The Effect of Green Tea Polyphenol (EGCG) on Orthodontic Micro-Implant Stability: An experimental study

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Abstract

Background Increasing the orthodontic micro-implant stability and enhancing bone response around it, is a fundamental objective of successful therapy. Materials and Methods Thirty orthodontic micro-implants, 1.3 mm in width and 5 mm in length (Dentos, AbsoAnchor, South Korea), were used with three implants were inserted in the right tibias of ten New Zealand rabbits, the rabbits categories into two groups, five each. The primary stability was measured once the implant inserted, using Periotest. The experimental group fed (0.5% wt/vol/day) Green Tea, using the loading technique, for four weeks interval. At the end of the experimental period, the rabbits were sacrificed, and the secondary stability measurement was taken. Results Using paired t-test, the results showed a significant increase in secondary stability for the experimental group after four weeks experimental period when compared with the primary one. While the control group showed a non-significant increase in the secondary stability compared with the primary one. Conclusion This study concluded that the Green Tea could enhance bone response around orthodontic micro-implant within four weeks healing period and consequently increase the stability of orthodontic micro-implant

Key words: Green Tea, Micro-Implant, Stability.

Introduction

Anchorage is a daily problem in clinical orthodontic practice and, considered one of the most limiting factors of the orthodontic therapy (Luzi et al., 2007). Further predictable and satisfactory results were achieved with the use of orthodontic micro-implants (Baumgaertel et al., 2008). The success of the micro-implants depends on achieving and maintaining their stability throughout the orthodontic treatment (Wilmes et al., 2006). The bone remodeling process after insertion of a micro-implant needs time in order to provide a suitable support for the micro-implant (Rebaudi et al., 2011).

Many attempts to improve the bone response around the micro-implant, such as laser etching and micro arc oxidation (Guo et al., 2010), ultra violet (UV) treatment of implants surfaces (Gao et al., 2013), and chemically modified implant surfaces (Feller et al., 2015). Various studies proved the positive effects of the natural products on bone response, Green Tea considered one of the natural products that has several health benefits (Johnson et al., 2012, Chacko et al., 2010 and Cabrera et al., 2006). Green
Tea is rich in polyphenol flavonoids including (-)-epigallocatechin-3-gallate, (-)-epigallocatechin, (-)-epicatechin-3-gallate, and (-)-epicatechin (Byun et al., 2014). Epigallocatechin-3-gallate (EGCG) is the most abundant and potent green tea catechin. Epigallocatechin-3-gallate has been extensively studied for its beneficial health effects as a nutriceutical agent; EGCG is beneficial for bone regeneration and has a promising treatment strategy for bone repair therapies (Wang et al., 2016). Green tea bioactive components among these EGCG favor bone formation and suppress bone resorption (Shen et al., 2009).

Different methods used to assess implant stability among those: surgeon’s perception, insertion torque, seating torque, reverse torque, radiographs, percussion testing, periostest, implant mobility checker, and resonance frequency analyzer (Digholkar et al., 2014, Agarwal et al., 2016, Meredith et al., 1996, Mathieu et al., 2014 and Atsumi et al., 2007). The Periotest offers a reliable method of diagnosing micro-implant status by measuring levels of subclinical mobility in a reproducible manner (Van and Wilson, 1991).

According to the research published, scarce information available regarding the effect of natural products uses on bone response to the implant with special regards to micro-implants. This research hypothesized that green tea could enhance bone remodeling around orthodontic micro-implants in a period of four weeks through increasing implant secondary stability compared to the control group.

**Aim**

To determine the effect of green tea (EGCG) on the bone response around orthodontic micro-implant.

**Materials and Methods**

The experimental animals used in this research were ten, 8-10 months healthy New Zealand rabbits with a weight range between 2-2.3 Kg. The rabbits were distributed into two groups, five each, experimental and control. Each rabbit put in an individual cage under observation and supervision of the veterinarian for four weeks before the experiment to monitor the behavior, eating standard quantity and quality of diet, drinking, defecation, weight monitoring and activity. At the time of the surgery, each experimental animal was pre-medicated with an intramuscular injection of 0.2 ml/kg B.W. of Ketamine 10% (anesthetic solution) and 0.025ml/kg B.W. of the Xylazine 2% (muscle relaxant drug), this dose kept the animal anesthetized for about 1 hour.

A thirty sterile orthodontic micro-implants (Dentos Inc. 1-5, Galsan-Dong, Dalseo-Gu, Daegu, Korea 704-900) made of Ti-6Al-4V alloy; 1.3mm diameter and 5mm length were used. The site where the orthodontic micro-implants inserted, the medial surface of the right Tibia, was further anesthetized by 1ml of local anesthetic solution 2% Xylocaine (Mapara et al., 2012). After the anesthesia was confirmed, the hair at surgical site was shaved off; the exposed skin disinfected with 10% Povidone Iodine with sterile surgical gauze. An incision approximately 35 mm in length (Figure 1) down in the skin parallel to the longitudinal axis of the tibia, in the dorsal aspect, was performed. After blunt dissection of fascia and muscle, the periosteum was stripped and elevated denuding the bone on the medial aspect of the tibia.
Three small holes (Figure 2) approximately 10 mm apart were prepared, with a 1 mm rounded head drill, using implant surgical engine (Surgic XT – NSK/ U.K), at a rotational speed 1100rpm on «FWD-»< clockwise rotation, and a torque of 35newton/cm (Al-Sultan and AlKhatib, 2015). The micro-implants were inserted into the prepared holes keeping their long axes perpendicular to the external cortical tibia as possible (Figure 3). The primary stability of the inserted implants was tested using a Periotest M (Medizintechnik Gulden–Germany). During measurements, the Periotest M device held horizontally, with the starting bottom up, and the hand-piece long axis directed perpendicular to the micro-implant head with the tip of the hand piece kept at a distance about 2-3 mm from the micro-implant head (Figure 4). Three readings for each micro-implant were calculated (Mortensen, 2007). Then the deep fascia and skin repositioned in its place and sutured with an absorbable suture (Braided polyglycolic suture) (figure 5).
After recovery, the experimental animals carried to their cage, post-surgical medication includes a daily single dose of antibiotics Pen Vet 300 0.1ml/ rabbit (Procaine Benzyl penicillin, Alfasan International B.V., Woerden, Netherlands) given intramuscularly. The experimental animal feeding protocol was started at the day of surgery and continued for four weeks. The green tea dose of 0.5% wt/vol (Teavigo 90% EGCG, 186 mg, Swanson health products, USA) was used, the selection of which is equal to 400 mg/kg BW, (Shen et al., 2008) the selected dose was dissolved in five ml distilled water for each rabbit, and given as a loading dose by special oral gavage needle fitted to ordinary disposable syringe.

