Short Communication

Foal Deciduous Teeth Stem Cells Enhance Wound Healing in Rabbit Wound Model

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Abstract

The purpose of this study was to evaluate the potential use of deciduous teeth stem cells from horses for wound healing in animal wound model. This study assessed the efficacy and safety of foal deciduous teeth stem cells (fDSCs) administration in rabbit excisional wound model. Deciduous teeth stem cells were harvested from horses and full thickness excisional wound was made on rabbits by surgical removal of epidermis and dermis to the depth of subcutaneous fat. New Zealand White rabbits (n=8) were divided into a control group (n=4) and a treatment group (n=4). Multiple injections of fDSCs suspension were intravenously administered into the rabbits with excisional wounds in the treatment group. Wound sizes were recorded on days 3, 5, 7, 10, 14, 21 and 28. The administration of fDSCs enhanced wound healing in the rabbit wound model. The scar sizes were significantly smaller compared with the control group ($p<0.05$). The rabbits exhibited immediate recovery with no complications. The transplantation of fDSCs provided good choice in terms of wound regeneration and healing. Therefore, our results suggested that fDSCs might provide a new approach to clinical treatment of wounds in veterinary patients, especially in horses in the future.

Keywords: cell therapies, dental stem cells, wounds

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Introduction

Chronic wound is a common problem and causes significant distress to patients (Samantha et al., 2010; Sood et al., 2010; Whang et al., 2005). It is incapable of healing in conventional therapy in an orderly and timely process (Hocking, 2015). Components for wound healing process such as cytokines and growth factors are impaired in all types of chronic wounds (Braund et al., 2007). Several studies have proposed that stem cells might be the new promising source for cellular therapies (McDonald et al., 2015). It has been reported that stem cells promote tissue regeneration by enhancing the production of growth factors, cytokines and collagens (Fathke et al., 2004; Kim et al., 2005; Parikka et al., 2005). Recently, stem cells derived from dental tissue have revealed potential for alternative device in the treatment of musculoskeletal injuries such as bone regeneration and skin defects (Luisa et al., 2012, Riccardo et al., 2008). Stem cells derived from dental tissue contain several enriched sources of stem cells such as periodontal ligament, apical papilla and dental pulp including dental pulp from deciduous teeth (Gronthos et al., 2000). Dental tissue stem cells provide good sources of growth factors and cytokines that promote wound healing conditions (Nagamura et al., 2009; Pravin et al., 2015). Tissue repair processes can be achieved by transplanting cells that prepare excellent factors to modulate inflammation and to enhance the production of molecules that are essential for wound healing (Irena et al., 2013). In veterinary medicine, stem cells have been used for treatment of tendon, ligament and joint injuries in horses and dogs (Jose et al., 2013; Konig et al., 2014). However, only few studies have been conducted to investigate the therapeutic effects of dental tissue stem cells. Therefore, this study described the therapeutic effects of foal deciduous teeth stem cells isolated from horses for wound healing in rabbit excisional wound models.

Materials and Methods

Collection of foal deciduous teeth stem cells: Foal deciduous teeth from horses were collected and cells (fDSCs) at passages 1 and 2 were characterized by intracellular flow cytometry (Santa Cruze Biotechnology, CA, USA.) as previously described (Petchdee et al., 2014). In brief, teeth root was cut to reveal the pulp chamber. Stem cells from pulp tissue were removed and then resuspended in 5 ml Dulbecco's PBS (Gibco, Invitrogen). The cells were placed onto T25 culture flask and incubated for 4 days in a carbon dioxide incubator maintained at 37°C and 5% CO_2 until cell adhesion. Then, the cells were replaced with fresh DMEM media and were carried until 80% confluency was achieved as shown in Figure 1.

