Effectiveness of resonance frequency in predicting orthopedic implant strength and stability in an in vitro osseointegration model

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Abstract—Developing noninvasive tools that determine implant attachment strength to bone and monitor implant stability over time will be important to optimize rehabilitation protocols following insertion of osseointegrated implants in patients with limb loss. While resonance frequency has been previously shown to correlate with implant stability in dental implants placed in the mandible and maxilla, this tool has not been evaluated with implants placed in the medullary canal of long bones. In an in vitro model used to simulate irregular medullary canal implant contact and osseointegration, a strong positive correlation was determined between resonance frequency implant stability quotient values and the force required for implant pushout. The force required for implant displacement also correlated to the distance from the point of fixation to the transducer at the proximal end of the implant (point of resonance frequency monitoring).

Key words: bone, bone-implant interface, implant stability, limb amputation, mechanical testing, osseointegration, outcomes, rehabilitation, resonance frequency, skeletal fixation.

INTRODUCTION

The term “osseointegration” has been used to describe a structural and functional connection between living bone and the surface of a load bearing implant [1–2]. Successful osseointegration techniques have been previously demonstrated for individuals with limb loss [3–5], as well as in the areas of dentistry [1–2,6–8] and craniofacial reconstruction [6]. As a result of an increased desire for functionality for patients with transfemoral, transhumeral, and transtibial amputations [3,9–11], osseointegration technology has been developed for direct skeletal attachment of an exoprosthesis to the residual limb. With the osseointegration procedure for persons with limb loss, a biocompatible metal fixation is surgically inserted directly into the bone of the residual limb and serves as an attachment system for connecting and suspending a prosthesis to the residual limb [5,12–13].

One challenge with the use of natural biological skeletal fixation is allowing the bone to heal and osseointegrate with the implant surface, thereby attaining a strong skeletal

Abbreviations: ABS = acrylonitrile butadiene styrene, ISQ = implant stability quotient, RFA = resonance frequency analysis, SD = standard deviation, VA = Department of Veterans Affairs.

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interlock, a prerequisite for long-term implant function and stability [14–15]. To prevent mechanical loosening at the bone-implant construct, current osseointegration rehabilitation protocols require extensive periods of restricted load bearing to allow sufficient bone attachment and prevent overloading at the bone-implant interface [12,16–24]. Limiting the force on the periimplant bone following insertion of an osseointegrated implant is based on the principle that stress must be exerted gradually to promote firm skeletal attachment, since under- or overloading may compromise the integrity of the host bone. However, previous literature indicates that immediate implant loading may not compromise the integrity of the bone-implant interface or prevent osseointegration if micromotion is controlled in properly designed implants [15,17–19,23–27].

The inability to quantitatively diagnose implant fixation has driven scientists and physicians alike to develop clinical tools to assess primary and secondary implant stability. Primary implant stability occurs immediately after surgical placement [28], and successful osseointegrated implants result from proper implant fit and fill [29] and surgical techniques [30–32]. However, secondary implant stability is the result of bone healing and remodeling that occurs over time [33]. While initial implant fixation is required to prevent micromotion and fibrous encapsulation [16,19,34–40], the long-term success of osseointegrated implants requires firm skeletal connections that may not occur until 9 months postoperative in human cancellous bone [16] and would be more representative of both primary and secondary implant stability.

Since no quantitative measures exist to determine optimal skeletal attachment in vivo, current European and Australian rehabilitation programs for transfemoral amputees with osseointegrated implants require slow, progressive weight bearing [41–42]. Methods for assessing osseointegration strength and stability have included mechanical testing, light microscopy, and scanning electron microscopy [43–46]; however, these techniques for evaluating skeletal fixation cannot be used in dental applications and for patients with limb loss because of the invasiveness [46–47]. Therefore, alternative means of assessing implant stability have been developed and include radiographs, percussion tests, and resonance frequency. Radiographs, however, are not sensitive for determining the extent of osseointegration [48] since a reduction in bone mineralization of 40 percent [49] is required before bone mineral loss can be accurately determined. An examination of radiolucencies with plain radiographs has shown to result in approximately 2 percent false-positive diagnoses of inadequate osseointegration and implant loosening [50]. In addition, traditional radiographs require standardization with threaded implants [21], since reproducibility is difficult and essential in preventing unnecessary surgical revisions [50]. Advanced imaging techniques such as computed tomography scans are also not practical because of expenses, image artifact generated from metal implants, and high exposure to radiation [51].

