Physicochemical and osteogenic properties of chairside processed tooth derived bone substitute and bone graft materials

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INTRODUCTION

Alveolar bone grafting has emerged as an integral part of a comprehensive oral rehabilitation process that by restoring the lost architecture of resorbed edentulous ridge provides improved form, function and esthetics to the patient1-5. The bone grafting materials that are currently being used in dentistry include autogenic, allogenic, xenogeneic bones, and synthetic or alloplastic materials6-9. Autogenous bone graft has excellent osteogenic properties and the ability of rapid healing with the least immune rejection. However, limited availability, donor site defect, and morbidity, patient discomfort in cases of distant extraoral grafts have hindered its use. This has prompted many clinicians to use an allograft, xenograft, or synthetic bone graft materials since these materials have provided satisfactory results6. Nevertheless, allografts, xenografts, and alloplastic materials also possess some drawbacks. Allografts are deemed expensive; may pose risks of infection and most of the donor's information is restricted or inadequate. Xenografts in addition to these could also be a source of zoonotic diseases5. Xenografts have also raised some ethical and religious concerns and hence their use should be considered for different religions and individuals7-8. Synthetic or alloplastic materials lack osteogenic and osteoinductive properties. These shortcomings have led to finding a novel source of autogenous bone graft processed from a human tooth.

The resemblance in the composition of dental structures especially dentin and cementum with alveolar bone has driven the use of teeth as a potential graft material10-13. X-ray diffraction analysis (XRD) have confirmed the presence of hydroxyapatite (HA), and small amounts of tricalcium phosphate (TCP), amorphous calcium phosphate (ACP), and octacalcium phosphate (OCP) in a different area of the tooth11-13. The surface of the autogenous tooth has shown homogenously scattered dentinal tubules with a visible dense collagen matrix while the phase of calcium phosphate apatite showed extensive calcium dissolution, similar to autogenous bones12. Another remarkable property of dentin and cementum is the presence type I, type III collagen and of several growth factors including BMP, IGF-II, cementum is the presence type I, type III collagen and of several growth factors including BMP, IGF-II, and TGF-β, which play a major role in promoting bone remodeling14-17. Majority of proteins found in bone such as osteopontin (OPN), osteocalcin (OC), bone sialoprotein (BSP), osterix, type I collagen, and Cbfa1 (Runx2) have also been identified in dentin, which could make it a viable alternative for alveolar grafting since these proteins are reportedly involved in bone formation and resorption18-21. Numerous animal studies have shown the potential of processed dentin as a viable grafting material for preserving the alveolar bone18,22. Besides,
the application of demineralized dentin matrix (DDM) was reported to induce new bone formation as early as 4 weeks than non-demineralized dentin or calcified dentin after implantation\(^{14,23}\).

Although several studies have shown the effectiveness of tooth as a grafting material, the procedure may be time-consuming, requiring specialized skill and laboratory processing\(^{24,25}\). Hence, the TDBS processed in our study is a complete non-demineralized whole tooth which is different from other studies. Therefore, in this study, we performed physicochemical and biological properties compared with allograft, xenograft and synthetic bone, as well as microbial analysis of bacterial contamination of immediately extracted and chair-side processed TDBS.

**MATERIALS AND METHODS**

**Ethics statement**

This study is laboratory-based, although human teeth and bone samples that would be regarded as clinical waste were collected and the whereabouts of the donors were kept confidential. Thereby, this study was conducted without the violation of any human rights and ethics. All methods and experimental protocols were approved and carried out per the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board (COE.No.MU-DT/ PY-IRB 2017/044.2010).

**Bone grafts materials**

Allografts (OraGRAFT\(^{\text{®}}\), LifeNet Health, VA, USA) (DO BONE\(^{\text{®}}\), Biotem, Seongnam-si, Korea), Xenograft (BioOss\(^{\text{®}}\), Geistlich Pharma, Wolhusen, Switzerland), Alloplast (BoneCeramic\(^{\text{®}}\), Straumann Holding, Basel, Switzerland). Human tooth and mandibular ramus bone that would be discarded as clinical waste (Table 1).