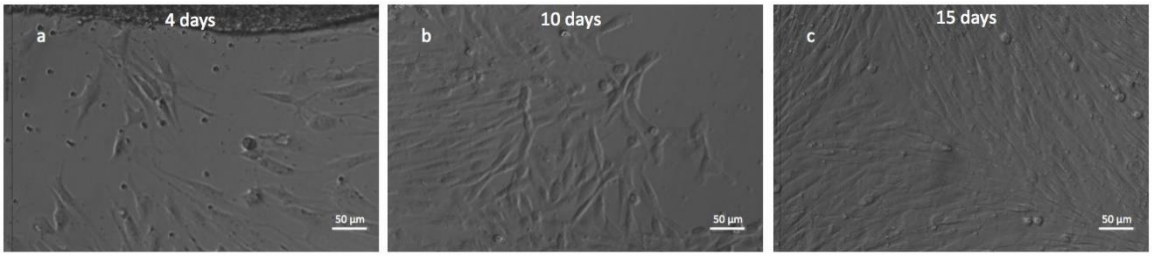

Figure 1 Morphology of foal deciduous teeth stem cells (fDSCs) isolated from the inner part of horse teeth. Phase contrast microscopy showed plastic adherent and fibroblast like cells seen in primary culture (a), becoming semi-confluence (b) and reaching 100% confluence in 25 cm^2 flasks in 15 days (c).

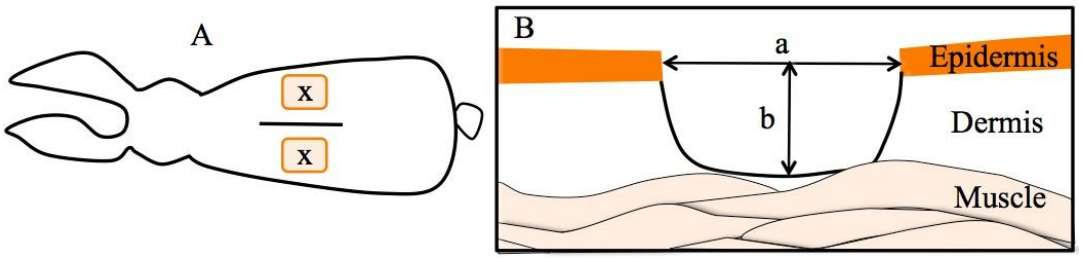

Figure 2 Incision area of approximately 5x5 cm^2 (x) was made through the right and left flank area of rabbits (A). Full thickness excision was made as shown in B, (a) represents the distance between the borders of the wound and (b) represents the depth of the wound.

Animals: The study was conducted in New Zealand White rabbits and approved (ACKU 03459) by the Ethical Committee for Animal Experiments, Kasetsart University, Thailand. The rabbits were randomly divided into two experimental groups consisting of the control group, rabbits given conventional wound care alone without stem cell administration (n=4); and the treated group, rabbits given fDSCs ($1x10^6$) injected through marginal ear vein on day 0 and day 14 after surgically induced full-thickness wound (n=4). Clinical evaluation consisted of physical examination, and complete blood cell counts were performed. The animals were anesthetized with isoflurane (5% induction, 2% maintenance) intubated and connected to a ventilator. Ventilation was done with a tidal volume of 50 ml, at frequency of 36 bpm.

Approximately 5x5 cm² skin excision was performed along the right and left flank region (Fig 2A). Epidermis and dermis were cut to the depth of subcutaneous fat as shown in Figure 2B. The animals were maintained for 2 months for investigation into the wound healing processes.

