Retraction: *Morinda citrifolia* leaves enhance osteogenic differentiation and mineralization of human periodontal ligament cells

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doi:10.4012/dmj.2012-053   JOI JST.JSTAGE/dmj/2012-053

This article has been retracted by the Editorial Board of *Dental Materials Journal* due to violation of our publishing policies and procedures as of December 1, 2013.
**Morinda citrifolia** leaves enhance osteogenic differentiation and mineralization of human periodontal ligament cells

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This present study investigated the potential of *Morinda citrifolia* leaf aqueous extract to induce osteogenic differentiation and matrix mineralization of human periodontal ligament (hPDL) cells. Human periodontal ligament cells were cultured in complete medium, ascorbic acid with β-glycerophosphate, or *Morinda citrifolia* leaf aqueous extract. *Morinda citrifolia* leaf aqueous extract significantly increased alkaline phosphatase activity compared to culturing in complete medium or ascorbic acid with β-glycerophosphate. Matrix-containing mineralized nodules were formed when the cells were cultured in the presence of *Morinda citrifolia* leaf aqueous extract. These nodules showed positive alizarin red S staining and were rich in calcium and phosphorus according to energy dispersive X-ray analysis. In conclusion, *Morinda citrifolia* leaf extract promoted osteogenic differentiation and matrix mineralization in human periodontal ligament cells, a clear indication of the therapeutic potential of *Morinda citrifolia* leaves in bone and periodontal tissue regeneration.

**Keywords:** *Morinda citrifolia*, Osteogenic differentiation, Mineralization, Periodontal ligament cells

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**INTRODUCTION**

Periodontitis is a chronic infectious disease of the periodontium affecting children, adolescents, adults, and the elderly. It is characterized by irreversible loss of connective tissue attachment and supporting alveolar bone1. Restoration of lost periodontal tissues requires a combination of cells, signaling molecules such as osteoinductive growth factors, and osteoconductive scaffolds. Cells provide the machinery for new tissue growth and differentiation. Growth factors or morphogens modulate cellular activities, and provide stimuli to the cells to differentiate and create extracellular matrix for the developing tissue. Three-dimensional scaffolds act as templates to guide and facilitate the above processes which are critical for tissue regeneration2.

The periodontal ligament (PDL) is an unmineralized connective tissue located between the cementum and alveolar bone. Its functions are to sustain the teeth within the jaw and facilitate movement between tooth and bone3. PDL cells play an integral role in the maintenance, repair, and regeneration of periodontal tissue. These PDL cells are multipotent cells composed of heterogeneous cell populations —including fibroblasts which are the principal cells of PDL — that can differentiate into either cementum-forming cells (cementoblasts) or bone-forming cells (osteoblasts)4-6. PDL have many osteoblast-like properties. For example, they produce type I collagen and non-collagenous extracellular matrix proteins such as osteopontin and bone sialoprotein in vitro7,8. As an indication of their ability to promote mineralization, PDL cells produced the alkaline phosphatase (ALP) enzyme in response to stimulation by cytokines and growth factors such as parathyroid hormone, insulin-like growth factor, bone morphogenic proteins, and 1,25-dihydroxyvitamin D38,9-12. Bone-like and/or cementum-like mineralized nodules were formed when cultured in a medium containing ascorbic acid and dexamethasone13,14.

Currently, diverse osteoconductive and osteoinductive bone graft substitutes —in place of autografts—are commercially available to promote cementum, PDL, and alveolar bone regeneration in periodontal therapy15,16. Nonetheless, growth factors are natural agents most capable of promoting PDL cell proliferation and migration17,18. Therefore, many osteoinductive materials have been extensively evaluated in search of one that could combine low cost with the ability to induce osteogenic differentiation and mineralization for periodontal tissue regeneration.

*Morinda citrifolia*, a small evergreen tree, is one of the traditional folk medicinal plants also known as noni or Indian mulberry. A number of major components essential for our health have been identified in the noni plant: namely, scopoletin, octoanoic acid, potassium, vitamin C, terpenoids, alkaloids, anthraquinones, β-sitosterol, carotene, vitamin A, flavone glycosides,
linoleic acid, amino acids, calcium, and phosphorus. The noni plant was also reported to have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, antihelmin, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects.