**Inclusion and exclusion criteria**

Twelve teeth extracted for orthodontic treatment, embedded as well as impacted teeth from non-smokers and non-alcoholic patients aged between 18 and 60 years were included for preparing TDBS. After acquiring the consent, a discarded piece of mandibular ramus bone was obtained from two healthy patients undergoing orthognathic surgery. Patients with infectious disease such as Hepatitis B, Hepatitis C or HIV that could be transmitted through tooth or bone; teeth with large restorations, extensive caries, periodontitis, endodontically treated with prosthetic crown, malformation or congenital anomalies such as enamel hypoplasia, fluorosis, amelogenesis imperfecta, dentinogenesis imperfecta, and dentin dysplasia were excluded from our study.

**Tooth processing**

The TDBS was prepared following the Smart Dentin Grinder\(^{\text{®}}\) protocol (SDG\(^{\text{®}}\), KometaBio, USA) without any modifications. Immediately after extraction, the tooth was cleaned with a high-speed bur to remove any soft tissue and debris. Completely cleaned tooth with both crown and roots was air-dried and placed in SDG\(^{\text{®}}\). The grinding and sorting yielded the particles between 300–1,200 \(\mu\)m (Fig. 1a). The particulates were then placed in the solution provided by the manufacturer for 10 min. Solution 1 is basic alcohol, comprised of 0.5 M of NaOH and 30% alcohol. The particulates were then washed in solution 2, which is sterile phosphate-buffered saline (PBS), for 3 min. The entire process of the chemical

<table>
<thead>
<tr>
<th>Grafts used</th>
<th>(n)</th>
<th>Brand and company name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allograft</td>
<td>2</td>
<td>OraGRAFT(^{\text{®}}) (LifeNet Health, VA, USA)</td>
</tr>
<tr>
<td>Xenograft</td>
<td>2</td>
<td>BioOss(^{\text{®}}) (Geistlich Pharma, Wolhusen, Switzerland)</td>
</tr>
<tr>
<td>Alloplast</td>
<td>2</td>
<td>BoneCeramic(^{\text{®}}) (Straumann Holding, Basel, Switzerland)</td>
</tr>
<tr>
<td>Autogenous bone</td>
<td>2</td>
<td>Mandibular ramus</td>
</tr>
<tr>
<td>Human tooth</td>
<td>12</td>
<td>—</td>
</tr>
</tbody>
</table>

![Fig. 1](image_url) Physical characteristics of (a) TDBS preparation using dentin grinder; (b) SEM image of TDBS at ×25 and (c) SEM image at ×50
Microbial analysis
After extraction, the tooth was cleaned using a high-speed bur to remove soft tissue and calculus, then dried and transported to the microbiology laboratory in the sterile test tubes. Samples reached the laboratory within 10 to 15 min and the sample processing commenced immediately. A tooth was ground in the hood (NU-440-600E, NUAIRE™, MN, USA) and before chemical treatment, 0.10 g of tooth particulates was transferred to the test tube containing 2 mL of Reduced Transport Fluid (RTF). Two hundred µL was pipetted to 1.8 mL RTF and 10-fold dilutions were further carried out (10⁻¹ to 10⁻⁸). One hundred µL of the supernatant from each dilution was spread on anaerobic basal agar (Oxoid, CM0972, UK) supplemented with 5% sheep blood (ABA) for isolation of anaerobic and facultative anaerobes. All ABA was anaerobically incubated at 37°C for 72 h.

The remaining tooth particulates were subjected to chemical treatment with solutions 1 and 2, then 0.10 g of the tooth particulates underwent the same procedure as mentioned earlier. The efficacy of decontamination with the chemical treatment was also confirmed by incubating the tooth particulates in anaerobic basal broth (ABB). After the incubation period, bacterial growth was observed. Colony-forming units (CFU) per gram of the tooth sample was calculated using the formula from the previous study by Kürkçü et al.²⁷.