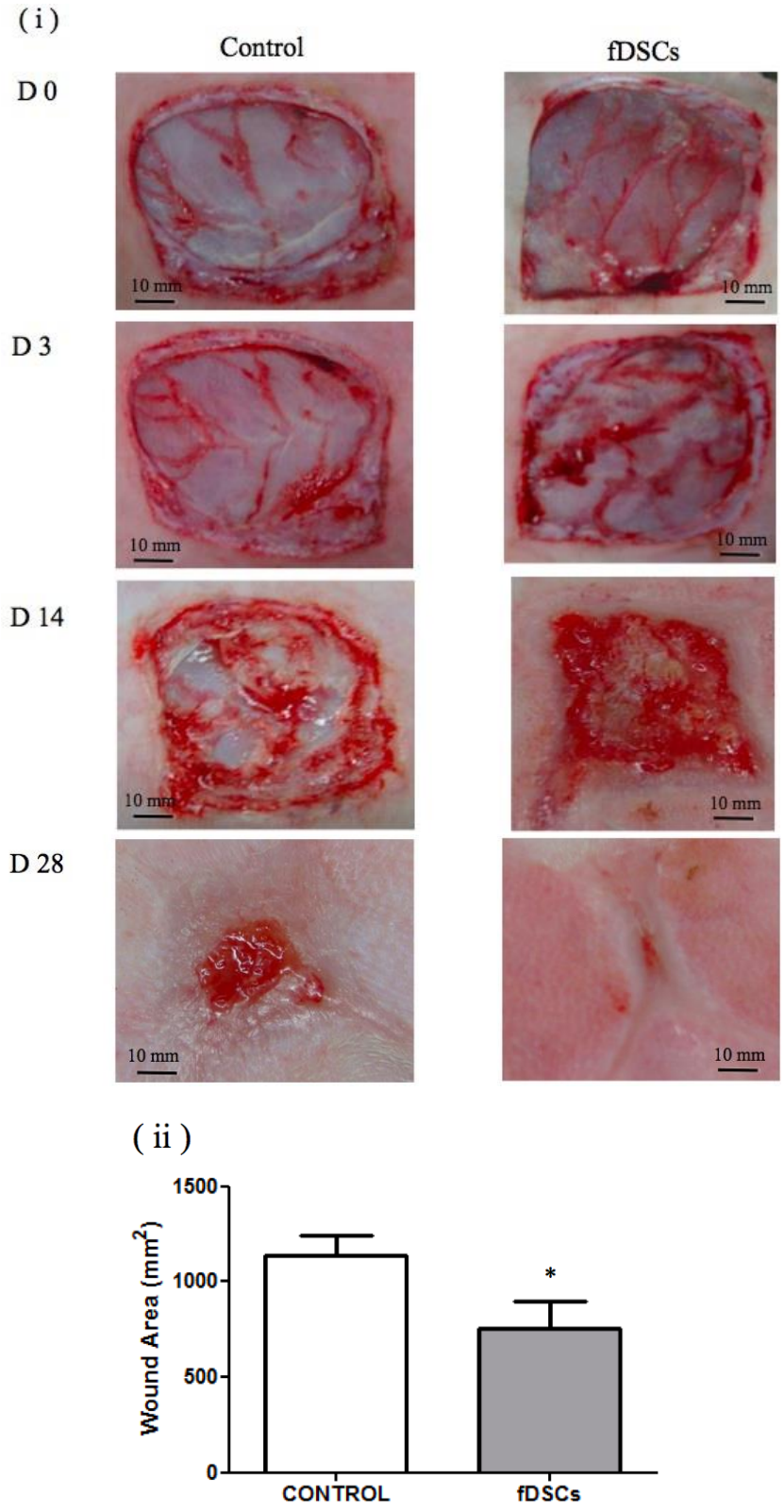

Figure 3 Images of wound areas prior to and following treatment with foal deciduous teeth stem cells (fDSCs). The wound areas were analyzed on days 0, 3, 5, 7, 10, 14, 21 and 28 in the control and treatment groups. Gross appearance of wounds on day 3 (D3) showed higher vascularization of wound in the treated group (fDSCs) (i) and significantly faster wound healing (*p<0.05) was observed on day 14 in the treatment group compared to the control group as shown in (ii).

