Comparison of Push-In versus Pull-Out Tests on Bone-Implant Interfaces of Rabbit Tibia Dental Implant Healing Model

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ABSTRACT

Purpose: This study aimed to investigate whether push-in and pull-out tests measure mechanical properties of the bone–implant interface differently, and which test is more sensitive to changes over the healing period.

Materials and Methods: Two identical self-threading dental implants (3.3 × 8.5 mm) were placed in medial surface of the proximal condyles of left and right tibias of 20 rabbits (40 implants total). Five rabbits each were sacrificed after 1, 4, 8, and 12 weeks of healing. Push-in test was performed on one side's tibia implant and pull-out on the other side's implant, at a rate of 6 mm/min. Primary and secondary implant stabilities and tibia weight were measured on all implants.

Results: The push-in test generated significantly higher failure load ($p = .0001$; 530 N vs 279 N), lower displacement at failure ($p = .0003$; 0.436 mm vs 0.680 mm), and higher interface stiffness ($p < .0001$; 1,641 N/mm vs 619 N/mm) than pull-out test. Failure load, stiffness, and secondary implant stability were significantly higher for longer compared with shorter healing periods, while displacement, tibia weight, and primary stability were not. Failure load and stiffness differed significantly for four healing times for the push-in but not for the pull-out test. Failure load was significantly correlated with secondary implant stability for both push-in ($r = 0.66$) and pull-out ($r = 0.48$) tests, but stiffness was significantly correlated with secondary stability only for the push-in test ($r = 0.72$; pull-out test $r = 0.40$).

Conclusion: The push-in test appeared more sensitive than pull-out to changes in mechanical properties at bone–implant interfaces during healing in rabbit tibia model.

KEY WORDS: bone–implant interface, pull-out test, push-in test, rabbit tibia healing model

INTRODUCTION

Dental implant therapy has been based on the concept of osseointegration, which is defined as a direct structural and functional connection between ordered, living bone and the surface of a load-bearing implant.[1] Osseointegration is used to describe the bone–implant interface that healed successfully after implant-placement surgery and is functioning under clinical loading. Accurate biomechanical characterization of the bone–implant interface during healing, once healed completely from surgery and after clinical loading, is important to understand how the healing process responds to various conditions, to predict the biomechanical consequences on the bone–implant interface from clinical overloading, and to develop biomaterials that can be used at the bone–implant interface. For research in this area, various animal models with different skeletal bones, testing techniques and loading rates, and outcome measures have been used (Table 1).[2–10] Numerous studies have been published using different animal models and testing techniques, but there is very little consensus available for selection of proper testing techniques (push-in, pull-out,

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TABLE 1 Examples of Animal Models Used to Test Interface Mechanical Properties

Authors	Species/Bone	Test/Rate	Implant	Healing Time/Load/Stress/ Modulus
Li et al. (1995)[10]	Rabbit/femur	Push-out/1 mm/min	HA/Al2O3 implant, 2.8 × 6 mm	3-months healing/ 15 MPa
Giavaresi et al. (2004)[5]	Rabbit/femur	Push-out/1 mm/min	PMMA/HA/glass implant, 2 × 5 mm	12-week healing/Max. force 96 N, shear strength 8.5 MPa
Müller et al. (2006)[2]	Rabbit/femur	Push-out/1 mm/min	Laser-textured implant, 3.5 × 5.5 mm	12-week healing/shear modulus: 25–41 MPa
Brosh et al. (1995)[9]	Dog/mandible	Push-in/10 mm/min	Dental implant, 4 × 14 mm	3-months healing/1194 N, 0.69 mm
Ogawa et al. (2000)[7]	Rat/femur	Push-in/1 mm/min	Acid etched versus turned implant, 1 × 2 mm	8-week healing/59 N vs 29 N
Zaffe et al. (2003)[6]	Rabbit/tibia	Push-in/NA	TiO implant, 3.7 × 10 mm	120 days healing/NA
Baker et al. (1999)[8]	Rabbit/tibia	Pull-out/0.05 mm/min	Dual etched vs machined implant, 3.3 × 4 mm	8-week healing/115 N vs 36 N
Gan et al. (2004)[4]	Rabbit/femur	Pull-out/1 mm/min	CaP coated vs machined implant, 3.5 × 9 mm	9 days healing/156 N vs 126 N
Huja et al. (2005)[3]	Dog/ant. vs post. mandible	Pull-out/ 3 mm/min	Orthodontic bone anchor screw, 2 × 6 mm	Fresh cadaver/135 N vs 388 N

ant. = anterior; min = minute; post. = posterior.

push-out), loading rates, and outcome measurements to properly characterize the bone–implant interface.