The primary indigenous use of the noni plant is to apply the leaves as a traditional topical treatment to promote wound healing. Recently, Nayak et al. used an animal model to demonstrate that noni leaf extract had therapeutic benefits in wound healing. On bone healing, the crude extract of noni leaf has been traditionally used in patients with bone fractures or dislocation to promote connective tissue repair and reduce inflammation.

Bone and periodontal tissue repair or regeneration requires growth factors or morphogens to induce precursor cells to differentiate and produce mineralized nodules. For noni leaf extracts, there was no experimental evidence to support its ability to induce osteogenic differentiation and mineralization. The aim of this study, therefore, was to investigate the potential osteoinductive effect of noni leaf extract on human PDL cells through in vitro alkaline phosphatase activity and matrix mineralization assays.

MATERIALS AND METHODS

Moringa citrifolia leaf extraction
Fresh noni leaves were collected from Nakhon Pathom province during the rainy season (July to October). After washing with sterilized deionized water, they were blended with cold sterilized deionized water in an electrical blender until finely chopped. The herbal mixture was processed using the cold infusion method by incubation at 4°C for 48 h. The infusion was strained and squeezed through a fine muslin cloth, and then centrifuged at 3,000 rpm for 10 min. Supernatant was filtered through a 0.22-µm cellulose acetate membrane filter. The crude extract of noni leaf was freeze-dried with a lyophilizer and stored at −80°C. Freeze-dried noni leaf extract was dissolved in phosphate-buffered saline (PBS). It was then minced into small pieces and placed in a tissue culture flask (Nunclon, Roskilde, Denmark). The explants were cultured in DMEM with 10% fetal bovine serum in an environment of 5% CO2 and 95% humidified air at 37°C. Culture medium was changed after 1 week and then every 2–3 days until cells grew out of the explants and reached confluence. Human PDL (hPDL) cells from passages 3 to 5 were used in this study.

Mouse calvaria-derived pre-osteoblastic cell line, MC3T3-E1 (a gift from Associate Professor R. Surarit, Department of Physiology and Biochemistry, Faculty of Dentistry, Mahidol University), was cultured under the same conditions as those for hPDL cells.

Cell proliferation assay
To determine the optimal concentration of noni leaf extract which could promote cell growth, the oxidative activity of mitochondria was measured by MTT assay (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide). hPDL cells were seeded at a density of 2×104 cells/well in a 96-well plate (Nunclon, Roskilde, Denmark). After 24-h incubation, confluent cells were treated with varied concentrations (namely, 0.025, 0.625, 1.25, 2.5, and 5%) of noni leaf extract diluted in growth medium, at 37°C for 24 h. Cells were washed with PBS and then incubated with 0.05% MTT (Sigma Co., St. Louis, MO, USA) for 4 h at 37°C protected from light.

At the end of incubation period, untransformed MTT was removed by aspiration and 100 µL of DMSO (Dimethylsulfoxide, Merck, Darmstradt, Germany) was added to each well. The plate was shaken vigorously to ensure complete solubilization of blue formazan. Optical density of each well was measured at 540 nm wavelength using an automatic multiwell microplate spectrophotometer (Maxline Microplate Reader, Molecular Device Corp, Menlo Park, CA, USA). Optical density of formazan produced from MTT was directly proportional to the number of living (viable) cells. Cell proliferation rate of each treated group compared to the control after 24-h noni treatment was calculated using the following formula:

Cell proliferation rate = [OD of treated cells/OD of control cells]×100%

ALP activity assay
hPDL cells, at a density of 2×105 cells/well, were seeded in a 24-well plate (Nunclon, Roskilde, Denmark). After 24 h, the cells were incubated in one of the following media up to 6 weeks: (a) complete medium; (b) complete medium supplemented with 50 µg/mL of ascorbic acid and 2 mM β-GP; or (c) medium containing the optimal concentration of noni leaf extract which did not affect the growth of hPDL cells after 24-h treatment. At the end of each week, incubated cells of all the three medium groups were washed with PBS three times, collected by scraping with a rubber spatula, and centrifuged at 1200 rpm for 4 min. Precipitate was mixed with 200 µL of a cold lysis buffer (containing NaCl, EDTA and Triton X-100 in Tris-HCl, pH 7.5) and allowed to lyse at 4°C.
ALP activity was assayed using a modified Lowry method to detect cell differentiation. 20-µL suspension of each sample was added to 100 µL of 125 mM carbonate-bicarbonate buffer (pH 10.3) at 37°C for 5 min, then mixed with 100 µL of a substrate solution that contained 2 mM MgCl₂ and 2 mM p-nitrophenyl phosphate (Sigma Co., USA). After 15-min incubation at 37°C, reaction was terminated by adding 100 µL of 0.2 M NaOH. Liberated p-nitrophenol was measured at 405 nm wavelength. ALP activities were normalized to cellular protein levels and expressed in nmol/mg protein/min. Total amount of protein in lysed cells was determined using a Bradford Protein Assay kit (Sigma Co., USA), by adding 5 µL of each suspension sample to 250 µL of dye reagent in a 96-well plate. Bovine serum albumin was used as the standard protein. Optical density was measured at 595 nm wavelength.

