Implant Osseointegration in Circumferential Bone Defects Treated with Latex-Derived Proteins or Autogenous Bone in Dog's Mandible

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ABSTRACT

Background: In sites with diminished bone volume, the osseointegration of dental implants can be compromised. Innovative biomaterials have been developed to aid successful osseointegration outcomes.

Purpose: The aim of this study was to evaluate the osteogenic potential of angiogenic latex proteins for improved bone formation and osseointegration of dental implants.

Materials and Methods: Ten dogs were submitted to bilateral circumferential defects (5.0 × 6.3 mm) in the mandible. Dental implant (3.3 × 10.0 mm, TiUnite MK3™, Nobel Biocare AB, Göteborg, Sweden) was installed in the center of the defects. The gap was filled either with coagulum (Cg), autogenous bone graft (BG), or latex angiogenic proteins pool (LPP). Five animals were sacrificed after 4 weeks and 12 weeks, respectively. Implant stability was evaluated using resonance frequency analysis (Osstell Mentor™, Osstell AB, Göteborg, Sweden), and bone formation was analyzed by histological and histometric analysis.

Results: LPP showed bone regeneration similar to BG and Cg at 4 weeks and 12 weeks, respectively ($p \geq .05$). Bone formation, osseointegration, and implant stability improved significantly from 4 to 12 weeks ($p \leq .05$).

Conclusion: Based on methodological limitations of this study, Cg alone delivers higher bone formation in the defect as compared with BG at 12 weeks; compared with Cg and BG, the treatment with LPP exhibits no advantage in terms of osteogenic potential in this experimental model, although overall osseointegration was not affected by the treatments employed in this study.

KEY WORDS: bone regeneration, circumferential bone defects, *Hevea brasiliensis*, implant osseointegration, latex proteins

INTRODUCTION

Natural latex extracted from the rubber tree *Hevea brasiliensis* has proven to be an innovative biomaterial. Because of its high biocompatibility,[1,2] natural latex

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DOI 10.1111/j.1708-8208.2009.00238.x

biomembrane[2] has been used to correct tympanic defects,[3] and as a substitute for the pericardium[4] and blood vessels[5] in experimental protocols. In humans, current clinical trials have been conducted using the natural latex to cover skin ulcers in patients with diabetes,[1,6,7] to reconstruct eye conjunctiva,[8] and to stimulate bone repair of dental sockets in rats.[9]

The application of natural latex in vivo induces tissue vascularization probably because of the presence of growth factors in the latex.[1] Immunohistochemical analysis from biopsies of venous ulcers covered with latex membranes demonstrated higher expression of vascular endothelial growth factor and transforming growth factor-β1 in contact with the material. Recently, increased osteogenesis in initial periods of dental socket repair when filled with latex granules was reported, probably as a consequence of higher vessel proliferation observed in the experimental group compared with control.[9] This may suggest a direct action of latex proteins involved with angiogenic properties. In fact, chromatographic purification of natural latex followed by gel electrophoresis confirmed the presence of a group of latex angiogenic proteins pool (LPP) with angiogenic activity present in the biomaterial.[10] Subsequently, patent registrations for latex derivatives were obtained (PI 0207426-5/2002 and 0506041-9/2005), and commercial products have become available for medical/pharmaceutical application.

Neoangiogenesis can be an indirect pathway to improve bone formation. It is well known that angiogenesis is essential for the osteogenic process, as bone regenerative process requires the participation of a number of growth factors and cellular components that lead to osteoblastic differentiation and bone matrix mineralization.[11–21] However, the benefit of angiogenic latex-derived proteins to osseointegration of titanium dental implants in vivo has not been evaluated. Sufficient bone volume and quality are primary requirements to successful dental implant osseointegration.[22,23] Therefore, several regenerative procedures and bone grafting materials have been employed to improve bone formation and dental implant osseointegration.[24] Autogenous bone has been widely considered the gold-standard grafting material in bone reconstructive surgery.[25] However, drawbacks such as morbidity, availability, time-consuming treatment, and unpredicted graft resorption have led to the search for more suitable bone substitutes.[24,26–28] The aim of the present study is to evaluate the osteogenic capacity of LPP in dental implant osseointegration placed in circumferential bone defects by using particulate autogenous bone graft (BG) as a reference.

