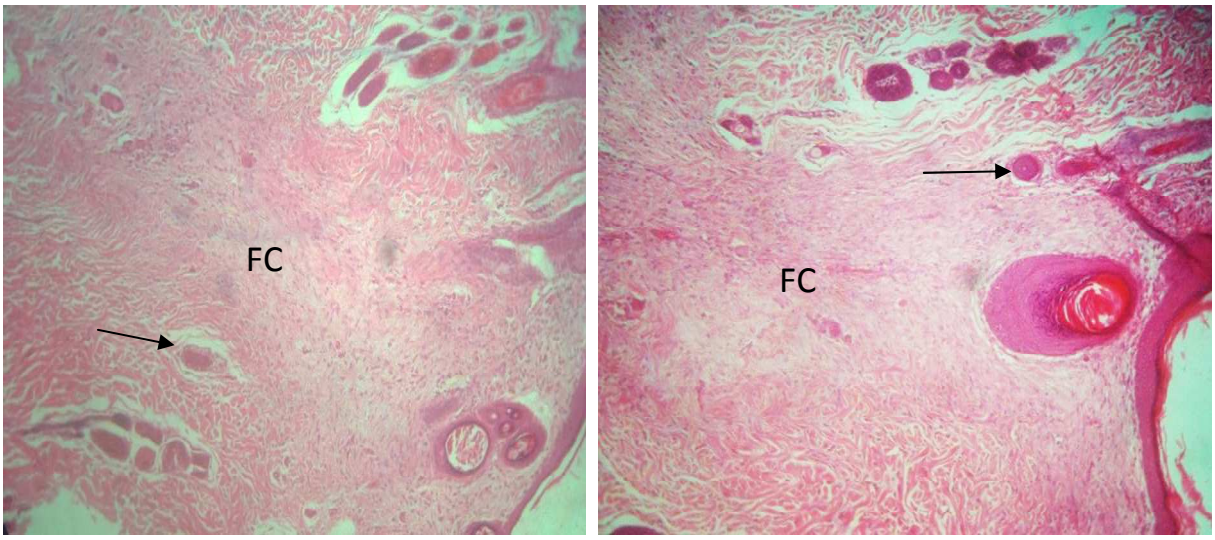


Figure 3: A section of skin wound sutured with silk (Grp A = left) and CHT (Grp B = right) at day 10 post-surgery showing high fibroblast proliferation and collagenization (FC) and blood vessels (arrow) (H&E x400).

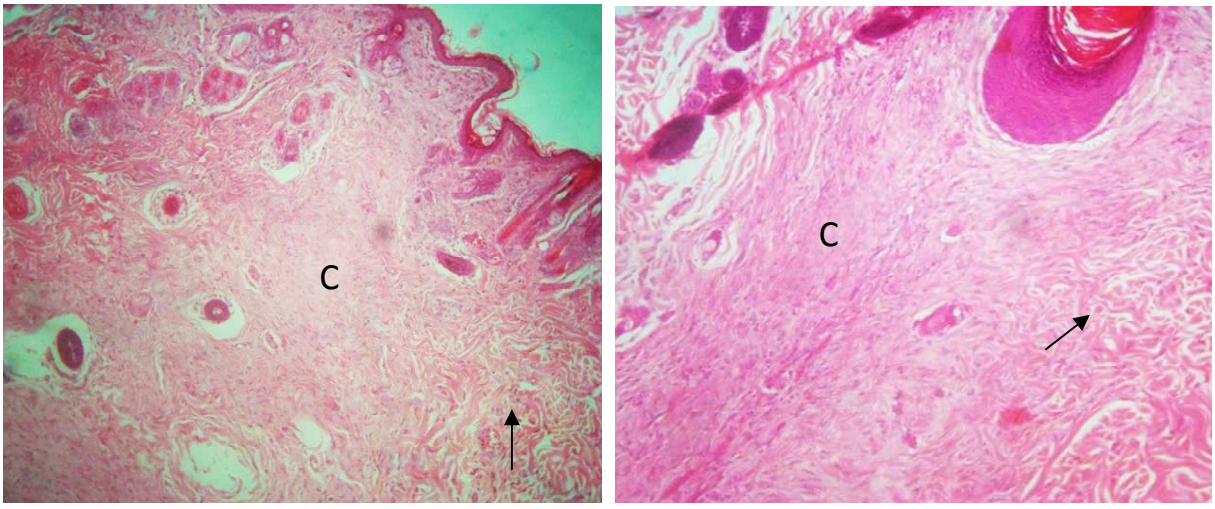


Figure 4: A section of skin wound sutured with silk (Grp A = left) and CHT (Grp B = right) at day 14 post-surgery showing fibroblasts (arrow) and more collagenization (C) (H&E x100).