To investigate the effects of different testing techniques (push-in, pull-out, push-out) on measurement of bone–implant interface mechanical properties, a rabbit long bone model was selected, as it has been widely used to test dental implant materials,[5] surface treatments,[2,8] and surgical and/or loading protocols.[11] The advantage of the rabbit model is that it is the smallest animal that can accept commercially available dental implants in long bones, the tibia and the femur. To simulate common clinical overloading conditions such as clenching and bruxing, a loading rate of 6 mm/min was selected, faster than the more commonly used 1 mm/min loading rate.[4,7,10] During a pilot study, the decision to exclude the push-out test from the current study was made. The push-out test had inconsistencies in the test sample preparation because of the difficulty in blindly cutting away bone from the apex of the implant perpendicular to the long axis of the implant without damaging the implant, and because push-out strength depends on the size of the hole on the supporting table.[12]

The purpose of this study was to investigate whether and how push-in and pull-out tests measure mechanical properties of healing bone–implant interfaces differently in the rabbit tibia model, and which test is more sensitive to changes in the mechanical properties of the bone–implant interface as healing progresses. This study also evaluated how secondary (after-healing) implant stability, measured with a resonance frequency device (Osstell™ mentor, Osstell AB, Göteborg, Sweden) at the animal's sacrifice, was related to mechanical properties of the bone–implant interface.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Minnesota. The experiments were conducted following animal welfare regulations and guidelines by the United States Department of Agriculture and adequate measures were taken to minimize pain or discomfort of animals. Twenty female New Zealand white rabbits with a weight of approximately 4 kg received a total of forty 3.3-mm diameter, 8.5-mm long dental implants (USII, Osstem Implant Co., Busan, Korea). Following a standard implant surgical protocol, two 3.3-mm diameter implants per rabbit were self-threaded in underprepared (3.0 mm in diameter) 8.5-mm deep bony sockets; one each in the medial surface of the right and left tibia proximal condyle. Immediately after the implant placement, initial (primary) implant stability was measured four times per implant from four different directions with a resonance frequency device. After implant-placement surgery, the rabbits were allowed to recover and sutures were removed 14 days after the surgery.

Five randomly selected rabbits were sacrificed after each of 1, 4, 8, and 12 weeks of healing. Right and left tibias with dental implants were extracted carefully and all the flesh was removed. Secondary implant stability was measured using a resonance frequency device. Tibia weight was then measured using a digital scale.

On the same day as euthanasia, push-in and pull-out failure tests using a hydraulic-loading machine (MTS 810, MTS Systems Corporation, Oak Ridge, TN, USA) were performed at a rate of 6 mm/min to measure the mechanical properties of dental implant–bone interfaces. In each rabbit, the right or left tibia-implant sample was used for either the push-in or pull-out test, in random balanced fashion. After weight measurement, the tibia-implant sample was cross sectioned out from the rest of tibia. Digital periapical radiographs were taken using a dental surveyor and a custom-made rod, which was screwed into the internal threads of the dental implant within the tibia and attached to the dental surveyor. Using the dental surveyor and rod, the tibia-implant sample was mounted over an aluminum plate with quick-set plaster (Snow White Plaster, Kerr Corp., Orange, CA, USA). The long axis of the dental implant was perpendicular to the aluminum plate and parallel to the push/pull rod of the MTS machine when the aluminum plate was positioned on the MTS table (Figure 1). Before testing, samples were allowed to rest for 15 minutes to allow complete setting of quick-set plaster. To confirm that the strengths of the aluminum plate and quick-set plaster are strong enough to support tibia-implant samples during interface failure tests using push-in and pull-out methods, a series of pilot tests were performed. Compression tests performed up to 1,000 N on aluminum plate only and plaster patty on aluminum plate without tibia-implant samples showed the linear increase of the force without interruption in force-displacement graphs. Their slopes (stiffness) were 6,024 N/mm and 4,166 N/mm, respectively, indicating adequate strengths of the aluminum plate and plaster as supporting system for tibia-implant samples whose interface stiffness ranged 430–2,185 N/mm (Table 2). After completion of the push-in/pull-out tests, digital periapical radiographs of the failed tibia-implant samples were taken again using the dental surveyor and rod (Figure 2).