MATERIALS AND METHODS

LPP Extraction

Natural latex was extracted from *Hevea brasiliensis* (rubber tree) and diluted in 2.2% acetic acid (1:2) under constant stirring. The solution was set to rest at room temperature for 30 minutes for latex coagulation. The polymerized rubber was then pressed to obtain the serum that was further mixed with an ammonium hydroxide solution (1 M). Because of the precipitation of proteins during the pH setting process, the serum was filtered in 1 μm pore diameter filters (Millipore, Bedford, MA, USA). Ion-exchange chromatography (7 mL/min flow) was used to separate the latex fractions present in the serum using a 0.01 M buffer solution of ammonium carbonate in increasing sodium chloride gradient. The latex fractions were analyzed in a spectrophotometer (U-2000 model, Hitachi®, Tokyo, Japan) at 280 ηm wavelength. Once the angiogenic fraction of natural latex was detected, according to its chromatographic profile, the material was dialyzed against distilled water, lyophilized, and then associated with bovine collagen gel (collagen type I, Sigma-Aldrich, São Paulo, SP, Brazil) and bacterial hyaluronic acid from *Escherichia coli* (Nikko Chemicals Co. Ltd., Shanghai, China) in a final concentration of 2.5% of collagen, 2.5% of hyaluronic acid, and 0.01% of latex angiogenic protein. All procedures were carried out under a sterile environment. The biomaterials were kept in disposable ethylene oxide-sterilized syringes and stored at −20°C until use.

Animals

Ten young mongrel male dogs (20–30 kg) were used in this study. The animals were vaccinated, vermifuged, and received vitamins during quarantine. The animals were kept in individual cages, under adequate veterinary care, and have free access to water and balanced chow during the entire experimentation period. The study protocol was approved by University of São Paulo's Animal Research Ethics Committee (no. 2006.1.1070.53.0).

Surgical Procedures

All animals underwent two surgery procedures, one for bilateral extraction of the first, second, third, and fourth

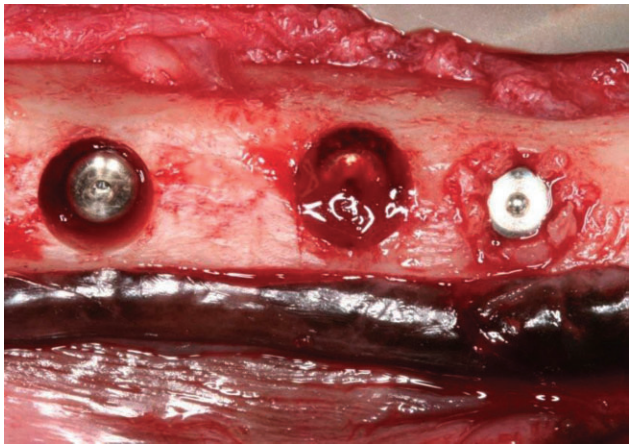

Figure 1 Implant installed into circumferential gaps filled with coagulum, LPP, or bone graft (left to right direction).

inferior premolars, and the other for implantation of the materials. The animals were submitted to external antisepsis with Polyvinylpyrrolidone Iodine (PVPI™) dye (Riodeine Tópico – Laboratório Biossintética Ltda., Ribeirão Preto, SP, Brazil), and intraoral antisepsis with PVPI™ topic (Riodeine Tópico – Laboratório Biossintética Ltda.); pre-anesthetized with Amplictil® 25 mg/mL intravenously (Rhodia Farma Ltda., São Paulo, SP, Brazil); induced to anesthesia with 0.1 mL/kg intramuscular Zoletil® 50 (Virbac do Brasil, São Paulo, SP, Brazil); and anesthetized with inhaling Forane® (Abbott Laboratórios do Brasil Ltda., São Paulo, SP, Brazil). Twelve weeks following tooth extraction, three circumferential defects (6.3 mm in diameter and 10.0 mm in depth) were created using a trephine bur in each mandible side. Bone tissue was collected during this procedure and stored in 0.9% sterile saline to be used later as particulate autogenous BG. Following drilling, three titanium implants (3.3 mm in diameter and 10 mm in length; TiUnite MK3, Nobel Biocare™ AB, Göteborg, Sweden) were installed in the center of each defect. The implant cover screws were placed, and the circumferential bone defects were filled with coagulum (Cg), LPP, and particulate autogenous BG (Figure 1). The circumferential bone defects were covered by a mucoperiosteal flap. The wounds were sutured with interrupted nonabsorbable (Silk Ethicon™ 4-0, Johnson & Johnson, São José dos Campos, SP, Brazil) stitches at extraction and implantation surgeries, and removed after 7 postoperative days.