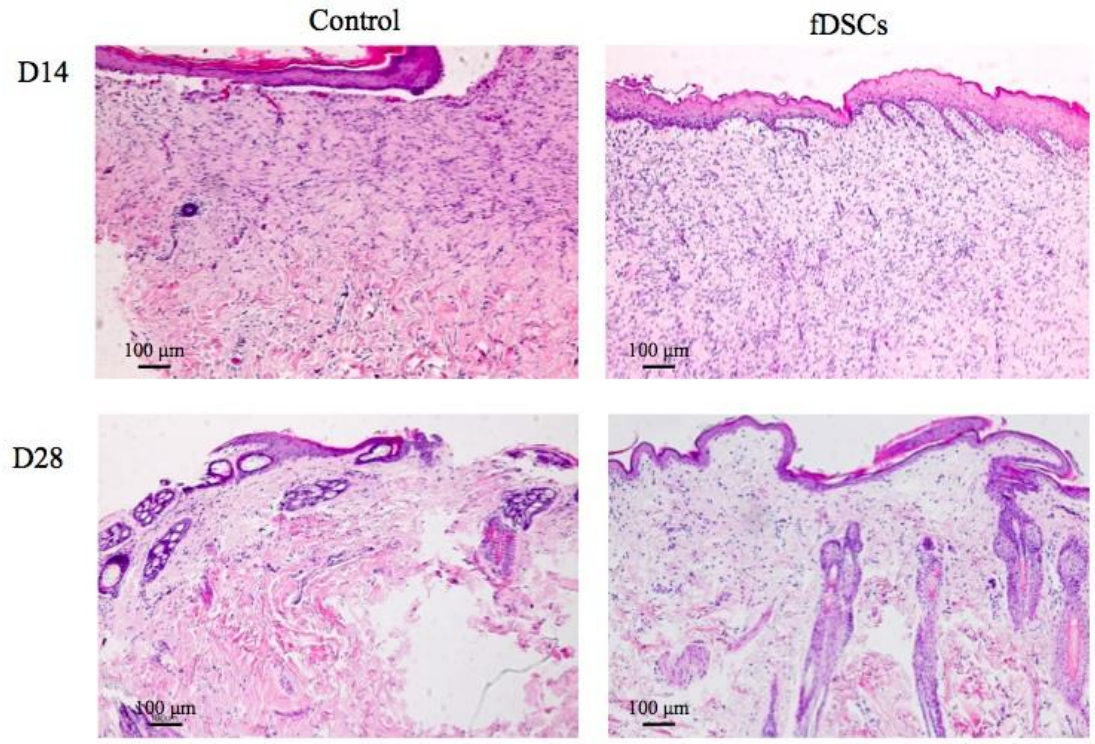

Figure 4 Histological images of wounds on days 14 and 28. H&E staining showed re-epithelialization in the fDSCs-treated group compared to the control group. The granulation tissue appeared to be thicker and larger in the fDSCs treated group.

Deciduous teeth stem cells administration: Foal deciduous teeth stem cells (fDSCs) from passage 2 were intravenously administered to the rabbits with excisional wounds. fDSCs were freshly prepared and $1x10^6$ cells were diluted in PBS and administered intravenously through the marginal ear vein of the rabbits. The second injection was made 2 weeks after the first injection.

Histological evaluation: Skin biopsies from rabbits of the control and treatment groups were performed on days 3, 5, 7, 10, 14, 21 and 28. The skin biopsy samples were processed for histological evaluation. The skin biopsy sections were embedded in paraffin blocks. Sections of the rabbit skin were cut and stained with Hematoxylin and Eosin (H&E) for further evaluation.

Wound analysis: Wound images were taken and analyzed on days 3, 5, 7, 10, 14, 21 and 28 after the surgically full-thickness excision. Wound area was measured by digital caliper and percentage of wound contraction was calculated as: {(Area of original wound - Area of actual wound) / Area of original wound} x 100%.

Statistical analysis: Mean values ± SEM were calculated using GraphPad Prism version 5.0. Student paired T-test was used to compare the percentages of wound areas and wound contraction. P values less than 0.05 ($p<0.05$) were considered statistically significant.

