e-ISSN: 2637-0611

Available online at
https://journal.uitm.edu.my/ojs/index.php/COS/index

Compendium of Oral Science 12(1) 2025, 82 - 95

Compendium of Oral Science

Surface Characteristics of Calcium Chloride Surface-Treated Mini Implant

Noor Ainnilwahida Alias[1], Saraswathy Devi Sinniah[1], Nornizar Anuar[2], Siti Nurul'ain Yusop[2], Mizaton Hazizul Hasan[3], Nor Amlizan Ramli[3], Mohamed Ibrahim Abu Hassan[4], Maryati Md Dasor[1*]

[1]*Centre of Studies for Paediatric Dentistry and Orthodontics, Faculty of Dentistry, Universiti Teknologi MARA Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia*
[2]*School of Chemical Engineering, College of Engineering, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia*
[3]*Faculty of Pharmacy, Universiti Teknologi MARA, Bandar Puncak Alam, Selangor, Malaysia*
[4]*Faculty of Dentistry MAHSA, Jenjarom, Selangor, Malaysia*

ARTICLE INFO

Article history:
Received 02 February 2024
Revised 16 April 2024
Accepted 05 July 2024
Online first
Published 01 March 2025

Keywords:
orthodontic mini-implant
surface treated
primary stability
calcium chloride
crystallisation

DOI:
10.24191/cos.v12i1.5649

ABSTRACT

Objective: This study aimed to investigate the surface characteristics of surface-treated titanium orthodontic mini-implant (MI) with acid etching and immersion in different conditions (concentrations, temperature and duration) of calcium chloride ($CaCl_2$).

Materials and Methods: Eighty-four MI by Orthodontic Microimplant AbsoAnchor® System (Dentos Inc.) were utilised in this study. Surface roughness was assessed qualitatively and quantitatively with a scanning electron micrograph (SEM)(at 50x,500x and 1000x magnifications) and energy-dispersive spectrometer (EDS) (Fei Quanta FEG 450 Environmental Electron Microscope) of the surface-treated MI with different conditions of $CaCl_2$ immersions; concentrations (0.01 M, 0.1 M & 1.0 M), temperature (5°C,15°C and 25°C) and duration (1hour, 7 hours and 24 hours).

Results: Surface topography changes were observed due to increase in surface roughness of MI treated with acid etching and $CaCl_2$ immersions at various conditions . There was a significant difference in surface topographic changes seen by various irregularities with small cavities and protrusions were observed on surface of the MIs at different magnifications. The highest level of Ca^{2+} concentration detected was in 0.1 M at 5°C for 7 hours of $CaCl_2$ immersion.

Conclusion: MI surface modification using $CaCl_2$ surface treatment provided a significant difference in surface topographic changes seen between the MI groups. Hence, it increased the potential surface area of

[1*] Corresponding author. *E-mail address*: maryatidasor@uitm.edu.my

osseointegration and enhanced tissue renewal and repair. The physicochemical characteristics analysis showed a significant presence of calcium ions by % wt on the MI surface. Different temperatures, concentrations, and times of immersion did affect the availability of $CaCl_2$ on the MI surface and $CaCl_2$ condition of 0.1 M at 5°C for 7 hours of immersion was the optimum condition.

SURFACE CHARACTERISTICS OF CALCIUM CHLORIDE SURFACE-TREATED MINI IMPLANT

Orthodontic anchorage plays a critical role in the success of orthodontic treatment, and recent advancements have significantly improved the reliability and effectiveness of anchorage strategies.

1. INTRODUCTION

Orthodontic anchorage is defined by the resistance to unwanted tooth movement during orthodontic treatment (Proffit, Jr, Larson, & Sarver, 2019). The traditional orthodontic anchorage appliances, such as palatal arches and Nance appliances, are proven to be less reliable during the treatment process (Diar-Bakirly, Feres, Saltaji, Flores-Mir, & El-Bialy, 2017; Sandler et al., 2014; Stivaros, Lowe, Dandy, Doherty, & Mandall, 2010; Zablocki, McNamara, Franchi, & Baccetti, 2008).

