Al-Azhar Journal of Dentistry

Volume 8 | Issue 3 Article 12

Oral Medicine and Surgical Sciences Issue (Oral Medicine, Oral and Maxillofacial Surgery, Oral Pathology, Oral Biology)

7-1-2021

Monitoring of Stem Cell Differentiation by Glycogen Synthesis Kinase-3 (GSK-3) Inhibitors in Induced Palatal Ulcer in Rabbits

Heba Abdelfatah
Assistant Lecturer of Oral and Dental Biology, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt, heba.abdelfatah@azhar.edu.eg

Mostafa Abdel Ghani
Professor of Removable Prosthodontics, Vice President of the University Girls Branch, Al-Azhar University, Cairo, Egypt, mostafama1@hotmail.com

Heba Adawy
Professor and Head of Oral and Dental Biology Department, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt, hebaadawy@hotmail.com

How to Cite This Article

Abdelfatah, Heba; Abdel Ghani, Mostafa; and Adawy, Heba (2021) "Monitoring of Stem Cell Differentiation by Glycogen Synthesis Kinase-3 (GSK-3) Inhibitors in Induced Palatal Ulcer in Rabbits," *Al-Azhar Journal of Dentistry*: Vol. 8: Iss. 3, Article 12.
DOI: https://doi.org/10.21608/adjg.2021.25557.1242

AL-AZHAR
Dental Journal
For Girls

The Official Publication
of The Faculty of Dental
Medicine For Girls,
Al-Azhar University
Cairo, Egypt.

Print ISSN 2537-0308 • Online ISSN 2537-0316

ADJ-for Girls, Vol. 8, No. 3, July (2021) — PP. 443:449

Monitoring of Stem Cell Differentiation by Glycogen Synthesis Kinase-3 (GSK-3) Inhibitors in Induced Palatal Ulcer in Rabbits

Heba Abdelfatah[*1], Mostafa M. Abdel Ghany[2], Heba A Adawy[3]

Codex : 12/21.07

azhardentj@azhar.edu.eg

http://adjg.journals.ekb.eg

DOI: 10.21608/adjg.2021.25557.1242

Oral Medicine & Surgical Sciences
(Oral Medicine, Oral & Maxillofacial Surgery, Oral Pathology, Oral Biology)

ABSTRACT

Purpose: To monitor stem cell differentiation by glycogen synthesis kinase-3 (GSK-3) inhibitors in induced palatal ulcer in rabbits and its possible effect of regeneration. **Materials and Methods:** One hundred and sixty-two adult male rabbits were used. Rabbits were divided into 3 groups, then each group divided into 3 subgroups (18 for each subgroup) according to the date of scarification at 1, 7, 14 days postoperatively. Group (I): Control group, the rabbits weren't subjected to any surgical procedure. Experimental groups; Group (II): (-ve control), the palatal mucosal ulcer was induced and allowed to heal normally. Group (III): (+ve control), the palatal mucosal ulcer was induced and Tideglusib solution was applied daily. **Results:** at 1 and 7 days, the minimum imply has been recorded in group (I), after that group (II), although the highest amount has been documented in group (III). While at 14 days, the minimum amount has been documented in group (I), tracked closely by group (III), while the highest amount has been recorded in group (II). **Conclusion:** Within the limitation of this study, it was concluded that topical application of Tideglusib on the palatal mucosal ulcer reduced inflammation, increased collagen deposition, enhanced stratifications of the oral epithelium and accelerated wound closure. Therefore, Tideglusib therapy could be a promising therapeutic modality for oral ulcers and wounds.

KEYWORDS

Stem cells, Palatal ulcer, GSK-3 Inhibitors.

