Comparative evaluation of surface roughness of three different dental implant abutments using atomic force microscopy: An in vitro study

C Femil Jilta, R Ravichandran, Harsha Kumar K, Vivek V Nair, Kavitha Janardanan and Zeenath

Abstract
The resistance of the periimplant tissues to inflammation is evidently much lower with the implant than that of natural teeth. In order to maintain periimplant health, and prevent implant failure, it is important to understand the relationship between abutment surface characteristics and plaque attachment. The aim of this study is to assess whether the surface roughness of three commercially available dental implant abutments are less than or equal to the clinical threshold value $R(a) = 0.2\mu m$ for bacterial retention that may eventually contribute to periimplantitis. Abutments from Adin, Nobel Biocare, and Myriad implant systems are sectioned into $2\times2$ mm dimension samples using High Speed Stainless cutting tools. To investigate the surface microstructure of the samples, three dimensional imaging and numeric analysis are done using AFM (Atomic Force Microscope). The results showed that the surface roughness values of the three groups Adin, Myriad and Nobel Biocare are less than the clinical threshold value $R(a) = 0.2\mu m$ and among them Myriad has the least surface roughness. Since the surface roughnesses are less than 0.2 $\mu m$, it was concluded that professionals can give preference to any of the abutments systems.

Keywords: Atomic force microscope, implant abutment, plaque, periimplant tissues, inflammation

1. Introduction
The success or failure of an osseointegrated implant depends on the surrounding supporting tissues, which not only anchor the implant to the bone but also have the important function of providing a protective soft tissue seal. The implant abutment pierces the oral mucosa and establishes a transmucosal connection between the external environment and the inner dental implant. This peri-implant soft-tissue barrier surrounding the abutment has been considered essential for long-term success of oral implants [1]. Clinical studies have reported a positive correlation between the surface roughness ($Ra$) of implant abutments and the rate of supragingival and subgingival plaque attachment [2, 3]. A maximum surface roughness of $R(a) = 0.2\mu m$ has been suggested as a threshold value for bacterial retention below this value no further reductions in plaque accumulation were observed while over this value biofilm accumulation increased with increasing surface roughness [4]. The resistance of the periimplant tissues to inflammation is also evidently much lower with the implant than that of natural teeth, with consideration to the nature of the structures surrounding the implant [5]. Hence longstanding inflammation does have a pronounced response in the peri-implant tissues, leading to failure of osseointegration as well [5, 6].

Therefore attention should be paid to the surface roughness of the abutments for a better clinical outcome. This study evaluates the surface roughness of three commercially available implant abutments of Nobel biocare, Adin, and Myriad implant systems which are found to be commonly used in the clinical practice in Kerala using an Atomic Force Microscope. Atomic force microscopy is arguably the most versatile and powerful microscopy technology for studying samples at nanoscale. It is powerful because an AFM can generate images at atomic resolution with nanoscale resolution height information, with minimum sample preparation.
2. Methods

2.1 Study design

Experimental in-vitro study design

2.2 Sample and Sampling Technique

Three brands of commercially available implant system abutments (fig 1). Nobel Biocare implant system (Nobel Replace abutment), Adin implant system abutment and Myriad implant system abutment were selected. The transmucosal part of the abutment was sectioned to 2×2 mm size section (fig 2) using high speed steel cutting tools (fig 3). Thirty three samples were selected for the study with eleven in each group.

2.3 Procedure

To investigate the surface microstructure, three dimensional imaging and numeric analysis were done using AFM (Atomic Force microscope) (make: park system xe 100) (fig 4). Initially the specimen was cleaned using isopropyl alcohol in order to clean all the debris present on the scanning surface. Then it was loaded on the sample holder with the help of a tweezer and stabilised using wax (fig 5). The sample holder was placed on the x y stage of the AFM. Using an optical microscope the surface of the sample was identified and then the AFM scanning probe (cantilever tip etched silicone cantilever) approached the surface at a particular frequency. The surface morphology of the samples were scanned at a scan area (15x15µm) to grasp as much of the entire surface of the abutment as possible.

Fig 1: (a) Myriad, (b) Adin (c) Nobel Biocare abutments

Fig 2: Single sectioned Abutment

Fig 3: High Speed Steel cutting tools

Fig 4: Atomic Force Microscopy

The investigation was carried out in the non-contact mode to prevent scratching on the surface of the sample. In non-contact mode the distance between sample and the tip is ~10 nm. The three dimensional images of the samples were obtained and numerical analysis was done using software (fig 6). The outcome of surface roughnesses of three brands of abutments are obtained in nm (nanometer) which are converted to micrometers.