In the realm of orthodontic treatment, MI has emerged as the preferred approach for skeletal anchorage, surpassing traditional anchorage reinforcements due to their simplicity of placement and removal, low cost, and capacity for immediate force loading (Devadkar, Potnis, Toshniwal, Pharande, & Vinay, 2022). Nevertheless, the absence of stability could potentially undermine the efficacy of MI. Various types of MI have been developed with various methods to increase their stability through surface treatment.

In vivo studies have proven that the application of surface-treated MI leads to enhanced osseointegration, with the surface of the implant without compromising cutting ability or create bigger bony defect (Att et al., 2009; Jang et al., 2018). Over the years, MI failure rates have increased from 13% to 15.8% (Sheibaninia, 2020). Although the success rate of MI has increased beyond 55% (Crismani, Bertl, Celar, Bantleon, & Burstone, 2010) or higher than 80% (Reynders, Ronchi, & Bipat, 2009) it still depends on several other contributing factors, which could be patient-related, orthodontist/operator-related, or MI-related (Casana-Ruiz et al., 2020; Lai & Chen, 2014; Lee & Baek, 2010; Sheibaninia, 2020; Yao et al., 2015).

To date, various surface treatments for increasing MI surface roughness or changing the nano-surface or topography to mimic that of natural bone have been developed to improve stability in patients with a high risk of MI failure. The surface modifications can be classified as macro-, micro- and nanotopographies of the MI (Table 1), (Alghamdi, 2018).

Table 1. Classification of surface roughness based on level of topography changes

Classification	Size (scale)
Macro	Milimeter
Micro	1-100 µm
Nano	Nanometer

Source: (Matos, 2021)

https://doi.org/10.24191/cos.v12i1.5649

The new technology enhances MI stability with a surface coating agent that favours tissue response and stimulates earlier osseointegration to prevent early loosening. $CaCl_2$ is one of the most versatile of the basic chemicals. It is very soluble and can serve as a source of calcium ions in a solution and one of the most versatile basic chemicals that can be utilised as a material for surface treatment. (Att et al., 2009; Jang et al., 2018).Since 1962, various studies have been done in animals and humans regarding the biocompatibility, cytotoxicity and safety of $CaCl_2$ (Vinnikov et al., 1962).

Therefore, this study aimed to investigate the surface characteristics of surface-treated titanium orthodontic (MI) with acid etching and immersion in different conditions (concentrations, temperature and duration) of calcium chloride ($CaCl_2$).

References should be listed at the end of the paper, should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters “a”, “b”, “c”, etc., placed after the year of publication.

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2. MATERIALS AND METHODS

2.1 Study design and materials

This in-vitro study has involved the surface treatment by acid etchingof 84 MIs (1.4 mm in diameter and 8 mm thread length (code number SH 1413-08) by the Orthodontic Microimplant AbsoAnchor® System manufactured by Dentos Inc. based in Daegu, Korea) with 0.11 mol/l HCl at a temperature of 65˚C (EG). Eighty-one of the acid etched MIs were then immersed in $CaCl_2$ solution (ECG) at different temperatures (5°C, 15°C & 25°C) and concentrations (0.01 M, 0.1 M & 1.0 M) of $CaCl_2$ for different durations of time immersions (1 hour, 7 hours and 24 hours). Untreated machined surfaces (CG) are controlled.

MI acid etching procedure was performed by using 0.11 mol/l HCl at a temperature of 65˚C for 20 minutes. Eighty-one of the acid-etched MI then immediately immersed in the 1ml of different $CaCl_2$ solutions concentrations (0.01 M, 0.1 M & 1.0 M) at different temperatures (5°C, 15°C & 25°C) and duration of immersions (1 hour, 7 hours and 24 hours). Each MI was immersed in the $CaCl_2$ solutions in a single conical tube. The MI then been left in the oven at a temperature of 37°C for 20 minutes. These procedures were done in triplicates. The MI was sterilized using gamma sterilization and was vacuum sealed to prevent contamination (Jang et al., 2018).

