In Vivo Evaluation of a Novel Implant Coating Agent: Laminin-1

Kostas Bougas, DDS;* Ryo Jimbo, DDS, PhD;† Stefan Vandeweghe, DDS, PhD;‡ Nick Tovar, PhD;§ Marta Baldassarri, PhD;¶ Ali Alenezi, DDS;** Malvin Janal, PhD;†† Paulo G. Coelho, DDS, PhD;‡‡ Ann Wennerberg, DDS, PhD§§

ABSTRACT

Purpose: The aim of this study was to assess the effect of implant coating with laminin-1 on the early stages of osseointegration in vivo.

Materials and Methods: Turned titanium implants were coated with the osteoprogenitor-stimulating protein, laminin-1 (TL). Their osteogenic performance was assessed with removal torque, histomorphometry, and nanoindentation in a rabbit model after 2 and 4 weeks. The performance of the test implants was compared with turned control implants (T), alkali- and heat-treated implants (AH), and AH implants coated with laminin-1.

Results: After 2 weeks, TL demonstrated significantly higher removal torque as compared with T and equivalent to AH. Bone area was significantly higher for the test surface after 4 weeks, while no significant changes were detected on the micromechanical properties of the surrounding bone.

Conclusions: Within the limitations of this study, our results suggest a great potential for laminin-1 as a coating agent. A turned implant surface coated with laminin-1 could enhance osseointegration comparable with a bioactive implant surface while keeping the surface smooth.

KEY WORDS: biochemical coating, implants, laminin-1, titanium

INTRODUCTION

Dental implants have been used with great success in clinics as a long-term therapy against edentulism.[1–3] The increased knowledge inherited by the extensive implant experience has generated high expectations, thereby challenging the original surgical protocol, which demanded two-step surgery and long healing periods. This demand of increased implant performance has led to the development of new moderately rough surfaces by utilizing chemical modification methods, for example, fluoride etching,[4] alkali–heat treatment,[5] and anodization.[6] These techniques alter both the surface chemistry and topography, which both may contribute to chemical influence on bone tissue, a phenomenon defined as bioactivity.[7] The bioactively modified implants have demonstrated higher success rates in demanding cases, for example, early functional loading[8] and reconstructive jaw surgery,[9] as compared with the previously used turned implants.

When moderately rough surfaces remain within bone tissue, no differences with respect to microbial colonization are observed.[10] However, exposure of the implant surfaces to the oral environment may lead to spontaneous progression at least of experimental peri-implantitis as has been reported in dog studies.[11–13] The

*PhD student, Department of Prosthodontics, Faculty of Odontology, Malmö University, Malmö, Sweden; †associate professor, Department of Prosthodontics, Faculty of Odontology, Malmö University, Malmö, Sweden; ‡senior researcher, Department of Periodontology and Oral Implantology, Dental School, Faculty of Medicine and Health Sciences, University of Ghent, Ghent, Belgium; §adjunct professor, Department of Biomaterials and Biomimetics, New York University, New York, USA; ¶researcher, Department of Biomaterials and Biomimetics, New York University, New York, USA; **MSc student, Department of Prosthodontics, Faculty of Odontology, Malmö University, Malmö, Sweden; ††senior scientist, Department of Epidemiology and Health Promotion, New York University, New York, USA; ‡‡assistant professor, Department of Biomaterials and Biomimetics, New York University, New York, USA; §§professor and head, Department of Prosthodontics, Faculty of Odontology, Malmö University, Malmö, Sweden

Reprint requests: Mr. Kostas Bougas, Department of Prosthodontics, Faculty of Odontology, Malmö University, 20506 Malmö, Sweden; e-mail: kostas.bougas@mah.se

DOI 10.1111/cid.12037

implant surface characteristics have been reported to affect the possibility for debridement during the surgical procedure, thereby potentially influencing the outcome of treatment of experimental peri-implantitis.[14] Therefore, the idea of an implant combining the osteoconductive properties of a moderately rough surface with the accessibility for debridement of a turned surface may be of clinical interest on condition that the experimental peri-implantitis observations are clinically applicable.

In order to induce osteogenicity, bone-specific biomolecules[15–17] have been utilized as implant coating agents. Interestingly, even non-bone-specific molecules have demonstrated osteogenic potential.[18] In vitro studies have identified laminin-1 as a potential osteogenic agent. Laminins are heterotrimeric glycoprotein molecules that bind to a protein family known as integrins, especially β1 and β2 integrins.[19] Laminin-1 has been proposed to selectively recruit osteoprogenitor cells though integrin β1-mediated cell attachment effect[20,21] and to stimulate production of alkaline phosphatase by osteoblasts.[22] Theoretically, any effect of a protein coating is presumably more pronounced during the early stages of osseointegration.