Fig 5: Sample stabilised with wax
3. Results
Data analysis was done using computer Statistical Package For Social Sciences (SPSS) version 16.0 software. Mean and Standard Deviation of surface roughness, of all three groups Adin, Myriad and Nobel Biocare are 31.560 ±2.78 nm, 21.738±1.30 nm and 77.560±3.54 nm respectively. (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Minimum</th>
<th>Median</th>
<th>maximum</th>
<th>Mean</th>
<th>SD</th>
<th>Interquartile range</th>
<th>95% confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adin</td>
<td>11</td>
<td>29.126</td>
<td>30.456</td>
<td>36.625</td>
<td>31.560</td>
<td>2.780</td>
<td>5.193</td>
<td>33.42909- 29.69272</td>
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<td>Nobel Biocare</td>
<td>11</td>
<td>70.234</td>
<td>77.560</td>
<td>83.126</td>
<td>77.744</td>
<td>3.54</td>
<td>3.720</td>
<td>79.94089 34.80910</td>
</tr>
</tbody>
</table>

n= sample size, nm= nanometer
SD= Standard deviation

From the table it is found that the surface roughness of Nobel Biocare is maximum and Myriad has the minimum surface roughness. The probability distribution curve does not show a normal distribution so the non-parametric test Kruskal Wallis one-way ANOVA is used to compare surface roughness among the groups followed by two group comparisons by Mann-Whitney U test determine the statistical significance among the study groups. Kruskal Wallis one-way ANOVA among the study group showed highly significant differences (p-value < 0.001) in the surface roughness (Table 2)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>Kruskal Wallis one-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adin</td>
<td>11</td>
<td>30.456</td>
<td>31.560</td>
<td>2.780</td>
<td>df=2 p-value 0.0001*</td>
</tr>
<tr>
<td>Myriad</td>
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<td>22.082</td>
<td>1.300</td>
<td></td>
</tr>
<tr>
<td>Nobel Biocare</td>
<td>11</td>
<td>77.560</td>
<td>77.744</td>
<td>3.54</td>
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</tr>
</tbody>
</table>