INTRODUCTION

Stem cell treatment has turned into a hopeful new way into the field of regenerative medicine. The significant benefit in the biology of stem cells is worried about their potential for repair as well as differentiate

- Paper extracted from Doctor thesis titled. Monitoring of Stem Cell Differentiation by Glycogen Synthesis Kinase-3 (GSK-3) Inhibitors in Induced Palatal Ulcer in Rabbits

1. Assistant Lecturer of Oral and Dental Biology, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt
2. Professor of Removable Prosthodontics, Vice President of the University Girls Branch, Al-Azhar University, Cairo, Egypt
3. Professor and Head of Oral and Dental Biology Department, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt

* Corresponding author email: heba.abdelfatah@azhar.edu.eg

several cell kinds also, is critical for normal tissue repair and regeneration following the damage [1]. The most important clinical aim of stem cell use in wound treatment is to aim enhanced the quality of wound healing. The physician's might be able to predict in order to achieve speeded up in the treatment, avoidance of wound shrinkage and scar creation, earlier wound closure, as well as preferably regeneration of the wound defect utilizing stem cells. Though, establishing the optimal supplier, a technique of handling and management after the medical perspective, in addition to identifying functions of stem cells in the actual clinical condition, continues to be the remaining task of utilizing stem cells for their regenerative healing wounds [2].

The mucous membrane lining the mouth known as oral mucosa. It includes stratified squamous epithelium, referred to as oral epithelium, as well as the underlying connective-tissue called lamina propria. The oral cavity has occasionally referred to like a mirror that indicates the wellbeing of the person. Alterations revealing of the illness are thought to be changes in the oral mucosa, that could expose general conditions, like the diabetes either deficient in vitamin. The oral mucosa manages to heal more quickly besides less scar formation contrasted with the skin [3].

One important divergence among wound treatment and renewal is that every tissue has the ability of restoration, although repaired tissue may not necessarily have the same functionality or morphology as the missing tissue. Additionally, healing wounds is a defensive role of the body which concentrates on a rapid improvement, while the procedure of regeneration in antagonistic conditions requires extra time. Especially, the oral cavity is an extraordinary atmosphere where the wound healing happens in a humid oral fluid which includes lots of bacteria [4].

GSK-3 is an atypical serine/threonine kinase which is usually effective in accordance within active conditions as well as mainly governed by deactivation via different signaling ways. The roles of the two mammals' subtypes, GSK-3α and β were involved in a wide range of biological processes. Because of the essential function of intracellular kinases within the cell signaling, protein regulation, digestion or cellular transport kinase suppression has turned into one attractive goal for medicinal involvement [5].

Many inhibitors of GSK-3 were pinpointed, ATP-competitive and non-ATP-competitive inhibitors. In the early studies, some researchers were first to report thiadiazolidinones (TDZDs) as non-ATP–competitive GSK-3β inhibitors. It has been assumed that such materials are identify plus communicate with the oxyanion uniting position of GSK-3β. Those writers additionally proposed that the negative charge of TDZD was significant for oxyanion to be recognized [6,7]. Tideglusib, also known as NP031112/NP-12 was developed for the therapy of Alzheimer disease (AD) and progressive supranuclear palsy (PSP). Tideglusib binds permanently to the non-ATP–competitive GSK-3β location. An IC50 amount of 60 nM has been informed and the safety plus ability attributes of Tideglusib have been examined in individual patients who have AD along with PSP [7].

MATERIAL AND METHODS

1. Animals

One hundred and sixty-two adult male rabbits weighing an average of (2.5 - 3 kg) were used in this study. Animal manipulation followed the rules and regulations of the animal experimental studies that were approved by an ethical committee including their facilities, diet and method of scarification.