Percussion tests have also been used to assess osseointegration implant stability, but this technique is considered generally inadequate in the clinical setting. When implant stability is evaluated with percussion tests using dental implants, the procedure often results in “more information about the tapping instrument, and will at best only reveal poor qualitative information” [52]. Therefore, percussion tests are limited since the process only provides quick distinction between mobile and osseointegrated implants but does not reveal the degree of implant stability and thereby restricts the ability of rehabilitation specialist to monitor and advance progressive weight-bearing regimens [53].

A potentially useful noninvasive alternative is resonance frequency analysis (RFA), which has been shown to correlate with the degree of implant stability in dental implants [21,44,47–48,52–55]. Fluctuations in implant stability quotient (ISQ) values even mimic expected bone remodeling rates [20,54,56]. Several studies have also reported that ISQ values decrease within the first 3 months postoperative [20,57–58], as a result of osteoclast resorption, which is key to increased osteoblast activity and new bone formation [33,59]. The reliability of resonance frequency has been confirmed with ISQ values but is dependent on jaw position [44,53] and attributed to the differences in bone quality and type [17,37–38,43,60].

The resonance frequency system most commonly used with dental implants and reported in the literature is the Osstell Mentor® (Osstell AB; Gothenburg, Sweden). This device uses a compact battery system and magnetic pulse to monitor ISQ values [61]. The handheld probe is attached to a docking station, and a magnetic pulse is transmitted to a Smartpeg (Osstell AB) temporarily attached to the proximal face of the implant [41,44]. With use of a bode plot function [61], the response signal is translated into a numerical ISQ value, which may be used
to determine the degree of implant stability. Extensive review articles evaluating the performance of the Osstell device are also available but are not within the scope of this narrative [49,52]. While RFA has been shown to effectively determine osseointegration implant stability in edentulous mandible and maxilla models, ours is the first study to evaluate the utility of this device in an in vitro model simulating irregular osseointegration in a long bone medullary canal.

The objective of this study was to determine a biomechanical relationship between RFA ISQ values and the force required to displace a titanium alloy implant. A composite fixture was utilized to improve reproducibility, which arises from the use of a more uniform material type. Establishing a relationship between torque, attachment location, and resonance frequency with mechanical pushout forces would provide valuable information as a basis for further in vivo testing, because currently no biomechanical testing data directly relate resonance frequency to load bearing. Clearly, new developments in orthopedics and rehabilitation will continue to demand advanced tools for patients seeking osseointegrated implants following limb loss. Therefore, the ultimate goal of this research is to utilize resonance frequency for optimizing the speed and safety of rehabilitation for amputees with osseointegrated implants, since skeletal fixation will vary between persons [49], and individual rehabilitation programs will be required.

**METHODS**

**Testing Fixture and Implant Design**

An implant fixation construct was designed to simulate percutaneous, osseointegrated implant attachment in a medullary canal and was built in a three-dimensional printer (Dimension Elite, Stratasys Inc; Eden Prairie, Minnesota) utilizing layered acrylonitrile butadiene styrene (ABS) plastic. The completed fixture was 45 mm in height by 30 mm in width with a 4 mm hole centrally located for implant attachment. An ABS model was selected as the testing material because it excludes the inherent variability of human cadaveric bone [62–64]. In addition to variability between bone specimens, dehydrated bone will exhibit a higher modulus of elasticity caused by the diffusion of water into vacant spaces and stiffening of collagen fibers [65]. This decrease in water content, which may occur during biomechanical testing, will subsequently lead to the host bone becoming brittle and exhibiting reduced plasticity [65] and may lead to inaccurate data collection. Since the protocol was initially targeting the precision and accuracy of the resonance frequency technique for potential use in patients with osseointegrated implants, a standard material type was selected.

