Effect of Bisphosphonates on the Osteogenic Activity of Osteoprogenitor Cells Cultured on Titanium Surfaces

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Purpose: This study investigated the effects of bisphosphonates, namely, alendronate and zoledronate, on the osteogenic activity of osteoprogenitor cells cultured on titanium surfaces at therapeutic doses in order to assess if altered osteoblastogenesis could compromise osseointegration and contribute to etiopathogenesis of painful disorders such as bisphosphonates-related osteonecrosis of the jaw (BRONJ) following implant placement. ***Materials and Methods:*** MC3T3-E1 Subclone 4 cells were utilized in this study. Therapeutic doses of alendronate and zoledronate were calculated based on reported peak plasma concentrations. The viability, proliferation, adhesion, and mineralization potential of cells was assessed using a LIVE/DEAD stain, alamarBlue assay, immunofluorescence microscopy, and Alizarin Red S staining, respectively. ***Results:*** Therapeutic doses of zoledronate negatively affected cell viability, whereas therapeutic doses of alendronate significantly enhanced cell differentiation and the amount of bone formation compared with the control. ***Conclusion:*** The findings of this study may provide some insight into the pathogenesis of BRONJ development following implant placement in patients treated with zoledronate and may have promising implications toward improved wound healing and osseointegration in patients treated with alendronate. *Int J Oral Maxillofac Implants 2020;35:939–947. doi: 10.11607/jomi.8354*

Keywords: alendronate, bisphosphonates, dental implants, osseointegration, osteoblasts, zoledronate

Bone healing around modern titanium-based dental implants involves a complex chain of biologic events that results in predictable osseointegration.[1] Osteogenesis begins early in this process, approximately 4 days after placement of the implant, and continues for a further 3 months until bone-to-implant contact is achieved.[2] During osteogenesis, viable osteoprogenitor cells in the granulation tissue differentiate into bone-forming osteoblasts and deposit osteoid and minerals toward, and on, the implant surface.[3]

Bisphosphonates are a group of antiresorptive agents that are widely used to treat a variety of diseases characterized by excessive bone resorption, such as osteoporosis, multiple myeloma, Paget disease, hypercalcemia of malignancy, and cancer bone metastasis. These drugs significantly improve a patient's quality of life by preventing events such as fractures and limiting bone pain and metastatic spread.[4] In addition to their inhibitory effects on osteoclasts, bisphosphonates have been shown to affect several other cells, including epithelial cells, lymphocytes, macrophages, myelomas, and breast cancer cells. Furthermore, the mechanism of action of these drugs on bone may not be completely understood.[5] Recent studies suggest that based on the type of bisphosphonates used and the multiple experimental protocols, bisphosphonates may also have a direct action on the bone-forming capabilities of osteoblasts.[6]

Bisphosphonates, due to their association with an osseodestructive condition called medication-related osteonecrosis of the jaw (MRONJ) have been extensively studied in recent literature.[7,8] MRONJ is characterized by exposed necrotic bone in the maxillofacial region that has persisted for more than 8 weeks in patients with a history of treatment with anti-resorptive or anti-angiogenic drug therapy, and where there has been no history of radiation therapy to the jaw or no obvious metastatic disease to the jaws. Dentoalveolar surgery is considered a major risk factor for developing MRONJ, including the procedure of placing dental implants. Several hypotheses have been proposed to explain the delayed bone healing seen in MRONJ; however, it is likely that the cause of the disease is multifactorial, with each of the drugs having slightly different etiopathogenic mechanisms.[9] Since only bisphosphonates are used in the present study, the term bisphosphonates-related osteonecrosis of the jaw (BRONJ) is used instead of MRONJ.

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Submitted February 16, 2020; accepted April 14, 2020.

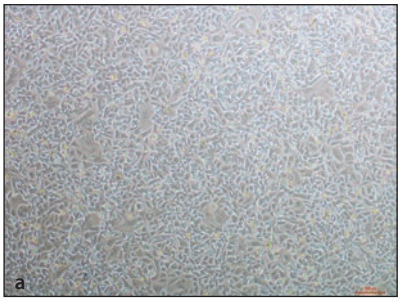


Fig 1 MC3T3-E1 cells viewed at *(a)* ×10 and *(b)* ×20 magnification. Images were taken prior to passaging at 95% confluence. Sample size = 3 replicates per group. Scale bar = 100 px.

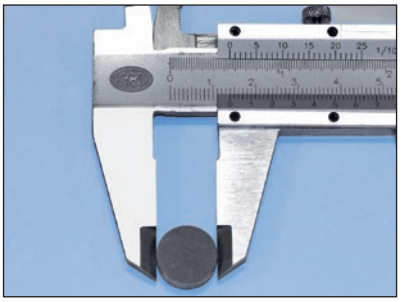


Fig 2 Example of a titanium disk after sandblasting. The diameter of the titanium disk (14 mm) was designed to be less than the diameter of the well (22.1 mm) to facilitate manipulation of the disk during the imaging experiments.