Osteogenic properties
1. hFOB cell culture
An established human fetal osteoblastic cell (hFOB 1.19) line was purchased from American Type Culture Collection (ATCC), maintained according to the guideline, in Dulbecco's modified Eagle medium (DMEM) (Hyclone, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin-streptomycin (RTF). Two hundred µL was pipetted to 1.8 mL DMEM supplemented with 2% FBS. Two hundred µL hFOB in 100 µL DMEM supplemented with 2% FBS were seeded into the filter of the upper chamber (pore size 0.8 µm) of the transwells. 100 mg TDBS or allograft from the human cortical bone (DO BONE®) or none (blank control) were added to the lower chambers with 600 µL of the same medium. After 3-day and 5-day incubation, the migrated hFOB located at the bottom part of the upper chamber were fixed with 4% paraformaldehyde and stained with crystal violet for 10 min. The optical density (OD) of each well was measured with a microplate reader (Bio-Tek, USA) at wavelength 570 nm. The migration was performed independently in triplicate.

2. Osteoblast migration
Cell migration assay was evaluated using a 2-chamber Transwell system (Corning, USA). Briefly, 1×10³/well hFOB in 100 µL DMEM supplemented with 2% FBS were seeded into the filter of the upper chamber (pore size 0.8 µm) of the transwells. 100 mg TDBS or allograft from the human cortical bone (DO BONE®) or none (blank control) were added to the lower chambers with 600 µL of the same medium. After 3-day and 5-day incubation, the migrated hFOB located at the bottom part of the upper chamber were fixed with 4% paraformaldehyde and stained with crystal violet for 10 min. The optical density (OD) of each well was measured with a microplate reader (Bio-Tek, USA) at wavelength 570 nm. The migration was performed independently in triplicate.

3. Enzyme-linked immunosorbent assay of released bone morphogenetic protein-2 (BMP2)
Three samples of 100 µg of each TDBS and allograft was added into 24-well plate containing 600 µL of the same conditioned medium with cell migration experiment, incubated at 34°C for 1, 3, 5, 10 days. At day1, 500 µL...
medium from each well was collected and kept under −80°C for further measurement. The new 500 µL of the same medium was added into the graft materials. The same procedure was repeated, and the supernatant was collected until day10. The BMP2 level was detected using Enzyme-linked immunosorbent assay (ELISA) kits (Sigma-Aldrich, USA) according to manufacturing protocols.

**Statistical analysis**
All data were expressed as the mean±standard deviation (SD). Statistical Package for the Social Sciences statistical software (SPSS, USA) was used in this study. The migration assays were analyzed by repeated measures analysis of variance (ANOVA) and Tukey's test for individual comparison. The BMP2 release was analyzed by unpaired t-test analysis after the data distribution test. The level for statistical significance was set at *p*<0.05.

**RESULTS**

In total 12 teeth from 12 different patients and mandibular ramus from 2 patients were taken for this study. However, for physicochemical analysis 6 teeth were randomly chosen and analyzed, the results demonstrated that all samples from TDBS showed the same tendency.

**Scanning electron microscopy (SEM) analysis**
The surface characteristics of TDBS as seen in lower magnification is depicted in Figs. 1b and c. TDBS surface structure before chemical treatment under SEM from longitudinal and cross-sectional views, in lower and higher magnifications, is shown in Figs. 2a, b, e, and f. The longitudinal section showed a regular pattern of dentinal tubules with some organic contents occluded between the tubules. These occlusions make the dentinal tubules pattern unclear. This observation was more obvious and clear under SEM before the chemical treatment of TDBS as shown in Figs. 2a and b. Similar observation applied to the cross-sectional segment of the TDBS (Figs. 2e and f). The crushing force of the dentin grinder did open the dentinal tubules; however, it was after the chemical treatment that the tubules were visible and appeared homogenous as shown in Figs. 2c, d, g, and h. In higher magnification, the area surrounding the dentinal tubules had an irregular and coarse surface structure as seen after the chemical treatment. Although, before chemical treatment dentinal tubules appeared more occluded, the surrounding was smoother as shown in Figs. 2a, b, e, and f.

