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EFFECT OF VITAMIN E ON HUMAN PERIODONTAL LIGAMENT FIBROBLASTS

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INTRODUCTION

The periodontal ligament fibroblasts plays an essential role in the organization and maintenance of the connective tissue during development and in response to injuries and diseases. They are also responsible of the migration and differentiation of the variety of cells that takes part in the osteogenesis in response to external forces (1, 2). Age related changes include decreased fibroblasts density and cellular activity which slows orthodontic tooth movement due to prolonged response of the connective tissue to external forces which poses a potential risk in orthodontic treatment (3, 4).

Vitamin E had been studied worldwide due to its health benefits in the fields of chronic diseases and ageing such as the anti-inflammatory and anti-osteoporotic effects (5, 6). Vitamin E consists of 2 major isoforms: tocopherols and tocotrienols, each with four distinct analogues (alpha, beta, gamma, and delta). Tocopherols are saturated forms of vitamin E, and tocotrienols are the unsaturated forms, distinguishable by the three double bonds in the tails of tocotrienols (7). Studies showed that tocotrienol is superior for its antioxidant properties as well as increasing cells viability and proliferation (8, 9). The current project aim to evaluate the response of human periodontal ligament fibroblasts (HPdLF) upon exposure to various concentrations of tocotrienols rich fraction (TRF) conditioned medium.

MATERIALS AND METHOD

Human periodontal ligament fibroblasts cells (HPdLF, LONZA BASEL, SCHWEIZ) were cultured and supplemented with DMEM (10% FBS, 1% antibiotic-antimycotic).Tocotrienols rich fraction (TRF) was a gift from Sime Darby (Gold Tri E 50, SIME DARBY BIOGANIC, MALAYSIA). TRF contained α-tocopherol 161 mg/g, α-tocotrienol 170.4 mg/g, β-tocotrienol 24.9 mg/g, γ-tocotrienol 154.9 mg/g, δ-tocotrienol 71.8 mg/g. TRF was mixed with 100% ethanol to create a stock solution of 250 mg/ml. Serial dilution was created by mixing the TRF/Ethanol stock solution with DMEM (10% FBS, 1% antibiotic-antimycotic) to produce multiple concentrations of 0.031 mg/ml, 0.062 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml, 1mg/ml. Additional concentration of 0.75 mg/ml was created separately. The multiple concentrations of TRF were incubated at 37°C, 5%CO_2 overnight before administrating it to the cells. The

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HPdLF cells were seeded at 1 x 10^4 cells/cm^2 in four replicates of 96-well plates and incubated with 200 µl of TRF concentration at 37ºC, 5% CO_2. The medium was changed every 48 hours. HPdLF cells viability was assessed on days 1, 2, 4, and 7 using Alamar Blue assay. Alamar Blue (LIFE TECHNOLGIES, USA) 10% (v/v) was prepared in DMEM medium. HPdLF cells were incubated with 150 µl Alamar Blue for 2 hours then 100 µl of AB reaction was transferred into black 96 well plate. The fluorescence intensity was read at 544 nm excitation and emission at 590 nm emission using microplate reader.

Results

The response of HPdLF cells viability toward TRF had increased gradually from Days 1 to 4 followed by a decrease in viability on Day 7 for all concentrations (Figure.1). Lower TRF concentration with dose from 0.031 mg/ml had a favourabl effect on HPdLF viability while higher concentration with dose 1mg/ml is lethal (Figure.2). Cells showed poor response for TRF concentration with doses from 0.5 mg/ml and 0.75mg on days 1, 2, and 4 followed by complete death on day 7.

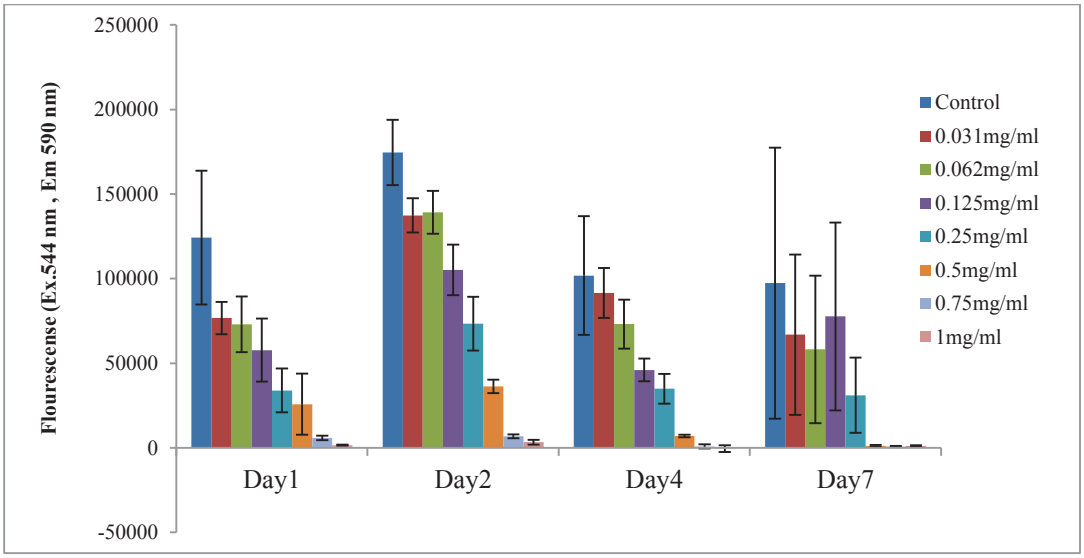

FIGURE 1: Effect of different concentrations of TRF on HPdLF cells viability on using Alamar Blue in days 1, 2, 4, and 7.

