

RESEARCH ARTICLE

## ***In vitro* evaluation of coenzyme Q10 on primary fibroblast culture**

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### ABSTRACT

Chronic inflammation in periodontitis results in continuous production of Reactive Oxygen Species (ROS), so the levels are excessive, causing destruction of the gingiva, periodontal ligament, and alveolar bone through a variety of mechanisms including DNA damage and the formation of proinflammatory cytokine. One way to prevent periodontal tissue damage caused by excessive ROS formation is by administering antioxidants. Coenzyme Q10 is a powerful antioxidant that is beneficial for inhibiting free radicals to prevent the progression of periodontal tissue destruction and accelerate healing processes. The purpose of this study was to evaluate the fibroblast proliferation of the combination of Coenzyme Q10 and vegetable glycerin compared to PerioQ. Materials used were made of original Coenzyme Q10 dissolved in glycerin that was prepared in a ratio of 2:8 and 1:9, and Perio Q as the control. Each group consisted of six samples (n = 6). Primary fibroblasts were derived from healthy gingival tissue. Observations on day -1, -3, and -5 using MTT assay at a wavelength of 550 nm. Statistical analysis used a Two-Way ANOVA test followed by a Post Hoc test. The experiment showed the absorbance values were high in all the groups, the highest value was on day 3, namely Coenzyme Q10 at a concentration of 2:8, followed by Coenzyme Q10 at a concentration of 1:9, and PerioQ. The statistical tests showed significant differences in the 3 groups ( $p < 0.05$ ). It is concluded that Coenzyme Q10 in 1:9 and 2:8 concentrations were both as viable as Perio Q towards primary gingival fibroblast culture.

**Keywords:** coenzyme Q10; fibroblast proliferation; reactive oxygen species

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### INTRODUCTION

Further damage due to periodontal disease needs to be prevented. Conventionally, mechanical prevention by removal of local factors such as scaling, has been widely carried out. In some cases, mechanical debridement is not sufficient, some agent has to be added to achieve an optimum result. The use of topical agents, such as antibiotics, has its own risk, including bacterial resistance.<sup>1-2</sup> Another agent is introduced to increase the host ability to maintain its cellular activity and regulation towards free radicals, i.e., Coenzym Q10. Coenzyme Q10 is known as ubiquinone because of its presence in nature and its quinone structure is similar to that of vitamin K. Coenzyme Q10 functions as an intercellular antioxidant. This enzyme functions as an endogenous antioxidant and increasing its concentration in the inflamed gingiva will effectively suppress advanced periodontal inflammation.

The reduction in Coenzyme Q10 in the gingival tissue can occur either independently or due to periodontal disease.<sup>3</sup>

Free radicals and reactive oxygen species (ROS) are very important in normal biological processes. At low concentrations, free radicals will stimulate the growth of fibroblasts and epithelial cells in culture, but at higher concentrations they can cause tissue damage.<sup>4</sup> ROS reactivity can be prevented by antioxidants by donating an electron so it becomes a stable atom. If the antioxidant levels are insufficient, the periodontal tissue is unable to cope with oxidative stress, resulting in an increase in the severity of periodontal tissue damage.<sup>5</sup>

In an *in vivo* study on rats, application of Coenzyme Q10 ointment after tooth extraction increased collagen density and suppressed socket inflammation.<sup>6</sup> The topical application of PerioQ, which contains Coenzyme Q10 mixed with glycerine, has been introduced to treat patients

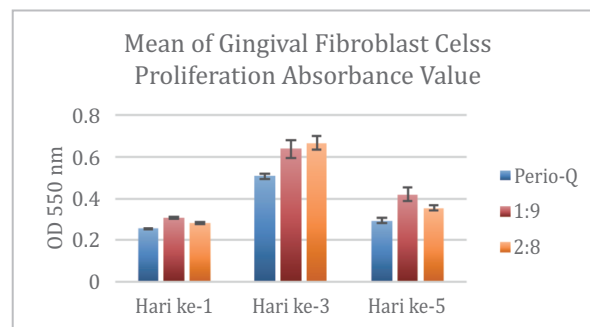
with gingivitis and mild to moderate periodontitis.<sup>7</sup> However, the product or regiments are not available in the research area. This research will investigate the effect of mixture of Coenzyme Q10 with different concentrations compared with PerioQ on fibroblast proliferation. The research hypothesis is Coenzyme Q10 in 1:9 and 2:8 concentrations increases fibroblast proliferation higher than PerioQ.