Table 1 Blood profiles of rabbits in the control and fDSCs-treated groups

Parameter	Normal range	Control Mean ± SEM		fDSCs Mean ± SEM	
		D0	D14	D0	D14
RBC (*10^6/mm^3)	3.8-7.9	5.69±0.28	6.24±0.21	6.44±0.43	6.00±0.22
HGB (g/dL)	9.4-17.4	12.33±0.51	13.10±0.32	13.65±0.49	12.90±0.31
HCT (%)	33-50	38.95±1.98	40.80±0.53	41.9±1.60	41.0±1.45
MCV (mm^3)	50-75	68.50±1.21	66.70±3.05	66.58±2.80	68.50±2.48
MCH (pg/cell)	18-24	21.70±0.20	21.40±0.25	21.32±0.72	21.50±0.65
MCHC (%)	27-34	31.70±0.60	32.10±0.61	32.58±0.45	31.50±0.36
PLT (*10^9/l)	200-650	388.25±4.6	378.00±7.42	356.5±2.83	492±7.17
WBC (*10^9/l)	5-13	9.65±0.87	8.78±0.63	7.72±1.26	8.18±1.05
NEUTROPHIL (%)	34-70	30.65±4.33	2.70±0.22	26.10±1.91	2.71±0.73
LYMPHOCYTE (%)	43-80	55.40±4.87	4.90±0.52	62.13±3.89	4.50±0.84
MONOCYTE (%)	0-4	9.08±0.66	0.79±0.02	7.53±1.47	0.69±0.13
EOSINOPHIL (%)	0-2	2.98±0.76	0.13±0.01	2.90±0.74	0.08±0.01
BASOPHIL (%)	0-0.84	1.90±0.34	0.26±0.07	1.35±0.13	0.20±0.04
CREATININE (mg/dl)	0.5-2.6	1.32±0.07	1.11±0.06	1.40±0.01	1.0±0.07
ALT (U/L)	14-80	55.25±7.40	59.50±17.26	47.25±3.71	50.3±4.33
TOTAL PROTEIN (g/l)	50-75	7.30±0.13	7.40±0.25	6.90±0.19	7.20±0.12

Results and Discussion

This is the first study to show the application of foal deciduous teeth stem cells (fDSCs) to treat wound in rabbit wound model. We provided evidence that fDSCs administered to excisional wounds through an intravenous route exert beneficial therapeutic effects without complications. Blood test results of the rabbits in the control and fDSCs-treated groups are shown in Table 1. The surgical wound model in rabbit was used in this study to assess the use of stem cells in wound repair processes. Multiple injections of $1x10^6$ of fDSCs were intravenously administered into the marginal ear vein of the rabbits. fDSCs in the circulation release chemotactic agents. Furthermore, previous studies have shown that dental stem cells can migrate to the damaged tissue. The growth factors and paracrine factors such as SDF-1, HGF and VGEF from stem cells act as chemotactic and homing signals (Briques et al., 2015; Pravin et al., 2015). Animal excisional wound models are associated with a greater sensitivity to be infected, therefore a proper wound dressing is recommended to assist appropriate wound healing (Roy et al., 2014). In this current study, conventional care for wound was applied to the wounds in the control group, the wounds were debrided and cleaned with 0.5% povidone iodine and normal saline. Normal saline dressings were changed daily in the first 3 days and then every 2 days thereafter. Our investigations showed that the administration of fDSCs accelerated wound healing and contraction (Figs 3 and 4) (Table 2). The granulation tissues were formed and the wound contracted within 4-5 days after the fDSCs administration in all rabbits in the treatment group. This was a significant improvement ($p<0.05$) compared with the control group, which used conventional wound management. There are several reports which suggest that cytokines and growth factors may be beneficial as a supplement for wound healing processes (Briquez et al., 2015). Gross appearance of the skin wound represented higher vascularization in the wounds of the treatment group as shown in Figure 3. One possibility for the therapeutic effects of stem cells is the promotion of wound healing by suppression of inflammatory cells and stimulation of repair mechanism, including angiogenesis (Turner et al., 2014). The skin sections of the rabbit wound are shown in Figure 4. Noticeably, H&E staining showed an increasing number of cells in stratum basale layer in the treated samples compared to the control samples. The amount of connective tissue cells in reticular layer was higher in the treated group when compared with the control group. To restore and prevent the breakdown of the extracellular matrix and to add deficient components such as growth factors and collagens may be the therapeutic mechanism of fDSCs (Shah et al., 1999; Falanga et al., 2000; Martin et al., 2013). The results from our study suggested that stem cells derived from deciduous teeth of the horse might be a novel approach for wound care and might be applied in clinical treatment of non-healing and large wounds. However, the treatment with stem cells from deciduous teeth requires further investigation to understand the underlying mechanisms of cytokine, growth factors and progenitor elements which contribute to the wound healing processes. This preliminary investigation suggests that fDSCs have the potential to promote wound healing in rabbit excisional wound models. However, further clinical application of a standardized model and appropriate controls should confirm the results of therapeutic effects of stem cells. This study is encouraging but not yet conclusive, the information obtained from further research will inform novel developments in wound management in the future.

