

Original Article**Effect of Four Different Media on Periodontal Ligament Cells Viability of Dry- Stored Dog Teeth**Fariborz Moazzami¹, Bahar Asheghi¹, Safoura Sahebi¹¹ Dept. Endodontic, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.**KEY WORDS**

Aloe vera;
 Cell viability;
 Periodontal ligament;
 Soy milk;
 Tooth avulsion;

Received December 2015;
 Received in Revised form April 2016;
 Accepted July 2016;

ABSTRACT

Statement of the Problem: The maintenance of viable periodontal ligament cells is the most important issue in the long-term preservation of avulsed teeth.

Purpose: The aim of this study was to assess aloe vera as a new storage media in maintaining the cell viability of dry-stored teeth in comparison with soy milk, Hank's balanced salt solution (HBSS), and milk.

Materials and Method: Twenty one extracted dog premolar teeth were dried for 30 minutes and stored in soy milk, HBSS, milk, and aloe vera extract (50%) for 45 minutes (n=6 for each). Furthermore, positive and two negative control groups (n=6), corresponding to 0 min, 30 min, and 2-hour drying times were also prepared respectively. The number of viable cells was counted following storage using Trypan blue exclusion. Data were statistically analyzed using the one-way ANOVA and post hoc Tukey-HSD test.

Results: Statistical analysis showed no significant differences in cell viability among aloe vera, soymilk, and HBSS- stored teeth; however, they were all superior to milk.

Conclusion: Aloe vera extract can be recommended as a suitable storage media for avulsed teeth.

Corresponding Author: Asheghi B., Dept. Endodontic, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran. Email: bahar64a@gmail.com Tel: +98-71-36263193-4

Cite this article as: Moazzami F., Asheghi B., Sahebi S. Effect of Four Different Media on Periodontal Ligament Cells Viability of Dry- Stored Dog Teeth. *J Dent Shiraz Univ Med Sci.*, 2017 March; 18(1): 24-29.

Introduction

Tooth avulsion is the complete displacement of a tooth from its socket as a result of trauma [1-2] The reported incidence of complete avulsion ranges 1-16% of all traumatic injuries to the permanent dentition. [3] The prognosis of a replanted tooth depends on the existence of viable periodontal ligament cells on the root surface. In order to minimize the risk of post-replantation resorption of an inflammatory or replacement nature, the suggested treatment is immediate replantation of an avulsed tooth. [1, 4-5] However, such treatment it is not always feasible. Thus, extra alveolar time interval always exists before the patient arrives at the dental office for replantation. [6]

Extra oral dry time and the storage medium are two of the most critical factors that affect the prognosis

of an avulsed tooth after replantation. [7]

The delayed replantation of dry teeth covers different clinical management and different prognosis from the delayed replantation of teeth kept in storage solutions from clinical point of view. [8-10] The duration of the extra-alveolar period and the nature of the storage conditions affects the survival rate of avulsed teeth following replantation. [11-12] The ability of the storage medium to maintain periodontal ligament cell viability may be even more important than the extra-alveolar period before tooth replantation. [13-14] Therefore, the assessment of cell recovery after exposure to the various solutions is important to increase the regenerative capacity of periodontal ligament (PDL) cells remaining on the avulsed tooth. [12]

An ideal storage medium for avulsed teeth must

have a low bacterial content, physiologic osmolality, a neutral pH and contain essential nutrients. [15] Different storage media, such as Hank's balanced salt solution (HBSS), milk, and saline have been used with some success, but an ideal storage medium remains to be developed. [16] The American Association of Endodontics has suggested HBSS as the storage medium of choice for avulsed teeth. [5] HBSS is considered superior regarding cell viability and vitality preservation due to its non-toxicity, balanced pH, and osmolality that allows cell growth. [12, 17]

Milk has also been shown to be a suitable storage medium for avulsed teeth, mainly due to its physiologic osmolality, neutral pH, essential nutrient content, and lack of active toxic components. [18] Recent studies have also shown that soy milk can maintain cell viability. Moazzami *et al.* [19] showed that soymilk is as effective as HBSS and can maintain PDL cell viability for up to 8 hours. Moura *et al.* [20] indicated that soymilk can preserve cell viability for up to 24 hours. Furthermore, Silva *et al.* [21] assessed the cytotoxicity of soymilk and concluded that soymilk can maintain cell viability at levels similar to solutions considered the gold standard for avulsed teeth such as whole milk and HBSS.