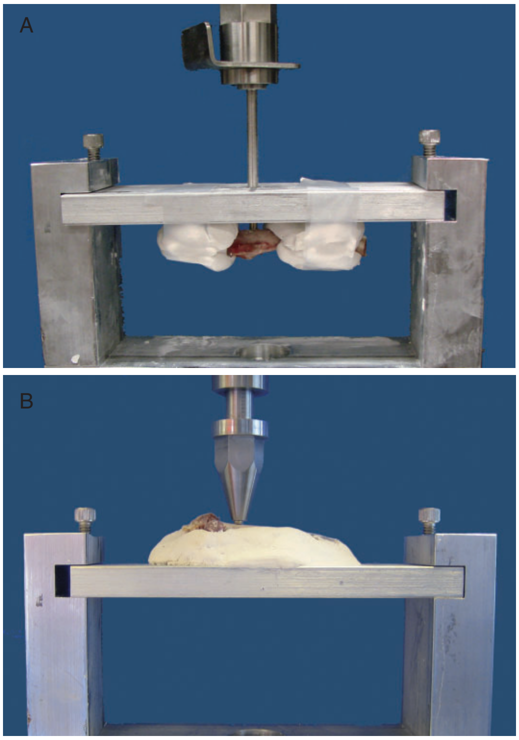

Figure 1 (A) Pull-out test. (B) Push-in test.

The force-displacement graph was visualized in real time on a monitor and if a failure point was observed, push-in/pull-out loading tests were continued a further 20 seconds before stopping to make sure the failure point information was included in the data set. Data were graphed as force versus displacement and the failure load was found from the graph (Figure 3). In addition to failure load, the displacement up to the failure point and interface stiffness were also calculated from the graph.

Comparisons of test methods and healing times used mixed linear models (a generalization of repeated-measures analysis of variance, ANOVA). For each outcome, the fixed effects were healing time (1, 4, 8, 12 weeks), test (push-in vs pull-out), and their interaction. For failure load, displacement, interface stiffness, and tibia weight, there was one random effect, namely rabbit; for initial and secondary implant stability, there were two random effects, rabbit and leg within rabbit. *p* Values and confidence intervals were not adjusted for multiple comparisons. Standard errors associated with adjusted estimates were computed using variance-component