Table 1 Selected Drugs and Drug Concentrations Used in This Study

Drug	Concentration
Control	N/A*
Alendronate	0.1 μM 0.2 μM (therapeutic dose)[11]
Zoledronate	0.5 μM 1 μM (therapeutic dose)[12]

*No treatment.

Osteogenesis is an integral part of hard tissue healing around dental implants, and it is well accepted that bisphosphonate therapy could induce predisposition for premature loss of implants and BRONJ. While Alqhtani et al[10] previously reported that low doses of bisphosphonates (less than 1,000th of clinical doses) could enhance the osteogenic activity in vitro, it is not known if similar effects are observed with therapeutic doses of bisphosphonates. With this in mind, the aim of this in vitro study was to investigate how therapeutic doses of bisphosphonates could affect viability, proliferation, adhesion, and differentiation of osteoprogenitor cells cultured on titanium surfaces.

MATERIALS AND METHODS

Cell Culture

MC3T3-E1 Subclone 4 (ATCC CRL-2593) mouse calvarium–derived osteoprogenitor cells were utilized in this study. Cells were expanded at 37°C with complete growth media comprising Dulbecco's Modified Eagle's Medium (DMEM) admixed with 10% fetal calf serum and 1% penicillin-streptomycin (Sigma-Aldrich) placed within a 5% CO_2 incubator. The media was replenished every 4th day, and cells were passaged at 95% confluence as confirmed using an inverted microscope (Nikon Eclipse TS100, Nikon Instruments; Fig 1). Only cells from passages 3 to 9 were used in the experiments, and all experiments were conducted using a triplicate sampling protocol.

Titanium Disk Preparation

Commercially pure titanium disks (14 × 3.5 mm; Fig 2) were placed into the wells of 12-well tissue-culture plates (one disk per well). Prior to the first experiment, the titanium disks were roughened with a sandblaster for 3 minutes (150- to 200-μm alumina particles; Korox 110, BEGO). Between the experiments, the titanium disks were physically cleaned using a soft nylon brush, placed in an ultrasonic bath for 30 minutes, rinsed with ultrapure water (Milli-Q), and then sterilized in an autoclave at 134°C for 20 minutes.

Experimental Groups

Two commonly prescribed bisphosphonates were assessed, alendronate and zoledronate (Sigma-Aldrich), at concentrations equivalent to their respective therapeutic doses (Table 1). These drug concentrations were based on reported human plasma concentrations attained after administering a single therapeutic dose of oral alendronate (70 mg) or intravenous zoledronate (2 to 4 mg).[11,12] To further investigate whether there was a direct correlation between the potency of bisphosphonates and their effects on the cells, half of these concentrations were also used.

Stock Solutions

Stock solutions were prepared by dissolving alendronate and zoledronate in phosphate-buffered saline (PBS) at pH 7.2 and storing at –20°C. Osteogenic media was prepared according to Sharma et al and comprised complete growth media supplemented with β-glycerophosphate (10 mM), ascorbic acid (200 μM), and dexamethasone (100 nM).[8] Prior to the

experiments, alendronate and zoledronate solutions were diluted with complete growth media or osteogenic media to the aforementioned concentrations.

Cell Viability

Cells were seeded onto the titanium disks (200 µL at 1 × 10^6 cells/mL) in complete growth media and left to attach for 30 minutes in an incubator at 37°C with 5% CO_2. Wells were then topped up with complete growth media containing cells (1.4 mL at 3.25 × 10^5 cells/mL), and cells were incubated for an additional day. To ensure that cells attached to the walls of tissue well plates did not influence the experimental findings, the titanium disks were moved to fresh 12-well tissue-culture plates on the subsequent day. Complete growth media supplemented with the different concentrations of alendronate or zoledronate was then added to the wells (1.6 mL per well) and refreshed every 3 days. Viability of cells was assessed at the end of days 3 and 10 using LIVE/DEAD stain. Titanium disks were moved to 6-well tissue-culture plates (3 disks per well). A working solution of 20% CytoPainter (ab219941, Abcam) and 2% Propidium Iodide (P1304MP, Thermo Fisher Scientific) in PBS was used at 0.4 mL/well. Cells were incubated at 37°C and refrigerated at 4°C for 0.5 hour each and washed with PBS (twice) before fluorescence was visualized using an Olympus IX53 inverted epi-fluorescence microscope (Olympus Australia).