Aloe vera (*Aloe Vera L.Xanthorrhoeaceae*) is a member of the liliaceae family and a cactus-like plant, which grows in hot and dry climates. [22-23] Since it can live and even bloom without soil, the Egyptians named it the plant of immortality. [24] The aloe vera leaves are filled with a transparent viscous gel. This gel consists of 96% water and 75 active components such as vitamins, enzymes, minerals, sugar, salicylic acid, and amino acids. [22] The use of aloe vera in modern medicine was first recorded in the 1930s which was used to heal radiation burns. [25] Since then, aloe vera has been used as an anesthetic, anti-bacterial, antifungal, antiviral, anti-inflammatory, and antioxidant [22, 26] as well as in wound healing. [27-28] Aloe vera has recently gained some popularity in the field of dentistry and is extremely helpful in the treatment of gum diseases like gingivitis and periodontitis. [29-30] Aloe vera has been shown to exert antimicrobial effects against resistant microorganisms found in the pulp space (*Enterococcus faecalis*) and the oral cavity (*Streptococcus mutans* and *S. mitis*). [31] Gontijo *et al.* [32] showed that aloe vera

was not cytotoxic and could maintain cell viability. They showed an increasing concentration of aloe vera resulted in increased cell viability. A recent study on rat pulp tissues has shown that the application of freeze-dried aloe vera in direct contact with the mechanically exposed pulp had acceptable biocompatibility and could lead to tertiary bridge formation. [33] Furthermore, Badakhsh *et al.* [34] concluded that increasing concentration of aloe vera (10%, 30%, and 50%) lead to increased preservation of PDL cells viability. However, none of these studies considered the dry time before storage in aloe vera solutions.

The aim of the present study was to compare aloe vera with soy milk, milk, and HBSS- to preserve PDL cell viability following a 30 min dry time.

Materials and Method

This study was approved by the animal ethics committee of Shiraz University of Medical Sciences, Shiraz, Iran, # 1663.

A total of 21 two- root premolars from five adult dogs were selected. The animals were anesthetized by intravenous injection of ketamine at 10 mg/kg of body weight (Imanan& Saba company; Shiraz, Iran) with xylazine 2% at 0.2-0.5 mg/kg of body weight (Imanan& Saba company; Shiraz, Iran).The dogs also received a local anesthetic (lidocaine 2% with epinephrine1/100000).The premolar teeth were extracted as atraumatically as possible such that the teeth, in their socket, were sectioned on the furcation region using diamond burs (Teledyne Densco, USA).The teeth were extracted in 15 s. following extraction, the teeth were held with forceps by the coronal region, and the coronal 3mm of PDL was scraped with a curette to remove cells that might have been damaged during extraction. The 42 premolar roots were randomly divided into four experimental groups, a positive control, and two negative control groups (n=6 in each group). The experimental storage solution groups were HBSS (Biosera-xc-s2065, Biosera Ltd, Ringmer, East, Sussex, UK) as group 1, Milk (Maxoy; Tehran, Iran) as group 2, soy milk (Maxoy; Tehran, Iran) as group 3, and Aloe vera extract 50% as group 4.

Aloe vera plants (*Aloe vera L.Xanthorrhoeaceae* with code: Pm 766) were obtained from the Pharmacology Faculty of Shiraz University of Medical Sci-

ence. The leaves were rinsed with normal saline and disinfected with 70% alcohol. The pulp of the leaves was then removed and filtered through a polystyrene filter for homogenization, yielding a 100% aloe vera extract. Then 50mL of distilled water was added to 50mL of this extract with shaking for 15 min, obtaining aloe vera extract 50%. The mixture was stored at room temperature.

The teeth in the experimental groups were kept in dry petri dish in the lab environment for 30 min followed by a 45-min immersion in the corresponding storage solution according to the assigned group. The positive control teeth were immediately treated with collagenase and dispase without any dry or solution storage. The negative control teeth were bench-dried for 30 min and 2 h, with no follow-up storage solution time, and then placed in the collagenase and dispase to count the PDL cells.

