論　文　題　目

Effects of tooth storage media on periodontal ligament preservation

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[Introduction]

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 and vascular and nerve structures (Andreasen & Andreasen, 2001; Barrett and Kenny 1997; Cohenca et al., 2006) 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 (Krasner, 1992). Commercially available Hank's Balanced Salt Solution (HBSS) has been used as a tooth storage medium (Trope & Friedman, 1992). Egg white is also reported to be comparable with milk as a storage medium for avulsed teeth (Khademi et al., 2008), 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.

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

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.

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]

Bio-physiological parameters

Normal physiological cellular osmolality is in the range 230–320 mOsm/kg, and the osmolality of HBSS, milk, and egg white was all within this range, and is thus considered to be physiological.

The concentration of Na^+ in HBSS was 160 mM, similar to the physiological concentration (150 mM), whereas it was present at much lower levels in milk (17 mM) and egg white (78 mM). The K^+ concentration of HBSS was identical to the physiological concentration (5 mM), but was considerably higher in milk and egg white (38 mM and 30 mM, respectively).

The normal physiological pH range is 7.35–7.45. The pH of HBSS was within this range (7.30–7.45), but milk was slightly more acidic (pH 6.60–6.70) and egg white was much more variable around the physiological average (pH 7.15–7.65).

Effects of immersion in different storage media

Cell numbers and cell layers

After immersion for 1 h in the three tooth storage media, the thickness of the PDL in the bifurcation area was variable. In the control group, the PDL cells were compact, whereas in the HBSS group, the cells were diffuse. In the egg white group, slightly diffuse cells were observed in the PDL. The thinnest PDL was observed in the milk group. There was no

significant difference in the number of cells and cell layers between the HBSS group and the control group. The number of cells and cell layers were significantly lower in the milk group than in the HBSS or control groups. There was no significant difference in the number of cells between the egg white group and either the HBSS or the control groups; but there was a significant difference in the number of cell layers between the egg white group and both the HBSS and the control groups.

Number of cells in storage media

After immersion for 1 h in the storage media, 5–8 times more living cells were observed in the milk group than in the other groups.

Immunohistochemistry

In the immunohistochemical analysis, periostin was prominently detected in the extracellular matrix of the control, HBSS and egg white groups, whereas staining in the milk group was generally pale with some intense punctate labeling. The distribution of cytokeratin-14 was co-localized with the epithelial rests of Malassez (ERM), which were found in the PDL as clusters of 2–4 cells.

Effects of immersion for 1 h, 3 h and 6 h

Immersion in egg white and HBSS for 1 h produced minimal variation in the results for all the groups. However, the PDL in the milk group was considerably thinner after immersion for 3 h, and almost absent after 6 h.

Characteristics of teeth after transplantation

Four days after transplantation

Four days after transplantation, early granulation tissue formation was observed in the bifurcation area in all groups. The interface between the PDL and the forming granulation tissue was indistinct. The acellular cementum seemed intact, and inflammatory cells were observed together with fibroblasts in the maturing granulation tissue. As before, periostin was detected only faintly in the PDL of the milk group compared with the intense staining seen in other groups. Thus, the interface between the PDL and the forming granulation tissue was clearly visible. CD68-positive cells were found in greater numbers in the PDL of the milk group than in that of other groups. ERM cells labeled with cytokeratin-14 were large and round in shape in all groups.

Seven days after transplantation

Seven days after transplantation, the bifurcation area was filled with granulation tissue containing fibroblastic cells and many newly formed blood vessels in all groups. The acellular cementum was intact in the control, HBSS and egg white groups, but began to shows signs of roughening of the acellular cementum in the milk group. Some cathepsin K-positive cells were observed on the rough root surface of teeth in the milk group. Large clusters of ERM cells were observed in the HBSS, milk and egg white groups, which were confirmed by cytokeratin-14 staining. In the immediate transplantation group, the ERM cells were reduced

in size, being comparable to those seen after immersion for 1 h.