Cell Proliferation

Cells were seeded onto the titanium disks (200 µL at 1 × 10^6 cells/mL) in complete growth media and left to attach for 30 minutes in an incubator at 37°C with 5% CO_2. Wells were then topped up with complete growth media containing cells (1.4 mL at 3.25 × 10^5 cells/mL), and cells were incubated for an additional day. The next day, the titanium disks were moved to fresh 12-well tissue-culture plates, and complete growth media supplemented with the different concentrations of alendronate or zoledronate was added to the wells (1.6 mL per well) and refreshed every 3 days. Proliferation of cells was assessed at the end of days 3, 6, and 10 using the alamarBlue assay. A 10% v/v dye solution of resazurin sodium salt (Sigma-Aldrich) was added into each well, and cells were incubated for 5 hours at 37°C with 5% CO_2. Then, 100-µL aliquots of media were transferred into a 96-well clear plate to measure the absorbance values from test and control wells at 600 and 570 nm (Bio-Rad Laboratories). The proportion of viable cells and the proliferation rate were determined by calculating the percentage of reduction of alamarBlue reagent within each well.

Cell Adhesion

Cells were seeded in complete growth media onto the titanium disks (200 µL at 5 × 10^5 cells/mL) and left to attach for 30 minutes in an incubator at 37°C with 5% CO_2. Wells were then topped up with complete growth media supplemented with the different concentrations of alendronate or zoledronate (1.4 mL per well), and cells were incubated for an additional day. The next day, after PBS wash, the cells were fixed for 10 minutes at room temperature with 4% paraformaldehyde. Cells were then permeabilized using 0.05% Tween 20 (Sigma-Aldrich) for 10 minutes at room temperature. Following this, cells were blocked with 5% fetal bovine serum for 30 minutes at room temperature. Cells were then stained with Flash Phalloidin Red (BioLegend) and 4′,6-Diamidino-2-Phenylindole (DAPI; BioLegend) for 20 minutes at room temperature. Fluorescence was visualized and imaged using an Olympus IX53 inverted epi-fluorescence microscope (Olympus Australia). Images were collected and processed using ImageJ software (U.S. National Institutes of Health), and quantitative analysis was performed by calculating the corrected total cell fluorescence of F-actin in three isolated cells per group using the same software.

Mineralization

Cells were seeded in complete growth media onto the titanium disks (200 µL at 1 × 10^6 cells/mL) and left to attach for 30 minutes in an incubator at 37°C with 5% CO_2. Wells were then topped up with complete growth media containing cells (1.4 mL at 1 × 10^6 cells/mL), and cells were incubated for an additional day. The next day, the titanium disks were moved to fresh 12-well tissue-culture plates and osteogenic media supplemented with the alendronate, or zoledronate was then added to the wells (1.6 mL per well) that were refreshed every 4 days. Mineralization was assessed on day 14 using Alizarin Red S staining as previously described by Reinholz et al.[5] Briefly, medium was aspirated from the wells, and cells were rinsed twice with PBS at room temperature and once with ice-cold PBS. Cells were then fixed with ice-cold 70% (v/v) ethanol for 1 hour. The ethanol was discarded, and cells were rinsed twice with deionized water. Then, 40 mM Alizarin Red S (Sigma-Aldrich) in deionized water (adjusted to pH 4.2) was added to stain the cells for 10 minutes at room temperature. Next, the Alizarin Red S solution was discarded and titanium disks were placed in new 12-well tissue-culture plates. Cells were rinsed 5 times with deionized water and then incubated in PBS for 15 minutes at room temperature on a Grant ES-20 Compact Shaker-Incubator orbital rotator (150 rpm; VWR International). The PBS was then discarded, and cells were rinsed once with fresh PBS before being de-stained with 10% (w/v) cetylpyridinium chloride in 10 mM sodium phosphate (pH 7.0) for