*p-value <0.05 is significant

4. Discussion
The oral rehabilitation of partially or completely edentulous patients using dental implants has become a common practice over the last decade, with reliable long term results. The documented high survival rate of osseointegrated root form dental implants has led them to be a realistic treatment alternative. Even though there are successes over a 5 year period, 0 to 14.4% of the dental implants demonstrated periimplant inflammatory reactions which were associated with crestal bone loss that may eventually lead to the loss of an implant [7]. Periimplant inflammatory reactions results in the loss of the supporting bone associated with suppuration, increased probing depth, mobility and radiographic bone loss [8]. Mombelli [9] reported that failing implant sites were found with pocket depth greater than 6 mm with bleeding on
probing and suppurative when compared to successfully Osseo integrated implants. Lindhe [8] suggested that implants have a less effective natural tissue barrier than natural teeth and are less resistant to infection. The reliability of a stable soft tissue attachment has not been confirmed, and the permucosal seal may be just a circular fiber arrangement around the implant. Bacterial infections play an important role in the failure of dental implants. Bacterial flora which are associated with periodontitis and peri-implantitis, are found to be similar [9]. Studies have shown that the bacterial infection is the prime reason for peri-implantitis [11] and bacterial flora at the failing implant sites consist of gram-negative anaerobic bacteria including Porphyromonas gingivalis, Prevotella intermedia and Actinobacillus actinomycetemcomitans, which resemble the pathogens in periodontal disease [9]. In partially edentulous patients there are more microflora surrounding implants and the teeth. However, there is a marked reduction in the number of periodontal pathogens around the implants in completely edentulous patients. It is because the natural teeth may serve as reservoirs for periodontal pathogens from which they may colonize the implants in the same mouth [12]. Kohavi [13] suggested when exposed in the oral cavity through the transmucosal abutment, an Osseo integrated implant provides a favorable surface for bacterial colonization. There are various factors that influence the attachment of oral bacteria to the implant surface, which includes surface roughness, surface free energy and hydrophobicity [14]. Among them the surface roughness plays an important role in bacterial adherence [15]. In an in vivo study, a smooth titanium abutment and a sandblasted titanium surface were evaluated for the biofilm accumulation. The results revealed that surface roughening harbored a lower percentage of the coccoid cells (64.2%) as compared to the smooth abutments (81%) [2]. In yet another previous study, Quirynen et al. [16] in a 96 hour supragingival plaque formation reported a positive correlation between the surface roughness and the plaque growth rate and pathogenicity. These studies show an important fact that the surface roughness has a significant contribution for the increased plaque accumulation. Bollen [17] indicated that a further reduction of the surface roughness, below a certain threshold R(a) 0.2 microns, has no major impact on the supra- and subgingival microbial composition. Quirynen [18] showed that a reduction in surface roughness less than a roughness of 0.2 micron had no major effect on the microbiologic composition, supragingivally or subgingivally. Surface roughness R(a)>0.2 μm leads to an increased rate of the biofilm formation and hence acts as the main etiology for the breakdown of the peri-implant soft tissue barrier. However, R(a)<0.2 μm has no impact on the quantity and quality supra and subgingival plaque formation. It has further been reported that R(a)<0.02 μm has further no quantitative or qualitative effect on the nature of the microflora. Liarimondini et al. [19] studied on the effect of surface roughness on early in vivo plaque colonization on titanium surface and concluded that a titanium surface with R(a) less than equal to 0.088 μm strongly inhibits accumulation and maturation of plaque at the 24-hour time period and recommended that such smoothness to be achieved in transgingival and healing implant components. Roughened abutments greater than 0.8μm result in a dramatic increase in sub gingival plaque 25 times greater as well as in its pathogenicity [16]. Even though there are many studies supporting the relationship between plaque and periimplantmucositis, there are also some controversial studies showing no relationship between plaque attachment and soft tissue reaction. Abrahamsson et al. [20] studied the soft tissue reactions to plaque formation at implant abutments with different surface topography in dogs and showed that the different surface characteristics of abutment made of commercially pure titanium failed to influence plaque formation and the establishment of inflammatory cell lesions in the periimplant mucosa. Similarly Wennberger [21] conducted an in vivo study on some soft tissue characteristics at implant abutments with different surface topography and reported that no relation was found between inflammatory response and abutment surface roughness after an evaluation time of 4 weeks in a human test model. On considering the studies supporting the relationship between plaque and periimplant soft tissue health, the current study compares whether the surface roughness of three commercially available dental implant abutments which are commonly used in Kerala are less than or equal to the clinical threshold value (0.2μm). To investigate and characterize surface topography, different studies have used various technologies like Scanning Electron Microscope (SEM), Confocal Laser Scanning Profilometer, Atomic Force Microscope. In the present study, we have used Atomic Force Microscope (AFM) for high resolution analysis at nanometer scale level. It provides high resolution imaging of surface structure combined with quantifying surface topography without any sample preparation. Studies comparing the surface roughness of abutments of various implant systems which are commonly used like Branemark, Nobel Biocare, Astra, Steri-Oss, IMZ have been done in Japan where we have selected three implant system abutments which are currently used widely in Kerala. The mean surface roughness values of the three groups Adin, Myriad and Nobel Biocare are .031μm, .021 μm, .077 μm respectively. Among the comparison groups, it is found that the surface roughness R(a) of Myriad is comparatively lesser than the other two groups . Myriad has the least surface roughness and Nobel Biocare has the maximum surface roughness. All R(a) values are way below 0.2 μm, which is suggested as the threshold roughness necessary to avoid a harmful influence of plaque accumulation. So any of the brands can be used as a reliable abutment for the clinical purpose. Since the roughness value of all the three abutments are <.088 μm, according to Liarimondini [19] all three brands inhibit the colonization and maturation of the plaque. On comparing the three groups the p value is <0.001 showing that the study is highly significant. Statistical analysis for comparison between two groups of three different combination also shows that the p value as <0.001 proving that the study is highly significant and the surface roughness of Nobel Biocare is more compared to Adin and Myriad, and the roughness of Adin is more when compared to Myriad. The study also has limitations like it is not an invivo study, and other parameters like surface features of the irregularities have not been carried out. Studies suggested that implants have a less effective natural tissue barrier than natural teeth and are less resistant to infection [22]. Based on the observations from the previous studies, it can be concluded that implants are more vulnerable to failure due to infection than natural teeth so it is very essential to control factors that promote bacterial attachment. Hence it is always safe and better to select abutment which has the highest smoothness and make sure that patients maintain good oral hygiene practices.

5. Conclusion
From the above study the following conclusions are drawn.

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1. The surface roughness values of all three abutments of Adin, Myriad, and Nobel Biocare implant system are less than the clinical threshold roughness value R(α)0.2 μm for plaque accumulation so any of the abutment systems can be preferred.

2. As implants are more vulnerable to failure due to infection than natural teeth, it is very essential to control factors that promote bacterial attachment. Hence it is always safe and better to select abutment which has the highest smoothness.

6. Abbreviations

<table>
<thead>
<tr>
<th>AFM</th>
<th>Atomic Force Microscope</th>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>R(α)</td>
<td>Surface roughness in terms of amplitude</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscope</td>
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<tr>
<td>SPSS</td>
<td>Statistical Package For Social Sciences</td>
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<tr>
<td>nm</td>
<td>nanometer</td>
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<tr>
<td>μm</td>
<td>micrometer</td>
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<tr>
<td>n</td>
<td>Sample size</td>
</tr>
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7. Acknowledgement

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8. Reference