Mineralized nodule staining
Formation of calcified nodules was monitored at the end of each week by visualization with alizarin red S staining. After 6 weeks of treatment with the optimal concentration of noni leaf extract, cells were washed three times with PBS prior to fixation with 70% ethanol for 30 min and rehydration with 1 mL of distilled water for 5 min. Cells were stained with 1% alizarin red S solution for 30 min and then rinsed twice with distilled water. Calcified nodules that appeared bright red in color were photographed under an inverted fluorescence microscope (Nikon Coolpix 4500, Japan). Mineralized nodule staining was conducted to rank group means when significant differences were found among the factors. All data were analyzed using SPSS for Windows (release 10) (SPSS Inc., Chicago, IL, USA). Throughout the experiment, statistical differences were declared to be significant at p<0.05.

RESULTS

Effect of noni leaf extract on hPDL cell proliferation
Cultured hPDL cells exhibited a spindle or polygonal morphology regardless of noni leaf extract concentration. To determine the effect of noni leaf extract on hPDL cell viability, as well as the optimal concentration of noni leaf extract for future experiments, a dose-response experiment employing MTT assay for cell proliferation was performed. To establish the range of noni leaf extract concentrations for investigation in the MTT assay in this study, a 24-h preliminary experiment was conducted using 0.01–10% of noni concentration on PDL cells. There was no impact on hPDL cells when noni leaf extract concentration was less than 0.025%. However, cells were completely dead at 7.5% noni concentration (data not shown).

To determine the optimal concentration of noni leaf extract that had no toxic effect on hPDL cells, an MTT assay was carried out on viable hPDL cells treated with varied concentrations (0.025–5% w/v) of noni leaf extract for 24 h. All noni concentrations, except 5%, promoted cell proliferation in the range of 116.8±13.0% to 147.0±15.2% (Fig. 1). It was apparent that 2.5% (w/v) of noni leaf extract was the highest concentration in this range which promoted hPDL cell proliferation. For this reason, 2.5% of noni leaf extract was used as the optimal dose for subsequent experiments.

Effect of noni leaf extract on ALP activity in hPDL cells
Bradford Protein Assay showed that all the three incubation media induced cellular protein synthesis. hPDL cells incubated with noni leaf extract produced statistically higher levels of protein than the other two incubation media, especially during weeks 2–5 (data not shown).

ALP activity of untreated hPDL cells slowly increased from 5 to 8 nmol/mg protein/min after 6 weeks of culture in complete medium. hPDL cells treated with 2.5% of noni leaf extract exhibited 2-fold increase in ALP activity at week 2 and reached the maximum level (3–4 fold increase) in weeks 3–4, then gradually decreased to a level similar to that of untreated group in week 6 (Fig. 2). When cultured in ascorbic acid with β-GP, ALP activity increased by approximately 2-fold in weeks 3–4, then gradually decreased to a level similar to that of untreated group in week 6 (Fig. 2). When cultured in ascorbic acid with β-GP, ALP activity increased by approximately 2-fold in weeks 3–4, then gradually decreased to a level similar to that of untreated group in week 6 (Fig. 2).
Dose-response effect of noni leaf extract (0.025%, 0.625%, 1.25%, 2.5%, and 5%) on hPDL cell proliferation, measured for 24 h by MTT assay. All concentrations, except 5%, significantly enhanced cell proliferation (*: *p* < 0.05) when compared to the control group (0%). Values are displayed as percentage of mean±SD from three independent experiments (n=4).