Fourteen days after transplantation

At 14 days after transplantation, formation of alveolar bone was observed in all groups, a finding confirmed by increasing radio-opacity observed in soft X-rays. In the HBSS and egg white groups, the PDL maintained a thickness similar to that in the control group immediately after extraction, but the PDL in the milk group was significantly thinner. However, ankylosis was observed in more than half of the specimens in the milk group. Many cathepsin K-positive cells were found around the alveolar bone and ankylosed areas in the milk group. After 14 days, ERM cells labeled with cytokeratin-14 were reduced in size to a level similar to that in the control group, whereas they remained larger than the control group cells in both the milk and egg white groups.

Low-fat milk

The histological and immunohistological features of the teeth stored in low-fat milk were similar to those of teeth stored in full-fat milk, with reduced PDL thickness after immersion for 1 h, faint labeling of periostin in the PDL, and profuse CD68-positive staining in the PDL at 4 days after transplantation. Large ERM cells were seen at 7 days after transplantation, and at 14 days after transplantation, formation of alveolar bone with many cathepsin K-positive cells and evidence of ankylosis were observed.

[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. For histological examination, the transplantation procedure offers the great advantage of being able to evaluate the preservation and regenerative potential of the PDL without these interferences. As previous transplant studies have demonstrated, alveolar bone formation occurs at 14 days after transplantation (Hamamoto et al., 2002). In the present study, at 14 day after transplantation, newly formed alveolar bone was observed in the upper side of the bifurcation area and no bone regeneration was observed on the outside surfaces of the transplanted teeth. The bifurcation area seems to provide a more favorable environment for alveolar bone formation because it is surrounded by PDL, which is a source of osteoblast progenitor cells. The outside surfaces of transplanted teeth are in contact with connective tissue, which may affect the PDL.

The thickness of the PDL after immersion for 1 h in egg white was found to be comparable with those at the same time points in the HBSS and control groups. Even over extended periods of time, such as immersion for 3 h and 6 h, the thickness of the PDL in the egg white group was comparable with the HBSS and control groups. Furthermore, after transplantation, the PDL thickness of the egg white group was not significantly different from the HBSS and control groups. The favorable results in the egg white group may be attributed to its natural

components, and specified and non-specified proteins may contribute to the efficacy of egg white as a storage medium for avulsed teeth. In present study, PDL thickness after immersion for 1 h varied between groups, and was thinnest in the milk group. The number of PDL cells was also reduced in this group, with a concomitant large increase in the presence of PDL cells in the milk medium, suggesting that PDL cells are released from the PDL and migrate to the media during immersion. The weak immunoreactivity of periostin observed in the PDL after immersion for 1 h and at 4 days after transplantation in the milk group can be interpreted as evidence of disturbance in collagen assembly, leading to degradation of the extracellular matrix in the PDL. This result indicates that the degradation of collagen and the extracellular matrix may cause thinning of the PDL. CD68 is expressed on macrophages, and the mobilization of large numbers of CD68-positive cells to the thinner PDL at 4 days after transplantation in the milk group suggests that the macrophages may actively engulf dead cells or degenerated extracellular matrix components.

At 7 days after transplantation, the surface of the cementum was observed to be roughened, with associated root resorption, suggesting that immersion for 1 in milk affects the cementum as well as the PDL extracellular matrix. Environmental induction of odontoclasts may also accelerate osteoclastogenesis in newly formed alveolar bone in the milk group, and this combination of degenerated cementum and accelerated bone remodeling might induce ankylosis. Alveolar bone formation was observed even in the thinner PDL in the milk group,

suggesting that osteoblast progenitor cells were still present in the remaining PDL, allowing alveolar bone formation to proceed.

Generally, inflammation increases the number of cells in the ERM. Rapid morphological change of the ERM cells after transplantation in this study might be caused by inflammation. Morphological change of the ERM cells was seen in all groups, but the PDL maintained an adequate thickness in all groups except for the milk group, in parallel with a high frequency of ankylosis. This histological study suggests that the function of ERM cells is not directly related to the preservation of the PDL or prevention of ankylosis.

[Conclusion]

As a tooth storage medium, egg white exhibits comparable efficacy with HBSS. Unlike milk, egg white maintains an adequate thickness of PDL, and ankylosis was not observed. Therefore, the easy availability and efficacy in preserving the PDL make egg white a favorable storage medium for avulsed teeth.

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