Titanium alloy (Ti-6Al-4V) implant analogs, 20 mm in length, were manufactured to investigate the utility of the resonance frequency device. Implants were fabricated as one unit and included a hexagonal head (5 mm in height by 8 mm in width) and a smooth post distally (15 mm in length and 3.5 mm in diameter). The hexagonal design included an 8 mm base to fit securely on the ABS device and prevent translation and rotation during data collection (Figure 1). Titanium alloy was also selected as the implant material based on its frequent successful use in total joint replacements [66–67], biocompatibility [68–69], and nonferromagnetic properties [70–71] that would not alter RFA recordings.

Three 3 mm holes were evenly spaced and drilled 5 mm apart in the ABS fixture to represent partial osseointegration along the titanium implant insert (Figure 1). Setscrews...
were fixed at multiple insertion torques and levels to prevent the smooth titanium implant from migrating and to simulate a series of nonuniform bone-implant interfaces and depths, since the quality of osseointegration cannot be assumed to be uniform along the bone-implant construct. In the ABS fixture, Hole A was located 20 mm from the most proximal aspect of the Smartpeg and each additional hole positioned 5 mm distally. Implants were secured with torques ranging from 5 to 12.5 N-cm, since 5 to 50 N-cm is regarded as necessary for primary implant stability [47]. Therefore, to ensure accurate data collection in this model and avoid exceeding the elastic limit of the setscrews, we chose 12.5 N-cm as the highest evaluated torque. A range of torques was also selected in the in vitro model, since the initial fixation required for successful osseointegration is unknown [47] and depends largely on bone quality and density [72].

**Resonance Frequency Analysis**

The Osstell measurement system (Ostell Mentor®) designed for oral cavity and craniofacial implants was used to obtain the resonance frequency values. Smartpegs were attached to the titanium alloy implants and received magnetic pulses to determine implant fixation strengths. The threaded Smartpegs (Type 1) were consistently torqued to 10 N-cm with a hand-held, digital torque meter (Advanced Force/Torque Indicator, Dillon Quantrol; Fairmont, Minnesota), and ISQ values were recorded before mechanical testing, based on recommendations from previous RFA literature [47]. An insertion torque of 10 N-cm was also selected since it was regarded as least likely to damage the Smartpeg’s screw threads [21] and would ensure accurate data collection.

Skeletal fixation was simulated with six variable torques (5.0, 7.0, 8.6, 10.0, 10.9, and 12.5 N-cm) at three separate fixation locations (Hole A, Hole B, and Hole C) (**Figure 1**). Screw torques were randomly selected within the predetermined range and verified with the digital torque meter before data collection. After recording three ISQ’s values on each orthogonal face to ensure implants were properly loaded on all axes, to demonstrate repeatability, and per manufacturer instructions [44], we removed the Smartpegs and positioned the implant below the crosshead of the mechanical testing apparatus (Model 8800, Instron Corp.; Norwood, Massachusetts). Smartpegs were discarded and replaced after three measurements to avoid screw thread wearing.

**Mechanical Testing**

Implants were secured in the ABS fixture, placed in the servohydraulic mechanical testing device, and preloaded to 30 N in a load-controlled testing mode. Load was increased steadily at a rate of 5 N/s (a recommended loading rate established by the mechanical engineering coauthors) until implant displacement occurred. All data were sampled continuously at 100 Hz throughout the mechanical-testing procedure (**Figure 2**). To prevent observer bias, we determined the exact point of implant displacement with a custom script (MATLAB, The MathWorks Inc; Natick, Massachusetts). The code was generated to determine a 3 percent change in slope for the continuous data, and the data point that corresponded with the initial change in slope was selected as the ultimate failure load. A 3 percent change in slope between position and time accounted for the specific tolerance of our mechanical testing system, as well as a safety factor of 1 percent. Therefore, a 3 percent difference in slope would likely account for measurement error and system noise and prevent subjective interpretations of implant displacement.

To make certain that the implant construct was not damaged from repeated mechanical-testing usage, the ABS fixture was carefully bivalved (Craftsman 10 in. Figure 2. After resonance frequency implant stability quotient values were recorded three times on each orthogonal face, Smartpeg was carefully removed and implant was preloaded with 30 N and pushed out of acrylonitrile butadiene styrene (ABS) fixture.
Direct Drive Band Saw, Sears Holding Corporation; Hoffman Estates, Illinois) and examined with a laboratory microscope (Nikon SMZ800, Nikon Inc; Melville, New York) equipped with imaging capturing software (Magnafire SP, Optronics; Goleta, California) at the conclusion of the pushout tests. Investigating the inner wall of the fixation device was important for ensuring that damage did not occur and alter the surface area for implant attachment or affect the predetermined loading conditions. Extensive examination of the construct did not reveal visible imperfections from mechanical testing and helped demonstrate repeatability and accuracy during data collection.