Effect of noni leaf extract on mineralized matrix formation in hPDL cells
To assess the efficacy of noni leaf extract in mediating osteoblastic differentiation and mineralization, culturing was extended up to a period of 6 weeks. There was no nodule formation for hPDL cells cultured in complete medium. For hPDL cells treated with 2.5% of noni leaf extract, cell clusters and nodules were observed after 3 weeks (Fig. 3a). These nodules gradually increased in size (Figs. 3b, c) and showed positive alizarin red S staining for calcium deposition (Figs 4b, c). In contrast, no matrix mineralization was observed when hPDL cells were cultured in complete medium alone (Fig. 4a). Similarly, hPDL cells cultured in ascorbic acid with β-GP revealed only cell aggregation with light alizarin red S staining after 6 weeks (Fig. 4d).

Effect of noni leaf extract on mineralized matrix formation in MC3T3-E1 preosteoblast cells
To further examine whether noni leaf extract would have similar effect on nodule formation in other osteogenic precursor cell types, MC3T3-E1 mouse preosteoblast cells were treated under the same conditions as those for hPDL cells.

After 3 weeks, no nodule formation was detected when cells were cultured in complete medium alone (Fig. 5a). In contrast, more prominent nodule formation was consistently found in the presence of noni leaf extract with bigger nodules (Fig. 5c) than when cultured in ascorbic acid with β-GP (Fig. 5b). After 6 weeks, there was still no nodule formation for cells cultured in complete medium (Fig. 5d). In contrast, the nodules increased in size and showed positive alizarin red S staining for calcium deposition for cells cultured in the presence of noni leaf extract (Fig. 5f) and in ascorbic acid with β-GP (Fig. 5e).

Effect of noni leaf extract on mineralized matrix formation in primary human bone cells
To determine the efficacy of noni treatment in matrix mineralization for future clinical applications, we carried out an additional experiment using primary...
Fig. 4  Phase contrast micrographs showing: (a) a monolayer of hPDL cells with a spindle-like morphology after 7-day culture in complete medium; (b, c) matrix mineralization in hPDL cells after 6-week treatment with noni leaf extract; and (d) cell aggregation with light alizarin red S staining after 6-week culture in ascorbic acid with β-GP.

Fig. 5  Phase contrast micrographs showing the formation of multilayered cell clusters of MC3T3-E1 cells and nodules after 3-week culture in: (a) complete medium; (b) ascorbic acid with β-GP; and (c) noni leaf extract. Nodule mineralization as demonstrated by alizarin red S staining after 6-week culture of MC3T3-E1 cells in: (d) complete medium; (e) ascorbic acid with β-GP; and (f) noni leaf extract.
human bone cells. Under the same conditions as those for hPDL cells, the consistent trend of more prominent nodule formation in the noni-treated group was observed after 3 weeks. After 5 weeks, positive alizarin red S staining showed the presence of calcium deposits within the nodules (data not shown).

Calcium phosphate is formed during matrix mineralization. For this reason, phosphate deposition within the nodules was visualized using Sirius red and von Kossa double staining technique after 5 weeks of incubation. Collagen is abundantly produced during matrix maturation. For this reason, Mallory’s collagen stain was used to visualize collagen deposition within the nodules after 5 weeks of incubation.

Primary human bone cells treated with noni leaf extract produced abundant nodules with positive staining

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Fig. 6  Phase contrast micrographs showing nodule formation as demonstrated by Sirius red and von Kossa double staining after 5-week culture of primary human bone cells in: (a) complete medium; (b) ascorbic acid with β-GP; and (c) noni leaf extract.

Collagen deposition as demonstrated by Mallory’s collagen stain after 5-week culture of primary human bone cells in: (d) complete medium; (e) ascorbic acid with β-GP; and (f) noni leaf extract.

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Fig. 7  Scanning electron micrographs showing the distributions of (a) calcium and (b) phosphorus after 6-week treatment with noni leaf extract, as well as the presence of matrix-containing mineralized nodules.
results for both phosphate and collagen deposition (Figs. 6c, f). In contrast, incubation in ascorbic acid with β-GP (Figs. 6b, e) and in complete medium (Figs. 6a, d) revealed markedly less nodule formation, with correspondingly less phosphate and collagen deposition.

Production of mineralized nodules by noni-treated hPDL cells according to EDX-SEM

EDX was applied in conjunction with SEM to demonstrate the distributions of calcium and phosphorus in noni-treated hPDL cells during mineralization. SEM micrographs showed that calcium (Fig. 7a) and phosphorus (Fig. 7b) were densely distributed within the nodules after 6 weeks of treatment with noni leaf extract. Nodules containing mineralized matrices which were produced by hPDL cells were also revealed by SEM (Fig. 7c).