Table 2 Time point of appearance of different wound contraction in days after administration of fDSCs

Days after fDSCs administration	% Wound contraction	
	Control	fDSCs
0	0	0
3	13.77±3.23	11.07±2.96
7	42.64±3.98	43.58±2.56
10	64.55±3.31	64.69±1.79
14	88.95±1.77	90.60±1.40
21	98.98±0.66	99.15±0.10

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References

Braund R, Hook S, Medlicott NJ 2007. The role of topical growth factors in chronic wounds. Curr Drug Deliv. 4(3): 195-204.

Briquez PS, Hubbell JA, Mikael MM 2015. Extracellular matrix inspired growth factor delivery systems for skin wound healing. Adv Wound Care. 4(8): 479-489.

Falanga V 2000. Classifications for wound bed preparation and stimulation of chronic wounds. Wound Repair Regen. 8: 347–352.

Fathke C, Wilson L, Hutter J, Kapoor V, Smith A, Hocking A 2004. Contribution of bone marrow-derived cells to skin: collagen deposition and wound repair. Stem Cells. 22(5): 812-22.

Gronthos S, Mankani M, Brahim J, Robey PG, Shi S 2000. Post natal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci. USA, 97 (25):13625-13630.

Hocking D 2015. Therapeutic applications of extracellular matrix. Advances in wound care. 4(8): 441-443.

Irena P, Olivera S, Natalie CY, Horacio R, Aron GN, Andrew S, Shailee BP, Laiqua K, Rivkah RI, Marjana TC 2013. Epithelialization in Wound Healing: A Comprehensive Review. Advances in wound care, 2013; 3(7): 445-464.

Jose MV, Manuel M, Angelo S, Giuseppe S, Mónica R, Belen C, Ramón C, Jose MC 2013. Controlled, blinded force platform analysis of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells associated to PRGF-Endure in osteoarthritic dogs. BMC Vet Res. 9(131): 1-6.

Kim DH, Yoo KH, Choi KS, Choi J, Choi SY, Yang SE 2005. Gene expression profile of cytokine and growth factor during differentiation of bone marrow-derived mesenchymal stem cell. Cytokine. 31(2): 119-26.

Konig L, Klopfleisch R, Kershaw O, Gruber AD 2015. Prevalence of Biofilms on Surgical Suture Segments in Wounds of Dogs, Cats, and Horses. Vet Pathol. 52(2): 295-297.

Luisa AD 2012 Oral Stem Cells: The Fountain of Youth for Epithelialization and Wound Therapy? Advances in wound care. 3(7): 465-467.

Martins GM, Petreaca M, Wang L 2013. Chemo- kines and their receptors are key players in the orchestra that regulates wound healing. Adv Wound Care. 2:327–347.

McDonald CA, Oehme D, Pham Y, Kelly K, Itescu S, Gibbon A, Jenkin G 2015. Evaluation of the safety and tolerability of a high-dose intravenous infusion of allogeneic mesenchymal precursor cells. Cytotherapy. 17:1178-1187.

Nagamura S, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M 2009. Stem cell proliferation pathway comparison between human exfoliated deciduous teeth (SHED) and dental pulp stem cells (DPSCs) by gene expression profile from promising dental pulp. J. Endod. 35:1536-1542.

Parikka V, Väänänen A, Risteli J, Salo T, Sorsa T, Väänänen HK 2005. Human mesenchymal stem cell derived osteoblasts degrade organic bone matrix in vitro by matrix metalloproteinases. Matrix Biol. 24(6): 438-47.

Petchdee S, Pattanapon N, Bootcha R, Srivattanakul P, Songserm T, 2014. Dental Tissue-Derived Stem Cells exerts therapeutic effects on chronic myocardial infarction model of rabbit. The Cardiology. 9(1): 1-6.

Pravin DP, Yogita DJ. 2015. Human dental pulp stem cell: Application in future regenerative medicine. World J Stem Cells. 7(5): 839-851.