In a previous study,[23] we have investigated the potential of laminin-1-coated titanium discs to precipitate calcium phosphates in vitro. The results from that study suggested enhanced precipitation of calcium phosphates on laminin-1 coated compared with non-coated surfaces. Additionally, alkali- and heat-treated surfaces, which have been claimed to be bioactive,[24] seemed to favor the rate of calcium phosphate precipitation. Nevertheless, because the in vivo environment is more complex in terms of protein interactions[25] and desorption of the coating agent,[26] in vivo validation is regarded most important.

The purpose of this in vivo study is to investigate the effect of a novel implant coating agent, laminin-1, on osseointegration.

MATERIALS AND METHODS

Implants and Laminin-1 Coating

In total, 88 threaded titanium implants (Neodent, Curitiba, Parana, Brazil) with turned surface were used (diameter: 3.5 mm, length: 7 mm). Half of the implants ($n = 44$) were bioactively modified by alkali and heat treatment as described in the literature.[5] In brief, the implants were soaked in 5 M aqueous NaOH for 24 hours at 60°C, rinsed with distilled water, and dried at 40°C for 24 hours. The implants were heated to 600°C by increasing the temperature by 5°C/min in an electrical furnace (Bitatherm, Bita Laboratory Furnaces, Ramat Gan, Israel) and were kept at 600°C for 2 hours. The implants were left in the furnace until they reached room temperature. Prior to surgery, half of the turned ($n = 22$) and half of the alkali- and heat-treated implants ($n = 22$) were coated with laminin-1 and served as the test group. In brief, laminin-1 (L2020, Sigma-Aldrich, Stockholm, Sweden) was diluted to a concentration of 100 μg/mL in Dulbecco's phosphate-buffered saline without $CaCl_2$ or $MgCl_2$ (14190-094; Gibco, Invitrogen Corporation, Grand Island, NY, USA). The implants were subsequently incubated in 48-well plates (Nunclon Surface, Nunc, Roskilde, Denmark) containing 250 μL of the laminin-1 solution per well for 1 hour at room temperature. As investigated with ellipsometry on thin vapor-deposited titanium surfaces,[27] the incubation resulted to protein thickness corresponding to 26 Å. The remaining uncoated implants served as the controls.

Surface Characterization

The surface topography of the implants was characterized with an optical interferometer (MicroXam, ADE Phase Shift, Tucson, AZ, USA) operating in a wavelength of $\lambda = 550$ nm. According to the suggested guidelines for implant surface characterization,[28] three implants from each group were randomly selected and each measured in nine regions (three thread tops, three thread valleys, and three flank regions). A 50×50 μm Gaussian filter was applied to separate roughness from form and waviness. The following three topographical parameters were evaluated: Sa (μm) = the arithmetic average height deviation from a mean plane; Sds (μm^{-2}) = the density of summits; and Sdr (%) = the developed surface ratio. Thus, the three selected parameters described the variation in height, the spatial dimension, and the overall surface enlargement; the latter is dependent on vertical and height variations, thereby representing a so-called hybrid parameter.

Animals and Surgical Procedure

The study was approved by the Malmö/Lund, Sweden, Regional Animal Ethical Committee (approval number: M282-09) and included 22 lop-eared rabbits (11 per time point) of mixed sexes with an average weight of 4.07 kg.

Prior to surgery, the animals were sedated by intramuscular injections of a mixture of 0.15 mL/kg

of medetomidine (1 mg/mL Dormitor; Orion Pharma, Sollentuna, Sweden) and 0.35 mL/kg of ketamine hydrochloride (50 mg/mL Ketalar; Pfizer AB, Sollentuna, Sweden). The hind legs were shaved and disinfected with 70% ethanol and 70% chlorhexidine. Lidocaine hydrochloride (Xylocaine; AstraZeneca AB, Gothenburg, Sweden) was administrated as local anesthesia at each insertion site at a dose of 1 mL. Four groups of implants were defined as the following: T: turned; TL: turned and laminin-1 coated; AH: alkali and heat treated; and AHL: alkali and heat treated and laminin-1 coated. Two implants were inserted in each tibia. Laminin-1-coated implants (TL and AHL) were always placed in the same leg to avoid errors due to possible diffusion of laminin-1 to the surrounding bone. However, the position of TL and AHL within the tibia was randomly selected, as was the case for T and AH. The operator was blinded regarding the implant insertion scheme. After the operation, buprenorphine hydrochloride (0.5 mL Temgesic; Reckitt Benckiser, Slough, UK) was administered as an analgesic for 3 days. After 2 and 4 weeks, the rabbits were sacrificed with an overdose (60 mg/mL) of pentobarbital natrium (Apoteksbolaget AB, Stockholm, Sweden).