The crushed autogenous ramus blocks from both patients showed the same tendency under SEM, which are homogenous wave and honeycomb-like pattern. At higher magnification, the honeycomb had various sizes with different depths and no occlusion was seen (Figs. 3a and b). Bio-Oss® showed a compact and regular arrangement of a small tubular structure. Braided rope-like patterns were observed in higher magnification (Figs. 3c and d). The Oragraft® is a mineralized ground cortical hence the pattern showed many Fmicron-and nano-topographies. However, the pattern was even somewhat like the collagen fibril arrangement seen in the SEM of bone (Figs. 3e and f). BoneCeramic® surfaces had the smoothest surface among all the samples. The surface showed two distinct patterns one looked like moon surface with crater and the other as seen in higher magnification had smooth but raised surface giving a cobblestone-like appearance (Figs. 3g and h).
Fig. 3  Different representative bone graft materials as seen under SEM. (a) Low (×500) and (b) high (×2,000) magnification of ramus bone; (c) Low (×500) and (d) High (×2,000) Bioss®; (e) Low (×500) and (f) high (×2,000) magnification of OraGRAFT®; (g) Low (×500) and (h) high (×2,000) magnification of BoneCeramic®

Energy dispersive X-ray spectroscopy (EDS) analysis
All grafting materials contained oxygen (O), carbon (C), calcium (Ca), phosphate (P), sodium (Na) and magnesium (Mg) with different percentage, except BoneCeramic® which had no Na and Mg in its configuration (Table 2). The chemical composition of TDBS showed that O accounted for 50.59% of its configuration with 21.98% (C), 15.71% (Ca) and 9.82% (P). Trace amounts of Na and Mg were also detected in TDBS at 1.36% and 0.55%, respectively. The composition of mandibular ramus bone was observed with the highest concentration of C at 41.70% and O at 40.05%. OraGRAFT® had C and O at 34.02% and 34.39%, respectively, while the Ca/P ratio was the highest level at 2.17. Bio-Oss® had the highest composition of O (53.99%) while BoneCeramic® showed the highest levels of Ca at 30.62% and P at 16.68% with Ca/P ratio of 1.83. In addition, the Ca/P ratio of TDBS is 1.60 similar to mandibular ramus bone (1.58) and Bio-Oss® (1.63).

X-ray diffraction (XRD) analysis
The XRD analysis was used to determine the crystal structure and crystallinity of the bone graft materials (Fig. 4). TDBS contained HA and OCP, which was also found in Bio-Oss®, OraGRAFT®, and mandibular ramus bone (Figs. 4b–d). Nevertheless, BoneCeramic® had only HA and TCP (Fig. 4a). The patterns of XRD analysis from TDBS and Bio-Oss® were similar, and on closer inspection, both had broad peak patterns giving them some resemblance to OraGRAFT® and mandibular ramus bone. The Scherrer equation was applied to assess the crystalline size of each bone grafts materials and the result showed that BoneCeramic® with the narrowest peak displaying a very sharp pattern had maximum 2-Theta ° at 31.81 and FWHM at 0.1918 (Fig. 4a). TDBS had second narrowest peak with maximum 2-Theta ° value at 31.79 and FWHM at 0.2735, followed by Bio-Oss® showed maximum 2-Theta ° at 31.90 and FWHM

Table 2  Chemical composition (atomic %) and respective Ca/P ratio of representative samples (Mean±SD) as analyzed by SEM-EDS of different bone graft materials