2.2 Scanning Electron Microscopy

To evaluate differences in surface topography between the 3 types of MIs (CG, EG and ECG), their surfaces were observed via scanning electron microscopy at magnifications of 50x, 500x and 1000x (Fei Quanta FEG 450 Environmental Electron Microscope). Carbon conductive adhesive was placed on a stage for each MI before inserted into the SEM for analysis. The surface chemical components were evaluated by means of energy-dispersive spectrometer (EDS). The 15 kV voltage was utilized to identify the peak and background ratio for elements. The working distance for EDS analysis was in a range of 9-13 mm. The middle 1/3 was observed for each surface type.

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2.3 Statistical analysis

The qualitative and quantitative results have been statistically analysed in this study. The descriptive and analytic analysis was performed using Statistical Package for Social Sciences (SPSS) software version 28 (IBM, Armonk, New York). The significance level was set at $p < 0.05$ with 95% confidence interval. Data distribution was performed using the Shapiro-Wilk test. Comparison of the surface roughness of the surface of MI between groups was done by visual identification of different levels of surface roughness at different levels of magnification (Matos, 2021). The physicochemical characteristics of MI surface treatment and cytotoxicity effect of $CaCl_2$ treatments with different concentrations were analysed using Friedman two-way Analysis of Variance ANOVA followed by pairwise comparison by Wilcoxon Signed-Ranked Test to identify the source of the significance. The significance level was set at $p<0.05$. Further analysis was done using Bonferroni adjustment.

3. RESULTS

3.1 Comparison of the surface roughness of the surface of MI between groups: CG, EG and ECG

Scanning electron microscopy analysis revealed different surface topography of the three types of MI, with EG & ECG MI surface treatment evidently changed the surface morphology resulting in a rough surface.

In Fig. 1. the comparison of surface roughness among three types of MI (CG, EG, and ECG) imaged at magnifications ranging from 50x to 1000x reveals surface topography changes resulting from increased surface roughness due to various surface treatments, including acid etching (EG) and different conditions of $CaCl_2$ immersions. Small cavities and protrusions were observed on the surface of the MIs, where the sizes were detected at all magnifications. This condition shows various irregularities in the surface structure of the MIs.