Removal Torque (RTQ)

The skin above the implants was incised, and 64 implants (eight of each group and time point) were screwed out of the bone with a RTQ device. The RTQ equipment is an electronic device with a strain gauge transducer where the torque necessary to loosen the implant from the bed is registered immediately at the moment of loosening. The highest value for each implant was recorded as the RTQ value.

Histomorphometry

The samples were processed for undecalcified ground sectioning.[29] In brief, after a series of dehydrations and infiltrations in resin, the samples were embedded in light-curing resin (Technovit 7200 VLC; Heraeus Kulzer, Wehrheim, Germany). One central ground section was prepared from each implant by using the Exakt sawing and grinding equipment. The sections were ground to a final thickness of approximately 10 µm and histologically stained with toluidine blue mixed with pyronin G.

Histological evaluations were performed using a light microscope (Eclipse ME600; Nikon Co., Tokyo, Japan), and the histomorphometrical data were analyzed by an image analysis software (Image J v. 1.43u; National Institutes of Health, Bethesda, MD, USA). The implant surface between two consecutive thread tops was defined as the area of interest. The bone-implant contact (BIC) percentage and the bone area (BA) percentage along the implant for total bone and new bone were calculated with ×10 objectives. Additionally, the osteoinductive capacity of the various implants was estimated in terms of BIC (new BIC) and BA (new BA) for the newly formed bone.

Nanoindentation

The remaining portion blocks embedded in resin were processed in the same manner as the histological sections; further polishing was performed to remove both scratches and staining using diamond suspensions of 9- to 1-µm particle size (Buehler, Lake Bluff, IL, USA). The final thickness of these sections was approximately 100 µm. Nanoindentation ($n = 30$/specimen) was performed using a nanoindenter (Hysitron TI 950, Minneapolis, MN, USA) equipped with a Berkovich diamond three-sided pyramid probe. A wax chamber was created above the acrylic plate around the implant-in-bone perimeter, so that tests were performed in distilled water. A loading profile was developed using a peak load of 300 µN at a rate of 60 µN/s, followed by a holding time of 10 seconds and an unloading time of 2 seconds. The extended holding period allowed bone to relax to a more linear response, so that no tissue creep effect was occurring in the unloading portion of the profile (ISO 14577-4). Therefore, from each indentation, a load–displacement curve was obtained.[30]

For each specimen, mechanical testing was performed in the threaded region (cortical area), in which generally new bone formation is present at early observation time points. This region was subdivided into four bone quadrants. Bone tissue was detected by imaging under the light microscope (Hysitron TI 950, Minneapolis, MN, USA) and indentations were performed in the selected areas. From each analyzed load–displacement curve, reduced modulus (GPa) and hardness (GPa) of bone tissue were computed and its elastic modulus E (GPa) was calculated as follows:

$$\frac{1}{E_r} = \frac{1-\nu^2}{E} + \frac{1-\nu_i^2}{E_i}$$

where E_r is the reduced modulus (GPa), ν (0.3) is the Poisson's ratio for cortical bone, and E_i (1140 GPa) and

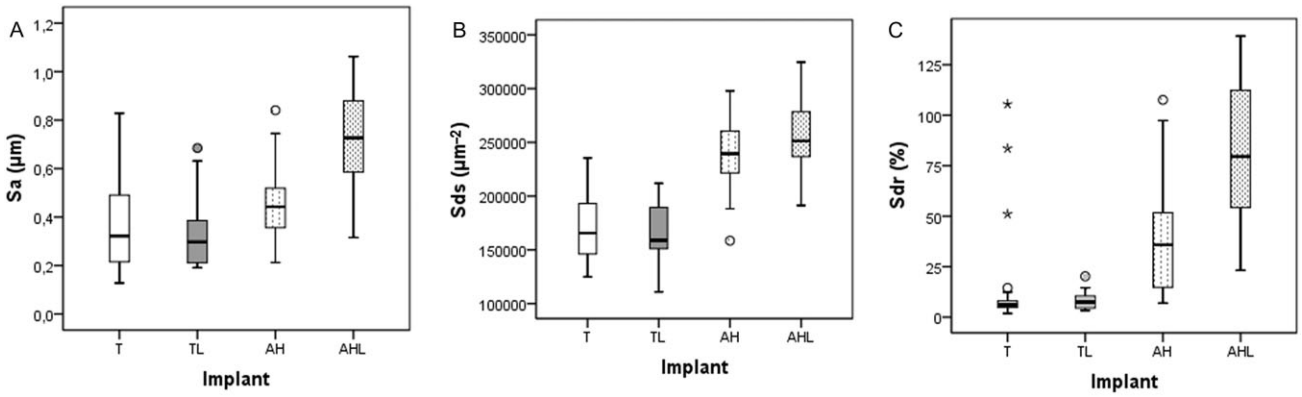

Figure 1 Surface topography parameters prior to operation: Sa (A), Sds (B), and Sdr (C). *p*-values are presented in Table 1. AH = alkali and heat treated; AHL = alkali and heat treated + laminin-1; T = turned; TL = turned + laminin-1.