Statistical Analysis

A multiple linear regression was used to correlate the outcome of mechanical pushout force with the predictor variables (screw torque, fixation distance, resonance frequency ISQ, interaction between resonance frequency and screw torque, and distance from Smartpeg). In one model, the distance from the Smartpeg was included as a continuous variable; in the second model, it was investigated as two dummy variables to account for the three points of fixation. In each case, the interaction between screw torque and resonance frequency was included. To avoid overfitting, at least 10 observations for each predictor variable were required in the model. In this study with a sample size of 45, four to five variables were included and would not lead to misinterpretation of data. $R^2$ values are reported with each model along with the adjusted $R^2$ values, which represent the correlation without problems due to overfitting. All statistical comparisons were conducted with commercially available software (SPSS, Inc; Chicago, Illinois).

RESULTS

Descriptive statistics (mean and standard deviation [SD]) based on mechanical displacement forces for fixation location and screw torque are provided in Table 1. When determining statistical significance, we excluded torques exceeding 10.9 N-cm, since inconsistent data indicated that the elastic limit of the screws was exceeded and inclusion would have provided speculative correlations. Therefore, a total of 45 data points was collected for three fixation locations and five torques on the single fixture. Further interpretation of Table 1 demonstrates that the maximum pushout forces were consistent for Hole B, which may have resulted from fixture construction and the point of contact along the implant shaft. Additionally, data in this table suggest that the elastic limit of the setscrews was exceeded even at 10.9 N-cm, since pushout forces decreased when torque increased from 10.0 to 10.9 N-cm for Holes A and B, but this trend was not evident with Hole C and therefore these quadrants must be treated as outliers in the data set.

A multivariable linear regression model was fit to the outcome variable (mechanical pushout forces) as shown in Table 2. The significant predictor variables were screw torque ($B = -13.40, p = 0.049$), resonance frequency ($B = -2.89, p = 0.012$) and the interaction of screw torque and resonance frequency ($B = 0.38, p = 0.004$). Distance from the point of fixation, included in the model as a continuous variable, was not significant ($B = -4.00, p = 0.36$). The rationale for including distance as a continuous variable was that previous reports indicated that implant stability linearly decreases with increasing distances from the Smartpeg [55].

For verification of the linear reduction in resonance frequency ISQ values with increasing distance from the Smartpeg, a second model was fitted and distance was included in the model as dummy variables (Table 3). The first dummy variable was located 20 mm from the Smartpeg (Hole A).

Table 1.
Mean ± standard deviation mechanical pushout forces independently assessed as a function of screw torque and distance from Smartpeg ($n = 3$ per combination, total $N = 45$).

<table>
<thead>
<tr>
<th>Screw Torque (N·cm)</th>
<th>2.0 mm, Hole A</th>
<th>2.5 mm, Hole B</th>
<th>3.0 mm, Hole C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>$26.83 \pm 4.21$</td>
<td>$35.80 \pm 3.76$</td>
<td>$33.37 \pm 3.72$</td>
</tr>
<tr>
<td>7.0</td>
<td>$26.19 \pm 8.74$</td>
<td>$46.13 \pm 3.14$</td>
<td>$26.19 \pm 4.15$</td>
</tr>
<tr>
<td>8.6</td>
<td>$41.60 \pm 12.46$</td>
<td>$47.95 \pm 9.55$</td>
<td>$47.95 \pm 1.57$</td>
</tr>
<tr>
<td>10.0</td>
<td>$73.40 \pm 0.00$</td>
<td>$79.90 \pm 3.76$</td>
<td>$61.13 \pm 4.21$</td>
</tr>
<tr>
<td>10.9</td>
<td>$58.82 \pm 15.46$</td>
<td>$70.61 \pm 18.10$</td>
<td>$68.79 \pm 4.71$</td>
</tr>
</tbody>
</table>
and the second dummy variable was located 30 mm from the Smartpeg (Hole C). The third position on the testing fixture was located 25 mm from the Smartpeg (Hole B) and acted as the reference point for the two dummy variables, since it was centrally located and the midpoint between fixation points (Figure 1). The significant predictor variables in this model were resonance frequency \( (B = -2.92, p = 0.008) \), interaction between resonance frequency and screw torque \( (B = 0.35, p = 0.005) \), and distance from Hole C to Hole B \( (B = 0.35, p = 0.005) \). However, the distance from Hole A to Hole B was not significant \( (p = 0.12) \). The predictor variable screw torque had a significant trend in which increased torques resulted in higher ISQ values and added to the interaction term, but this was not statistically significant alone \( (p = 0.07) \). A positive correlation between resonance frequency ISQ values and screw torque has also been previously reported in an in vitro model [60] and demonstrates the reproducibility of our work with previous studies.