https://doi.org/10.24191/cos.v12i1.5649

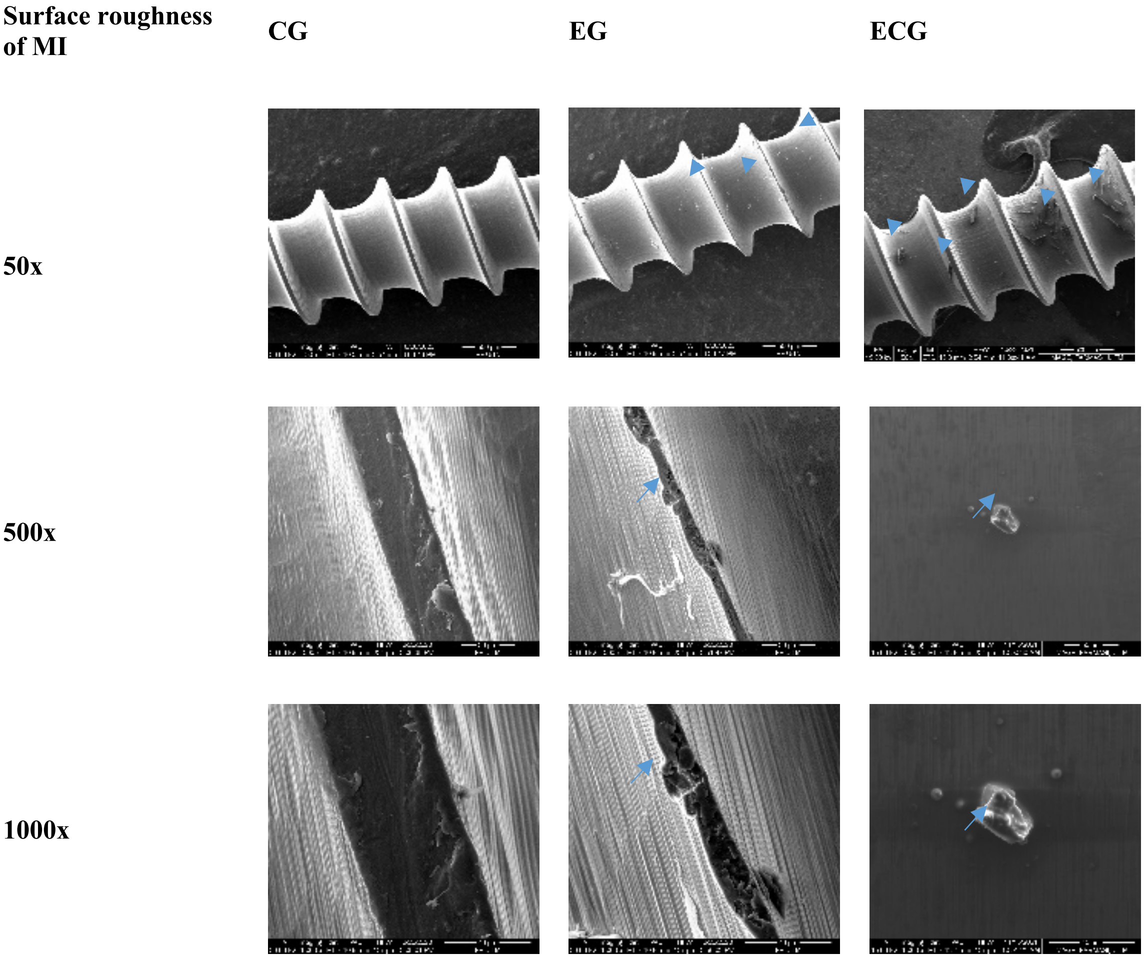

Fig. 1. Surface roughness of machined surfaces (CG) MI, surface treated machined surfaces with acid etching (EG) and surface treated machined surfaces with acid etching immersed in $CaCl_2$ solution (ECG) at 50x ,500x and 1000x magnification.

3.2 Comparison of physicochemical characteristics of $CaCl_2$ grown on the surface of MI between each condition for ECG

3.2.1 Identification of calcium ions on MI surface in different concentrations, temperature and time of immersion (ECG).

Data were not normally distributed within the groups as observed by the physicochemical EDS analysis of the calcium (Ca) element in different conditions of $CaCl_2$ immersion in various concentrations of $CaCl_2$ at various temperatures (5°C, 15°C, and 25°C) and time points (1 hour, 7 hours, and 24 hours) (Table 2). The median concentrations of calcium ion (Ca^{2+}) ranged from 0.10 to 8.79 at 5°C, from 0.18 to 2.49 at 15°C, and from 0.31 to 3.93 at 25°C. The IQR values for Ca^{2+} ranged from 0.31 to 10.35 at 5°C, from 0.87 to 9.95 at 15°C, and from 0.55 to 10.24 at 25°C.

https://doi.org/10.24191/cos.v12i1.5649

Table 2. Median and interquartile (IQR) of calcium element in EDS analysis of the MI immersed in different conditions of $CaCl_2$.

Conditions	Ca
	Median (IQR)
0.01 M 5 °C	
1 hour	0.43 (3.71)
7 hours	3.23 (7.26)
24 hours	1.20 (3.40)
0.1 M 5 °C	
1 hour	0.61 (4.91)
7 hours	8.79 (10.35)
24 hours	2.52 (7.15)
1 M 5 °C	
1 hour	2.49 (3.43)
7 hours	7.59 (6.38)
24 hours	2.28 (11.05)
0.01 M 15 °C	
1 hour	0.10 (0.64)
7 hours	0.62 (1.97)
24 hours	0.0 (0.14)
0.1 M 15 °C	
1 hour	0.37 (0.87)
7 hours	2.88 (9.95)
24 hours	1.53 (2.26)
1 M 15 °C	
1 hour	0.18 (1.07)
7 hours	2.08 (3.15)
24 hours	0.73 (0.31)
0.01 M 25 °C	