Following the drying and soaking stages, the teeth were washed three times in 0.1 M phosphate- buffered saline, and incubated for 30 min in 15-mL Falcon tubes (BD Bio Sciences; San Jose, Canada) with a 2.5-mL solution of 0.2 mg/mL of collagenase CLS II (Sigma; St. Louis, Mo, USA) and a 2.4 mg/mL solution of dispase grade II (Roch; Mannheim, Germany). Following incubation, 20 μ L of fetal bovine serum was added to each tube for enzyme inactivation. All tubes were then centrifuged (Eppendorf centrifuge 5702r, USA) for 4 min at 1,000 rpm. The supernatant was removed with sterile micro pipettes and the cells were stained with 0.4% trypan blue to assess viability. The number of viable periodontal ligament cells was counted under a light microscope (Optikal Nikon; Japan) with a hemocytometer (QIUJING, China) at 20 \times magnification. For each tooth, two independent counts were performed by a single calibrated blinded examiner. In the protocol presented herein, viable cells were those with a clear cytoplasm whereas non-viable cells were those with a blue cytoplasm. The results were statistically analyzed using one-way ANOVA and Tukey-HSD test. The level of significance was 5% ($p < 0.05$).

Results

The results of this study indicated that aloe vera kept 98% of PDL cell viability. The percentages of viable cells in different storage media are shown in Table 1.

Table 1: The percentage of viable cells in different storage media. HBSS, Hanks Balanced Salt solution

Groups	Cell viability (%)
Control positive	99.14 \pm 0.51 ^a
Control negative 2h	6.94 \pm 1.83 ^b
Control negative 30min	61.64 \pm 4.66 ^c
Aloe vera	98.15 \pm 0.67 ^a
Soy milk	94.20 \pm 2.68 ^a
HBSS	95.13 \pm 2.60 ^a
Milk	65.54 \pm 4.05 ^c

The Post hoc Tukey-HSD test showed no significant differences between aloe vera, soy milk, HBSS, and the positive control groups. However, these groups showed significantly more viable cells than the two negative control groups. There was no significant difference between milk and the 30 min negative control group, but milk was superior to the 2 h negative control group (Figure 1).

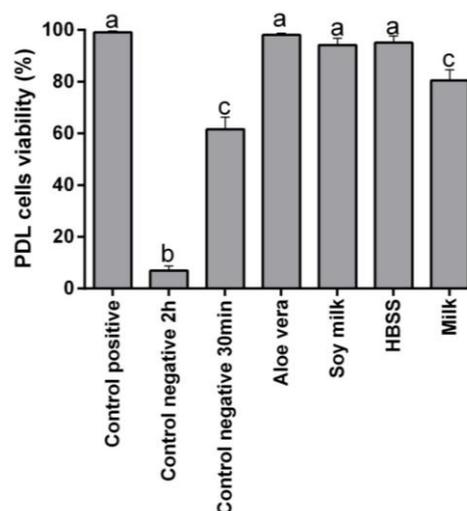


Figure 1: Means and standard deviations for the percentage of viable cells. a, b, c: Different superscript letters show significant different between groups ($p < 0.05$)

Discussion

Dental avulsion is the most severe type of traumatic tooth injuries because it damages several structures and results in the complete displacement of the tooth from its socket in the alveolar bone. [1-2] The ideal situation is to replant the tooth immediately after avulsion since the extraoral time is a determinant factor for treatment success and for a good prognosis. [7] However, this is not always possible since there is a time interval between accident and treatment and most patients come to clinic after 30 minutes. The success of replantation depends on a number of factors that may contribute to accelerate or minimize the occurrence of

root resorption or ankylosis, among which is the type and characteristics of the medium used for temporary storage during the time elapsed between avulsion and replantation. [3, 7] Maintaining the tooth in an adequate wet medium to preserve the vitality of the periodontal ligament cells that remain on root surface is the key to success of replantation. [7] Recent studies introduced the development of storage media that produce conditions that closely resemble the original socket environment. [17-19] Although these storage media can now be purchased in the form of retail products, the most common scenario is that such a product will not be readily available at the moment of the accident.