DISCUSSION

Bone and periodontal tissue regeneration is a process that depends upon a sequence of biological events including cell proliferation, osteogenic differentiation, attachment to extracellular matrix components, organic matrix formation, and matrix mineralization. The ability to grow and manipulate stem cells within the periodontal ligament is of considerable clinical significance, especially for developing novel mechanisms to achieve bone and periodontal tissue regeneration.

Previous findings have indicated that hPDL cells possess several osteoblast-like properties, including the ability to form mineralized nodules in vitro when cultured in the presence of growth factors such as enamel matrix derivative (EMD) and transforming growth factor-beta1 (TGF-β1). However, not only is EMD costly, clinical and cell culture studies have yielded controversial and conflicting results. Therefore, in this study, we sought to determine whether noni, a natural herb that has been widely and safely used in tropical folk medicine could provide additional scientific advantages for cellular events associated with bone and periodontal tissue regeneration. Noni leaves were an indigenous Polynesian medicine used for traditional bone injury treatment or applied as a topical treatment for wound healing. Scientific reports have emerged on the usefulness of noni leaves in promoting soft tissue wound healing and mitigating UVB-induced injuries to the skin. However, there was a lack of scientific evidence to support the application of noni leaves to bone or periodontal tissue repair and regeneration.

In a study by Nayak et al., which evaluated the wound-healing activity of noni leaf extract using a rat model, more fibroblasts were found in the granulation tissue obtained from rats treated with the leaf extract as opposed to more inflammatory cells found in the control rats. In the present study, treatment with noni leaf aqueous extract enhanced hPDL cell proliferation by almost 1.5-fold when compared to the complete medium. Therefore, noni leaf extract not only promoted fibroblast cell proliferation, but also proliferation of hPDL cells.

Our preliminary data showed that both noni leaf extract and noni fruit aqueous extract (data not shown) were capable of enhancing ALP activity in rat dental pulp cells (RPC-C2A donated from Prof. S. Kasugai), MC3T3-E1 cells, and human bone cells in vitro. In addition, noni leaf extract strongly increased matrix mineralization in MC3T3-E1 preosteoblasts. While ALP activity is a definitive early marker of osteoblast differentiation and activity in bone formation, mineralized nodule formation is a marker of the late stage of osteoblast differentiation. Therefore, noni leaf extract was chosen over noni fruit aqueous extract to be the focus of investigation in this study for two reasons: it was capable of promoting differentiation and mineralization of both PDL and preosteoblast cell types as well as stimulating osteoblast differentiation and maturation.

Another parameter to be determined during our preliminary study was the optimal concentration of noni leaf extract for hPDL cells. MTT assay, a standard method to determine cell viability or cell proliferation, was used to establish the range of noni leaf extract concentrations to be used in this study. In a 24-h preliminary experiment, hPDL cells were treated with noni concentrations ranging from 0.01% to 10% g/mL and then analyzed by MTT assay. There was no alteration in hPDL cell function or viability for noni concentration less than 0.025%, but cells were completely dead at 7.5% and 10% (data not shown). In the present study, noni extract at 5% appeared to be toxic to hPDL cells (Fig. 1). In contrast, other concentrations were biocompatible to hPDL cells and significantly promoted cell proliferation. Therefore, the noni leaf extract concentration used in this study ranged between 0.025% to 2.5%, a biologically safe concentration range as determined by MTT assay.

The non-toxic concentration of noni leaf aqueous extract at 2.5% not only stimulated total protein synthesis, but also ALP activity. This enzymatic activity increased 3–4 fold at weeks 3–4 before it gradually decreased. ALP activity pattern correlated well with nodule formation in that the latter was observed after 3 weeks of culture — the week at which ALP activity peaked when treated with 2.5% of noni leaf extract. Bellows et al. also demonstrated that ALP played a crucial role in the differentiation and mineralization of cells with osteoblastic or cementoblastic phenotypes. And not only in hPDL cells, Aronow et al. also observed this pattern of ALP expression in other mammalian osteoblast cultures during the various stages of osteoblast differentiation to maturation in vitro. Taken together, noni leaf extract was efficacious in inducing cell proliferation, ALP activity, and mineralized nodule formation of hPDL cells in vitro.

