Fibroblast Cell Viability Effectiveness between the Highlands and Lowlands Coconut Water (Cocos Nucifera L.)

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Abstract

Tooth avulsion is a tooth that completely detached from its socket. The purpose of this study is to determine the differences of the effectiveness of coconut water in the dwarf variety from highlands and lowlands on fibroblast cell viability. Coconut water from each habitat was picked randomly. Samples were divided into five groups. Group 1 was the media control, group 2 was the cell control, group 3 was HBSS, group 4 was the coconut water from highlands, and group 5 was the coconut water from lowlands. To determine the BHK-21 fibroblast cell viability after 4 hours of submerging, MTT assay was used. The absorbance was read by ELISA reader with wavelength 620nm.

The results were analyzed using One-way ANOVA with α=0.05. There are significant differences in each group. Coconut water from highlands has cell viability percentage of 8%, while the coconut water from lowlands has 54%. Based on the CD50% parameter, coconut water from the lowlands is more effective than coconut water from the highlands. The coconut water in the dwarf variety from the lowlands is more effective than the coconut water from the highlands towards BHK-21 fibroblast cell viability for 4 hours.

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Introduction

Dental trauma is a trauma on face which causes tooth to fracture, to get luxation or even to get avulsion that it affects masticatory function, aesthetic and psychology. The prevalence of dental trauma was reported accounted for nearly 64% with the largest number of children less than 4 years.[1] Tooth replantation is the primary tooth avulsion treatment by attaching the tooth back to its socket.[2] The prognosis is affected by periodontal ligament cell viability, the period the tooth is outside its socket and the storage media.[3] Periodontal ligament fibroblast cell viability plays the biggest role in regenerating periodontal tissue since fibroblast cell can proliferate and differentiate to be osteoblast-like cell and cementoblast-like cell.[3,4] Cell viability in the surface of avulsed tooth root will decline along time and after 2 hours the cell viability will vanish.[5] If tooth replantation cannot be done within 30 minutes, then the tooth avulsion has to be stored in a storage with pH 7.2-7.4 and osmolarity of 290-330 mOsm/L.[6,7,8]

American Academy of Pediatric Dentistry recommended Hank's Balanced Salt Solution (HBSS) as the best storage media for tooth avulsion since it is biocompatible with the periodontal ligament cell. Furthermore, HBBS contains essential nutrition, pH 7.2, and osmolarity of 320 mOsm/L.[9,10] HBBS can be found easily in cities and particular places such as pharmacies and drug stores (for example, there is one store named "Save-A-Tooth"), but difficult to get in district area.

Coconut water can be used as an alternative to store tooth avulsion as it contains essential nutrition, has biocompatible characteristic and reasonable price. Coconut water has osmolarity of 372 mOsm/L and pH 5.6. Therefore, it is adequate since cell can live in osmolarity of 230-400 mOsm/L and pH 6.6-7.8.[8,11]. Previous study showed that coconut water holds the same effectiveness as HBBS for 15-20 minutes.[12]

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Coconut water in the dwarf variety contains the highest and most stable glucose substance compared to those from the tall variety and the hybrid variety. Glucose substance is used as cell energy source, such as molecule active transport through cell membrane, cell growth and development, as well as other physiological functions to maintain cell's survival.[13] Coconut plants can be found in both highlands and lowlands, but they grow better in lowlands, and the difference of the height of the habitat can influence coconut water nutrient.[14,15]

Materials and methods

This study was an experimental laboratory research with the post-test only control group design. The sample is BHK-21 (Baby Hamster Kidney-21) fibroblast cell culture obtained from PUSVETMA (Pusat Veterinaria Farma) laboratory Surabaya, was incubated for 24 hours.

Coconuts from highlands and lowlands which contain 6-7 months coconut water in the dwarf coconut variety (*Cocos nucifera L.*) with the weights exceeding 600 gram are skinned for the fiber and shell part. Then, the coconut water was extracted using 10 ml injection needle and filtered with 0.20 µm millipore.

Fibroblast cell culture in roux bottle which was washed with PBS and flaked using versene trypsine was replaced with new Eagle media. Afterward, the cell culture was moved to 96 well microplate and incubated in CO_2 incubator for 24 hours at 37°C. Samples were divided into 5 groups and each group received 8 repeating treatments. Group 1 was given 10 µ Eagles media as media control; group 2 was given 10 µ Eagles media as the cell control; group 3 was given 10 µl HBSS; group 4 was given 10 µ highlands coconut water; and group 5 was given 10 µ lowlands coconut water. Then, the samples were incubated at 37°C in regular incubator for 4 hours. The microplate was washed using PBS and filled with Eagles media and FBS. Tetrazolium (MTT) was dropped in each well approximately 10 µl and then the wells were incubated again in the same temperature for 3 hours. Later every well was added with 50 µl DMSO (dimethyl sufoxide) and the microplate was stirred using a shaker for 5 minutes until formazan crystals were liquefied.[16]

The outcome of formazan crystals absorption examination on BHK-21 (Baby Hamster Kidney-21) fibroblast cell culture using ELISA reader at 620 nm wavelength is stated as the value of optical density (OD). Cell viability percentage is counted using the following pattern.[16]

$$\text{Cell viability (\%)} = \frac{\text{OD treatment - OD media control}}{\text{OD cell control - OD media control}} \times 100\%$$

All experiments were performed with One-way ANOVA statistic test and continued with LSD (Least Significant Difference) test p value < 0.05.