The use of extracted dog teeth to stimulate avulsion has been recommended in previous studies. [20, 35-36] In an attempt to mimic the typical situation during which the teeth may remain dry before being placed into a storage medium, the dog teeth were left in dry conditions after extraction. [37-38]

Various techniques have been used to quantitate the number of viable PDL cells. Herein, the root surface was treated with collagenase and dispase grade II according to Pileggi *et al.* [39] This procedure allowed rapid cell retrieval and maintained maximum cellular integrity, as was demonstrated by the positive control samples. [39] This method is more representative of the actual clinical situation since the cells are not subjected to long processing times to determine their viability status. [40]

The trypan blue exclusion staining technique was chosen for its fast and easy application, and since it distinctively differentiates non-viable cells from viable cells. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes. [41]

It is important to show the ability of media to revive the PDL cells on the root surface since these healthy PDL cells can proliferate on the root surface after replantation. [12] Herein, the ability of the media to revive the PDL cells was determined. Cells were counted following 30 min of dry time and an increase in the number of cells after exposure to the different solutions was observed. Thus, this differentiates the present results from those of similar studies [19-21] regarding aloe vera, where the number of viable cells was determined only after exposure to different solu-

tions. Furthermore, since the surfaces area of all teeth roots are not equal, the percentage of vital cells with regards to the whole cell population was calculated instead of cell numbers per se as stated by Moura and Silva *et al.* [20-21] Nevertheless, the present study confirmed the results of the studies of Moazami [19] and Silva *et al.*, [21] that soy milk showed similar behavior to HBSS in the maintenance of PDL cell viability. Additionally, there were no significant differences between aloe vera and HBSS or soy milk, whereas all three were superior to milk. And in this study, we considered an average amount of saturation for aloe vera since it was shown that aloe vera 50 % could preserve more PDL cells alive. [34]

The maintenance of viability of PDL cells in aloe vera medium is probably due to the nutrients contained such as vitamins, amino acids, minerals, and sugars, which nourish cells and maintain their viability. [22] Several studies have assessed the healing capacity of aloe vera in different fields of medicine [30, 42-46] and found to be due to an increase in blood supply and, increased oxygenation, which both stimulate fibroblast activity and collagen proliferation. [28] Aloe vera also contains vitamins A, C, E, and B12, and folic acid; vitamin C is involved in collagen synthesis. [47] Tanwar *et al.* [24] studied the constituents and biological effects of aloe vera and showed that the healing properties of aloe vera could be related to Glucomannan, a mannose-rich poly-saccharide, and Gibberellin, a growth hormone. These can interact with growth factor receptors on fibroblasts, thereby stimulating their activity and proliferation which in turn, significantly increases collagen synthesis.

Fibroblast cell viability and proliferation is a critical factor in the prognosis of avulsed teeth in avulsion injuries. Thus, aloe vera might be useful in the replantation of avulsed teeth due to its fibroblast stimulating substances.

Further studies should address the different concentrations and immersion times following the dry period in order to produce the best aloe vera media for avulsed teeth; such a product would be commercially marketed.

Conclusion

Based on the favorable results obtained in this study,

aloe vera extract can be recommended as a suitable storage media for avulsed teeth.

Conflict of Interest

None to declare.

