Acceleration of epithelialization in different application of scaffolds and growth factors post tooth extractions controlled by a mobile application

M M Damayanti¹*, B S Hernowo²

¹ Department of Anatomic Pathology, Faculty of Medicine, Universitas Islam Bandung, Taman Sari 22, 40116, Bandung, Indonesia
² Department of Anatomic Pathology, Hasan Sadikin General Hospital, Bandung, Indonesia

*metamaulida@unisba.ac.id

Abstract. Tissue engineering technology is widely used that involves the use of patients own biologically active proteins, growth factors and biomaterial scaffolds for therapeutic purposes. Epithelialization is an essential component of wound healing used as a defining parameter of a successful wound closure. The purpose of this research was to analyse differences of wound healing process post tooth extraction between application of platelet-rich fibrin and platelet-rich plasma as a growth factors and hydroxyapatite as a scaffold seen from thickness of epithelialization. This research was an animal experiment. 18 rabbits randomly divided into 2 groups of treatment (K1) apply platelet-rich fibrin post tooth extraction and (K2) apply of platelet-rich plasma plus hydroxyapatite post tooth extraction. Then each treatment group was divided randomly into 3 groups of observation time (Day 3, 7 and 14). Tissue biopsy to see histopathologic pattern is analysed based on thickness of epithelialization. The results showed that thickness of epithelialization in group PRP+HA is similar with group PRF (P = 0.304). Thickness of epithelialization base on observation time had a significant difference in each group (P<0.001). It was concluded that application of platelet-rich fibrin, platelet-rich plasma and hydroxyapatite can accelerate the wound healing of tooth extraction socket by means of thickness of epithelialization increasing.

1. Introduction

Traumatic dental injuries usually imply wound healing processes in the periodontium, the pulp and sometimes associated soft tissue. The general response of soft and mineralized tissues to surgical and traumatic injuries is a sensitive process, where even minor changes in the treatment procedure may have an impact upon the rate and quality of healing. This healing process is basically the same in all tissue, but may vary clinically according to the tissues involved. Thus wound healing after dental trauma is complicated by the multiplicity of cellular systems involved. Wound healing is a dynamic, interactive process involving cells and extracellular matrix and is dependent on internal as well as external factors. Classically, the events taking place after wounding can be divided into the three phases, namely the inflammation, the proliferation and the remodeling [1,2].

Epithelialization will not be complete before granulation tissue has developed. Epithelial cells will use this bed for subsequent migration. Depending upon the size of wound, the surface will subsequently
be covered by a scar epithelium which is thin, and lacks strong attachment to the underlying dermis as well as lacking Langerhans cell and melanocytes. One factor that has strong influences upon epithelial healing is the depth of the wound [2,3].

Epithelium covers all surfaces of the body, including the internal surfaces of the oral mucosa, gastrointestinal, respiratory and genitourinary tracts. The major function of epithelium is to provide a selective barrier between the body and the environment. The epithelial barrier is the primary defense against threats from the environment and is also a major factor in maintaining internal homeostasis. Physical and chemical injury of the epithelial layer must therefore be repaired quickly by cell proliferation. After injury to the epidermis, wound protection is provided two steps, within minutes there is a temporary coverage of the wound by coagulated blood which serves as a barrier to arrest the loss of body fluids. The second step is movement of adjacent epithelium beneath the clot and over the underlying dermis to complete wound closure. Re-epithelialization of an injured surface is achieved either by movement or growth of epithelial cells over the wound healing the most important process is cell migration, which is dependent of cell division [4,5].

One of therapeutic efforts in regenerating periodontal tissues and alveolar bone is by tissue engineering. The three basic components in tissue engineering are progenitor cell, scaffold, and growth factor [6].