<table>
<thead>
<tr>
<th>Elements</th>
<th>TDBS</th>
<th>Mandibular Ramus (Autogenous)</th>
<th>OraGRAFT®</th>
<th>Bio-Oss®</th>
<th>Bone Ceramic®</th>
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<tr>
<td>C</td>
<td>21.98±5.85</td>
<td>41.70±6.97</td>
<td>34.02±8.68</td>
<td>11.30±3.23</td>
<td>7.20±2.54</td>
</tr>
<tr>
<td>O</td>
<td>50.59±5.02</td>
<td>40.05±3.52</td>
<td>34.99±4.95</td>
<td>53.99±7.52</td>
<td>45.50±12.75</td>
</tr>
<tr>
<td>Ca</td>
<td>15.71±7.14</td>
<td>10.70±5.33</td>
<td>21.28±8.56</td>
<td>20.99±7.64</td>
<td>30.62±11.48</td>
</tr>
<tr>
<td>P</td>
<td>9.82±3.15</td>
<td>6.78±2.70</td>
<td>9.79±2.83</td>
<td>12.85±2.98</td>
<td>16.68±3.29</td>
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<tr>
<td>Na</td>
<td>1.36±0.19</td>
<td>0.55±0.11</td>
<td>0.38±0.04</td>
<td>0.38±0.05</td>
<td>0</td>
</tr>
<tr>
<td>Mg</td>
<td>0.55±0.09</td>
<td>0.21±0.09</td>
<td>0.20±0.09</td>
<td>0.50±0.07</td>
<td>0</td>
</tr>
<tr>
<td>Ca/P ratio</td>
<td>1.60</td>
<td>1.58</td>
<td>2.17</td>
<td>1.63</td>
<td>1.83</td>
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</table>
Fig. 4 The results of XRD analysis showing different crystalline structures and maximum 2-Theta° present in (a) BoneCeramic®; (b) Bio-Oss®; (c) Oragraft®; (d) mandibular ramus bone; (e) TDBS

Ca/P ion dissolution test using Inductively coupled plasma mass spectrometer (ICP-MS) and Ion chromatography (IC)

The dissolution of Ca from TDBS and mandibular ramus bone were gradually increased with time and showed an accumulative value of 0.59 mg/L and 0.47 mg/L, respectively. Oragraft®, on the other hand, showed an extensive calcium dissolution at the early stage, with a decreasing trend. These grafts showed much higher total calcium dissolution in comparison to Bio-Oss® and BoneCeramic®. Meanwhile, the dissolution of P from all materials including TDBS, measured on days 3, 7, and
Table 3 Calcium ion (mg/L, Mean±SD) and phosphorus ion (%) dissolution of each bone graft materials at day 3, 7 and 14

<table>
<thead>
<tr>
<th>Day</th>
<th>BoneCeramic®</th>
<th>Bio-Oss®</th>
<th>OraGRAFT®</th>
<th>Mandibular ramus bone</th>
<th>TDBS</th>
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<tr>
<td></td>
<td>Ca</td>
<td>P</td>
<td>Ca</td>
<td>P</td>
<td>Ca</td>
</tr>
<tr>
<td>3</td>
<td>0.095±0.007</td>
<td>0.885±0.021</td>
<td>0.060±0</td>
<td>1.005±0.007</td>
<td>0.105±0.120</td>
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<tr>
<td>7</td>
<td>0.125±0.007</td>
<td>0.850±0</td>
<td>0.060±0</td>
<td>0.855±0.007</td>
<td>0.175±0.007</td>
</tr>
<tr>
<td>14</td>
<td>0.090±0</td>
<td>0.845±0.007</td>
<td>0.090±0.014</td>
<td>0.530±0.014</td>
<td>0.13±0.014</td>
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Table 4 Microbial analysis of tooth samples before and after chemical treatment