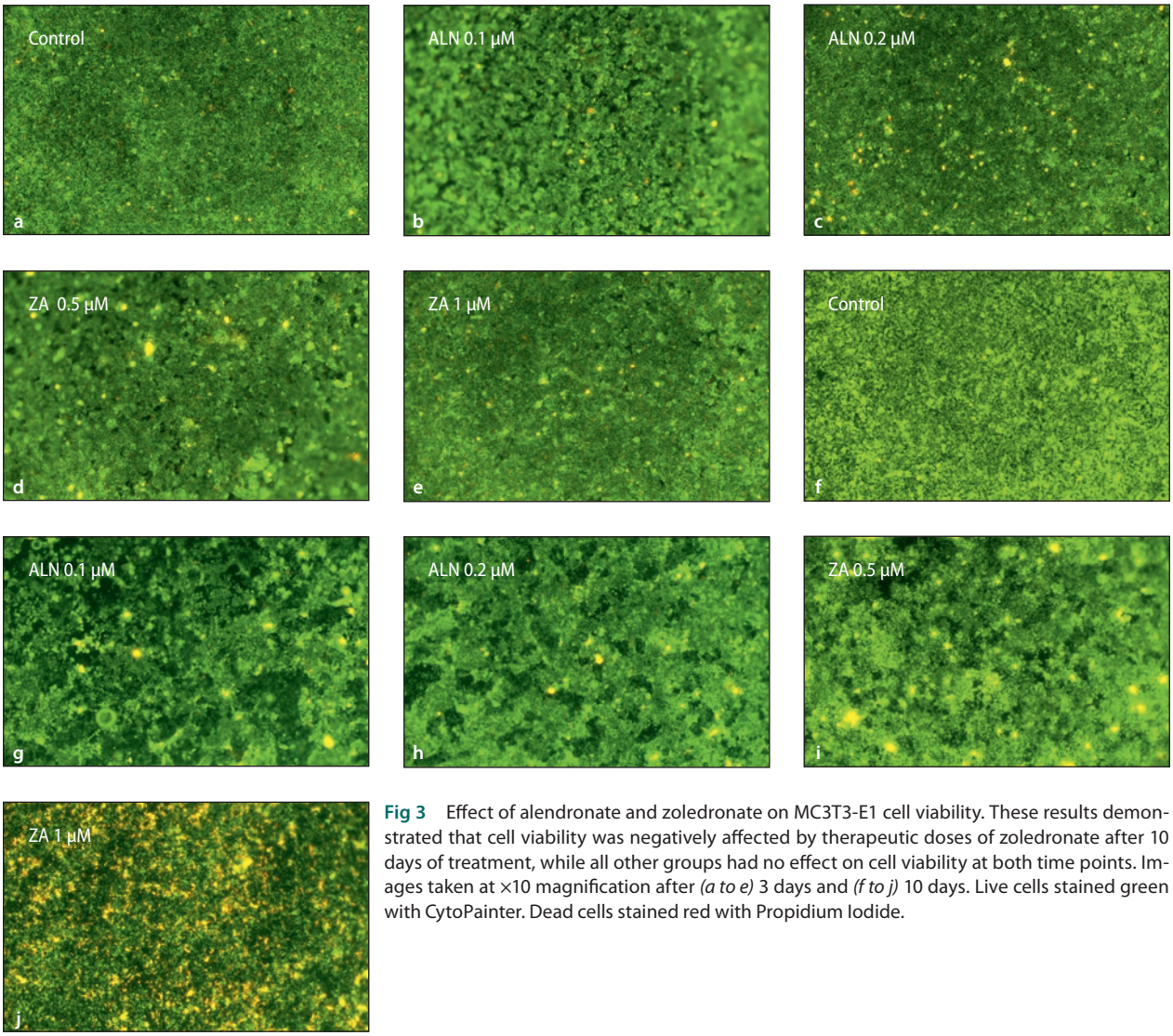


Fig 3 Effect of alendronate and zoledronate on MC3T3-E1 cell viability. These results demonstrated that cell viability was negatively affected by therapeutic doses of zoledronate after 10 days of treatment, while all other groups had no effect on cell viability at both time points. Images taken at ×10 magnification after *(a to e)* 3 days and *(f to j)* 10 days. Live cells stained green with CytoPainter. Dead cells stained red with Propidium Iodide.

15 minutes at room temperature on an orbital rotator (150 rpm). The extracted stain was then transferred to a 96-well clear plate to measure absorbance at 570 nm (Bio-Rad Laboratories).

Statistical Analysis

All the acquired data were expressed as mean ± SD. Comparisons were between the experimental groups and the control and were conducted using analysis of variance (ANOVA, two-tailed, post hoc test: Dunnett). The software SPSS 25.0 for Windows (IBM) was used for calculations, and results with *P* less than .05 were considered to be statistically significant.

RESULTS

Cell Viability

The viability assay acted as a preliminary experiment to identify the cytotoxic effects of the bisphosphonates on the cells at the selected drug concentrations. After 3 days of incubation, no cytotoxic effects were observed, as titanium disks were almost completely confluent with live cells in all groups (Fig 3). By day 10 of culture, however, cytotoxic effects were evident in the groups treated with therapeutic doses of zoledronate. This negative effect that zoledronate had on cellular viability was found to be dose- and time-dependent, as notably more dead cells were present with the therapeutic dose compared with the half-dose and after 10 days compared with that after 3 days.