ν_i (0.07) are the elastic modulus and the Poisson's ratio for the indenter.[31–33]

Statistical Analyses

The statistical calculations were performed with SPSS® (version 18, Chicago, IL, USA). The normality of the data was assessed by the Shapiro-Wilk test. The statistical comparison for the topographic parameters Sa, Sds, and Sdr, as well as for RTQ, was assessed by Friedman's two-way analysis of variance by ranks. Pairwise comparisons were executed with the nonparametric Wilcoxon signed-rank test. The statistical comparison for the mean values of BIC, new BIC, BA, and new BA was assessed by one-way analysis of variance combined with the Bonferroni post-hoc test. For the statistical analysis of the nanoindentation, a generalized randomized block was used. The level of statistical significance was set at $p \leq .05$.

RESULTS

Surface Characterization

Coating of the turned implants with laminin-1 (TL) induced no statistically significant differences of the initial (T) topography at the micrometer level of resolution (Figure 1, A–C). In contrast to the T surface, once the AH surface was subjected to laminin coating (AHL), the height deviation from the mean plane (Sa) was increased and the total surface of the implant (Sdr) was enlarged significantly. On the contrary, the coating process had no effect on the density of summits (Sds) of the alkali- and heat-treated surfaces. All the investigated surface topography parameters were significantly lower for T and TL as compared with both AH and AHL (Table 1).

RTQ

After 2 weeks, the implants TL and AH demonstrated significantly higher RTQ as compared with T and AHL. No significant differences were detected among the four implant groups after 4 weeks (Figure 2).

Histomorphometry

When measuring the osteoconductivity of the surfaces, significantly higher BA (Figure 3B) was detected after 4 weeks for TL as compared with all other groups (Table 2). For the same observation time, new BIC (Figure 3C) was also higher for TL, but the difference was not significant ($p = .258$).

After 2 ($p = .353$) and 4 weeks ($p = .459$), no significant differences were concluded among the four implant groups in BIC (Figure 3A). New BA was not found to differ among the groups (Figure 3D) at any time point

TABLE 1 *p*-Values for the Pairwise Comparisons Regarding Surface Topography Parameters among the Implant Types

	Sa (*p*-Value)	Sds (*p*-Value)	Sdr (*p*-Value)
T–TL	.528	.647	.574
T–AH	.033*	<.001***	<.001***
T–AHL	<.001***	<.001***	<.001***
TL–AH	.002**	<.001***	<.001***
TL–AHL	<.001***	<.001***	<.001***
AH–AHL	<.001***	.131	<.001***

*$p \leq .05$; **$p \leq .01$; ***$p \leq .001$.
AH = alkali- and heat-treated implant; AHL = alkali- and heat-treated implant coated with laminin-1; T = turned implant; TL = turned implant coated with laminin-1.