All animals were medicated with analgesics (subcutaneous tramadol, Anangon™ – Laboratórios Biosintética Ltda., São Paulo, SP, Brazil), oral Ketofen (Merial Saúde Animal Ltda., Paulínia, SP, Brazil), and antibiotic therapy using oral Stomorgyl 10™ (Merial Saúde Animal Ltda.) during 5 days. Five animals were sacrificed at 4 weeks and 12 weeks, respectively, after implant installation, using 1% thiopentax™ (sodium thiopental, Cristália, Produtos Químicos Farmacêuticos LTDA, Itapira, SP, Brazil) and 19.1% potassium chloride at 1 mL/kg (10 mL dose – Samtec Biotecnologia, Ribeirão Preto, SP, Brazil) intravenously.

Implant Stability

Implant stability was measured by means of resonance frequency analysis (Osstell Mentor™, Osstell AB, Göteborg, Sweden) at 0 week, 4 weeks, and 12 weeks. The average of two measurements was used to express an implant stability quotient (ISQ) for each implant.

Histological Processing

Bone blocks of the experimental sites were fixed in 4% buffered formaldehyde for 48 hours. The samples were dehydrated in graded concentrations of alcohol and embedded in acrylic resin (LR White®, London Resin Company Ltd., Berkshire, England, UK). The blocks were then sectioned buccal-lingually with a precision saw (Microslice 2™, Ultra Tec Manufacturing Inc, Santa Ana, CA, USA) and mounted onto glass slides for histological and histomorphometric analysis of the circumferential bone defect region (Leica DMLB®, Leica Microsystems GmbH, Wetzlar, Germany). The histometric analysis was performed to evaluate the bone-implant contact (BIC) and bone area in a standardized rectangle (BAR) (Figure 2) using the Leica Qwin Pro® software version 3.4.0 (Leica Microsystems GmbH).

Statistical Analysis

Data were analyzed using the analysis of variance, and orthogonal contrasts posttest were used for multiple comparisons to determine statistical significance among the experimental groups. Statistical significance was considered for $p \leq .05$.

RESULTS

Clinical Evaluation

Any tissue dehiscences, exposure of biomaterial, or dental implant was not detected. The wound edges were closed, and tissue healing progressed uneventfully.

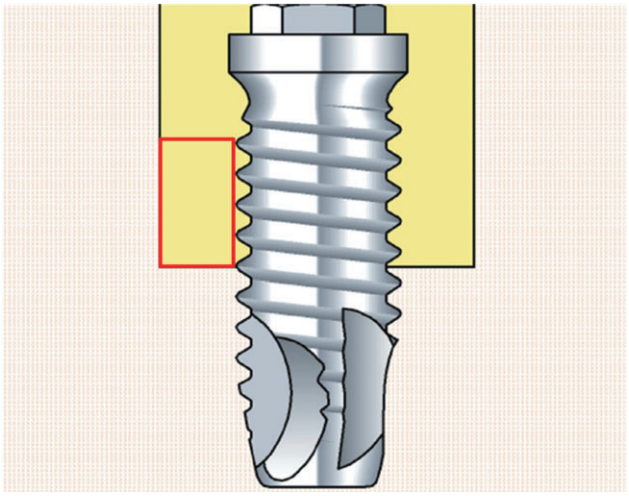

Figure 2 Schematic drawing of a dental implant installed in a circumferential bone defect (6.3 mm in diameter and 5.0 mm in depth). BAR region is highlighted in red.