Results

The analysis of nutrient content of coconut water using a spectrophotometer, found differences in nutrient content between the amount of coconut that grows in the high and lowlands (tabel 1) and HBBS substance based on manufacturer's specification(Gibco)(table 2).[17]

Substance	Unit	Result	
		High-lands coconut water	Low-lands coconut water
Glucose	%	2.18	2.77
Calcium (Ca)	mg/kg	2.642	24.67
Natrium (Na)	mg/kg	14.728	438.69
Potassium (K)	mg/kg	1.723	1.983
Chlorine (Cl)	mg/kg	10.642	75.89
Magnesium (Mg)	mg/kg	1.059	0.922
pH	-	6	6.64

Table 1. Glucose, minerals, and pH substances in the dwarf variety coconuts from highlands and lowlands.

HBBS substance	Concentration
Calcium Chloride (CaCl2)	140 mg/kg
Magnesium Chloride (MgCl2-6H2O)	100 mg/kg
Magnesium Sulfate (MgSO4-7H2O)	100 mg/kg
Potassium Chloride (KCl)	400 mg/kg
Potassium Phosphate monobasic (KH2PO4)	60 mg/kg
Sodium Bicarbonate (NaHCO3)	350 mg/kg
Sodium Chloride (NaCl)	8000 mg/kg
Sodium Phosphate dibasic (Na2HPO4)	48 mg/kg
D-Glucose (Dextrose)	1000 mg/kg
pH	6.7 – 7.8
Osmolarity	270 - 305 mOsm/kg

Table 2. HBBS substance.

Optical density percentage from BHK-21 fibroblast cell viability test which was measured using ELISA reader at 620nm wavelength. The treatment group which was given HBBS has the highest optical density average compared to coconut water treatment groups. Lowlands

coconut water treatment group has 0.242 on average for the optical density while the highlands has 0.114 (table 3).

Storage Media	Calculation after 4 hours
	X ± SD
Media Control	0.092 ± 0.009
Cell Control	0.368 ± 0.017
HBSS	0.307 ± 0.012
Lowlands coconut water	0.241 ± 0.009
Highlands coconut water	0.114 ± 0.006

Table 3. Optical density average value and BHK-21 fibroblast cell standard deviation.

Storage media	Media control	Cell control	HBBS	Highland coconut water	Lowland coconut water
Media control	-	.00*	.00*	.00*	.00*
Cell control		-	.00*	.00*	.00*
HBBS			-	.00*	.00*
Highland coconut water				-	.00*
Lowland coconut water					-

Note: *= with the significant differences

Table 4. LSD test between the treated groups and the control group.

Optical density value was counted using a formula to determine BHK-21 fibroblast cell viability percentage.

Cell viability of HBBS media is 78%, highlands coconut water the percentage is 8%, and lowland coconut water is 54% (Figure 1).

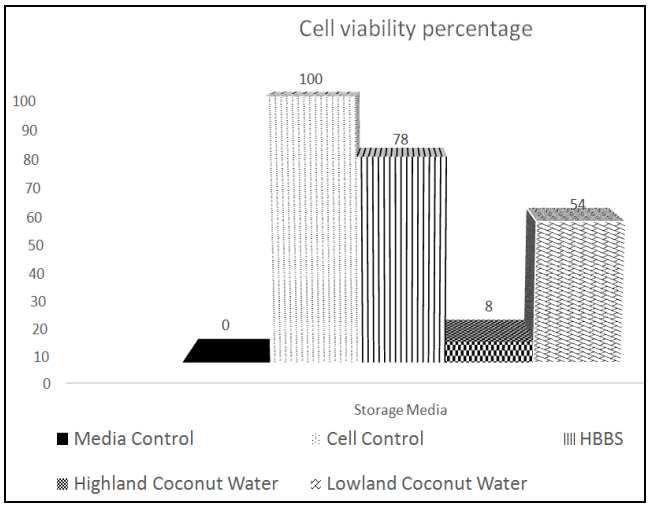

Figure. Cell viability percentage of BHK-21 fibroblast.