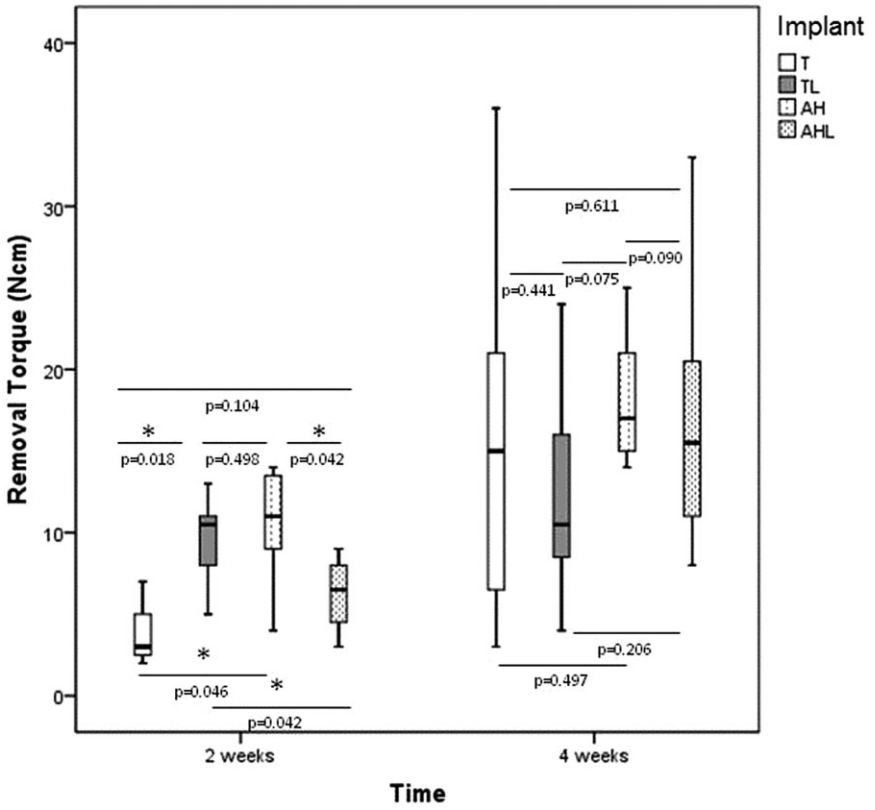

Figure 2 Removal torque values after 2 and 4 weeks. AH = alkali and heat treated; AHL = alkali and heat treated + laminin-1; T = turned; TL = turned + laminin-1.

($p = .938$ and $p = .218$). Significant differences were detected neither for new BIC (Figure 3C) ($p = .288$) nor for BA (Figure 3B) ($p = .566$) after 2 weeks.

Nanoindentation

The micromechanical properties of the bone surrounding the four tested implant types did not present any significant differences in elastic modulus or in hardness after 2 ($p = .647$ and $p = .418$, respectively) and 4 ($p = .739$ and $p = .523$, respectively) weeks.

DISCUSSION

In the present study, the knowledge from the previous in vitro results[23] was implemented in the choice of the surface modifications. Evaluation of the selected topographical parameters did not reveal any alterations of the turned implant surface profile as a result to laminin-1 coating. Hence, the response of the bone tissue to TL implant may be explained by the molecular influence of laminin-1. Although coating with laminin-1 did not affect the surface topography of the turned surface significantly, it did elevate the average height deviation from a mean plane (Sa) and the developed surface ratio (Sdr) of the alkali- and heat-treated surface. The structure of alkali- and heat-treated surfaces has been described before[5] as spike-like structures. The protein deposition on the surface may have further increased the length of the spike-like structures. This could have resulted in higher Sa and subsequent increase of the total implant surface (Sdr). On the other hand, the protein coating did not result in significant changes in Sds. It might have been expected that the protein molecules would be detected as prominences on the titanium surfaces. However, such prominences could not be detected in the current resolution and hence did not result in any changes in the density of summits (Sds).

Reflecting the optimal surface properties for osseointegration,[34] higher RTQ values for the AHL implant may have been expected because its surface properties are closer to the optimal ones. Nevertheless, important surface chemistry properties[5] may have been altered by the laminin-1 coating. This may explain why the RTQ performance of AHL implants corresponded to that of T implants. However, the intriguing finding was that laminin-1 coating of the turned surfaces promoted the RTQ response to a level equivalent to RTQ for the