Riccardo A, Gianpaolo P, Gregorio L, Antonio G 2008 Dental Pulp Stem Cells: A Promising Tool for Bone Regeneration, Stem Cell Rev. 4: 21–26.

Roy I, Evans DB, Dwinell MB 2014. Chemokines and chemokine receptors: update on utility and challenges for the clinician. Surgery. 155 (6): 961-73.

Samantha JW, Steven LP 2010. Chronic Equine Wounds: What Is the Role of Infection and Biofilms? Wounds. 22(6): 138–145.

Shah M, Revis D, Herrick S, Baillie R, Thorgeirson S, Ferguson M 1999. Role of elevated plasma transforming growth factor-beta1 levels in wound healing. Am J Pathol. 154:1115–1124.

Sood R, Roggy D, Zieger M, Balledux J, Chaudhari S, Koumanis DJ, Mir HS, Cohen A, Knipe C, Gabehart K 2010. Cultured epithelial autografts for coverage of large burn wounds in eighty-eight patients: the Indiana University experience. J Burn Care Res. 31: 559-568.

Turner NJ, Badylak SF 2014. The use of biologic scaffold in the treatment of chronic non-healing wounds. Advances in wound care. 4(8): 490-500.

Whang KK, Kim MJ, Song WK, Cho S 2005. Comparative treatment of giant congenital melanocytic nevi with curettage or Er: YAG laser ablation alone versus with cultured epithelial autografts. Dermatol Surg. 31: 1660-1667.

บทคัดย่อ

เซลล์ต้นกำเนิดจากฟันน้ำนมลูกม้าในการรักษาบาดแผลในกระต่าย

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วัตถุประสงค์ของการศึกษานี้คือเพื่อประเมินศักยภาพของเซลล์ต้นกำเนิดจากฟันน้ำนมม้า ในการรักษาบาดแผลในสัตว์ทดลอง การศึกษานี้นำเสนอประสิทธิภาพและความปลอดภัยของการรักษาบาดแผลที่เกิดจากการผ่าตัดในกระต่ายด้วยเซลล์ต้นกำเนิด จากฟันน้ำนมลูกม้า (fDSCs) ทำการผ่าตัดกระต่ายเพื่อให้เกิดบาดแผลโดยการผ่าตัดผิวหนังส่วนของหนังกำพร้า และหนังแท้ออกโดยมีความลึกไปจนถึงชั้นของไขมันใต้ผิวหนัง แบ่งกระต่ายขาวนิวซีแลนด์ (n=8) ออกเป็นกลุ่มควบคุม (n=4) และกลุ่มการรักษา (n=4) โดยทำการรักษาด้วยเซลล์ต้นกำเนิดจากฟันน้ำนมลูกม้าโดยการฉีดเข้าทางหลอดเลือดดำที่หูของกระต่าย ทำการบันทึกขนาดของบาดแผลในวันที่ 3, 5, 7, 10, 14, 21 และ 28 จากการทดลองพบว่า เซลล์ต้นกำเนิดจากฟันน้ำนมลูกม้าสามารถช่วยรักษาบาดแผลในกระต่าย ขนาดของบาดแผลเป็นมีขนาดเล็กอย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มควบคุม ($p<0.05$) กระต่ายแสดงการหายของแผลทันที และไม่พบภาวะแทรกซ้อนจากการรักษาด้วยเซลล์ต้นกำเนิดจากฟันน้ำนมลูกม้า (fDSCs) เซลล์ต้นกำเนิดจากฟันน้ำนมลูกม้าให้ผลลัพธ์ที่ดีในแง่ของการฟื้นฟูและการรักษาบาดแผล ผลจากการศึกษานี้ชี้ให้เห็นว่า เซลล์ต้นกำเนิดจากฟันน้ำนมลูกม้า (fDSCs) อาจเป็นทางเลือกใหม่ในอนาคตในการรักษาบาดแผลในสัตว์ โดยเฉพาะอย่างยิ่งการรักษาบาดแผลในม้า

คำสำคัญ: เซลล์บำบัด เซลล์ต้นกำเนิดจากเนื้อเยื่อทันตกรรม บาดแผล

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