p-11 The verification of the MTT assay on the viability of periodontal ligamental cells in rat molars through the histologic examination

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Ⅰ. Objectives

The purpose of this study is to examine the viability of PDL cells in rat molars by using MTT assay and to verify the MTT assay through the histologic observation.

Ⅱ. Materials and Methods

1) Pretreatment of experimental animals

Thirty of Sprague-Dawley white female rats of 4-weels old with a body weight of about 100 grams were used. To facilitate the extraction, the rats were fed on powdered Purina rat diet containing 0.4% β-aminoproprionitrile for 3 days before the extraction. On the day of the experiment, under Ketamine anesthesia, the maxillary right and left molars were extracted with minimal trauma to surrouding tissue after performing peritomies with sharp explorer.

2) Preparation for MTT assay

Ten teeth of each group were immersed in 200㎕ of yellow MTT solution 0.05 mg/ml in each well of 96-well plate. Groupings are as follows

Group A: Positive control group(n=10)

Ten teeth were immersed in MTT solution after extraction immediately

Group B: Negative control group(n=10)

Ten teeth were immersed in MTT solution after drying for an hour under warm dry condition.

Group C: 1hour ViaSpan treated group(n=10)

Ten teeth were immersed in MTT solution after storing them in ViaSpan at 4 for 1 hour

3) Preparation for histologic observation

Ten teeth of each group were treated as same as above and replanted to the original socket of experimental animals. After two weeks of replantation, hearts of all the experimental animals were exposed by surgical incision under Ketamine anesthesia. And after fixation by perfusion of 40ml of 10% formalin through the heart, maxillary jaw was extracted. Extracted maxillary jaw was dimineralized for five days in 5% nitric acid, and after washing it with 0.1M sodium cacodylate buffer, it was dehydrated using 50% to 100% ethanol in steps. After precipitating it with Xylene, it was embedded in paraffin. Serial section by 5㎛ and parallel to occlusal plane was carried out for observation under light microscope, and for construction of specimen, hematoxylin-eosin dye was used.

Histologic slides were stored using Matrox Intellicam(Matrox Graphics Inc., Quebac, Canada), the photos of specimen were transferred to Microsoft Powerpoint(Microsoft Co., USA) and central point was marked at the center of the root. Center of oblique lines of 8 directions were established to meet at the marked center point, and then, existence of root resorption was evaluated at points where the oblique lines meet with outline of the root.

Ⅲ. Results

1) MTT assay

The mean MTT measurement of group A(positive control) is 2.81 and the mean measurement of group B(negative control) is 0.98 which is significant differnt($P<0.05$). The mean measurement of group C is 2.65 and there is significant difference between group B and C($P<0.05$). However, there is no difference between group A and C.

2) Histologic observation

The average resorption points of group A is 2 points. In the group B, average 5.7 points resorption and 2.5 points showed resorption in the group C. Unlike with MTT assay, there was no significant difference between the group A and C.

Ⅳ. Conclusion

The usage of MTT assay as a viable cell marker may give us a better indication of the maintenance of periodontal ligament cell vitality.