https://doi.org/10.24191/cos.v12i1.5649

Conditions	Ca
	Median (IQR)
1 hour	0.31 (0.55)
7 hours	2.58 (6.48)
24 hours	0.58 (0.29)
0.1 M 25 °C	
1 hour	3.93 (7.81)
7 hours	1.86 (10.24)
24 hours	1.21 (5.21)
1 M 25 °C	
1 hour	1.32 (4.13)
7 hours	1.21 (2.46)
24 hours	3.00 (10.20)

3.2.2 Identification of comparison of Ca element in different $CaCl^2$ immersion conditions.

A Friedman two-way ANOVA was performed to analyse the effect of different time duration immersion on wt% Ca. (Table 3). This analysis indicated that rankings of time duration varied significantly across the three different time duration, χ^2 (df = 2) = 34.44, p <.001. Between group comparison Friedman test revealed that there were statistically significant conditions to wt% Ca at 3 different conditions; for concentration of 0.01M at 5°C (p =0.032), concentration of 0.01 M at 25°C (p =0.009) and concentration of 0.1M at 15°C (p = 0.032). Pairwise comparisons by the Wilcoxon signed-ranked test on different combinations of related groups with α = 0.006 after Bonferroni adjustment further reveals not statistically significance in different conditions of immersions. The difference between the ranked 7 hour to 24 hours in concentration of 0.01M at 25°C (Mean Rank = 2.78) and difference between the ranked 1 hour to 7 hours in concentration of 0.1M at 15°C (Mean Rank = 2.56) which were approached significance (p = 0.011, p =0.015) respectively. This effect size can be described as “large” as there were with r = .84 and r=.81.

Table 3. Comparison of Ca at 3 different time duration of MI immersions.

Condition	Friedman test[a]		Pairwise comparison[b]					
			1 hour – 7 hours		1 hour – 24 hours		7 hour – 24 hours	
	X^2 stats	P value	Z	P value	Z	P value	Z	P value
0.01M, 5°C	6.89	0.032*	-2.073	0.038	-0.077	0.441	-1.599	0.110
0.01M, 15°C	3.185	0.203	-	-	-	-	-	-
0.01M, 25°C	9.314	0.009*	-2.310	0.021	-1.262	0.207	-2.547	0.011
0.1M, 5°C	2.889	0.236	-	-	-	-	-	-
0.1M, 15°C	6.889	0.032*	-2.429	0.015**	-2.073	0.038	-1.718	0.086
0.1M, 25°C	0.889	0.641	-	-	-	-	-	-
1M, 5°C	4.222	0.121	-	-	-	-	-	-
1M, 15°C	4.222	0.121	-	-	-	-	-	-
1M, 25°C	0.0	1.000	-	-	-	-	-	-

[a] Friedman Test (within group analysis) , Statistically significant ($p \leq 0.05$)*
[b] Pairwise comparison by Wilcoxon Signed-Ranked Test with $\alpha = 0.006$ after Bonferroni adjustment. Approached significant**

The rankings of Ca varied significantly across the 26 conditions of $CaCl_2$ immersions: χ^2 (26) =91.08 for Ca (Table 4).

Table 4. Friedman two-way ANOVA test analysis for overall Ca element.