References

- [1] Andreason JO, Andreason FM, Andreason L. Text book and color atlas of traumatic injuries the teeth. 4th ed. Oxford: Blackwell MunKsgaard; 2007. p. 444-488.
- [2] Ingle JI, Bakland LK, Baumgartner JC. Ingle's endodontics 6th ed. Hamilton, ON: B. C. DeckerInc; 2008. p. 1348-1351.
- [3] Guideline on Management of Acute. Dental Trauma. American Academy of Pediatric Dentistry. 2011; 33: 220-228.
- [4] McCulloch CA, Bordin S. Role of fibroblast subpopulations in periodontal physiology and pathology. J Periodontal Res. 1991; 26(3 Pt 1): 144-154.
- [5] Flores MT, Andersson L, Andreasen JO, Bakland LK, Malmgren B, Barnett F, et al. Guidelines for the management of traumatic dental injuries. II. Avulsion of permanent teeth. Dent Traumatol. 2007; 23: 130-136.
- [6] Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. 4. Factors related to periodontal ligament healing. Endod Dent Traumatol. 1995; 11: 76-89.
- [7] Martin MP, Pileggi R. A quantitative analysis of Propolis: a promising new storage media following avulsion. Dent Traumatol. 2004; 20: 85-89.
- [8] Trope M. Avulsion of permanent teeth: theory to practice. Dent Traumatol. 2011; 27: 281-294.
- [9] Andreasen JO, Andreasen FM, Skeie A, Hjørting-Hansen E, Schwartz O. Effect of treatment delay upon pulp and periodontal healing of traumatic dental injuries -- a review article. Dent Traumatol. 2002; 18: 116-128.
- [10] Andersson L, Andreasen JO, Day P, Heithersay G, Trope M, Diangelis AJ, et al. International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. Dent Traumatol. 2012; 28: 88-96.
- [11] dos Santos CL, Sonoda CK, Poi WR, Panzarini SR, Sundefeld ML, Negri MR. Delayed replantation of rat teeth after use of reconstituted powdered milk as a storage medium. Dent Traumatol. 2009; 25: 51-57.
- [12] Sigalas E, Regan JD, Kramer PR, Witherspoon DE, Opreman LA. Survival of human periodontal ligament cells in media proposed for transport of avulsed teeth. Dent Traumatol. 2004; 20: 21-28.
- [13] Andreasen JO. Effect of extra-alveolar period and storage media upon periodontal and pulpal healing after replantation of mature permanent incisors in monkeys. Int J Oral Surg. 1981; 10: 43-53.
- [14] Söder PO, Otteskog P, Andreasen JO, Modéer T. Effect of drying on viability of periodontal membrane. Scand J Dent Res. 1977; 85: 164-168.
- [15] Souza BD, Lückemeyer DD, Felipe WT, Simões CM, Felipe MC. Effect of temperature and storage media on human periodontal ligament fibroblast viability. Dent Traumatol. 2010; 26: 271-275.
- [16] Macway-Gomez S, Lallier TE. Pedialyte promotes periodontal ligament cell survival and motility. J Endod. 2013; 39: 202-207.
- [17] Trope M, Friedman S. Periodontal healing of replanted dog teeth stored in Viaspan, milk and Hank's balanced salt solution. Endod Dent Traumatol. 1992; 8: 183-188.
- [18] Blomlöf L. Milk and saliva as possible storage media for traumatically exarticulated teeth prior to replantation. Swed Dent J Suppl. 1981; 8: 1-26.
- [19] Moazami F, Mirhadi H, Geramizadeh B, Sahebi S. Comparison of soymilk, powdered milk, Hank's balanced salt solution and tap water on periodontal ligament cell survival. Dent Traumatol. 2012; 28: 132-135.
- [20] Moura CC, Soares PB, de Paula Reis MV, Fernandes Neto AJ, Zanetta Barbosa D, Soares CJ. Potential of coconut water and soy milk for use as storage media to preserve the viability of periodontalligament cells: an in vitro study. Dent Traumatol. 2014; 30: 22-26.
- [21] Silva EJ, Rollemberg CB, de Souza Coutinho-Filho T, Zaia AA. A multiparametric assay to compare the cytotoxicity of soy milk with different storage media. Dent Traumatol. 2013; 29: 319-322.
- [22] Joseph B, Raj SJ. Pharmacognostic and phytochemical peoperties of Aloe vera linn- An overview. Int J Pharmaceutical Sciences Review and Res. 2010; 4: 106-110.
- [23] Sikarwar Mukesh S, Patil MB, Shalini S, Vishnu B. Aloe vera: plant of immortality. Int J Pharm Scien Res. 2010; 1: 7-10.
- [24] Tanwar R, Gupta J, Asif S, Panwar R, Heralgi R. Aloe vera and its uses in Dentistry. Indian Journal of Dental Advancements. 2011; 3: 656-658.
- [25] Collins CE. Alvagel as a therapeutic agent in the treat-