## MATERIALS AND METHODS

The research took place at the Integrated Research and Testing Laboratory (LPPT) Unit II Universitas Gadjah Mada, and has been ethically approved by the Ethic Committee of Faculty of Dentistry, Universitas Gadjah Mada (00554/KKEP/FKG-UGM/EC/2020). The test materials, namely Coenzyme Q10 1:9 and 2:8, were obtained by mixing the Coenzyme Q10 powder (Bioquinone, China) and vegetable glycerin solvent (Sigma Aldrich, USA) in the mentioned ratios. The gel should be used within 48 months from the date of manufacture and stored in a dry place, away from light and heat sources.<sup>7</sup>

Primary fibroblast culture was derived from healthy gingival tissue preparation which was derived after crown lengthening procedure. The patient with excess gingiva had short clinical crown. Excision was intended to reveal the ideal crown

length. The patient's consent for the culture of the gingival fibroblasts was requested. Tissue excised was rinsed using sterile PBS three times, then minced and moved into the solution of Dulbecco's Modified Eagle's Medium (DMEM), mixed with 10% FBS supplement, 2% penicillin-streptomycin, and 0.5% fungizone inside the culture plate. The culture plate was then placed into a 37 °C CO<sub>2</sub> incubator, examined daily and the medium was replaced as needed, two times in a week or once in three days. The culture was subcultured when the culture reached a 70-80% confluent state. The culture was separated from the medium using 0.25% EDTA-trypsin and harvested in the fourth passage. The harvested cells were moved into a 96-well microplate, in which each well contained 2.5 x 10<sup>3</sup> cells then incubated for 24 hours. MTT Assay was



**Figure 1.** Graph of the absorbance value of gingival fibroblast cell proliferation

**Table 1.** Mean and standard deviation of primary gingival fibroblast cell culture proliferation absorbance value

Group	n	Day-1	Day-3	Day-5
Perio-Q	6	0.255 ± 0.003	0.507 ± 0.012	0.294 ± 0.011
1:9	6	0.308 ± 0.005	0.638 ± 0.041	0.420 ± 0.031
2:8	6	0.282 ± 0.006	0.667 ± 0.032	0.354 ± 0.014

\*Read from a microplate reader using MTT assay a wavelength of 550 nm

**Table 2.** Two-Way ANOVA test of absorbance value of gingival fibroblast cell proliferation

Group	F	Significance
Material tested	111.316	0.000*
Observation Time	1069.932	0.000*
Material tested *Observation Time	18.728	0.000*

\*Significance p < 0.05

**Table 3.** Post hoc LSD

Time	Type of Application	D1			D3			D5		
		1	2	3	1	2	3	1	2	3
D1	1	-	0.000*	0.038*	0.000*	0.000*	0.000*	0.003*	0.000*	0.000*
	2		-	0.048*	0.000*	0.000*	0.000*	0.292	0.000*	0.001*
	3			-	0.000*	0.000*	0.000*	0.341	0.000*	0.000*
D3	1				-	0.000*	0.000*	0.000*	0.000*	0.000*
	2					-	0.023*	0.000*	0.000*	0.000*
	3						-	0.000*	0.000*	0.000*
D5	1							-	0.000*	0.000*
	2								-	0.000*
	3									-

\*Significance  $p < 0.05$

1= Perio Q

2= 1 : 9

3= 2 : 8

performed on days-1, 3, and 5 using the standard protocols. The data were evaluated by Two Way ANOVA followed by Least Significant Difference (LSD) Post Hoc Test using statistical software.

## RESULTS

The evaluation of Coenzyme Q10 on fibroblast culture proliferation is shown below (Table 1). Table 1 displays the mean of gingival fibroblast culture proliferation value (measured in absorbance unit). As shown in Table 1, the absorbance value observed in all the groups increased, on days 1, 3, and 5. The trends are shown in Figure 1. Figure 1 illustrates the mean of gingival fibroblast proliferation absorbance value.