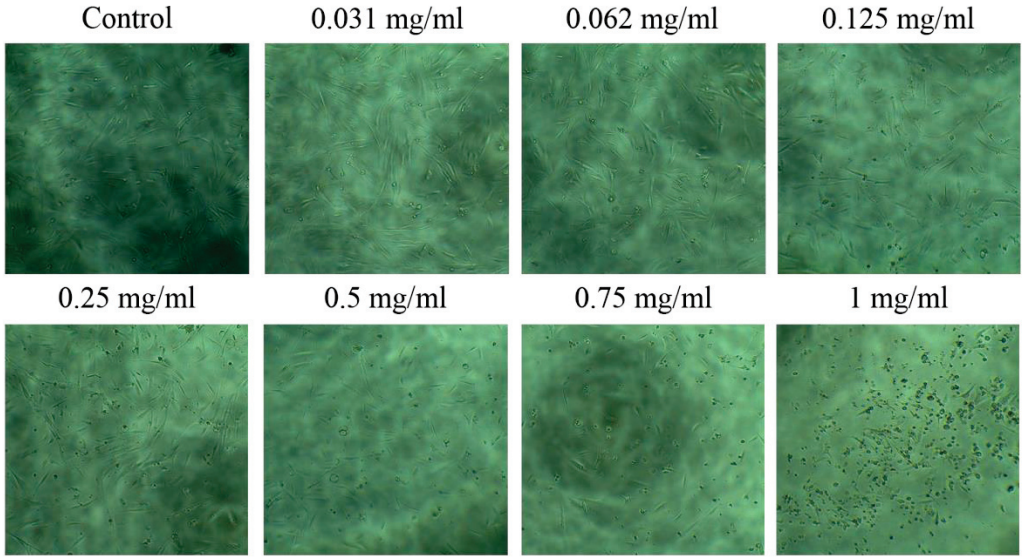

FIGURE 2: Effect of different concentrations of TRF on HPdLF cells viability on Day 2. Magnification x20 using inverted microscope (OLYMPUS, CK X 41, JAPAN).

Discussion

Successful orthodontic treatment relies essentially on the periodontal condition status and its response to external forces. The number of adult patients seeking improvement of dentofacial aesthetics and functionality is increasing (10). These patients may have altered periodontal condition attributed to aging (11). It is prudent to take the periodontal status in consideration and the complex response to orthodontic tooth movement which starts with the periodontal ligament fibroblasts (2). Little is known about its effect vitamin E on periodontal tissues but it started gaining some attention of orthodontists over the past few years (12, 13).

Vitamin consists of two isoforms: tocopherols and tocotrienols. α – tocopherol is the most active and abundant form of Vitamin E. But it was found that when used individually it does not improve cell growth (14) which is why TRF was the choice of this study. It is worthy to mention that (15) addressed the issue of proliferation using a combination of α- tocopherols and selenium on gingival and periodontal fibroblasts using an in vitro model that mimics periodontal wound-healing process. It was found that the α- tocopherols and selenium combination increased the rates of proliferation and viability compared when using of α-tocopherols individually.

To our knowledge there are no studies had addressed the effects of tocotrienols on periodontal ligament fibroblasts. The present study evaluated the effects of TRF on HPdLF cells viability. The data shows that HPdLF viability decreased with TRF treatment with high doses. We observed clear changes in cell morphology in response to high TRF dose. The cells spindle shape was lost and cells became rounded and black colored.

It appears the HPdLF are more sensitive to TRF and react differently to high TRF doses unlike other types of fibroblasts. One study showed an increase in viability on human skin fibroblasts in a dose dependent manner suggesting that TRF may protect skin fibroblasts against H_2O_2-induced oxidative stress (16). Another

study showed that TRF had good impact on senescent Human Diploid Fibroblasts (HDF) viability compared with young HDF cells either when the entire tocotrienol isoforms it administered to the cells or as separate analogue in which case γ-tocotrienol had the most effect on senescent cells (8). It is also found that tocotrienol increase myoblasts proliferation rate after 24 hours incubation at 37∘C and remained constant between the concentration of 50 µg /ml and 200 µg/ml (17).

In our current study all TRF concentrations used did not exceed the control which indicate that TRF may be mildly toxic to the cells in a dose dependent manner. However cells shows recovery in the following days. Naturally , further investigation with lower concentrations will be carried out in this ongoing study.

CONCLUSION

The findings indicated that the HPdLF response favourably to TRF concentrations below 0.5mg/ml. Lower TRF concentration appear to be the most effective in promoting gradual viability for HPdLF cells for a short period of time followed by gradual decrease upon prolonged exposure.

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