When treated with ascorbic acid and β-GP, the ALP activity of hPDL cells was 2-fold greater than that of the control group at weeks 2–3, and then decreased to a level similar to that of the control. When compared to the ALP activity of hPDL cells treated with noni leaf extract, that of ascorbic acid and β-GP was significantly lower from...
week 3 to week 6. There was also an absence of nodule formation and matrix mineralization: only aggregated cells with light alizarin red S staining was observed for hPDL cells treated with ascorbic acid and β-GP. These results well agreed with previous studies which suggested that nodule formation of hPDL cells mostly occurred in certain condition such as in the presence of dexamethasone, estradiol, or EMD with TGF-β1,2,3,26,30.

To confirm the potency of noni leaf extract in inducing osteogenic differentiation and matrix mineralization, a mouse preosteoblast cell line (MC3T3-1) was treated with the optimal concentration of 2.5% of noni leaf extract. Nodule formation was observed at week 3, which was also observed when the cells were treated with ascorbic acid and β-GP. However, in the control complete medium, nodule formation was absent throughout the 6-week experimental period. These results well agreed with previous studies which reported that ascorbic acid with β-GP was sufficient for the induction of MC3T3-E1 differentiation and mineralization.34,35 These data also indicated that noni leaf extract is a promising osteoinductive material for preosteoblasts and hPDL cells to stimulate differentiation and mineralized nodule formation.

An additional experiment using primary human bone cells was carried out to assess the applicability of noni leaf extract to the clinical setting. Like the results obtained for hPDL and MC3T3-E1 cells, calcium deposits were present within the nodules formed by primary human bone cells after treatment with noni.

Although the expressions of osteogenic marker genes usually confirm osteogenic differentiation, it is not necessary that up-regulation of these genes is a definitive indication of complete osteogenic differentiation. A previous study by Kawase et al.36 showed that platelet-rich plasma (PRP) increased the ALP activity of hPDL cells and the expression of osteogenic markers. However, PRP did not induce full differentiation into bone cells, and thus a clear indication of incomplete mineralization.

Instead, mineralized nodule formation is an important hallmark of complete osteogenic differentiation. Collagen is the most abundant protein in extracellular matrix accounting for matrix maturation. Therefore, in this study, mineralized nodule formation assay using Mallory’s collagen stain was carried out to assess the collagen content of nodules formed by primary human bone cells. Phosphorus deposition also occurs during matrix maturation, which was visualized using Sirius red and von Kossa double staining in this study. Positive staining results confirmed the presence of both collagen and phosphorus within nodule formation (Fig. 6), and thus a clear indication of matrix mineralization. These results obtained with primary human bone cells were consistent with those obtained for hPDL cells when they were treated with noni leaf extract.

It has been widely reported that ascorbic acid can promote interaction of type I collagen with α2β1 integrin interaction, resulting in increased ALP activity.37 In the present study, hPDL cells exhibited only a low level of ALP activity when cultured in complete medium which was devoid of ascorbic acid. Therefore, adding ascorbic acid to noni treatment might further enhance the ALP activity of hPDL cells via the pathway of ascorbic acid-induced collagen production and integrin expression. This could be followed by the accumulation of noni-induced calcium and phosphorus deposits on collagen fibrils to form a mineralized matrix.

Phytochemical constituents of noni leaves might play a role in the induction of osteogenic differentiation and matrix mineralization. Components of noni leaves which were reported to have a stimulatory effect on bone formation included vitamin C, triterpenes, and flavonoid23,26. Triterpenes reportedly stimulated proliferation, protein synthesis, and ALP activity through TGF-β1. Flavonoids were shown to promote the osteogenic differentiation of human bone marrow-derived mesenchymal stem cells. Therefore, the components of noni leaves warrant more in-depth analysis in future studies to identify and confirm the active ingredients which contribute to the osteogenic process.

CONCLUSIONS

The present study provided evidence that Morinda citrifolia leaf aqueous extract was efficacious in inducing cell proliferation, protein synthesis, alkaline phosphatase activity, and matrix mineralization in vitro. Therefore, Morinda citrifolia leaves are a promising osteoinductive agent for bone and periodontal tissue regeneration.

ACKNOWLEDGMENTS

The authors would like to thank Associate Professor R. Surarit (Mahidol University) for the donation of MC3T3-E1 cells used in this study. The authors also thank Drs. S.R. Gill, R. Dziak (The State University of New York at Buffalo), and B. Foster (University of Washington) for kindly reviewing this manuscript. This study was supported by funding from the Faculty of Dentistry, Mahidol University, Bangkok, Thailand.

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