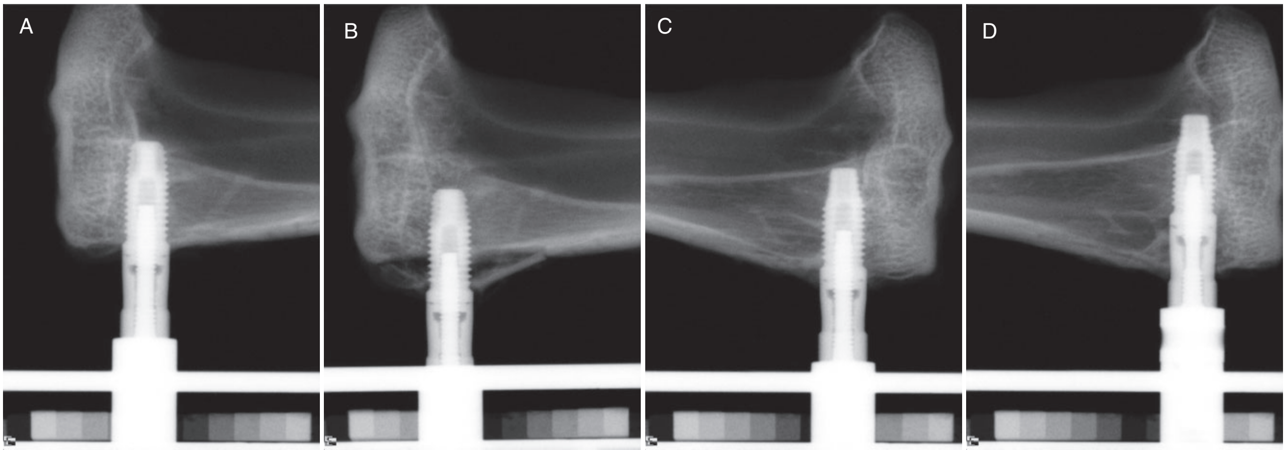

Figure 2 Radiographic images of 12-week healed tibia-implant samples. (A) Before pull-out test. (B) After pull-out test. (C) Before push-in test. (D) After push-in test.

estimates from the mixed-linear model analysis. Analyses used the restricted-likelihood method implemented in JMP (v. 7, SAS Institute Inc, Cary NC, USA). Pearson's correlation (r) was computed between secondary implant stability and mechanical properties of bone–implant interface; p values are from testing the slope in the corresponding linear regression.

RESULTS

All 20 rabbits survived and all 40 self-threaded dental implants showed good secondary implant stability at euthanasia. The push-in mechanical data of two samples in 1-week healing group were excluded because of the mishap during the surgery and the mechanical test. The most common complications were seroma arising from the surgical trauma, which was present in 14 out of 40 legs, though none of the seromas needed incision and drainage and all rabbits managed well until the scheduled sacrifice.

Both the weight of the tibia measured at sacrifice and initial implant stability measured immediately after implant-placement surgery were similar between push-in and pull-out test groups and among the 1-, 4-, 8-, and 12-week healing groups (Table 2). The secondary

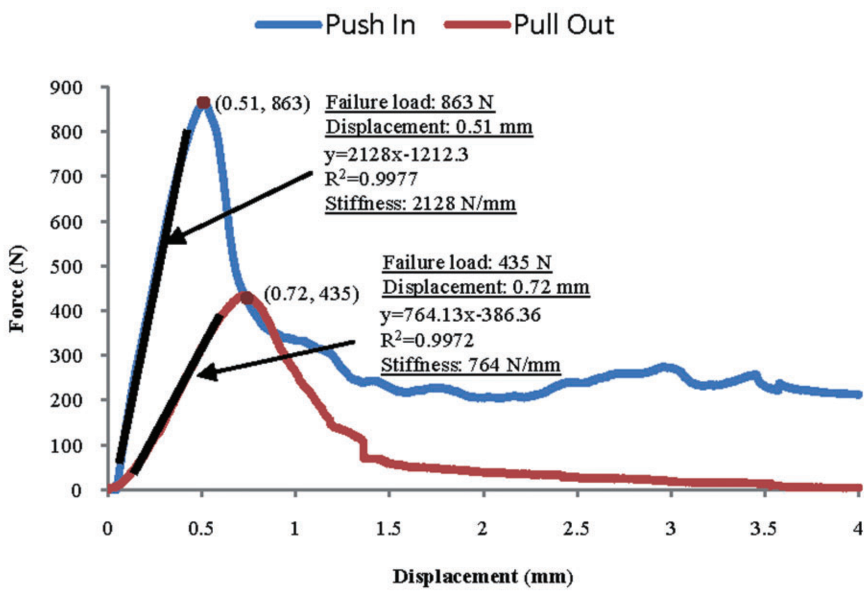

Figure 3 Force-displacement graphs for both pull-out (red: left tibia) and push-in (blue: right tibia) tests for rabbit number 12 from the 12-week healing group. The slope of the force-displacement graph, which is the stiffness, was determined by finding the longest linear portion ($R^2 = 0.9972$ and 0.9977) of the graph slopes.