	N	Chi-square	df	Asymp. Sig.
Ca	9	91.08	26	< 0.001*

Friedman test analysis
Significant level set at p-value p<0.05
Significant*

A Friedman two-way ANOVA was performed to analyse the effect of different concentrations of immersion on wt% Ca (Table 5). This analysis indicated that rankings of concentrations varied significantly across the three different concentrations χ^2 (df = 2) = 30.77, p <.001. Between group comparison Friedman test revealed that there were statistically significant conditions to wt% Ca at 3 conditions: at temperature of 5°C for 24 hours (p=0.032), temperature of 15°C for 7 hours (p =0.015), and temperature of 15°C for 24 hours (p =0.004). Pairwise comparisons by the Wilcoxon signed-ranked test on different combinations of related groups with $\alpha = 0.006$ after Bonferroni adjustment further reveal no statistical significance in different conditions of immersion. The difference between the ranked condition with temperature at 15°C for 7 hours in concentrations between 0.01 M and 0.1 M (mean rank = 2.61) and the difference between the ranked condition with temperature at 15°C for 24 hours in concentrations between 0.01 M and 0.1 M (mean rank = 2.78) approached significance (p = 0.012, p =0.008) respectively. This effect size can be described as "large", r = .84 and r=.89.

https://doi.org/10.24191/cos.v12i1.5649

Table 5. Comparison of Ca at 3 different concentrations of MI immersions.

Condition	Friedman test[a]		Pairwise comparison[b]					
			0.01 M – 0.1 M		0.01 M – 1 M		0.1 M – 1 M	
	X^2 stats	P value	Z	P value	Z	P value	Z	P value
5°C, 1 Hour	2.667	0.264	-	-	-	-	-	-
5°C, 7 Hour	0.889	0.641	-	-	-	-	-	-
5°C, 24 Hours	6.889	0.032*	-1.718	0.086	-1.599	0.110	-0.178	0.859
15°C, 1 Hour	0.867	0.648	-	-	-	-	-	-
15°C, 7 Hours	8.400	0.015*	-2.521	0.012**	-1.955	0.051	-1.718	0.086
15°C, 24 Hours	10.889	0.004*	-2.666	0.008**	-2.310	0.021	-2.073	0.038
25°C, 1 Hour	3.600	0.165	-	-	-	-	-	-
25°C, 7 Hours	0.889	0.641	-	-	-	-	-	-
25°C, 24 Hours	2.889	0.236	-	-	-	-	-	-

[a] Friedman Test (within group analysis) , Statistically significant ($p \leq 0.05$)*
[b] Pairwise comparison by Wilcoxon Signed-Ranked Test with $\alpha = 0.006$ after Bonferroni adjustment. Approached significant**

A Friedman two-way ANOVA was performed to analyse the effect of different temperatures of immersion on wt% Ca (Table 6). This analysis indicated that rankings of concentrations varied significantly across the three different concentrations χ^2 (df=2) = 30.71, p <.001. Between group comparisons, the Friedman test revealed that there were statistically significant conditions for wt% Ca at conditions of concentration of 0.01 M for 7 hours and 24 hours (p =0.016, p=00.7) respectively, concentration of 0.1 M for 1 hour (p=0.032), concentration of 1 M for 1 hour and 7 hours with p-value of (p=0.018 and p =0.050) respectively. Pairwise comparisons by the Wilcoxon signed-ranked test on different combinations of related groups with $\alpha = 0.006$ after Bonferroni adjustment further reveal no statistical significance in different conditions of immersion. The difference between the ranked temperature at 5°C and 15°C in concentration of 0.01 M for 24 hours (mean rank = 2.56) approached significance (p = 0.008) whereas in temperature at 5°C and 15°C for concentration of 0.01 M for 7 hours p=0.011. This effect size can be described as "large" with r = .89 and r=.85, respectively.

Table 6. Comparison of Ca at 3 different temperatures of MI immersions.