2. Tideglusib

Tideglusib solution was prepared as 3μg of Tideglusib powder (Sigma –Aldrich pharmaceutical company) dissolved in 1 μl of dimethyl sulfoxide

solution (DMSO), (Sigma –Aldrich pharmaceutical company), and 9 µl of distilled water, all were mixed and applied using microliter syringe. (Fig. 1-a)

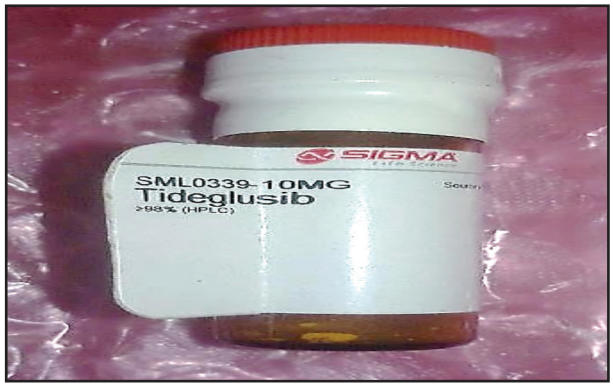

Figure (1-a): 10 mg of Tideglusib powder (SIGMA)

3. Wound and Treatment

The rabbits were anaesthetized intramuscularly using 0.1 ml / 200 g of rabbit's weight using equal parts of ketamine and xylazine [8]. The palatal mucosa was sterilized with a cotton swab covered by 0.12% chlorhexidine di-gluconate [9]. Then the palatal mucosa was carefully separated by a surgical instrument. The ulcer area was 0.5 x1 cm (width - length), and 1.5 mm in thickness [10]. Then in-group (III), 150 ng / animal of Tideglusib solution was injected into each side of the operated area by using an insulin syringe, daily till the date of scarification. (Fig. 1-b)

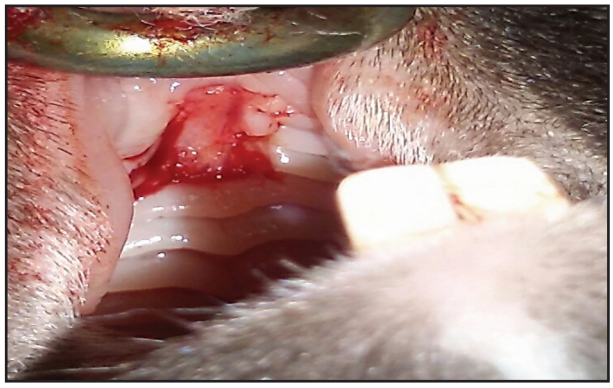

Figure (1-b): Palatal wound dimension before and after preparation

4. Sample collection

By the end of the experimental period for each subgroup, rabbit scarification was done by slaughter. And their heads were immediately dissected to obtain a standard biopsy of the wounded area in the palate. The specimens processed for routine histological examination by Hematoxylin and Eosin stain (H&E).

5. Specimens preparation for histological examination

The specimens were usually fixed, dehydrated, cleared and paraffin wax embedded. Then 4-micron thickness sections were cut, stained by H&E stain and examined by a light microscope at different magnifications [11].

RESULTS

A. Clinical Results

In-group (I) the normal shape of the hard palate of the rabbit showed transverse palatine plicae. Each plica was covered with a smooth mucosa without any undulations. Gaps between the plicae were generally not long but resembled a notch of a saw or serrations. A picture was taken at days 1,7, and 14 postoperatively, clinical examination revealed that the wound region reduced significantly quicker in group (III) in comparison with the group (II). In Addition, the time period needed for maximum healing of the ulcer has been reduced in group (III). Furthermore, no hematoma or inflammations were observed in rabbits after surgery.

B. Histological Results

In-group (I) the three main tissue components of the palatal mucosa were a stratified squamous epithelium, an underlying connective tissue layer, and submucosa. The epithelium either orthokeratinized or parakeratinized stratified squamous. The lamina propria comprises of 2 sub-layers, papillary layer that contains a loose group of collagenous fibers along with deeper layer by even additional tightly packed collagenous fibers. The submucosa includes the main arteries, veins, as well as nerves of the palate. (Fig. 2)