TABLE 2 ***p*** **Values and Adjusted Estimates (Standard Error) For Fixed Effects and Interactions**

	Adjusted Estimates (Standard Error)*					
Effect	Failure Load (N)	Displacement (mm)	Stiffness (N/mm)	Weight of Tibia (g)	Initial Stability (1–100)	Secondary Stability (1–100)
Test	$p = .0001$	$p = .0003$	$p < .0001$	$p = .3407$	$p = .9048$	$p = .4813$
Pull-out	278.9 (34.6)	0.680 (0.04)	619 (69)	10.7 (0.3)	61.6 (2.0)	68.9 (0.9)
Push-in	530.9 (37.7)	0.436 (0.04)	1,641 (75)	10.9 (0.3)	61.3 (2.0)	68.0 (1.0)
Healing time	$p = .0046$	$p = .1727$	$p = .0024$	$p = .9573$	$p = .8483$	$p < .0001$
Week 1	245.4 (58.2)	0.589 (0.07)	748 (124)	11.0 (0.6)	61.2 (3.2)	56.7 (1.4)
Week 4	366.1 (49.9)	0.621 (0.06)	996 (110)	10.9 (0.6)	62.8 (3.1)	67.4 (1.3)
Week 8	438.6 (49.9)	0.446 (0.06)	1,330 (110)	10.8 (0.6)	59.4 (3.1)	73.5 (1.3)
Week 12	569.5 (49.9)	0.575 (0.06)	1,445 (110)	10.6 (0.6)	62.6 (3.1)	76.1 (1.3)
Test*healing time	$p = .1958$	$p = .4193$	$p = .0377$	$p = .8886$	$p = .2657$	$p = .7959$
Pull-out week 1	187.9 (69.2)	0.656 (0.08)	430 (139)	11.0 (0.6)	58.7 (4.0)	58.4 (1.9)
Week 4	260.7 (69.2)	0.810 (0.08)	502 (139)	10.7 (0.6)	66.9 (4.0)	67.3 (1.9)
Week 8	315.3 (69.2)	0.524 (0.08)	837 (139)	10.7 (0.6)	57.4 (4.0)	73.8 (1.9)
Week 12	351.8 (69.2)	0.728 (0.08)	705 (139)	10.5 (0.6)	63.6 (4.0)	76.3 (1.9)
Push-in week 1	302.9 (91.6)	0.521 (0.10)	1,067 (181)	11.0 (0.6)	63.7 (4.5)	55.1 (2.1)
Week 4	471.6 (69.2)	0.432 (0.08)	1,490 (139)	11.0 (0.6)	58.6 (4.0)	67.5 (1.9)
Week 8	561.9 (69.2)	0.368 (0.08)	1,821 (139)	10.8 (0.6)	61.4 (4.0)	73.3 (1.9)
Week 12	787.2 (69.2)	0.422 (0.08)	2,185 (139)	10.6 (0.6)	61.7 (4.0)	76.0 (1.9)

*Standard errors associated with adjusted estimates are computed using variance-component estimates from the mixed-linear model analysis.

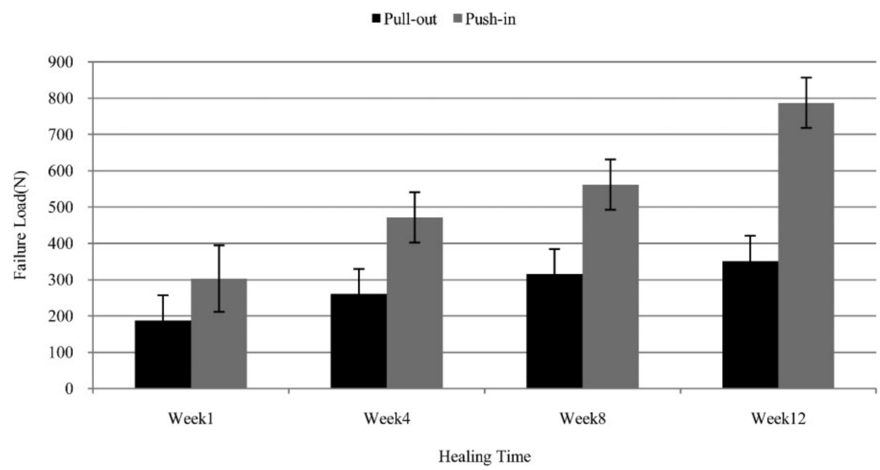

Figure 4 Failure load measured by pull-out (black) and push-in (grey) tests, with the different healing periods (1- to 12-week).

implant stability measurements were also similar between push-in and pull-out test groups at all sacrifice time points ($p = .48$), although secondary stability increased significantly as healing time increased from 1 to 12 weeks ($p < .0001$) (Table 2).