Condition	Friedman test[a]		Pairwise comparison[b]					
			5°C - 15°C		5°C - 25°C		15°C - 25°C	
	X^2 stats	P value	Z	P value	Z	P value	Z	P value
0.01 M, 1 Hour	1.543	0.462	-	-	-	-	-	-
0.01 M, 7 Hours	8.222	0.016*	-2.547	0.011**	-0.652	0.515	-2.429	0.015
0.01 M, 24 Hours	10.00	0.007*	-2.666	0.008**	-1.481	0.139	-2.254	0.024
0.1 M, 1 Hour	6.889	0.032*	-1.840	0.066	-0.889	0.374	-2.310	0.021
0.1 M, 7 Hours	0.667	0.717	-	-	-	-	-	-
0.1 M, 24 Hours	2.889	0.236	-	-	-	-	-	-
1 M, 1 Hour	8.00	0.018*	-2.073	0.038	-1.244	0.214	-2.312	0.021
1 M, 7 Hours	6.00	0.050*	-1.836	0.066	-2.073	0.038	-0.889	0.374
1 M, 24 Hours	2.667	0.264	-	-	-	-	-	-

[a] Friedman Test (within group analysis) , Statistically significant ($p \leq 0.05$)*
[b] Pairwise comparison by Wilcoxon Signed-Ranked Test with $\alpha = 0.006$ after Bonferroni adjustment. Approached significant**

4. DISCUSSION

Visual analysis of topographic showed changes in EG and ECG groups, various surface irregularities starting at 50x, 500x and 1000x magnifications. These changes showed a significant difference as compared to CG group (Ye et al., 2022). Further analysis through EDS analysis revealed that, element of calcium and chloride were noted on surface on MI which is correlated with other studies (Ding et al., 2020; Doe et al., 2020). MI smooth surface has been modified from the turned relatively smooth surface to the dominating moderately rough surfaces. Acid etching of MI surface followed by immersion of the MI in various $CaCl_2$ solutions done in this study did alter the surface roughness. The characteristics of an implant surface have generally been recognised as a crucial component in achieving and maintaining osseointegration (Baser & Ozel, 2023; Ravi, Duraisamy, Rajaram, Kannan, & Arumugam, 2023). $CaCl_2$ crystallisation can be detected mostly at the flute area of MI at the middle third of cutting edges. Even though there was no study specifying the location of surface treatment location. Coincidence with this study was that the majority location of surface roughness with topography changes can be seen at the flute area which will improve the roughness at the thread depth area. Roughened surface results in textured implants surfaces exhibiting more surface area for integrating with bone via osseointegration process. This process will increase surface area on the implant improves bone-to-implant contact after the implant placement (Tiwari, Rajamanickam, Jain, & Vas, 2023). Roughening the Ti surface induces an excellent bone cell response to the surface (Baser & Ozel, 2023; Jain, Ponnada, & Chandrasekhar, 2022; Tiwari et al., 2023). Acid etched surface is altered primarily in topographical aspects, whereas surface treatment methods alter the implant's topographic features and chemistry (Att et al., 2009; Ding et al., 2020; Ravi et al., 2023).

In this study, selection of $CaCl_2$ concentrations for MI surface treatment based on significant findings from previous in vivo studies. Studies conducted by Doe et al. and Jang et al (Doe et al., 2020; Jang et al., 2018) demonstrated the materials' substantial potential in inducing osseointegration, thereby enhancing the clinical success rate of MI. Additional research further supports the successful improvement in cell integration and aggregation, contributing to the overall promotion of osseointegration (Att et al., 2009; Ding et al., 2020; Jang et al., 2018). The highest level of Ca^{2+} in this study was at a concentration of 0.1 M at 5°C for 7 hours of immersion. Chloride ions were not a direct element of interest during bone formation. Due to its biocompatibility, chloride ion was important during initial bonding with calcium to ensure availability of calcium ions in cells surrounding (Hoshyari et al., 2016; Liu et al., 2017). This study showed that surface modification as modified calcium, a technique where a large number of OH-groups directly reacts with calcium ions. Therefore, calcium probably exists in the form of calcium hydroxide, $Ca(OH)_2$ (Doe et al., 2020). Ca^{2+} evident to be available in this study, on the MI surface confirmed by EDS analysis and titration analysis. Ca^{2+} is a divalent ion and it plays an important role in osseointegration especially in aged MI surface (Att et al., 2009). This occurs because, by immersing the titanium sheet in the $CaCl_2$ solution, the charge state on the surface is changed (Haraguchi et al., 2020). The divalent calcium ion can be used as a bridge ion to adsorb the negative charge on the surface of pure titanium (Doe et al., 2020).