Platelet concentrates, which are Platelet-Rich Plasma (PRP) and Platelet-Rich Fibrin (PRF) are used in various medical field, especially in oral and maxillofacial surgery. The concentrates contain high growth factors level, which is an important element in wound healing, especially in bone regeneration. Therefore, it can be considered as a new therapy adjuvant. Hydroxyapatite (HA) as a scaffold in bone tissue regeneration. HA (Ca\(_{10}\)(PO\(_4\))\(_6\)(OH)\(_2\)) is the main component of hard tissues, i.e. bones and teeth. HA is used mainly as a material to aid in bone regeneration because of its biocompatibility, does not cause excessive inflammation phenomena, non-toxic, good osteoconduction and bioaffinity and a synthetic material widely used in medicine and dentistry. Many studies have proven the use of tissue engineering, as was done by Tajesh Yelamali, D. Saikrishna who proved that the use of PRP and PRF in the process accelerates the wound healing process. Besides it, the research conducted by James L. Rutkowski, David A. Johnson, Nicholas M. Radio, et al, using PRP as a medium in the process of wound healing after tooth extraction the results were very good. The aim of this study was to determine the difference of wound healing process post tooth extraction in rabbits (*Oryctolagus cuniculus*) between the addition of platelet-rich fibrin and the addition of hydroxyapatite to platelet-rich plasma [7-13].

2. Method
The subjects of this study were rabbits (*Oryctolagus cuniculus*) which had wounds after tooth extraction. The selection of research subjects was done randomly according to the research criteria. The study used 2 treatment groups and 3 observation times. The treatment group were: (K1) after tooth extraction were given PRF and (K2) after tooth extraction were given PRP + HA. Observation time is Day 3, 7 and 14.

Epithelial thickness was measured by making periodontal tissue histology preparat which were cut vertically (interdental border) then hematoxylin eosin staining was carried out. Epithelial tissue is a network that has a tight arrangement, and is located above the basement membrane (stratum basale). Observation of epithelial thickness using optilab on an Olympus microscope with magnification of 400x in units of µm. The thickness of the epithelial layer was measured using the Image Raster software, from the stratum basale layer to the stratum corneum [14].

3. Results
Epithelial thickness using a 400x magnification light microscope on healing rabbit tooth sockets (*Oryctolagus cuniculus*) in groups 1 and 2 based on 3 observation times showed differences in epithelial thickness. Differences in epithelial thickness between K1 and K2 groups did not show a significant difference (P =0.304) (table 1.) (figure 1.) The epithelial thickness at group 1 and group 2 based on time observation shown significant differences (P <0.05). (table 2.). In response to epidermal injury, both PRF and PRF+HA are participating in re-epithelialization of the wound defect.
3.1. Epithelial thickness of group 1 and group 2

Table 1. Differences in epithelial thickness between K1 and K2 groups by independent t-test.

| No | Time | Group of treatment | K1* | K2*           | P value |
|----|------|--------------------|-----|---------------|---------|
|    |      |                    |     |               |         |
| 1  | Day-3|                    | 0,111(0,013) | 0,110(0,010) | 0,885   |
|    |      |                    | 0,09-0,124   | 0,098-0,124  |         |
| 2  | Day-7|                    | 0,203(0,023) | 0,226(0,020) | 0,90    |
|    |      |                    | 0,180-0,242  | 0,200-0,254  |         |
| 3  | Day-14|                   | 0,262(0,038) | 0,326(0,459) | 0,25    |
|    |      |                    | 0,218-0,306  | 0,256-0,370  |         |
| 4  | Total|                    | 0,192(0,0689)| 0,220(0,095) | 0,304   |
|    |      |                    | 0,090-0,306  | 0,098-0,370  |         |

Note: * Average deviation and Range values

Figure 1. Epithelial Thickness. An epithelial thickness at group 1 day 3. B epithelialization thickness of group 2 day 3. C epithelial thickness at group 1 day 7. D epithelial thickness at group 2 day 7. E epithelial thickness at group 1 day 14. F epithelial thickness at group 2 day 14.

3.2. Epithelial thickness based on observation time

Table 2. Comparison of each treatment group based on observation time by kruskal wallis test.

| Group of treatment | P value |
|--------------------|---------|
| K1                 | <0,001  |
| K2                 | <0,001  |

Epithelial thickness in groups 1 and 2 increased from the 3rd, 7th and 14th days indicating epithelialization occurred in all three groups. Increased epithelial thickness at each time of observation indicates the influence of giving growth factor and scaffold on PRP, PRF and hydroxyapatite can accelerate the wound healing process.

4. Conclusion

Group 1 who were given PRF showed lower epithelializing thickness results than group 2 given PRP + HA, but this difference was not significant. But based on observation time shows that the ability of PRF
and hydroxyapatite as a scaffold and PRF and PRP as growth factor is able to increase epithelial thickness which means accelerating the wound healing process.

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