Table 2 presents the ANOVA-type fixed-effect tests and adjusted estimates for failure load, displacement, and interface stiffness. Compared with the pull-out tests, the push-in tests generated significantly higher failure load ($p = .0001$; 530 N vs 279 N), lower displacement at failure ($p = .0003$; 0.436 mm vs 0.680 mm), and higher interface stiffness ($p < .0001$; 1641 N/mm vs 619 N/mm). Failure load and stiffness increased significantly as the healing period increased from 1 to 12 weeks, while displacement did not. Figures 4–6 show failure load, displacement, and stiffness measured by the pull-out and push-in tests after the different healing periods.

Further analyses were performed to study whether push-in and pull-out tests have different sensitivity in distinguishing bone–implant interface conditions as healing progresses (Table 3). The failure load and interface stiffness measured at the four healing times differed significantly for the push-in test ($p = .001$, $p < .001$, respectively) but not for the pull-out test ($p = .38$; $p = .17$) (Table 3A). Failure load and stiffness measured by the push-in test were significantly higher than those measured by the pull-out test at all healing times except for 1-week failure load (Table 3B).

Failure load was significantly correlated with secondary implant stability for both push-in ($r = 0.66$) and pull-out ($r = 0.48$) tests; however, stiffness was significantly correlated with secondary stability only for the push-in test ($r = 0.72$) (Table 4). Tibia weight and

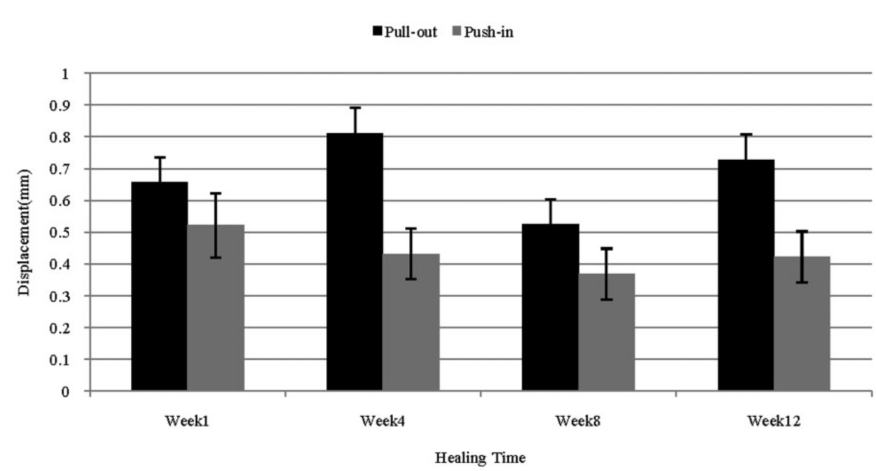

Figure 5 Displacement measured at the failure of the bone–implant interface under pull-out (black) and push-in (grey) tests, with the different healing periods (1- to 12-week).

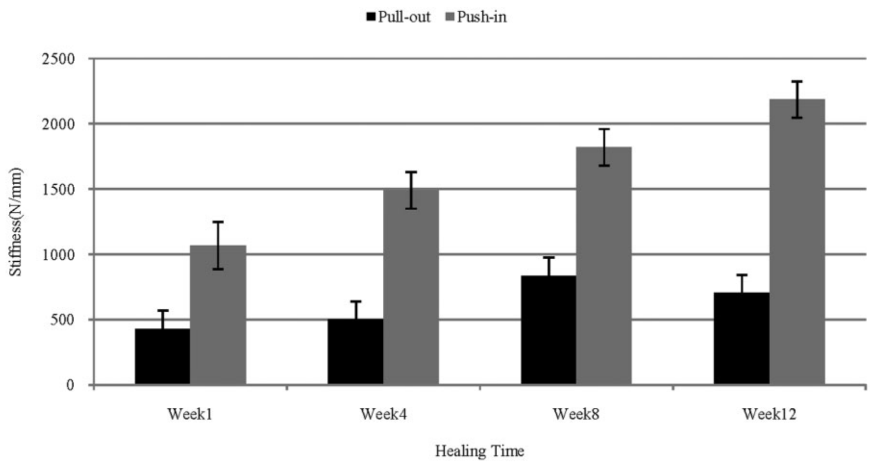

Figure 6 Stiffness of the bone–implant interface measured by pull-out (black) and push-in (grey) tests, with the different healing periods (1- to 12-week).

displacement were not significantly associated with either the push-in or the pull-out test.