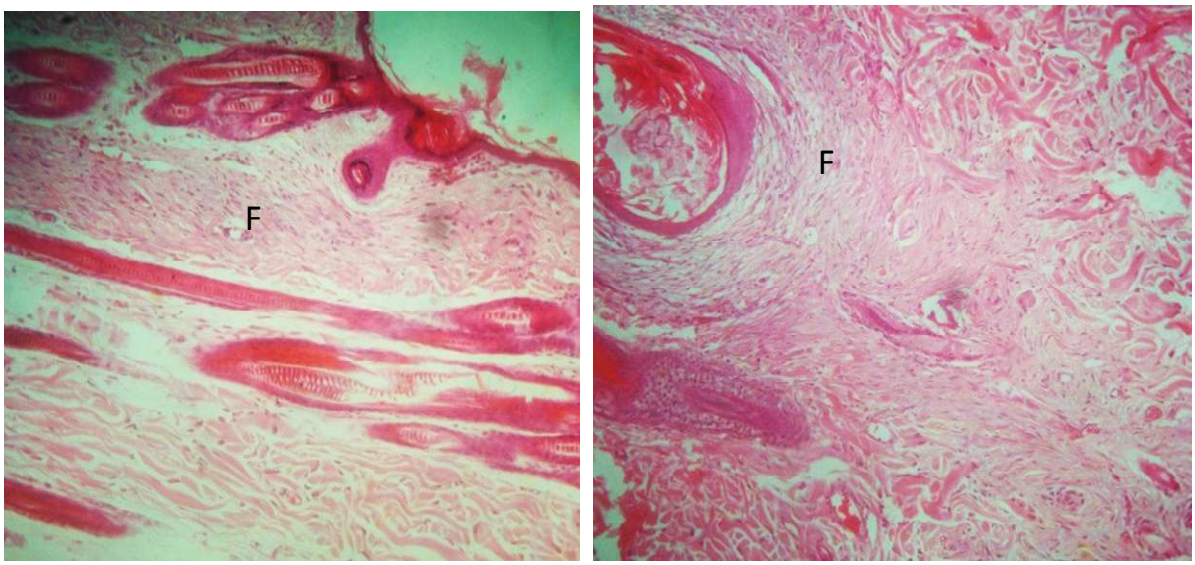


Figure 5: A section of skin wound sutured with Silk (Grp A - left) and CHT (Grp B = right) day 21 post-surgery showing high level of collagenization and fibroplasia (F) indicating complete union in both groups (H&E x100).

**DISCUSSION**

Re-epithelialization depicts a process of restoring an intact epidermis after cutaneous injury [6]. The results of this study showed little re-epithelialization and presence of high level of inflammatory cells (polymorphonuclear leucocytes) and absence of re-vascularisation in both groups A and B at day 1 post-surgery. The observed tissue reactions depict early stage of wound healing. The above observations were in agreement with the work of Li *et al*. [3] who stated that inflammatory cells (polymorphonuclear leucocytes) predominates the wound site within 24hrs post-wounding. Li *et al*. [3] also observed that re-epithelialization starts within 24hrs, peaking at 72hrs post-wounding in acute wound.

The absence of collagen at this early stage of wound healing is not unusual since its normal formation in an injured area commences within 3 to 4 days post-wounding [3]. Presence of thick and greater number of epithelial layers in both groups A and B at day 7 post- surgery suggested a marked level of re-epithelialization and progression to a more advance stage of wound healing.

At day 7 fibroblasts proliferation and revascularization became very prominent at this stage of wound healing. These observations agree with the work of Li *et al.* [3] who made similar observations 7 days post-wounding in the rat. In addition, Kurkinen *et al*. [4] also noted that fibroblasts in the wound edges begin to proliferate and by approximately day 4 start to migrate into the provisional matrix of the wound clot, where they lay down a collagen-rich matrix, including collagens, proteoglycans, and elastin.

However, at day 7, polymorphonuclear leucocytes and tissues macrophage were sparsely distributed in the two groups. This is in contrast with the work of Dahe *et al.* [13] who observed marked infiltration of polymorphonuclear leucocytes and macrophages in the wound sites of rats sutured with silk at day 7 post-surgery.