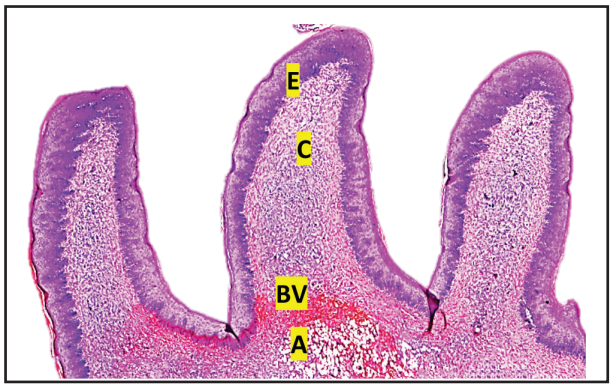

Figure (2): Normal (baseline) of the palate, showed normal palatal mucosa and submucosa, from top to bottom: Epithelium (E), connective tissue papillae (C), and upper part of the submucosal tissue. Note how the adipose tissue (A) and many blood vessels (BV) found in the submucosal layer. (H&E x40).

At day (1): in-group (II), the defect appeared wide involved the epithelium and connective tissue, the periphery of the epithelium showed mitosis. The connective tissue showed acute inflammatory cells as well as moderate size of fat cells. In-group (III) the epithelial margin migrated laterally and showed a widening of intercellular space, this observation did not show in in-group (II). The connective tissue showed an acute inflammatory reaction like group (II) with an interesting observation we noticed new fat cells in the healed wounds in-group (III) differs from normal fat cells in-group (II). (Fig.3:(a, b))