DISCUSSION

The tibia weight and initial implant stability data were very similar between push-in and pull-out test groups and among the four healing time groups, confirming that there was no systematic difference between implants and bones used for push-in and pull-out, even though this study's sample size (n = 5 per group, n = 40 in total) was not very large. Thus the results can be considered causal effects of the two testing techniques (push-in vs pull-out) and the healing times (1, 4, 8, 12 weeks).

Our results, showing an increase in failure load with increased healing time, agree well with studies done in rat femur,[7] dog mandible,[9] and goat mandible and maxilla.[13] A push-in test performed at a rate of 10 mm/min

TABLE 3 Comparing the Sensitivity of Push-in and Pull-Out Tests for Failure Load and Stiffness

A. Change From 1 Week By Test

	Failure Load: Estimate (SE)*		Stiffness: Estimate (SE)	
	Pull-Out Test	Push-In Test	Pull-Out Test	Push-In Test
4-week – 1-week	72.8 (97.8)	168.6 (114.8)	71.7 (196.5)	422.7 (228.3)
8-week – 1-week	127.4 (97.8)	259.9 (114.8)	407.1 (196.5)	754.8 (228.3)
12-week – 1-week	163.9 (97.8)	484.2 (114.8)	274.4 (196.5)	1,118.4 (228.3)
p Value	$p = .380$	$p = .001$	$p = .170$	$p < .001$

B. Push-In Minus Pull-Out, By Week

	Failure Load (N)		Stiffness (N/mm)	
	Estimate (SE)	*p* Value	Estimate (SE)	*p* Value
1-week	115.1 (113.1)	0.322	636.6 (205.6)	0.006
4-week	210.9 (95.8)	0.044	987.6 (169.7)	<0.001
8-week	246.6 (95.8)	0.021	984.2 (169.7)	<0.001
12-week	435.4 (95.3)	<0.001	1,480.3 (169.7)	<0.001

*Standard errors associated with adjusted estimates are computed using variance-component estimates from the mixed-linear model analysis.
SE = standard error.

TABLE 4 Correlation (r) and *p* Value between Secondary Stability and Mechanical Properties of Bone–implant Interfaces Measured by Pull-Out and Push-in Tests

		Pull-Out Test Group		Push-In Test Group	
Variable	By Variable	*r*	*p* Value	*r*	*p* Value
Secondary stability	Failure load	0.476	0.034*	0.656	0.003*
Secondary stability	Displacement	0.077	0.745	–0.283	0.255
Secondary stability	Stiffness	0.396	0.084	0.722	<0.001*
Secondary stability	Tibia weight	0.024	0.919	–0.026	0.916

*$p < .05$.

on 4-mm diameter, 14-mm long dental implants placed in dog mandibles after 3 months of healing[9] showed results similar to the present rabbit tibia push-in data at 12 weeks healing (Table 2) for failure load (1,194 N vs 787 N), displacement at failure (0.69 mm vs 0.42 mm), and interface stiffness (1,959 N/mm vs 2,185 N/mm).

The push-in test measured significantly higher failure load and interface stiffness than the pull-out test in the current study. This result was expected because the push-in load is resisted by bone at the apex of the implant and bone engaging the axial walls of the dental implant, while pull-out load is resisted only by bone engaging the axial walls of the dental implant. As a result, the pull-out test measures the shear strength of the bone at the interface while push-in test measures a combination of that same shear strength (measured in the opposite direction of force application from pull-out test) and compressive strength of the bone at the apex.