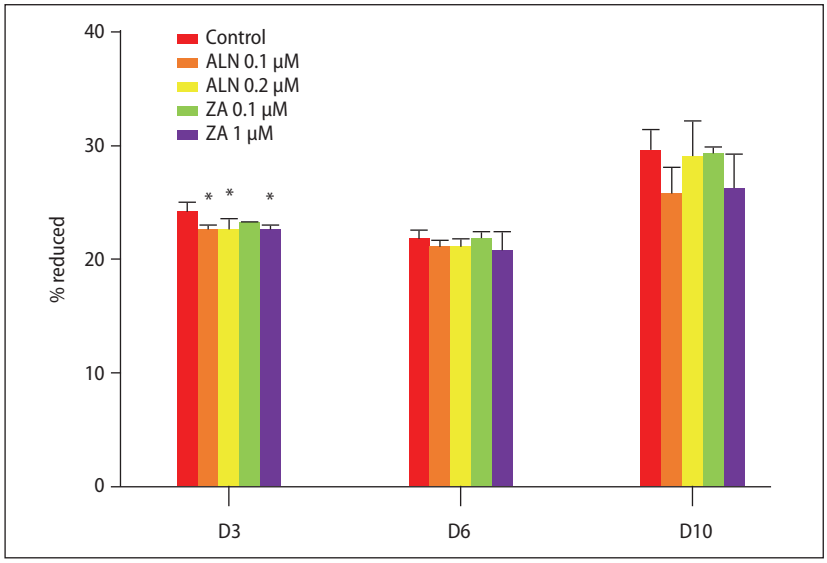


Fig 4 Effect of alendronate and zoledronate on MC3T3-E1 cell proliferation. These results demonstrated that cell proliferation was initially significantly inhibited by both doses of alendronate and the therapeutic dose of zoledronate, but then the cells recovered with no overall long-term effects, while half of the therapeutic dose of zoledronate had no significant effect on cell proliferation. Significant differences compared with control are indicated as $*P < .05$.

Cell Proliferation

The proliferation assay aimed to investigate the effects of alendronate and zoledronate on cell division and growth using the alamarBlue assay. Cells followed a typical growth curve in all the experimental groups, where the number of cells slightly decreased from day 3 to day 6 before reaching a maximum at the end of day 10 (Fig 4). Both doses of alendronate and the therapeutic dose of zoledronate significantly inhibited cell proliferation after 3 days (control 24.52% ± 0.77%, alendronate 0.1 μM 22.97% ± 0.23%, alendronate 0.2 μM 22.87% ± 0.92%, and zoledronate 1 μM 22.74% ± 0.48%; $P = .280$, $P = .020$, and $P = .013$); however, there were no significant effects on cell proliferation observed thereafter. Half the therapeutic dose of zoledronate showed no significant effects on cell proliferation throughout the 10 days of culture.

Cell Adhesion

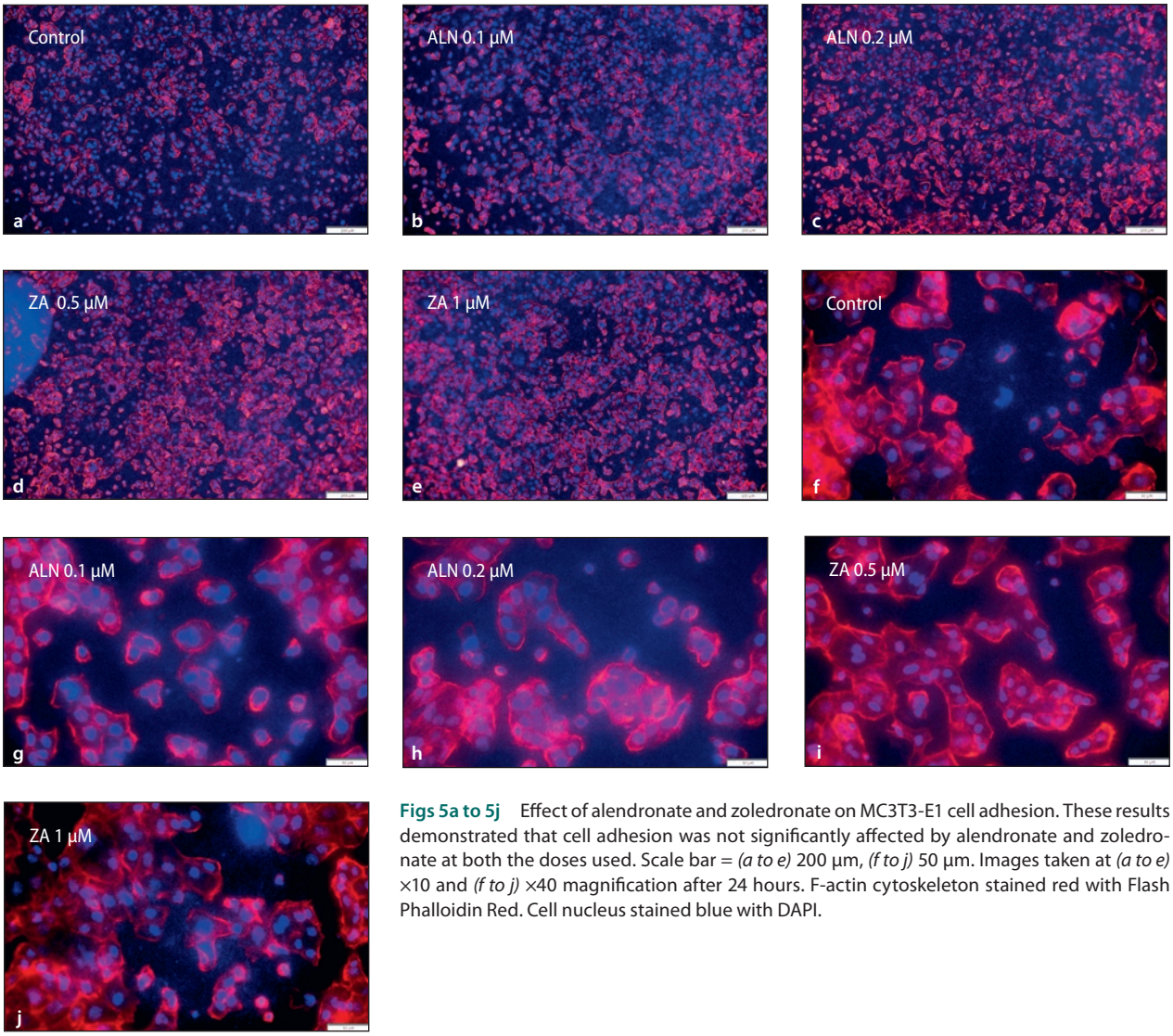
The adhesion assay aimed to investigate the effects of alendronate and zoledronate on cell attachment to the titanium disk using immunofluorescence microscopy to study the cytoskeletal protein F-actin. After 24 hours, no observable differences in cell morphology and spread were evident, and this was confirmed with statistical analysis where the amount of F-actin expression per viable cell was not significantly different between the groups and the control (Figs 5 and 6).