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Patients' age</th>
<th>Gender</th>
<th>Tooth number (FDI 2-digit system)</th>
<th>Tooth status</th>
<th>Chemical treatment (CFU/0.1 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>Female</td>
<td>13</td>
<td>Fully embedded</td>
<td>2.0×10⁴</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>Male</td>
<td>28</td>
<td>Fully impacted</td>
<td>2.0×10⁴</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>Male</td>
<td>38</td>
<td>Fully impacted</td>
<td>3.0×10⁴</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>Female</td>
<td>18</td>
<td>Fully impacted</td>
<td>3.0×10⁴</td>
</tr>
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<td>5</td>
<td>22</td>
<td>Female</td>
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<td>Fully impacted</td>
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<tr>
<td>6</td>
<td>27</td>
<td>Female</td>
<td>44</td>
<td>Fully erupted</td>
<td>2.2×10⁵</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>Male</td>
<td>18</td>
<td>Fully erupted</td>
<td>4.3×10⁵</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>Female</td>
<td>18</td>
<td>Fully erupted</td>
<td>5.1×10⁵</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>Female</td>
<td>38</td>
<td>Fully erupted</td>
<td>7.3×10⁵</td>
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<td>29</td>
<td>Male</td>
<td>48</td>
<td>Fully erupted</td>
<td>1.1×10⁶</td>
</tr>
<tr>
<td>11</td>
<td>29</td>
<td>Male</td>
<td>24</td>
<td>Fully erupted</td>
<td>1.7×10⁶</td>
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<tr>
<td>12</td>
<td>32</td>
<td>Female</td>
<td>14</td>
<td>Fully erupted</td>
<td>2.3×10⁶</td>
</tr>
</tbody>
</table>

NG=no growth

14 showed a similar amount of P release (Table 3).

**Microbial analysis**
This study included 12 human teeth extracted from 7 females and 5 male patients with a mean age of 25. Of these samples, 3 were a lower third molar, 5 were an upper third molar, 2 were upper premolar, 1 impacted canine, and 1 lower premolar. These teeth were then classified into two groups as 5 fully impacted or embedded teeth and 7 fully erupted teeth. The maximum colony forming unit per 0.1 g (CFU/0.1g) before chemical treatment was 2.3×10⁶ as shown in Table 4. After chemical treatment, no microorganism was detected. Noted that fully impacted or embedded teeth had a much lesser number of CFU/0.1 g and microbial contamination from the very beginning.

**Osteogenic properties**
To study the osteogenic property of TDBS, we investigated hFOB migration, cultured in condition with and without the graft materials. There was no sign for bacterial contamination nor detachment of the hFOB when cultured with the TDBS or allograft at all time points. hFOB could vertically migrate through the pored membrane to the bottom side of the transwells in all conditions including the blank control as observed on day 3 (OD at 0.54±0.16) and day 5 (OD at 0.70±0.12) as shown in Fig. 5. Notably, the number of hFOB migration was greatly increased when there was a presence of TDBS (OD at 0.78±0.07 or 44.4% increase) or allograft (OD at 0.86±0.52 or 59.2% increase) in the lower chamber, observed on day 3.

The release of BMP2 protein from each material was investigated in the same condition medium as the migrating experiment (Fig. 6). The results demonstrated that BMP2 was released from TDBS (53.81±1.36 pg/mL) and allograft (46.32±8.36 pg/mL) early at day 1, and gradually released until day10. Furthermore, BMP2 was released in a significant higher amount from TDBS than allograft, measured at day 5 (162.81±9.03 pg/mL vs. 132.15±3.85 pg/mL) and day 10 (212.53±9.11 pg/mL vs. 175.75±2.25 pg/mL); at p-value<0.05.
DISCUSSION

The use of extracted tooth, which is considered as biomedical waste and disposed of, unlocks the simple and readily available bone substitution material. The demineralized dentin matrix is biocompatible, with a property of both osteoinductive and osteoconductive, as have been highlighted in the previous studies\(^{23,28-31}\). The presence of type I, type III collagen, growth factors such as BMP, IGF-II, and TGF-\(\beta\)\(^{14,18,22,23}\), as well as proteins such as OPN, osteocalcin, BSP, osterix, type I collagen, and Cbfa1 (Runx2) in dentin makes it a potential bone grafting material since these reportedly involved in bone formation and resorption\(^{18-20,32}\).

