論　文　要　旨

Effects of tooth storage media on periodontal ligament preservation

平成 28 年度

北海道医療大学大学院歯学研究科

Md Riasat HASAN

[Purpose]

Dental avulsion is one of the most serious traumatic tooth injuries and is characterized by complete displacement of the tooth from its alveolar socket, causing severe damage to the supporting tissues along with vascular and nerve structures. The ideal treatment is immediate re-implantation, but this is not always possible. The primary goal in cases of tooth avulsion is to preserve the vitality of cell in the periodontal ligament (PDL) until re-implantation can be performed. Consequently, an easily available tooth storage medium is required to preserve the vitality of PDL cells. The American Association of Endodontics has recommended milk as a tooth storage medium to preserve the viability of PDL cells. Commercially available Hank's Balanced Salt Solution (HBSS) has been used as a tooth storage medium. Egg white is also reported to be comparable with milk as a storage medium for avulsed teeth, although there is few histopathological data for this method. The purpose of the present study was to evaluate histologically the effect of different tooth storage media on the periodontal ligament (PDL) of extracted teeth.

[Materials and Methods]

In the experiment HBSS, milk and egg white were used as tooth storage media. The egg white was separated from egg yolk for experimental purpose. For histological examination male 6 week-old Sprague-Dawley rats were used in these experiments. After giving general anesthesia with sodium pentobarbital one maxillary first molar from each rat was gently extracted.

1. Bio-physiological examination

For bio-physiological examination the osmolality levels were measured using an osmometer. Na^+ and K^+ meters were used to measure the Na^+ and K^+ concentrations. To determine the al-

kalinity or acidity of the HBSS, milk and egg white storage media pH indicator strips were used.

2. Cell counting procedure

For the cell counting after extraction, extracted tooth was immersed into three different tooth storage media for 1 h. After 1 h immersion the remaining HBSS, milk and egg white were centrifuged. Then supernatant was discarded and the cells were re-suspended with HBSS, and centrifuged again. This procedure was repeated twice. The same volume of 0.4% Trypan Blue solution was added to the cells. Finally, the number of cells was counted using a hemocytometer under the microscope.

3. Histological examination

In every sample six rat's extracted teeth were randomly assigned to the experiments. The rats were divided into no-transplantation procedure and transplantation procedure. Extracted teeth were immersed in three different tooth storage media for 1 h at room temperature and then transferred to the freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). In control group, extracted teeth were fixed immediately after extraction with freshly prepared fixative for one day. In transplantation procedure extracted teeth were immersed in three different tooth storage media for 1 h and then transplanted in a receiving pocket in the abdominal wall. At 4 days, 7 days and 14 days after transplantation, rats were anesthetized with pentobarbital. After perfusion fixation with same fixative described above, the teeth were carefully excised with the surrounding tissue and immersed in the fixative for one day. In no-transplantation procedure and transplantation procedure specimens were decalcified with EDTA and embedded in paraffin. Serial sections were obtained and stained with hematoxylin

and eosin (HE). For immunohistochemistry mouse anti-human cathepsin K, anti-OSF-2/Periostin, anti-rat CD68 and cytokeratin 14 were detected.

[Results]

Osmolality of HBSS (275-280 mOsm/kg), milk (275-280 mOsm/kg) and egg white (295-300 mOsm/kg) are within the normal physiological range (230-320mOsm/kg); although that of egg white may be slightly high for supporting an optimal growth rate (290-330 mOsm/kg). Milk is slightly acidic (pH 6.60-6.70) and egg white is slightly alkaline (pH 7.15-7.65). After 1 h immersion in three tooth storage media, various thickness of PDL in bifurcation area was observed. Among all, milk group showed thinnest PDL. There is no significant difference between HBSS group and control group in number of cells and cell layers. Egg white group showed slightly less but milk group showed significantly less number of cells and cell layers. In the media used for immersion, five to eight times more living cells were observed in milk than other solutions. In immunohistochemistry, periostin was detected prominent in control, HBSS and egg white group; while in milk group, the periostin was detected pale in general with some intense cluster. After 4 days, beginning of granulation tissue formation was observed in all groups. Periostin was detected to be pale and more CD68 positive cells were detected in the PDL of milk group. After 4 days and 7 days, epithelial cell rest of Malassez (ERM) rapidly increased and made large cluster in all groups. After 7 days transplantation, granulation tissue was formed in all groups. Many cathepsin K positive cells were observed in root surface of milk group. After 14 days transplantation, formation of alveolar bone was observed in all groups. This is also confirmed by the radio-opacity observed in soft X-ray. In case of HBSS and egg white, PDL maintained some thickness, which is similar to immediate control group. Moreover, in milk group thinner PDL and ankylosis were observed. Many ca-

thepsin K positive cells were found around the alveolar bone and ankylose area in milk group. After 7 days in HBSS group, ERM were reduced and became similar to the control group. In milk and egg group ERM were reduced but still large in size.

[Discussion]

Bio-physiological findings suggested that HBSS and egg white have better physiological properties as tooth storage media. HBSS is an established cell and tissue culture medium and it helps to maintain optimum physiological properties for cell growth. Thinnest PDL after 1 h immersion in milk may be due to the migration of the PDL cells into milk solution and degradation of extracellular matrix. Change in assembly of collagen bundles in milk also suggested by pale and uneven detection of periostin. Degeneration of extracellular matrix is also suggested by the appearance of many macrophages in PDL after 4 days transplantation in milk group. Accelerated activity of odontoclast and osteoclast in milk group may be related to the occurrence of ankylosis. Inflammation during transplantation might be the reason to increase the size of ERM. In HBSS and egg white group, in case of 1 h immersion and even in transplantation it preserved adequate thickness of PDL which is similar to the control group; that might be the reason to prevent ankyloses in HBSS and egg white group.

[Conclusion]

As a tooth storage media, HBSS and egg white were compatible compared to the control group. In this study, after 1 h immersion in milk, less number of PDL cells and cell layers were found; as a result after 14 days ankylosis might occur. This study suggested that due to easy availability and well preserving the PDL adequately, egg white might be a suitable storage medium for avulsed teeth.