The histological results at day 10 post-surgery in both groups A and B showed a considerable reduction in the polymorphonuclear leucocytes. The stability of the vascularization and fibroblasts that were noted in both groups depict advanced stage of wound healing. Li *et al*. [3] in evaluating the rate of wound healing post wounding in the rat noted similar observation at day 10 post-surgery.

**Table 1: Mean* scores of the semi-quantitative histological findings.**

| | REEPITH | PMNL | TH | FIBRO | VASCULA | COLLAG |
|---|---|---|---|---|---|---|
| **Day 1** | | | | | | |
| Grp A(silk) | 0.50 ± 0.29 | 1.00 ± 0.50 | 0.33 ± 0.29 | 0.67 ± 0.17 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Grp B(CHT) | 0.67 ± 0.17 | 0.83 ± 0.17 | 0.33 ± 0.29 | 0.67 ± 0.33 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| **Day 3** | | | | | | |
| Grp A(silk) | 1.17 ± 0.44 | 0.83 ± 0.17 | 0.83 ± 0.17 | 0.83 ± 0.17 | 0.67 ± 0.17 | 0.00 ± 0.00 |
| Grp B(CHT) | 0.83 ± 0.17 | 0.67 ± 0.17 | 0.83 ± 0.17 | 1.17 ± 0.44 | 0.67 ± 0.33 | 0.00 ± 0.00 |
| **Day 7** | | | | | | |
| Grp A(silk) | 1.33 ± 0.33 | 0.33 ± 0.17 | 0.50 ± 0.29 | 0.67 ± 0.17 | 0.83 ± 0.17 | 1.33 ± 0.33 |
| Grp B(CHT) | 1.17 ± 0.44 | 0.50 ± 0.29 | 0.67 ± 0.17 | 0.83 ± 0.17 | 0.87 ± 0.17 | 1.17 ± 0.44 |
| **Day 10** | | | | | | |
| Grp A(silk) | 0.83 ± 0.17 | 0.33 ± 0.17 | 0.33 ± 0.17 | 0.33 ± 0.17 | 0.50 ± 0.29 | 1.67 ± 0.33 |
| Grp B(CHT) | 1.00 ± 0.58 | 0.33 ± 0.33 | 0.33 ± 0.33 | 0.33 ± 0.33 | 0.67 ± 0.17 | 1.50 ± 0.50 |
| **Day 14** | | | | | | |
| Grp A(silk) | 0.50 ± 0.00 | 0.17 ± 0.17 | 0.33 ± 0.17 | 0.33 ± 0.17 | 1.33 ± 0.33 | 2.33 ± 0.33 |
| Grp B(CHT) | 0.50 ± 0.00 | 0.17 ± 0.17 | 0.33 ± 0.17 | 0.33 ± 0.33 | 0.17 ± 0.44 | 2.17 ± 0.83 |
| **Day 21** | | | | | | |
| Grp A(silk) | 0.33 ± 0.33 | 0.00 ± 0.00 | 0.17 ± 0.17 | 0.33 ± 0.17 | 0.83 ± 0.17 | 2.00 ± 0.00 |
| Grp B(CHT) | 0.33 ± 0.17 | 0.00 ± 0.00 | 0.17 ± 0.17 | 0.17 ± 0.17 | 0.67 ± 0.17 | 1.83 ± 0.73 |

*No significance differences ($p > 0.05$) between the means of the parameters. **Key:** REEPITH = Re-epithelialization; PMNL = Polymorphornuclear leucocytes; TM = Tissue macrophages; FIBRO = Fibroblasts; VASCULA = Vascularisation; COLLAG = Collagen.

The results at day 14 post-surgery showed that the epithelial lining of the sutured wounds in the two groups (A and B) was not considerably different and epithelialisation was complete in both groups. Absence of polymorphonuclear leucocytes and macrophages noted at this stage of wound healing were similar to the trends of reductions in polymorphonuclear leucocytes and tissue macrophages with time upon implantation of disulfide-crosslinked hyaluronan films in rats [14].

The regression of wound healing markers in groups A and B at day 21 post-surgery suggested a reasonable degree of wound healing. Thick collagen and keratinization that were observed in both groups correspond with high level of fibroblasts and keratinocytes which secrete the collagen and the keratin respectively. These changes are indicators of tissue transformation. This observation has been reported earlier for Poly scaffolds implanted subcutaneously in rats [15].

Furthermore, results of the semi-quantification of wound healing markers showed that wound healing progression or tissue reaction in the animals was similar ($p > 0.05$) in both groups of experimental

animals. Consequently, histopathological comparison of the two groups proved indistinguishable, indicating the biocompatibility of the cotton hair thread and silk as suture materials.

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