The data was analysed using one-way ANOVA test (p=0.05). The result showed that p significance value was 0.000 ($p<0.05$). Then LSD test had the significant differences less than 0.05 ($p<0.05$) among experimental groups.

Discussion

The result of MTT test reveals that percentages of BHK-21 fibroblast cell viability in highlands coconut waters was 8%, and 54% in lowlands and 78% in HBBS media. It shows that lowland and HBBS media could keep BHK-21 fibroblast cell viability for 4 hours.

The previous research has discovered that HBBS media has the same effectiveness as Eagle media in keeping periodontal ligament cell viability.[18] In this research, the percentage of BHK-21 fibroblast cell viability in HBBS is lower than in Eagle media. The reason behind this is that the period of HBBS storing used for experiment was rather long due to the lack of MTT substance in the laboratory which caused the research to be postponed for 2 months. The time of HBBS storing can change substance concentration. The longer HBBS is stored, the lesser its effectiveness to keep fibroblast cell viability.[19] The research shows that the percentage of BHK-21 fibroblast cell viability in HBBS is above 50%; thus, according to $CD_{50\%}$ parameter HBBS is still effective to use as the storage media for tooth avulsion.

$CD_{50\%}$ parameter is the percentage of the amount of BHK-21 fibroblast cell which survives over 50% after being submerged for 4 hours. It means that HBBS media is biocompatible, thus it can be used as tooth avulsion storage media.

Thomas (2008) stated that coconut water has the same effectiveness as HBBS in keeping BHK-21 fibroblast cell viability with 15-120 minutes submerging.[12] This research reveals that BHK-21 fibroblast cell viability percentage in HBBS media is higher than in the coconut water. This is because the nutrition and pH of HBBS are higher than of the coconut water. Also, the period of BHK-21 fibroblast cell submerging in this research is longer than the previous one which might cause cell viability reduction since the nutrition is falling.

The percentage of fibroblast cell viability in lowlands coconut water is higher than 50%, and counter to $CD_{50\%}$ parameter, the lowlands coconut water is effective to be used as storage

media for tooth avulsion. The highland coconut water, however, still can keep the BHK-21 fibroblast cell viability but less effective since its percentage is less than 50%.

The height difference of the habitat can influence coconut water nutrient. The bigger the substance concentration difference inside and outside the cell, the greater the diffusion speed becomes. [16] The glucose and mineral substance in highlands coconut water is lower than those in lowlands coconut water. The same case applies to the speed of glucose and mineral diffusion in the coconut water.

Glucose is used as cell energy source, such as molecule active transport through cell membrane, cell growth and development, as well as other physiological functions to maintain cell's survival. Mg^{2+} controls enzyme metabolic activity, such as glucose 6-phosphatase in glucose metabolism and succinate and dehydrogenase glutamate enzymes in mitochondrial respirasion.[20] Mg^{2+} also acts as catalyst on ATPase function so that it can keep the balance of cellular ionic. Furthermore, Mg^{2+} in cytosol has roles to limit the Ca^{2+}-uptake to endoplasmic reticulum and the Ca^{2+} release from organelle through IP_3.[21]

Ca^{2+} ion takes roles in fibroblast cell adhesion on periodontal tissue remodeling process. Intracellular Ca^{2+} signal is initiated by cells which are contacting to each other until they trigger cadherin and catenin interaction with cytoskeleton actin filaments. Those interactions cause fibroblast cells adhesion.[21] Also, Ca^{2+} ion in cytoplasm which related to camoldulincan affects the ion transport system to maintain Ca^{2+} in physiological concentration. It can maintain the membrane cell permeability so that it can control other ions from going in and out by activating certain enzymes.[22]

Na^{+}-K^{+}active transport pump function to maintain the Na^{+}-K^{+}ion concentration differences inside and outside the cell and to control the liquid electrolyte balance and the cell volume. Cl^{-} has the function of sustaining extracellular fluid osmotic pressure by diffusing through chloride channel in membrane. The difference of Cl^{-} concentration between extracellular and intracellularfluid is caused by potential difference on cell membrane surface.[22]

The difference of BHK-21 fibroblast cell viability is also influenced by the pH. The optimum pH for cell to live is 7,2-7,4, but they can still survive in the pH of 6.6-7.8. In this research, highlands coconut water's pH is 6.01.It is slightly more acidic compared to pH of lowlands coconut water which is 6.64. Acidic condition will increase the H^{+} concentration which can cause the cell metabolic disorder with the consequences of producing non-optimal energy and decreasing cell viability. Therefore, fibroblast cell viability in highlands coconut water is lower than those in lowlands.

Conclusions

1. The cells living of tooth avulsion should be held in reserve to 4 hours with the lowland coconut water.
2. The fibroblast cell viability of the lowland coconut water 7 times more highly than highlands coconut water of the same variety.

Declaration of Interest

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