Different preparation methods of extracted teeth provide a potential for use as bone substitutes. Various published studies have shown the possibility of tooth derived bone graft materials. However, the drawbacks while using tooth derived bone grafts are its availability, limited indications, and issues associated with its preparation such as cleaning and time-consuming demineralization process\(^{35-36}\). Most of the previous studies focused on demineralized dentin matrix (DDM), our study approached a possible way to process extracted teeth to be readily used in clinical settings. In this study, we firstly investigated the physicochemical and osteogenic properties of a complete non-demineralized tooth or TDBS to approach a new way of using TDBS, which is more practical in a clinical setting.

The surface analysis using SEM showed TDBS after the chemical treatment had less occlusion of the dentinal tubules leading to diffusion of more growth factors and exposure of collagen matrix\(^{10,19,36}\) that might increase bone formation and resorption. This study also showed the resemblance of TDBS to Bio-Oss\(^{®}\) and OraGRAFT\(^{®}\) in terms of the density, roughness, and homogeneity. The organic and inorganic composition of crown and roots are diverse, which results in different healing mechanisms after been used as bone graft\(^{10,11,37}\). EDS analysis showed different percentages in terms of chemical composition hence Ca/P ratio had the values ranged from 1.27–1.83. Many researchers have speculated that these calcium phosphates react mutually causing favorable remodeling in vitro grafts\(^{11,13}\). The XRD analysis showed TDBS had HA, TCP, and OCP, which was similar to the previous studies\(^{11,38}\). In our study, these components were also present in Bio-Oss\(^{®}\), OraGRAFT\(^{®}\), and mandibular ramus bone suggesting the similarity and thereby an alternative for bone grafting.

The crystallinity of solid material analyzed by X-ray diffraction indicated that even if the chemical compositions of different materials are identical, their crystalline size might differ. Narrow XRD peaks are associated with high crystallinity, whereas wide or broad peaks are linked with low crystallinity\(^{27,30,41}\). In this study, TDBS showed a narrow peak which corresponds to high crystallinity, however, the pattern of arrangement was broader somewhat like mandibular ramus and allograft, thereby associated with low crystallinity and crystalline size (domain)\(^{42}\). This data could be attributed to the fact that in our study we used whole ground tooth that crown and root have different pattern\(^{11}\). Numerous studies have reported that that high crystalline apatite is insoluble, whereas low crystalline apatite has higher relative solubilities\(^{42,43}\). TDBS which showed a narrow peak and the broader arrangement, might contribute to the property of providing a scaffold for bone regeneration and also biological degradable. The mandibular ramus, allograft and BoneCeramic\(^{®}\) in our study agreed with a previous study that the first two had low crystallinity, whereas the latter had the narrowest peak showing high crystallinity\(^{11}\). Moreover, the in-vitro dissolution test of TDBS showed similar values to those of mandibular ramus bone and OraGRAFT\(^{®}\). It suggested that the mineral composites released calcium and phosphorous
ions, which in turn induces the reprecipitation of the apatite on the surfaces, therefore encourage the osseointegration of bioceramic composites\textsuperscript{(48)}.

The microbial analysis was conducted to determine the decontamination protocol set by SDG\textsuperscript{®}, since iatrogenic contamination of the collected bone may lead to possible infectious risk for grafting procedures in oral surgery\textsuperscript{26,44,45}. The extracted teeth were taken from healthy donors and free from pathology, \textit{i.e.}, carious lesions, periodontitis, however, the procedure of harvesting autogenous bone for grafting may easily be contaminated by the host oral flora which was confirmed by the presence of bacterial contamination of all extracted teeth before the decontaminating process. Noted that TDBS was completely free of microorganisms after treated with the chemical procedures in our study.