The relationship of resonance frequency and screw torque to mechanical pushout forces is graphically represented in Figure 3 and quantitatively described in Table 4 using the same raw data. The three-dimensional representation shows a general increase in the force required to displace the titanium implant as screw torque and resonance frequency increased. The majority of the data points collected in the model occurred at higher ISQ values \( (37/45) \), which is a trend also noted in previous dental applications [48]. For ease of understanding of the relationship, resonance frequency was subdivided into ranges of low, low-to-medium, medium, and high values, which were used to predict the degree of osseointegration. The mean and SD of each quadrant are presented in Table 4.

### DISCUSSION

The prolonged recovery and rehabilitation period that currently follows osseointegration implantation (approximately 12 months from postoperative to full weight bearing...
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Table 4.
Force (mean ± standard deviation) required for mechanical pushout subdivided by resonance frequency and screw torques, where “—” signifies that no data were collected in the quadrant (N = 45).

<table>
<thead>
<tr>
<th>Screw Torque (N·cm)</th>
<th>ISQ Range</th>
<th>Low, 30–40 (2 data points)</th>
<th>Low–Medium, 40–50 (6 data points)</th>
<th>Medium, 50–60 (25 data points)</th>
<th>High, 60–70 (12 data points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>30–40</td>
<td>—</td>
<td>34.8 ± 4.2</td>
<td>29.8 ± 5.3</td>
<td>—</td>
</tr>
<tr>
<td>7.0</td>
<td>40–50</td>
<td>—</td>
<td>28.0 ± 7.0</td>
<td>36.7 ± 13.1</td>
<td>—</td>
</tr>
<tr>
<td>8.6</td>
<td>50–60</td>
<td>30.7</td>
<td>—</td>
<td>47.6 ± 5.9</td>
<td>47.9 ± 9.6</td>
</tr>
<tr>
<td>10.0</td>
<td>60–70</td>
<td>58.7</td>
<td>73.1 ± 7.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10.9</td>
<td>70</td>
<td>41.6</td>
<td>71.5</td>
<td>67.9 ± 5.7</td>
<td>69.5 ± 15.0</td>
</tr>
</tbody>
</table>

ISQ = implant stability quotient.

[73]) in individuals with lower-limb amputations demonstrates the need for a simple, noninvasive tool that determines implant strength and stability. The use of an RFA-type of rehabilitation device is anticipated to quantitatively determine the integrity of the bone-implant interface during osseointegration and increase the loading rate on osseointegrated implants, thereby shortening current rehabilitation regimens. Therefore, the aim of this study was to determine whether a relationship existed between resonance frequency and mechanical pushout forces in a carefully controlled in vitro medullary canal osseointegration model.

The correlation between screw torque and resonance frequency, as demonstrated in Figure 3, confirms the reliability of the Osstell device for predicting mechanical pushout forces in a nonuniform osseointegration model. While several data points were in the low and low-to-medium ranges, the data collected were primarily in the medium and high ranges of ISQ values and fit the published values for stable osseointegration ISQs ranging from 40 to 70 [48]. The majority of data collected in the model was in the ISQ range of 50 to 70, and the torques tested were in the vicinity of previous in vivo dental applications. While the exact ISQ value for complete osseointegration is unknown, Zix et al. report an average ISQ of 57.66 ± 8.19 (ISQ range: 23–73) [48], Kessler-Liechti et al. report 64.50 ± 7.90 (ISQ range: 58–72) [53], and Zhou et al. report 53.90 ± 7.70 (ISQ range: 37–68) [46] in in vivo human and rabbit models. These values are in the locality of the overall mean and SD in this experiment, which was 55.74 ± 7.35.