Mineralization

The mineralization assay aimed to investigate the effects of alendronate and zoledronate on cell differentiation and subsequent mineralization using Alizarin Red S staining. At the end of 14 days, alendronate at both doses was found to significantly promote mineralization compared with the control (control 0.081 ± 0.009, alendronate 0.1 μM 0.108 ± 0.006, and alendronate 0.2 μM 0.118 ± 0.003; $P = .0044$ and $P = .0005$; Fig 7). This positive effect that alendronate has on mineralization was found to be dose-dependent, as the therapeutic dose increased the amount of calcium deposition by 45% compared to 33% with the half-dose. In comparison, zoledronate at both doses had no significant effect on mineralization.

DISCUSSION

Dental implant therapy in bisphosphonate-medicated patients could have two possible negative outcomes: implant loss due to failure in osseointegration and BRONJ within the implant surgical site. At present, there are mixed findings in the literature regarding the risk of these outcomes in bisphosphonate patients. A recent systematic review by Mendes et al[13] clearly delineated the variation in findings with a history of bisphosphonate therapy, with some studies reporting slightly lower survival rates and higher incidences of BRONJ, while other studies reported no remarkable differences in patients with a history of bisphosphonate therapy compared with healthy patients. Furthermore, a review of animal studies by Vohra et al[14] noted that most of the studies demonstrated that bisphosphonates enhanced osseointegration under osteoporotic conditions, wherein increased bone volume and bone-to-implant contact were observed in animals receiving systemic bisphosphonate doses, compared with control animals.[14] Given the variation in the clinical observations and animal experiments, further basic research into the possible mechanisms and pathways involved



Figs 5a to 5j Effect of alendronate and zoledronate on MC3T3-E1 cell adhesion. These results demonstrated that cell adhesion was not significantly affected by alendronate and zoledronate at both the doses used. Scale bar = *(a to e)* 200 µm, *(f to j)* 50 µm. Images taken at *(a to e)* ×10 and *(f to j)* ×40 magnification after 24 hours. F-actin cytoskeleton stained red with Flash Phalloidin Red. Cell nucleus stained blue with DAPI.

is warranted. To date, to the best of the authors' knowledge, there has only been one other similar study that investigated the role of bisphosphonate on the osteogenesis during osseointegration.[10] However, in their study, concentrations of bisphosphonates equivalent to 1/1,000th of therapeutic doses were used, and comparisons between the effects of other bisphosphonates, such as the widely used, highly potent zoledronate, were not included. Therefore, the present study aimed to investigate the effects of therapeutic doses of alendronate and zoledronate on the viability, proliferation, adhesion, and differentiation of osteoprogenitor cells cultured on titanium surfaces.

In the present study, cell viability appeared to be unaffected by therapeutic doses of alendronate, but negatively affected by therapeutic doses of zoledronate. The negative effect of zoledronate at therapeutic doses could be attributed to its potency, as zoledronate is the most potent bisphosphonate, approximately 20 times more potent than alendronate.[7] Furthermore, BRONJ is mainly observed in patients treated with zoledronate (intravenous), and less commonly with orally administered alendronate (lower potency).[8] Therefore, the fact that cell viability seemed to be adversely affected by therapeutic doses of zoledronate could provide some insight into the pathogenesis of BRONJ development following implant placement in patients treated with zoledronate, as there would be a reduction in viable cells to form new bone during the healing process. Previously, Huang et al and Thibaut et al reported a significant cytotoxic effect on MC3T3-E1 cells and human fetal osteoblasts with concentrations of zoledronate higher than 10 µM.[15,16] This may suggest that the negative effect of zoledronate on cell viability is only significant at concentrations higher than the therapeutic dose. The in vivo study by Pozzi et al supports this