As shown in Figure 1, the mean of absorbance values was high in all the groups, the highest value was on day-3, namely Coenzyme Q10 at a concentration of 2:8, followed by Coenzyme Q10 at a concentration of 1:9, and Perio-Q. The result of the Two-Way ANOVA Test is shown in Table 2.

As shown in Table 2, the Two-Way ANOVA Test showed a statistically significant result ( $p < 0.05$ ). The test was then followed by Least Significant Difference (LSD) Post Hoc test, shown in Table 3.

Table 3 shows that almost all the gingival cell proliferation was significantly different, except for; 1:9 concentration on day 1 with PerioQ on day 5 and 2:8 concentration on day 1 with PerioQ on day 5.

## DISCUSSION

The fibroblasts proliferated as early as the first day after the injury. The cell population was higher than the baseline, which continued to increase after day 3 and reached the peak on day 5.<sup>3,8,9</sup> The proliferation of fibroblasts can be observed using Methylthiazole Diphenyl-Tetrazolium Bromide (MTT) colorimetric assay. The MTT assay method is a reliable method for examining cell proliferation based on the reduction in tetrazolium salts.<sup>10</sup>

One of regenerative therapy goals is to pool the progenitor cells in the defect area, including fibroblasts. Fibroblasts have important roles in the healing and regenerative process of gingival tissue.<sup>8</sup> The effect of Coenzyme Q10 at a concentration of 1:9 and a concentration of 2:8 on the Optical Density of fibroblast proliferation was the same; this was related to its clinical use. Compared to Perio Q, the forms of Coenzyme Q10 1:9 and Coenzyme Q10 2:8 were superior which might be because of the source of Bioquinone

used. For external use, such as open wounds, a thick concentration is required (2:8), for closed wounds, for example, in periodontal pockets, it can be thinner (1:9).

Periodontal treatment needs to increase the resistance of the periodontal itself, so it not only eliminates the bacteria in infection but also acts as an antioxidant. Antibacterial and antioxidant are found in the body itself, namely Coenzyme Q10. During chronic infection, ROS are produced excessively, resulting in tissue damage. Coenzyme Q10 provides antioxidants so regeneration continues, namely by increasing the production of fibroblasts. The goal and target of Coenzyme Q10 are to release antioxidants and prevent further damage; an increased level of mitochondrial CoQ10 has been proven to effectively act as free radical eliminating agent.<sup>11,12</sup>

In patients with periodontal disease, there is an excessive level of Reactive Oxygen Species (ROS). Reactive Oxygen Species is a highly reactive oxidizing agent that functions to kill bacteria, but if chronic inflammation occurs, ROS will be produced continuously so the levels are excessive and cause destruction of gingival tissue, periodontal ligament, and alveolar bone by damaging DNA and stimulating the formation of proinflammatory cytokines.<sup>13</sup> The condition where ROS levels increase and antioxidant activity decreases is known as oxidative stress. Oxidative stress accelerates the formation of lesions in the periodontal tissue.<sup>14</sup> The extent to which the host response influences the pathogenesis of chronic periodontitis has led to the development of additional therapy methods to modify the host response, thereby increasing the effectiveness of SRP and curettage in patients with periodontitis.<sup>15</sup>

It was stated that ROS had an impact on the periodontal soft and hard tissues. Gingival epithelial cells exposed to ROS will experience lysis while collagen will experience fragmentation.<sup>16</sup> Coenzym Q10 is used to convert food into adenosine triphosphate (ATP) in mitochondria which serves as energy for the body's metabolism to accelerate tissue regeneration or healing.<sup>17</sup>

Reactive Oxygen Species reactivity can be prevented by antioxidants by giving an electron, so it becomes a stable atom. If the antioxidant levels are insufficient, the periodontal tissue is unable to cope with oxidative stress, resulting in an increased severity of periodontal tissue destruction. Coenzym Q10 (CoQ10) is a natural antioxidant in the human body which has the same structure as vitamin K. Coenzyme Q10 is an important vitamin-like substance that is found in every cell of the body and plays a role in energy production. In addition, CoQ10 plays a role in oxidation-reduction recycling. The reduced form is ready to give electrons to neutralize oxidants or block free radicals, thereby preventing cell damage; this shows a strong antioxidant activity.<sup>17</sup> The periodontal indices, including plaque index, pocket depth, gingival index, and clinical attachment loss, decreased in patients treated by topical intrapocket application of CoQ10.<sup>18</sup>