- ment of roentgen and radium burns. *Radiol Rev Chicago Med Rec.* 1935; 57: 137-138.
- [26] Tudose A, Celia C, Cardmone F, Vono M, Molinaro R, Paolini D. Regenerative properties of Aloe Vera juice on human keratinocyte cell culture. *Farmacia.* 2009; 57: 590–597.
- [27] Chithra P, Sajithlal GB, Chandrakasan G. Influence of Aloe vera on collagen characteristics in healing dermal wounds in rats. *Mol Cell Biochem.* 1998; 181: 71-76.
- [28] Davis RH, Leitner MG, Russo JM, Byrne ME. Wound healing. Oral and topical activity of Aloe vera. *J Am Podiatr Med Assoc.* 1989; 79: 559-562.
- [29] Grindlay D, Reynolds T. The Aloe vera phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. *J Ethnopharmacol.* 1986; 16: 117-151.
- [30] Bhat G, Kudva P, Dodwad V. Aloe vera: Nature's soothing healer to periodontal disease. *J Indian Soc Periodontol.* 2011; 15: 205-209.
- [31] George D, Bhat SS, Antony B. Comparative evaluation of the antimicrobial efficacy of aloe vera tooth gel and two popular commercial toothpastes: an in vitro study. *Gen Dent.* 2009; 57: 238-241.
- [32] Gontijo SML, Gomes ADM, Gala-Garcia A, Sinisterra RD, Cortés MD. Evaluation of antimicrobial activity and cell viability of Aloe vera sponges. *Eletron J Biotechnol.* 2013; 16: 1-10.
- [33] Gala-García A, Teixeira K, Mendes LL, Sobrinho A, Santos VR, Cortés ME. Effect of Aloe vera on rat pulp tissue. *Pharmaceutical Biology.* 2008; 46: 302–308.
- [34] Badakhsh S, Eskandarian T, Esmaeilpour T. The use of aloe vera extract as a novel storage media for the avulsed tooth. *Iran J Med Sci.* 2014; 39: 327-332.
- [35] Khademi AA, Atbaee A, Razavi SM, Shabani M. Periodontal healing of replanted dog teeth stored in milk and egg albumen. *Dent Traumatol.* 2008; 24: 510-514.
- [36] Buttke TM, Trope M. Effect of catalase supplementation in storage media for avulsed teeth. *Dent Traumatol.* 2003; 19: 103-108.
- [37] Huang SC, Remeikis NA, Daniel JC. Effects of long-term exposure of human periodontal ligament cells to milk and other solutions. *J Endod.* 1996; 22: 30-33.
- [38] Chamorro MM, Regan JD, Opperman LA, Kramer PR. Effect of storage media on human periodontal ligament cell apoptosis. *Dent Traumatol.* 2008; 24: 11-16.
- [39] Pileggi R, Dumsha TC, Nor JE. Assessment of post-traumatic PDL cells viability by a novel collagenase assay. *Dent Traumatol.* 2002; 18: 186-189.
- [40] Gopikrishna V, Baweja PS, Venkateshbabu N, Thomas T, Kandaswamy D. Comparison of coconut water, propolis, HBSS, and milk on PDL cell survival. *J Endod.* 2008; 34: 587-589.
- [41] Singla A, Garg S. Reimplantation: Clinical implications and outcome of dry storage of avulsed teeth. *J Clin Exp Dent.* 2010; 2: 38–42.
- [42] Davis RH, Donato JJ, Hartman GM, Haas RC. Anti-inflammatory and wound healing activity of a growth substance in Aloe vera. *J Am Podiatr Med Assoc.* 1994; 84: 77-81.
- [43] Choonhakarn C, Busaracome P, Sripanidkulchai B, Sarakarn P. The efficacy of aloe vera gel in the treatment of oral lichen planus: a randomized controlled trial. *Br J Dermatol.* 2008; 158: 573-577.
- [44] Mandeville FB. Aloe vera in the treatment of radiation ulcers of mucous membranes. *Radiology.* 1939; 32: 598–599.
- [45] Bovik EG. Aloe vera: Panacea or old wives tales? *Tex Dent J.* 1966; 84: 13–16.
- [46] Poor MR, Hall JE, Poor AS. Reduction in the incidence of alveolar osteitis in patients treated with the SaliCept patch, containing Acemannan hydrogel. *J Oral Maxillofac Surg.* 2002; 60: 374-379.
- [47] Yagi A, Egusa T, Arase M, Tanabe M, Tsuji H. Isolation and characterization of the glycoprotein fraction with a proliferation-promoting activity on human and hamster cells in vitro from Aloe vera gel. *Planta Med.* 1997; 63: 18-21.