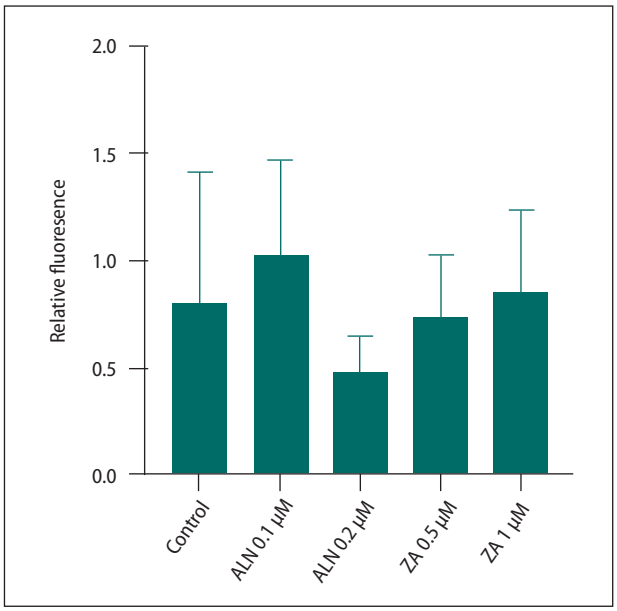


Fig 6 Quantitative analysis of cellular adhesion showing no statistically significant differences between alendronate and zoledronate group at any of the tested doses.

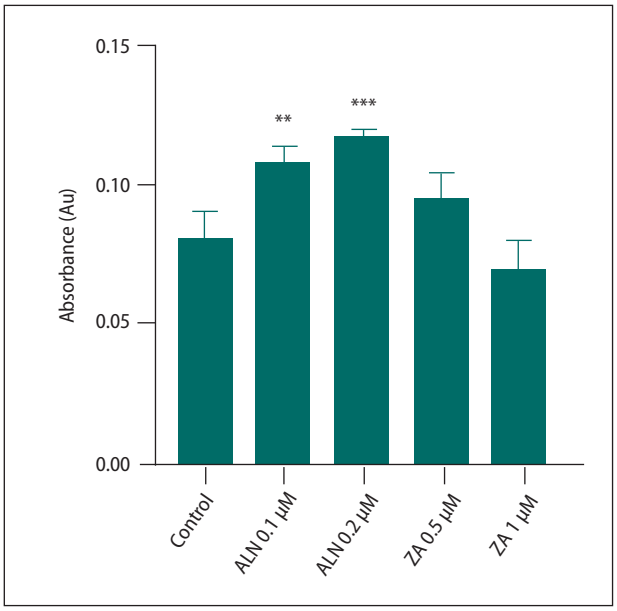


Fig 7 Effect of alendronate and zoledronate on MC3T3-E1 mineralization. These results demonstrated that mineralization was significantly enhanced by alendronate in a dose-dependent manner, while both doses of zoledronate had no significant effect on mineralization. Significant differences compared with control are indicated as $^{**}P < .01$, $^{***}P < .001$.

notion, where it was shown that therapeutic doses of zoledronate decreased the numbers of osteoblasts per bone perimeter in mice without any statistical significance being reached.[17] On the other hand, Pan et al reported that zoledronate induced cell death in human adult osteoblast-like cells at concentrations of 0.5 µM or greater, in a dose-dependent manner.[18] Taken together with the existing literature, the results of the present study may suggest that cell viability in the context of osseointegration could be negatively affected by therapeutic doses of zoledronate.

Cell proliferation was initially inhibited by therapeutic doses of alendronate and zoledronate, probably due to the initial shock of drug exposure, but then cells recovered with no overall long-term effects. In fact, Alqhtani et al reported that lower doses of alendronate (10 nM and 100 nM) significantly stimulated the proliferation of human mesenchymal stem cells cultured on titanium surfaces.[10] Similarly, Im et al and Xiong et al found that alendronate significantly increased cell numbers over the controls in primary human trabecular bone cell culture and the MG-63 osteoblast-like cell line, respectively, with the greatest effect at 10 nM.[19,20] Low concentrations of zoledronate also appear to induce proliferation of human mesenchymal stem cells, as von Knoch et al reported an increase in the number of cells that were treated with 10 nM of zoledronate.[21] On the other hand, a significant anti-proliferative effect on human term placental mesenchymal stem cells was found in Sharma et al, with concentrations of alendronate and zoledronate higher than 2 µM and 1 µM, respectively.[8] Orriss et al also reported a significant inhibition of primary rat osteoblast cell growth and function with zoledronate at concentrations beyond 1 µM.[22] The findings of the present study may suggest that at therapeutic doses, alendronate and zoledronate do not affect the proliferation of osteoprogenitor cells during osseointegration; however, given the findings available in the literature, it is plausible that lower doses may enhance proliferation while higher doses may induce cytostasis.