Protein carbonyl (PC) is one of the biomarkers of oxidative stress in gingival sulcus fluid (CSG), which is the end result of oxidation of ROS which can reflect cellular damage induced by ROS. In healthy conditions, there are low PC levels. Increased PC levels can be used as a sign of increased oxidative stress and are also correlated with the development and severity of a disease.<sup>19</sup> Increased PC levels in gingival crevicular fluid indicate increased damage and severity of periodontal disease.<sup>20</sup>

The healing and repair of periodontal tissue require sufficient energy production, which partly depends on an adequate supply of CoQ10.<sup>21</sup> In addition, CoQ10 increases phagocytic activity of macrophages, granulocyte proliferation in fighting infection, CD4/CD8 ratio, IgG levels, and oxygen in gingival cells.<sup>22</sup> The research continued on the application of Coenzyme Q10 in vivo in experimental animal models with periodontitis to observe the proliferation of periodontal tissue osteoblasts. The limitations of the study is looking for a mixture of CoQ10 and glycerin as a comparison, before finally a concentration of 2:8 was obtained. It is necessary to conduct future research on hard tissues as represented by osteoblasts.

**CONCLUSION**

Coenzyme Q10 in 1:9 and 2:8 concentrations and Perio Q increased fibroblast proliferation.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest with the data contained in the manuscript.

**REFERENCES**

- Mahuli SA, Zorair AM, Jafer MA, Sultan A, Sarode G, Baeshen HA, Raj AT, Sarode S, Patil S. Antibiotics for periodontal infections: biological and clinical perspectives. *J Contemp Dent Pract.* 2020; 21(4): 372–376.
- Johnson DK, Perez M. Local delivery of chemotherapeutic agents in periodontal therapy. Naval Postgraduate Dental School, National Naval Dental Center, Bethesda, Maryland. 2010; 15-17.
- Sale ST, Parvez H, Yeltiwar RKR, Vivekanandan G, Pundir AJ, Jain P. A comparative evaluation of topical and intrasulcular application of coenzyme Q10 (Perio Q™) gel in chronic periodontitis patients: A clinical study. *J Indian Soc Periodontol.* 2018; 18(4): 461-465. doi: 10.4103/0972-124X.138690
- Dahiya P, Kamal, R, Gupta R, Bhardwaj R, Chaudhary K, Kaur S. Reactive oxygen species in periodontitis. *J Indian Soc Periodontol.* 2013; 17(4): 411-416. doi: 10.4103/0972-124X.118306
- Liu C, Mo L, Niu Y, Li X, Zhou X, Xu X. The role of reactive oxygen species and autophagy in periodontitis and their potential linkage. *Front Physiol.* 2017; 8: 439. doi: 10.3389/fphys.2017.00439
- Yoneda T, Tomofuji T, Kwabata Y, Ekuni D, Azuma T, Kataoka K, Kunitomo M, Morita M. Application of Coenzyme Q10 for accelerating soft tissue wound healing after tooth extraction in Rats. *Nutrients.* 2014; 6(12): 5756-5769. doi: 10.3390/nu6125756
- Pitale U, Khetarpal S, Peter K, Pal V, Verma E, Gupta P. Evaluation of efficacy of coenzyme Q<sub>10</sub> in Management of gingivitis and slight periodontitis-a clinical study. *Int J Curr Pharm.* 2012; 4(4): 33-38.
- Smith PC, Martinez C, Martinez J, McCulloch CA. Role of fibroblasts populations in periodontal wound healing and tissue remodeling. *Front Physiol.* 2019; 10: 270. doi: 10.3389/fphys.2019.00270
- Miron RJ, Fujioka-Kobayashi M, Hernandez M, Kandalam U, Zhang Y, Ghanaati S, Choukroun J. Injectable Platelet-Rich Fibrin (i-PRF): Opportunities in Regenerative Dentistry?. *Clin Oral Investig.* 2017; 21(8): 2619-2627. doi: 10.1007/s00784-017-2063-9
- Gamal AY, Al-Berry NN, Hassan AA, Rashed LA, Lacono VJ. In vitro evaluation of the human gingival fibroblast/gingival mesenchymal stem cell dynamics through perforated guided tissue membranes: cell migration, proliferation and membrane stiffness assay. *Journal of Periodontal Research.* 2017; 52(3): 628-635. doi: 10.1111/jre.12431
- Rathod S, Khan F, Kolte A. Coenzyme Q10 gel in periodontal diseases - a wonderful remedy. *International Journal of Pharmaceutical Science Invention.* 2013; 2 (6): 47-50.
- Marchegiani F, Kordes S, Cirilli I, Orlando P, Silvestri S, Vogelsang A, Möller N, Blatt T, Weise J, Damiani E, Tiano L. Coenzyme Q10 as an anti-ageing agent in human dermal fibroblast. Comparison of bioavailability and efficacy of the reduced and oxidized form. *Free Radical Biology and Medicine.* 2021; 177: S113. doi: 10.1016/j.freeradbiomed.2021.08.175
- Kanzaki H, Wada S, Narimiya T, Yamaguchi Y, Katsumata Y, Itohiya K, Fukaya S, Miyamoto Y, Nakamura Y. Pathways that Regulate ROS scavenging enzymes, and their role in defense against tissue destruction