Branemark estimated shear stress with the pull-out test using a simple cylinder approximation as a function of measured pull-out force, outer diameter of the implant, and effective length of the implant in contact with the bone.[14] For the push-in test, however, because of the difficulty in distinguishing shear and compressive components of the measured total force, no model has been proposed to accurately estimate separate compressive and shear strengths. That may be the reason why more pull-out/push-out than push-in tests have been used for interface characterization studies and why most studies employing push-in tests report the actual measured total force and displacement, not estimated compressive and shear stresses. However, even with this limitation, the push-in test has the most clinical relevance because chewing, clenching, and bruxing are all more similar to the push-in test than to the pull-out or push-out tests.[15]

For failure load and interface stiffness, the push-in test appears to be more sensitive to changes over the healing period than the pull-out test – it not only measured higher failure load and stiffness than pull-out but also showed more striking increases as healing progressed (Table 3). On the other hand, the amount of displacement measured under both push-in and pull-out tests may not be a good indicator of progression of healing process. Even though the pull-out test induced on average 240 µm more displacement at failure than the push-in test, there was no average trend over healing periods in displacement (Table 2, $p = .17$), nor do the testing methods differ in their (non-existent) trends over the healing periods (test-by-healing period interaction, $p = .42$). With the pull-out test, failure load, displacement, and stiffness did not change significantly as healing progressed from 1 to 12 weeks. Unfortunately, no literature was found comparing push-in and pull-out/push-out tests in animal models. A study by Ogawa reported that failure load increased significantly over 0–8 weeks of healing and was significantly higher with rough surfaced implants than machine-surfaced implants.[7] Ogawa's study supported the validity of the push-in test in rats as a rapid and sensitive biomechanical assay system for implant osseointegration.

The implant stability value (ISQ, implant stability quotient) measured by resonance frequency analysis (RFA) reflects the stiffness of the bone–implant interface.[16,17] During the healing period, an increase of implant stability as measured by RFA devices has been reported in rabbit tibia studies[18,19] and human clinical studies.[16,20] The current results agree with previous studies, showing that secondary implant stability increased significantly over the 12-week healing period, and the p value ($p < .001$) was comparable to those of failure load ($p = .005$) and stiffness ($p = .002$) (Table 2).

As a result, RFA-based secondary implant stability measurement may be a sensitive predictor for the bone–implant interface stiffness along with mechanical tests, such as the push-in or pull-out. Earlier, Johansson showed that biomechanical testing was more sensitive in terms of predicting the stability of the bone–implant interface than histomorphometric analyses.[21]

Failure load was significantly correlated with secondary implant stability for both push-in ($r = 0.66$) and pull-out ($r = 0.48$) tests. Interface stiffness measured by the push-in test had the highest correlation ($r = 0.72$) with secondary stability by RFA, which is presumed to reflect interface stiffness. However, stiffness measured from the pull-out test was not significantly correlated with secondary implant stability ($r = 0.40$); this is consistent with our finding that the push-in test may be more sensitive than the pull-out test in measuring changes of mechanical properties at bone–implant interfaces during healing in the rabbit tibia model. A sheep mandible study measured ISQ values, maximum pull-out forces, and bone–implant contact of micro-/nano-textured surface and machined surface implants after 12 weeks of healing, but no correlations between ISQ values and maximum pull-out forces were reported.[22] A human cadaver study showed significant positive correlations between pull-out resistance of implants and bone density measured by quantitative computed tomography, but implant stability was not measured.[23]

In conclusion, push-in and pull-out tests measured mechanical properties of healing bone–implant interfaces differently in the rabbit tibia model. The push-in test was more sensitive than the pull-out test in capturing change in the failure load and stiffness of the bone–implant interface as healing progressed. Clinically, the push-in test simulates in vivo occlusal loading such as chewing, clenching, and bruxing more closely than the pull-out test. Thus, the push-in test may be better suited than the pull-out test to measuring mechanical properties of bone–implant interfaces in a rabbit long bone model. A future fresh human cadaver study of dental implants which have been supporting prostheses in different areas of the jawbone, using the same test techniques of the current push-in test, is warranted in an effort to establish the relevance of rabbit long bone model data to human clinical jawbone data, even though limitations still exist such as bony architecture differences between long bone and jaw bone and loading pattern differences between push-in test and clinical chewing, clenching, and bruxing.

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CONFLICT OF INTEREST

The authors have no conflict of interest in this research.

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