Cell adhesion to the titanium disk was assessed by analyzing the expression of F-actin, a cytoskeletal protein involved in regulating cellular shape change and force generation in activities such as migration, attachment, and division.[23] Firm adherence of osteoprogenitor cells is an important factor for cell survival and differentiation into osteoblasts, which over time become mature and produce fibronectin, an extracellular glycoprotein that regulates the adhesion, differentiation, and function of various adherent cells.[24] The present data showed that the amount of F-actin expression per viable cell remained unaffected by the addition of therapeutic doses of alendronate and zoledronate to the culture media. This may suggest that as with cell proliferation, cell adhesion to the titanium implant surface is not affected by therapeutic doses of alendronate and zoledronate.

Cell differentiation and subsequent osteogenesis, as measured by mineralization, was stimulated by therapeutic doses of alendronate. The ability of alendronate to increase mineralization was not surprising, as previous in vivo studies have demonstrated that local drug delivery of bisphosphonates by way of implant coatings improves the fixation of titanium implants in human bone and increases peri-implant bone density in osteoporotic sheep.[25,26] Furthermore, several in vitro studies have shown that alendronate can affect osteoblastogenesis depending on its concentration, with a stimulatory effect observed at lower doses and an inhibitory effect at higher doses.[6] The results of the present study are in agreement with those reported by Alqhtani et al,[10] suggesting that the anabolic effect of alendronate on osteoblasts (in osseointegration) is maintained at therapeutic doses. Kim et al found that the osteogenic differentiation of multipotent mouse mesenchymal stem cells was also enhanced after treatment with alendronate at concentrations higher than in the present study.[27] This may suggest that the stimulatory effect on osteoblast bone formation during osseointegration is also present at stronger concentrations than the therapeutic dose. However, Idris et al found that alendronate inhibits bone nodule formation in mice osteoblast cells above 2 to 10 μM, thereby demonstrating that a ceiling effect on osteoblast formation exists at concentrations approaching this range.[28] Pan et al reported that zoledronate enhanced mineralized matrix formation in human adult osteoblast-like cells at concentrations higher than 0.5 μM, with an inhibitory effect observed at concentrations exceeding 5 μM.[18] This was contrary to the findings of the present study and may be due to a range of factors, including different cell type, duration of treatment and therefore cumulative dose of zoledronate, and the presence of a titanium disk in the present study. Nevertheless, the findings of this study may suggest that therapeutic doses of alendronate could enhance the mineralization potential of osteoblasts to improve osseointegration and implant success, whereas the same could not be confirmed for the therapeutic doses of zoledronate.

Although the present study used a well-established model to study osseointegration in vitro, there are three major limitations worth noting. The first limitation is that it was not possible to select drug concentrations that exactly correlate to physiologic conditions. This is because the extent of cellular exposure within the body, particularly of osteoblasts to bisphosphonates, is yet to be ascertained.[5] As previously mentioned, the concentrations of bisphosphonates used in this study were selected based on the concentrations found in patient blood plasma after a single intake of the drug at therapeutic doses. While the peak plasma levels are considered to be transitory, bisphosphonates are taken up quickly and at increased concentrations into the osseous tissues, with very small amounts released into peripheral circulation during turnover (long half-life drugs).[18] Therefore, it is possible that osteoblasts in the bone microenvironment are exposed to bisphosphonates at concentrations several times higher than the patient's peak plasma level. In fact, one report estimated that therapeutic doses of alendronate could give rise to local concentrations as high as 1 mM in sites of active bone resorption.[29] The second limitation is that a mouse cell line (MC3T3-E1) was used. James Cook University kindly donated these cells, as they have been using them for other similar studies. While these cells provide a good and reliable model to study osteoblast biology, they do not represent human tissue as accurately as primary human cells would.[30] The third limitation is that statistical analysis could not be performed for the viability assay, as there were too many cells present, making a reliable and reproducible quantification impossible. Similar experiments utilizing primary human osteoblasts along with a range of seeding densities could be performed to allow for quantification of the live and dead cells.

CONCLUSIONS

This study using therapeutic concentrations of alendronate and zoledronate on osteoprogenitor cells cultured on titanium showed the positive effect that alendronate had on mineralization, which could have promising implications toward improved wound healing and osseointegration around dental implants. In contrast, a possible negative effect on cell viability caused by therapeutic doses of zoledronate may infer a greater risk of implant loss in terms of reduced osseointegration and BRONJ in these patients. Currently, minimal in vivo studies exist evaluating the effects of systemic bisphosphonates on bone healing around dental implants, and the findings within the existing studies vary significantly. Therefore, further research investigating the effects of systemic bisphosphonates on bone healing around dental implants, with an emphasis on animal and human studies, is warranted.

ACKNOWLEDGMENTS

The authors wish to acknowledge the James Cook University College of Medicine and Dentistry Honours program and the Australian Dental Research Foundation Colin Cormie Grant for funding this study. The authors declare that there are no conflicts of interest.

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