At the end of experimental period (four weeks), the experimental animals were sacrificed with an over does of ketamine hydrochloride (Jimbo et al., 2013). A full thickness flap reflected exposing the micro-implant, and the secondary stability reading using Periotest M was taken by the same technique followed as that of the primary one.

The statistical analysis of this study was done using SAS system.

**Results**

The descriptive statistics of the primary stability of the control (Pr.C) and experimental (Pr.GT) groups are shown in the Table (1). While the descriptive statistics of the secondary stability for the control (Sc.C) and experimental (Sc.GT) group are shown in the Table (2).

Table 1: Descriptive Analysis (Mean, Standard Deviation, Standard Error, Minimum and Maximum Values) for the Primary Stability

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Err.</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pr.C</td>
<td>15</td>
<td>-2.7333</td>
<td>0.4467</td>
<td>-3.4000</td>
<td>-1.9000</td>
</tr>
<tr>
<td>2</td>
<td>Pr.GT</td>
<td>15</td>
<td>-2.8600</td>
<td>0.4372</td>
<td>-3.9000</td>
<td>-2.000</td>
</tr>
</tbody>
</table>

Table 2: Descriptive Analysis (Mean, Standard Deviation, Standard Error, Minimum and Maximum Values) for the Secondary Stability

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Err.</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sc.C</td>
<td>15</td>
<td>-2.8067</td>
<td>0.8498</td>
<td>-4.1000</td>
<td>-1.4000</td>
</tr>
<tr>
<td>2</td>
<td>Sc.GT</td>
<td>15</td>
<td>-5.5200</td>
<td>0.3448</td>
<td>-6.2000</td>
<td>-5.1000</td>
</tr>
</tbody>
</table>

The Paired t-test results showed a non-significant differences between the values of primary (Pr.C) and secondary (Sc.C) stability of the control group, while of the Green Tea group showed a highly significant increase (P<0.0001) in Periotest values for the secondary stability (Sc.GT) when compared with the primary one (Pr.GT), as shown in
Table 3: Comparison between Primary and Secondary Stability for the Control and Green Tea Group

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Pr.C vs. Sc.C</td>
<td>0.7684</td>
</tr>
<tr>
<td>2 Pr.GT vs. Sc.GT</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Discussion

Maintaining the stability of micro-implant to withstand an orthodontic load without fear of anchorage misplace considered a challenge for orthodontist. After four weeks healing period, the control group showed a non-significant increase in the secondary stability when compared to the primary one, which is possibly due to the insufficient duration of the healing period. This result is comparable with the results of Serra et al., (2010), who concluded that bone contact and bone area percent were very few in 1 or 4 weeks period. The results are possibly agreed with the Shin et al., in (2012), who concluded that maximum removal torque of orthodontic micro-implants was slightly higher in the 6-week group than in the 1-week group, but statistically non-significant.

A significantly higher secondary stability value in the experimental group could be explained by the effect of Green tea polyphenol supplementation which had been proved to increase the percentage of bone mineral density and strength (Shen et al., 2012). Epigallocatechin-3-gallate (EGCG) is the major polyphenol in green tea (Lee et al., 2010), which evidenced to have the most potential anti-oxidation effect (Liao et al., 2001). It has been reported that EGCG can induce the apoptotic cell death of osteoclasts (Nakagawa et al., 2001). Others have shown that EGCG inhibits osteoclast formation (Yun et al., 2004). A study conducted on rat, in which 0.5% wt/vol of Green tea polyphenols have been shown to improve bone mass and bone microarchitecture (Shen et al., 2008).

The consumption of green tea, a rich source of (EGCG), has been found to be accompanied with increased bone mineral density, however; the straight effects of such flavonoid on mineralization of bone still unknown (Vali et al., 2007). Further results demonstrated that EGCG can increase the potential of osteoblastic terminal differentiation by increasing alkaline phosphatase activity, and finally stimulating mineralization (Chen et al., 2005). These above findings possibly could support the research results.

Among the study limitations, the dose of green tea used may be considered comparatively high if attempts are made to use it in the human diet, the micro implants in the current study were not loaded as in usual orthodontic treatment. In addition, based on the condition of OMSIs exposure, the closed method which has been used in this study is not compared with the open method that makes OMSIs faces the oral cavity environments. This study only involved biomechanical study. Histopathological examination was not included in the study for logistic reasons.
Conclusion
It could be concluded that Green tea polyphenols (EGCG) could enhance bone response around orthodontic micro-implants that may add to increase in its stability.

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Conflicts of Interest
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References