Several systematic reviews have shown the efficacy of DDM\textsuperscript{19,46,47}. According to reports from Koga et al. and Minamizato et al., autogenous partial demineralization dentin matrix (APDDM) was shown to be the most effective healing in rat calvarial defect when compared to non-demineralized or complete demineralized dentin matrix (UDD, CDDM) and the size of the particle could greatly affect the healing period. It was suggested that APDDM at 1,000 \textmu m can be used as an option for bone substitutes since laboratory and clinical studies have shown to be successful\textsuperscript{33,34}. In this study, we investigated the osteogenic properties of a complete non-demineralized tooth or TDBS. A primary cell line like Human Fetal Osteoblast (hFOB 1.19) was reported to have minimal chromosome abnormalities along with matrix producing properties similar to differentiated osteoblasts. These properties make hFOB the ideal model for osteoinduction, osteoconduction, and osseointegration\textsuperscript{(48-50)}.

Osteoblast migration is an essential cell chemotaxis process in bone remodeling or regeneration, in response to specific chemoattractants such as BMP2, PDGF, VEGF, and IGF\textsuperscript{11-34}. In the present study, hFOB could migrate through the membrane to the bottom side in the presence of grafting materials including TDBS implied that the nature of our TDBS is compatible with the cellular environment and did not cause any bacterial contamination during the cell culture. Besides, the number of migrated osteoblasts was significantly greater than the blank control, which showed strong chemotaxis of the TDBS. This osteoconductive effect of TDBS was firstly evidenced in our present study. While partial demineralized dentine matrix (PDDM) was reported to promote osteoblast migration when compared to other materials such as xenograft\textsuperscript{55}, the present study provided initial evidence of the non-demineralized TDBS could show good biocompatibility as well as inducing the hFOB migration. In addition, the liquid nitrogen treated non-demineralized dentin graft had brought a satisfactory bone induction compared to cortical bone graft\textsuperscript{56}. Taken together, it highly indicated that non-demineralized both from dentin or whole tooth have bone regeneration properties comparable to those of autogenous bone.

The release of BMP2 protein was related to human primary osteoblasts migration in a dose-dependent manner, suggesting it might be involved in the chemoattractiv recruitment of osteoblasts during bone formation in addition to its function as a differentiation enhancer\textsuperscript{57}. The BMP2 also was found preserved in mammal teeth, which can be gradually released even long after extraction or post-chemical processing\textsuperscript{58,59,60} making a successful clinical outcome for DDM or TDBS as a grafting material\textsuperscript{24,33,60-62}. In the present study, BMP2 protein was found in supernatant containing TDBS prepared chairside after decontaminating steps, ensuring that this novel technique of preparing TDBS can preserve substantial proteins in dentin matrix \textit{i.e.}, BMP2, and this protein could also be released from the matrix without the demineralizing process. Nevertheless, many other proteins and growth factors are found after demineralization (from DDM) such as BMP2, Collagen 1, TGF-\beta1, fibroblast growth factors (FGF)\textsuperscript{55,57}. In the present study, the total level of BMP2 from TDBS was greater than the commercial allograft. It gradually released in a similar amount to those of the allograft from early days which may explain the similar efficacy on hFOB migration between the TDBS and allograft on days 3 and 5. The relationship between new bone formation and BMP2 level was consistently reported in the calvarial defect model using histomorphometric analysis and TDBS\textsuperscript{63}. Here, we evidenced a biological property of TDBS processed chairside by demonstrating that TDBS continuously release BMP2 and could induce osteoblast migration. With our preparation technique, we speculate that the TDBS could have a property to preserve a greater amount of other growth factors that are known to aid bone remodeling. Further investigations are urged to examine the effect of TDBS on bone cells interaction in the complex physiology such as bone healing.

In conclusion, the present study verified the chairside-preparation of TDBS demonstrated physicochemical and osteogenic properties. The microbial decontamination of TDBS using chemical processing was one hundred percent effective. Carbon (C), Calcium (Ca), Oxygen (O), Phosphate (P), Sodium (Na) and Magnesium (Mg) were the elements presented in TDBS as well as the elements presented in DDM as well as all other bone graft materials. TDBS showed a similar Ca/P ratio to mandibular ramus bone and allograft. Moreover, TDBS demonstrated both osteoconductive and osteoinductive properties comparable to the allografts, therefore, this study proved that TDBS can be highly considered as an alternative for bone grafting material.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

REFERENCES