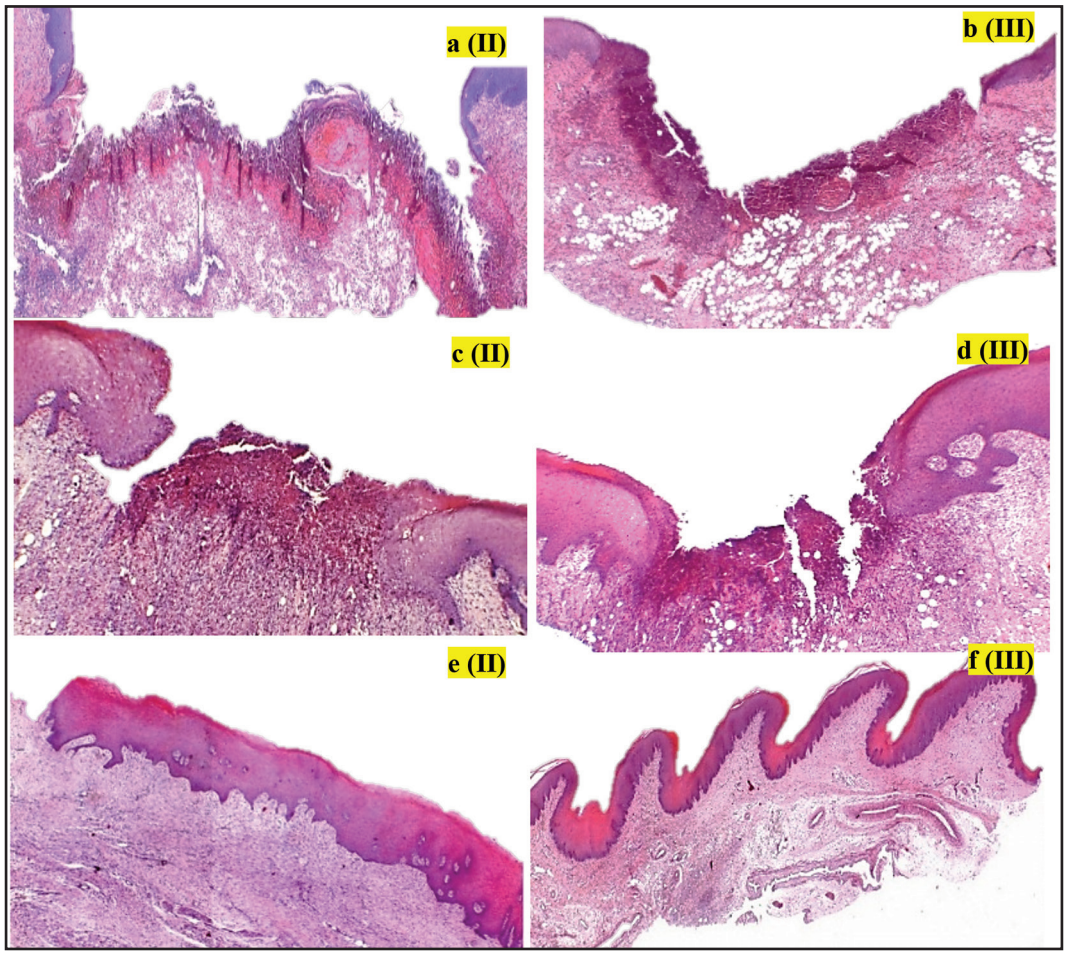

Figure (3): A photomicrograph of H&E stained sections (x40) from the palatal ulcers healing of experimental groups. (a, b) comparing sections of group II, III at (1) day, revealed that the defect appeared wide involved the epithelium and connective tissue. While, (c, d) showed that in-group (III), at (7) days, the wound defect was decreased compared with group (II). At (14) days, (e, f) group (II) showed quite thin parakeratinized stratified epithelium without formation of rete ridges or the palatine rugae. While in-group (III), relatively thick orthokeratinized stratified epithelium which regained its original thickness and shape.

At day (7): in-group (II), the palatal defect slightly decreased than before and the epithelium initiated to migrate laterally. There was mitosis at the periphery of the epithelium in the basal and spinous cell layers. The connective tissue revealed inflammatory cells with newly formed blood vessels. While in-group (III), the wound defect was decreased compared with a group (II), on both sides, a layer of the epithelium migrated below the granulation tissue. There was mitosis at the periphery of the epithelium like group (II) but increased in its amount. Moreover, there was a beginning of fibroblasts infiltration and the connective tissue filled with fat cells. (Fig.3:(c,d))

At day (14): Comparing among group (II) besides group (III). Group (II) showed relatively thin parakeratinized stratified epithelium with continuous basement membrane without the formation of rete ridges or the palatine rugae. While in-group (III), the palatal wound defect completely healed by relatively thick orthokeratinized stratified epithelium which recovered its unique thickness besides shape of the rete ridges and palatine rugae. (Fig.3:(e,f))

DISCUSSION

Stem cells hold a significant promising treatment of progressive sickness. Though, after thinking about stem-cell research, largely persons think about cell-substitution techniques, that are expensive as well as current with significant challenges into tissue integration as well as function. Minor molecule of medicines might be desirable to magnify stem cells group or direct them to the differentiation through affecting specific signaling pathways. Additionally, they may be applied to recognize the fundamental mechanisms controlling stem cell reconstruction as well as differentiation [12].

So, in our research we used Tideglusib drug since it is considered as inhibitors of GSK-3 with multiple therapeutic applications such as in patients with diabetes, GSK-3β inhibitors enhance sensitivity to insulin, glycogen production, as well as blood sugar metabolism in the skeletal muscles. GSK-3β is also particularly expressed in many kinds of cancers, like colon, ovary, and prostate-cancer, also aid in the therapy of Alzheimer's disease, stroke, and emotional problems, which includes the bipolar disorder, lately, it was used as a promotion of natural teeth repair [13,14].

The main objectives were also the main goal of wound healing to heal the injury in the shortest time with minimal pain and discomfort, in-group (III) our prepared drug was applied topically also detected next the 5th day, the dead tissue was detached, the inflammation besides tenderness were diminished when compared with group (II) which exhibited deeper ulcers, this indicates that the Tideglusib had tissue debride result on the wound site after 7 days. Those observations were concomitant with other scientists [15], who examined the activity of wound healing by circumcision, incision and dead space wound replicas on Swiss Albino rats by lupeol which contains GSK3- inhibitors and found that the wound area decreased much faster and the time required for healing the whole of ulcers is heavily in the lupeol processing range compared with the control group.