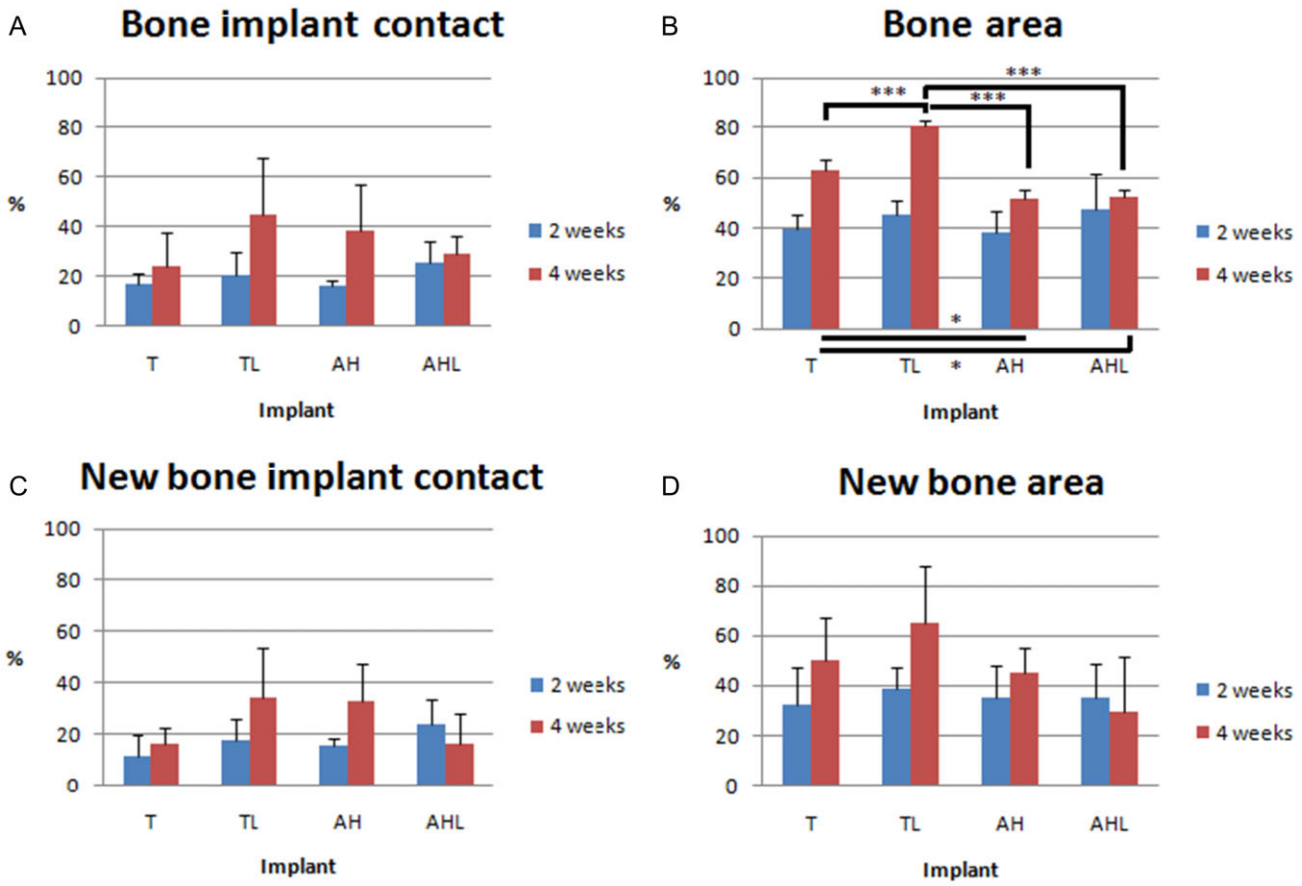

Figure 3 Bone-implant contact (A), bone area (B), new bone-implant contact (C), and new bone area (D) after 2 and 4 weeks measured by histomorphometry. *p*-values are presented in Table 2. AH = alkali and heat treated; AHL = alkali and heat treated + laminin-1; T = turned; TL = turned + laminin-1.

"bioactive" AH surfaces. To our understanding, this may be explained by the alterations in surface chemistry induced by laminin-1 because no alterations in surface topography were apparent. This seems to be in agreement with the in vitro explanation model proposing recruitment of pre-osteoblasts by an integrin β1 dependent cell attachment effect.[20,21]

As in vitro results show higher protein release during early incubation time points,[35] the biochemical coating is theoretically released more rapidly during the early time after implantation, and it may be assumed that the findings from histomorphometry should correspond to the RTQ values. Despite the differences in RTQ after 2 weeks, no statistically significant differences were detected in terms of BIC, new BIC, BA, or new BA. However, although a well-established method, histomorphometry may be assumed not to provide adequate information regarding the overall quality of the bone in a three-dimensional pattern because it represents a single central section. The higher BA detected after 4 weeks is more likely to contribute to enhanced pull-out strength than higher RTQ. This assumption is in agreement with the findings from a study evaluating bisphosphonate-coated implants in rats.[36]

TABLE 2 *p*-Values for the Pairwise Comparisons Regarding BA among the Implant Types after 4 Weeks

	BA 4 Weeks (*p*-value)
T–TL	.001***
T–AH	.015*
T–AHL	.019*
TL–AH	<.001***
TL–AHL	<.001***
AH–AHL	1.000

*$p \leq .05$; **$p \leq .01$; ***$p \leq .001$.
AH = alkali- and heat-treated implant; AHL = alkali- and heat-treated implant coated with laminin-1; BA = bone area; T = turned implant; TL = turned implant coated with laminin-1.