Changing the charge state on the surface of the biological material can change the static electricity between the protein and the biological material in this environment where positive charges and negatively charged proteins to quickly form early adhesion through electrostatic attraction and accelerates the enhancement of early osseointegration of implants (Ding et al., 2020; Ogawa, 2014). Surface modification may change the chemical composition of MI surface known as osteoconductivity apart from hydrophilicity property. Osteoconductivity achieved by means of anodised surface with different charges results in aged titanium consisting of cells inert and cell attractive terminals due to bridging effect by Ca^{2+} achieved from the earlier immersion of our MI in $CaCl^2$ solution. Only divalent cations such as Ca^{2+} function as bridging agents to attract anionic proteins (Att et al., 2009). This is important, because a newly produced implant surfaces are filled with titanium cations that attract negatively charged cells and proteins (Att et al., 2009; Hori et al., 2010). However, carbon compounds sticking to the implant surface during storage reduce surface hydrophilicity. Therefore, preventing adhesion of carbon compounds using calcium chloride solution preserved the bioactivity of titanium, thereby improving protein adhesion and implant maintenance (Att et al., 2009; Jang et al., 2018).

The idea behind this surface modification was that because the coat and the bone share many chemical properties. It might be possible to create a chemical bond between them. Where such bonds are present, bioactive material was indicated as having the potential to hasten and accelerate the healing of the implanted bone. Numerous ions integrated into the implant surface, including Ca, P, Sr, F, NaOH, and Mg, have produced a potent bone response in numerous experimental trials. Incorporating modified calcium surface treatments does not alter the surface structure of MI and no major damage was observed on MI in SEM images after removal (Doe et al., 2020; Jang et al., 2018).

5. CONCLUSION

In our study, distinct surface topographic changes were observed among different groups of MIs. Notably, the surface treatment applied to MIs resulted in discernible alterations, suggesting an enhancement of the surface area conducive to osseointegration potential. Furthermore, the comprehensive physicochemical analysis underscored a significant presence of calcium and chloride ions by %wt on the MI surface. This highlights the MI as a readily accessible source of calcium ions crucial for the bone integration process during osseointegration. Importantly, our findings elucidate the impact of varying temperatures, concentrations, and immersion times on the availability of $CaCl_2$ on the MI surface. These insights contribute to a deeper understanding of the factors influencing osseointegration, providing valuable information for the development of enhanced implant surfaces.

https://doi.org/10.24191/cos.v12i1.5649

ACKNOWLEDGEMENTS/FUNDING

The authors would like to acknowledge the support of Universiti Teknologi MARA (UiTM), Selangor, Kampus Sungai Buloh and Faculty of Dentristry, Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia for providing the facilities and financial support on this research.

CONFLICT OF INTEREST STATEMENT

The authors would like to declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

Noor Ainnilwahida Alias carried out the research, wrote and revised the article. **Maryati Md Dasor Nornizar Anuar, Mizaton Hazizul Hasan, Nor Amlizan Ramli and Siti Nurul'ain Yusop**, conceptualised the central research idea and provided the theoretical framework. **Maryati Md Dasor, Saraswathy Devi Sinniah and Mohamed Ibrahim Abu Hassan** designed the research, supervised research progress; **Maryati Md Dasor** anchored the review, revisions and approved the article submission.

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6. APPENDIX

A. About the Authors

Noor Ainnilwahida Alias obtained her Doctor of Dental Surgery (DDS) in 2010 from Universiti Kebangsaan Malaysia, Kuala Lumpur, and her MSc in Dentistry (2020) from Universiti Teknologi Malaysia. Her dissertation includes several methods for surface treatment of mini-implants and cell culture studies for cytotoxicity. She has showcased her work both locally and internationally through posters and oral presentations. She is currently working with the Oral Health Programme, Ministry of Health Malaysia.