At day (1): in Tideglusib group (III) we noticed new adipocytes cells within the healed wounds that different from normal fat cells in-group (II) in term of had a different form and size as well as increased in numbers. These histological results were consistent with those of other study [16], which found that adipocytes can play a role in tissue repair, such as skin fibroblasts and muscle adipocytes after trauma. In addition to known fat precursor cells, they can also differentiate into mature fat cells, which seem to help repair, because preventing differentiation leads to deficiencies in fibroblast migration and matrix deposition.

While at day (7): the histological findings in-group (III) illustrated the wound defect was decreased in size compared with a group (II), Moreover, there was the beginning of fibroblasts

infiltration and the connective tissue filled with new blood vessels formation combined with many of fat cells. This finding was in close agreement with researchers[17], in which the epithelial cells proliferate and migrate from the borders of the wound to close it, and loss of the extracellular matrix and cell-cell contacts. Also, coordinated through keratinocyte proliferation and migration that connected with the expression of p63.Initiation of Wnt signaling was mediated by the suppression of GSK-3 and b-Catenin was raised in mesenchymal cells during the proliferative phase of the wound during healing and believed to regulate fibroblasts proliferation rate and motility. Furthermore, activated platelets and macrophages stimulating the epithelial proliferation via transforming growth factor-alpha and epidermal growth factor [18].

While at day (14): when comparing with each other group (II) as well as group (III). Group (II) demonstrates relatively thin parakeratinized stratified squamous epithelium Whereas in-group (III), the palatal wound defect completely healed by relatively thick orthokeratinized stratified squamous epithelium, our study showed that p63 was a necessary condition to maintain the proliferation of multilayered epithelial cells. However, the thin parakeratinized stratified squamous epithelium that results in-group (II) due to a decrease of proliferative capability, even between the transit-amplifying cells, might disturb stratification, which confirmed with others [19], who found that used of inducible knockout model temporarily reduce p63 expression in tissue layers found that loss of p63 in adult mice was connected with low stratification of epithelial cells, increased the senescence promoted aging.

Small-molecule of GSK-3 inhibitors activates Wnt/b-catenin signaling through stabilization of b-catenin. It was found that GSK-3 inhibition was maintained pluripotency in mouse pluripotent stem cells (PSCs) and to stimulate self-renewal in mESCs[20]. These results demonstrated that modulation of the Wnt signaling via Tideglusib increased the palatal wound healing by stimulation and differentiation of PSCs.

CONCLUSION

So, from the previous investigation, and analysis of all studied groups, we offers a clear evidence that topical application of Tideglusib solution demonstrated to remain one of the powerful wound healing agents which revealed to cause palatal large ulcer defect to healed and regain its normal appearance better than normal ulcer healing without treatment through the illustration of beta-catenin dependent Wnt pathway which stimulates PSCs via inhibition of GSK-3.

ACKNOWLEDGEMENT

I am grateful to Dr. Ibrahim Ghalib, Lecturer of Pharmacology, Faculty of Pharmacy, Al-Azhar University, Cairo, for his essential role in drug preparation.

RECOMMENDATIONS

1. Evaluating multiple pro and anti-inflammatory effect of Tideglusib may be beneficial for future studies.
2. Further studies should be carried out with different concentrations to correlate the clinical outcome of Tideglusib with its biologic mechanisms.
3. This information will be used to develop therapies for prevention of scar formation in large palatal wound, surgical procedures, accidental trauma, even after skin burns.

Declaration statement

Authors declare no conflict of interest.

No funding was received for this study

REFERENCES

1. Dehkordi AN, Babaheydari FM, Chehelgerdi M, Dehkordi SR. Skin tissue engineering: wound healing based on stem-cell-based therapeutic strategies. Stem Cell Res Ther. 2019; 10: 111-5.