- in periodontitis. *Front Physiol.* 2017; 8: 351. doi: 10.3389/fphys.2017.00351
14. Talmaç AC, Çalışır M. Antioxidants and periodontal diseases. In: Ostwani AEOA, editor. *gingival disease - A Professional Approach for Treatment and Prevention.* London: Intech Open; 2019 [cited 2022 Dec 29]. Available from: <https://www.intechopen.com/chapters/66172>
  15. Pendyala G, Thomas B, Kumari S. The challenge of antioxidants to free radicals in periodontitis. *J Indian Soc Periodontol.* 2008; 12(3): 79-83. doi: 10.4103/0972-124X.44100
  16. Bhusari BM, Ridhima M, Shubhangi R, Pooja S. Reactive oxygen species and its role in periodontal disease. *IOSR Journal of Dental and Medical Sciences.* 2014; 13(8): 52-59.
  17. Al-Jureisy IT, Al-Wattar WT, Taqa GA. The topical effect of coenzyme q10 on wound healing in mice. *Al-Rafidain Dent J.* 2019; 19(1): 42- 51. doi: 10.33899/rden.2018.163600
  18. Attia AM, Edrees MF, Alghriany A. Clinical and immunological evaluation of coenzyme Q10 as an adjunct to nonsurgical periodontal therapy in chronic periodontitis patients. *J Periodontal Med Clin Prac.* 2016; 03:128-140.
  19. Baltacıoğlu W, Akalın FA, Alver A, Değer O, Karabulut E. Protein carbonyl levels in serum and gingival crevicular fluid in patients with chronic periodontitis. *Arch Oral Biol.* 2008; 53(8): 716-722. doi: 10.1016/j.archoralbio.2008.02.002
  20. Pradeep AR, Ramchandraprasad MV, Bajaj P, Rao NS, Agarwal E. Protein carbonyl: an oxidative stress marker in gingival crevicular fluid in healthy, gingivitis, and chronic periodontitis subjects. *Contemp Clin Dent.* 2013; 4(1): 27-31. doi: 10.4103/0976-237X.111589
  21. Saini R. A Clinical and microbiological study to evaluate the effect of dietary supplement of coenzyme Q10 in nonsurgical treatment outcome of chronic periodontitis patients after phase 1 periodontal therapy. *Eur J Gen Dent.* 2014; 3(3): 194-198.
  22. Darweesh M, El-Sharkawy H, Fadhil ON, Maria OM. Doxycycline and coenzyme Q10 treatment modalities for chronic periodontitis: a preliminary study. *Int J Oral Dent Health.* 2015; 1: 014.