As it regards the nanomechanical properties of the newly formed bone, the absence of significant difference on elastic modulus and hardness may have several explanations. The mechanical properties of newly formed bone (4 weeks) around implants in rabbits have been studied by nanoindentation[37] and micro-Brillouin scattering.[38] The results have demonstrated that newly formed peri-implant bone presents lower hardness, lower elastic modulus, higher biomechanical heterogeneity, and 13.5% less mass compared with mature bone (7 weeks). Taking into account the time points used in the present study (2 and 4 weeks), it may be speculated that any difference in bone response triggered by laminin-1 coating is limited on the implant-bone interface at the investigated time points. This may explain why RTQ testing most successfully detected differences in the biomechanical response among the tested surface modifications, although the mechanical properties of the surrounding bone tissue did not significantly differ among the tested groups, as evaluated with the nanoindenter. Hence, it could be of importance to examine the biological phenomena occurring in the implant-bone interface in a future study.

CONCLUSIONS

To our knowledge, this is the first time a study presents evidence that coating of turned titanium implants with laminin-1 enhances osseointegration in vivo. This finding is of importance because laminin-1 coating has great potential to enhance osseointegration compared with ordinary turned. As the mechanisms of coating clearance in vivo are complicated, it appears to be challenging to determine the appropriate time point to examine the peak of the phenomenon. This complexity results in an outcome highly depending on the choice of evaluation time points and methods. Therefore, further studies are needed to understand the underlying biological mechanisms in terms of gene expression and molecular signaling pathways.

CONFLICTS OF INTEREST AND SOURCE OF FUNDING

The authors declare no conflicts of interest. This project was funded by the Swedish National Graduate School in Odontological Science, the Swedish Research Council (K2009-52X-06533-27-3), the Hjalmar Svenson Research Foundation, the Sylvan Foundation, the Wilhelm and Martina Lundgren Science Foundation, the Swedish Knowledge Foundation, the Council for Research and Development in Södra Älvsborg, Sweden, and the Royal Society of Arts and Sciences in Göteborg. The authors would like to acknowledge Neodent (Curitiba, Parana, Brazil) for kindly providing the implants.

REFERENCES

1. Jemt T, Johansson J. Implant treatment in the edentulous maxillae: a 15-year follow-up study on 76 consecutive patients provided with fixed prostheses. Clin Implant Dent Relat Res 2006; 8:61–69.
2. Jemt T. Single implants in the anterior maxilla after 15 years of follow-up: comparison with central implants in the edentulous maxilla. Int J Prosthodont 2008; 21:400–408.
3. Astrand P, Ahlqvist J, Gunne J, Nilson H. Implant treatment of patients with edentulous jaws: a 20-year follow-up. Clin Implant Dent Relat Res 2008; 10:207–217.
4. Ellingsen JE. Pre-treatment of titanium implants with fluoride improves their retention in bone. J Mater Sci Mater Med 1995; 6:749–753.
5. Kim HM, Miyaji F, Kokubo T, Nakamura T. Preparation of bioactive Ti and its alloys via simple chemical surface treatment. J Biomed Mater Res 1996; 32:409–417.
6. Sul YT, Johansson CB, Albrektsson T. Oxidized titanium screws coated with calcium ions and their performance in rabbit bone. Int J Oral Maxillofac Implants 2002; 17:625–634.
7. Williams DF. The Williams dictionary of biomaterials. Liverpool: Liverpool University Press, 1999.
8. Jungner M, Lundqvist P, Lundgren S. Oxidized titanium implants (Nobel Biocare TiUnite) compared with turned titanium implants (Nobel Biocare mark III) with respect to implant failure in a group of consecutive patients treated with early functional loading and two-stage protocol. Clin Oral Implants Res 2005; 16:308–312.
9. Brechter M, Nilson H, Lundgren S. Oxidized titanium implants in reconstructive jaw surgery. Clin Implant Dent Relat Res 2005; 7 (Suppl 1):S83–S87.
10. Quirynen M, Van Assche N. RCT comparing minimally with moderately rough implants. Part 2: microbial observations. Clin Oral Implants Res 2012; 23:625–634.
11. Albouy JP, Abrahamsson I, Persson LG, Berglundh T. Spontaneous progression of peri-implantitis at different types of implants. An experimental study in dogs. I: clinical and radiographic observations. Clin Oral Implants Res 2008; 19:997–1002.
12. Albouy JP, Abrahamsson I, Persson LG, Berglundh T. Spontaneous progression of ligatured induced peri-implantitis at implants with different surface characteristics. An experimental study in dogs II: histological observations. Clin Oral Implants Res 2009; 20:366–371.
13. Albouy JP, Abrahamsson I, Berglundh T. Spontaneous progression of experimental peri-implantitis at implants