2. Shojaei F, Rahmati S, Banitalebi Dehkordi M. A review on different methods to increase the efficiency of mesenchymal stem cell-based wound therapy. Wound Repair Regen. 2019; 27:661-71.
3. Nanci A. Ten Cate's Oral Histology. 9th ed. Canada. Elsevier. 2018; 280-5.
4. Politis C, Schoenaers J, Jacobs R, Agbaje JO. Wound healing problems in the mouth. Frontiers in physiol. 2016; 7: 507-9.
5. McCubrey JA, Steelman L, Bertrand FE, Davis NM, Abrams SL, Montalto G, et al. Multifaceted roles of GSK-3 and Wnt/β-catenin in hematopoiesis and leukemogenesis: opportunities for therapeutic intervention. Leukemia. 2014; 28:15-33.
6. Sánchez-Cruz A, Martínez A, Enrique J, Hernández-Sánchez C. GSK-3 Inhibitors: From the Brain to the Retina and Back Again. Retinal Degenerative Diseases: Springer; 2019; 3:437-41.
7. Tolosa E, Litvan I, Höglinger GU, Burn D, Lees A, Andrés MV, et al. A phase 2 trial of the GSK-3 inhibitor tideglusib in progressive supranuclear palsy. Mov. Disord. 2014; 29:470-8.
8. Brizeno LAC, Assreuy AMS, Alves APN, Sousa FB, Silva PGdB, Sousa SCO, et al. Delayed healing of oral mucosa in a diabetic rat model: Implication of TNF-α, IL-1β and FGF-2. Life Sci. 2016; 155:36-47.
9. Cantín M, Olate S, Fuentes R, Vásquez B. Alveolar Ridge Conservation by Early Bone Formation After Tooth Extraction in Rabbits. A Histomorphological Study. Int J Morphol. 2015; 33:369-74.
10. Xu X, Cui N, Wang E. Application of an acellular dermal matrix to a rabbit model of oral mucosal defects. Exp Ther Med. 2018; 15:2450-56.
11. Slaoui M, Fiette L. Histopathology procedures: from tissue sampling to histopathological evaluation. Drug safety evaluation: Springer; 2011; 13:69-82.
12. Rao RC, Dedania VS, Johnson MW. Stem cells for retinal disease: a perspective on the promise and perils. Am J Ophthalmol. 2017; 179: 32-8.
13. Maqbool M, Mobashir M, Hoda N. Pivotal role of glycogen synthase kinase-3: a therapeutic target for Alzheimer's disease. Eur. J. Med. Chem. 2016; 3:107: 63-81.
14. Matsunaga S, Fujishiro H, Takechi H. Efficacy and Safety of Glycogen Synthase Kinase 3 Inhibitors for Alzheimer's Disease: A Systematic Review and Meta-Analysis. J Alzheimers Dis. 2019; 23:1-9.
15. Lin C, Li X, Zhang Y, Guo Y, Zhou J, Gao K, et al. The microRNA feedback regulation of p63 in cancer progression. Oncotarget. 2015; 6: 84-8.
16. Franz A, Wood W, Martin P. Fat body cells are motile and actively migrate to wounds to drive repair and prevent infection. Deve cell. 2018; 44: 460-70.
17. Wang T, Zhao N, Long S, Ge L, Wang A, Sun H, et al. Downregulation of miR-205 in migrating epithelial tongue facilitates skin wound re-epithelialization by derepressing ITGA5. Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol. 2016; 1862: 1443-52.
18. Shi Y, Shu B, Yang R, Xu Y, Xing B, Liu J, et al. Wnt and Notch signaling pathway involved in wound healing by targeting c-Myc and Hes1 separately. Stem Cell Res Ther. 2015; 6: 120-5.
19. Soares E, Zhou H. Master regulatory role of p63 in epidermal development and disease. Cell Mol Life Sci. 2018; 75: 1179-90.
20. Yoda K, Ohnuki Y, Masui S, Kurosawa H. Optimized conditions for the supplementation of human-induced pluripotent stem cell cultures with a GSK-3 inhibitor during embryoid body formation with the aim of inducing differentiation into mesodermal and cardiac lineage. J Biosci Bioeng. 2020; 129:371-8.