Since previous literature by Friberg et al. has shown that the density of the host bone bed and bone quality are factors affecting resonance frequency reporting [72], we selected an ABS material with an approximate density of 1.05 g/cm³. While this material resulted in a moderate to large coefficient of determination when screw torque, resonance frequency, fixation location, and the interaction between screw torque and resonance frequency were compared with mechanical pushout forces (Tables 2–3), the material type selected did not entirely replicate the density of cortical bone, which is reported to be 2.06 g/cm³ in human femurs [74]. The discrepancy in material selection may have accounted for the adjusted coefficients of determination of 0.67 in the multiple linear regressions, which are considered to be a moderate-to-high-positive correlation [75], but may have been even larger if a different material type had been selected to help validate the model. However, a positive correlation between ISQ values and screw torque has been previously reported in an in vitro model and demonstrates the consistency of our model with previous studies [60]. Further investigation using fresh cadaveric bone samples is planned in the future and will serve to validate our current model.

In our experiment, the force required for implant removal was affected by the points of fixation, as indicated by significance between Hole B and Hole C, respectively. While a statistical relationship was not found for all hole locations, varying degrees of implant stability based on the distance of the Smartpeg to the proximal bone bed surface have been reported previously [55]. According to manufacturer specifications, “a change of about 3 ISQ/mm should be expected if implants are placed in the same bone density” [55]. However, investigation of Table 1 indicates that this trend was not observed in our model and may be due to implant geometry, which has been reported as a factor affecting stiffness in dental and orthopedic implants [60,76]. The hexagonal head of the implant allowed the Smartpeg to stay within the same distance to the ABS material, but the fixation point differed
in 5 mm increments between hole locations. The rationale for including the hexagonal head in the model was that it allowed for reproducibility by preventing implant migration which would lead to confounding variables. However, there is reason to believe that a threaded design may have also allowed accurate data collection but would have required fabrication of multiple ABS constructs and screws because of anticipated damage to the screw threads during pushout tests.

While the data collected in the model was initially targeted at proving safety and efficacy in long bone medullary canals, high correlations between resonance frequency and mechanical pushout forces demonstrate the utility of the device for future use in short bones (digits) and low load-bearing implants (facial reconstruction). In all these cases, the biomechanical behavior of osseointegrated implants is vital for firm skeletal attachment [77], since stress must be applied gradually over time to prevent over- or underloading. In addition, to further reduce the likelihood of osseointegration failure due to high interfacial shear stresses, this model may provide useful boundary conditions for finite element models using our mechanical pushout forces as primary stability estimations.

Limitations of the ABS construct used in our model include the restrictions of torques exceeding 10.9 N-cm because of the elastic limit of the setscrews and the inability to determine the effect of resonance frequency on mechanical pushout with primary and secondary implant stability. The relationship determined in this study demonstrated a strong positive correlation between resonance frequency ISQ values and mechanical pushout forces but requires in vitro cadaveric validation and in vivo experimentation in long bones to monitor the degree of osseointegration over time with biomechanical testing.

CONCLUSIONS

The relationship between resonance frequency ISQ values and the force required for implant removal in a nonuniform bone-implant interface model had not been evaluated before this investigation. Orthopedic implants in human medullary canals often result in a nonuniform bone-implant interface [78], and this limits the speed of rehabilitation for individuals with limb loss. While our investigation used an in vitro testing modality, a clear distinction was evident from multiple linear regressions, which demonstrated that the interaction between resonance frequency and screw torque correlated with mechanical pushout forces. The simulated approach allowed for controlled loading at separate contact points to represent partial osseointegration, reaffirmed the utility of resonance frequency as an advocated nondestructive mechanical assessment of skeletal fixation, and demonstrated a direct relationship with implant displacements. While the exact force required for implant removal may not be necessary in dental applications, based on our findings in this in vitro model, RFA appears to hold promise for application as a rehabilitation tool for determining implant strength and stability following osseointegration implant placement, and this technology deserves further human cadaveric and in vivo investigation.

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