with different surface characteristics: an experimental study in dogs. J Clin Periodontol 2012; 39:182–187.
14. Albouy JP, Abrahamsson I, Persson LG, Berglundh T. Implant surface characteristics influence the outcome of treatment of peri-implantitis: an experimental study in dogs. J Clin Periodontol 2011; 38:58–64.
15. Thorey F, Menzel H, Lorenz C, Gross G, Hoffmann A, Windhagen H. Osseointegration by bone morphogenetic protein-2 and transforming growth factor beta2 coated titanium implants in femora of New Zealand white rabbits. Indian J Orthop 2011; 45:57–62.
16. Wikesjo UM, Huang YH, Xiropaidis AV, et al. Bone formation at recombinant human bone morphogenetic protein-2-coated titanium implants in the posterior maxilla (type IV bone) in non-human primates. J Clin Periodontol 2008; 35:992–1000.
17. Abtahi J, Tengvall P, Aspenberg P. A bisphosphonate-coating improves the fixation of metal implants in human bone. A randomized trial of dental implants. Bone 2012; 50:1148–1151.
18. Nyan M, Sato D, Kihara H, Machida T, Ohya K, Kasugai S. Effects of the combination with alpha-tricalcium phosphate and simvastatin on bone regeneration. Clin Oral Implants Res 2009; 20:280–287.
19. Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. Dev Dyn 2000; 218:213–234.
20. Roche P, Goldberg HA, Delmas PD, Malaval L. Selective attachment of osteoprogenitors to laminin. Bone 1999; 24:329–336.
21. Roche P, Rousselle P, Lissitzky JC, Delmas PD, Malaval L. Isoform-specific attachment of osteoprogenitors to laminins: mapping to the short arms of laminin-1. Exp Cell Res 1999; 250:465–474.
22. Vukicevic S, Luyten FP, Kleinman HK, Reddi AH. Differentiation of canalicular cell processes in bone cells by basement membrane matrix components: regulation by discrete domains of laminin. Cell 1990; 63:437–445.
23. Bougas K, Franke Stenport V, Tengvall P, Currie F, Wennerberg A. Laminin coating promotes calcium phosphate precipitation on titanium discs in vitro. J Oral Maxillofac Res 2011; 2(4):e5.
24. Brunette D, Tengvall P, Textor M, Thomsen P. Proteins at titanium interfaces titanium in medicine: material science, surface science, engineering, biological responses and medical applications. Berlin: Springer, 2001:457–483.
25. Hamouda IM, Enan ET, Al-Wakeel EE, Yousef MK. Alkali and heat treatment of titanium implant material for bioactivity. Int J Oral Maxillofac Implants 2012; 27: 776–784.
26. Fang F, Satulovsky J, Szleifer I. Kinetics of protein adsorption and desorption on surfaces with grafted polymers. Biophys J 2005; 89:1516–1533.
27. Linderback P, Harmankaya N, Askendal A, Areva S, Lausmaa J, Tengvall P. The effect of heat- or ultra violet ozone-treatment of titanium on complement deposition from human blood plasma. Biomaterials 2010; 31:4795–4801.
28. Wennerberg A, Albrektsson T. Suggested guidelines for the topographic evaluation of implant surfaces. Int J Oral Maxillofac Implants 2000; 15:331–344.
29. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. J Oral Pathol 1982; 11:318–326.
30. Oliver WC, Pharr GM. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. J Mater Res 1992; 7:1564–1583.
31. Doerner MF, Nix WD. A method for interpreting the data from depth-sensing indentation instruments. J Mater Res 1986; 1:601–609.
32. Hoffler CE, Moore KE, Kozloff K, Zysset PK, Brown MB, Goldstein SA. Heterogeneity of bone lamellar-level elastic moduli. Bone 2000; 26:603–609.
33. Hoffler CE, Guo XE, Zysset PK, Goldstein SA. An application of nanoindentation technique to measure bone tissue lamellae properties. J Biomech Eng 2005; 127:1046–1053.
34. Wennerberg A. Doctoral thesis: on surface roughness and implant incorporation. Göteborg 1996.
35. Lenza RF, Jones JR, Vasconcelos WL, Hench LL. In vitro release kinetics of proteins from bioactive foams. J Biomed Mater Res A 2003; 67:121–129.
36. Agholme F, Andersson T, Tengvall P, Aspenberg P. Local bisphosphonate release versus hydroxyapatite coating for stainless steel screw fixation in rat tibiae. J Mater Sci Mater Med 2012; 23:743–752.
37. Vayron R, Barthel E, Mathieu V, Soffer E, Anagnostou F, Haiat G. Nanoindentation measurements of biomechanical properties in mature and newly formed bone tissue surrounding an implant. J Biomech Eng 2012; 134:021007.
38. Mathieu V, Fukui K, Matsukawa M, et al. Micro-Brillouin scattering measurements in mature and newly formed bone tissue surrounding an implant. J Biomech Eng 2011; 133:021006.