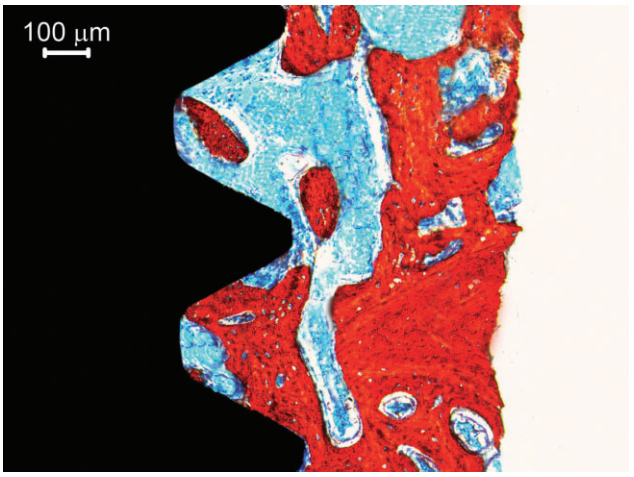

Figure 4 Photomicrography at 10× resolution. Histological image of the bone graft group at 4 weeks.

Histological Analysis

Four-Week Group. Osteogenesis was apparently favored when BG, LPP, and Cg were used. Woven-bone, bone matrix deposition, and presence of immature trabecular bone – which resembled cancellous bone – were found close to the surface of the implant. In the BG group, bone particles were embedded into the novel bone in contact with the implant. Also, sparse osteoclastic activity on the particles' surface was observed (Figures 3–5).

Twelve-Week Group. Compared with 4 weeks, more bone formation was observed in the circumferential defect area and toward the implant surface, regardless of the treatment used. At this stage, bone tissue exhibited a mature pattern, characterized by higher density and lamellar-like aspect, similar to trabecular bone. All groups presented a similar pattern of bone formation (Figures 6–8).

Histometric and Stability Analysis

Improved bone formation (BIC and BAR) was observed at 12 weeks compared with 4 weeks ($p \leq .05$), regardless of the materials used (Figure 9).

At 4 weeks, BG showed higher BAR values compared with the Cg treatment ($p \leq .05$), however, no difference was found between the BG and LPP groups ($p \geq .05$). Furthermore, no difference was found between Cg and LPP treatment comparison in the same

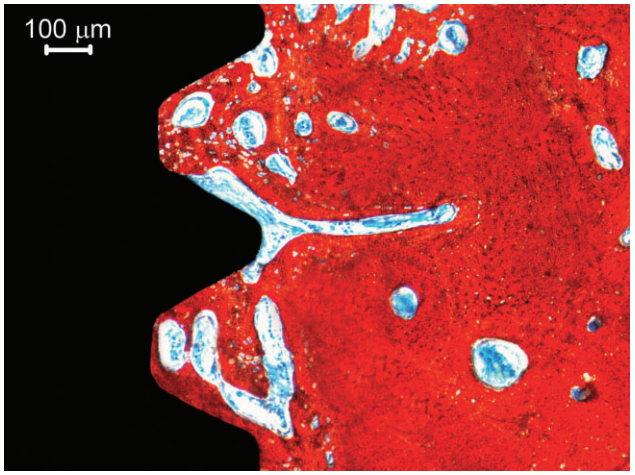

Figure 3 Photomicrography at 10× resolution. Histological image of the LPP group at 4 weeks.

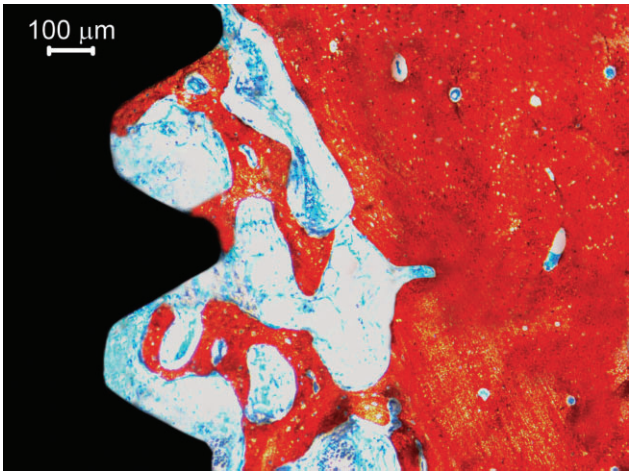

Figure 5 Photomicrography at 10× resolution. Histological image of the coagulum group at 4 weeks.

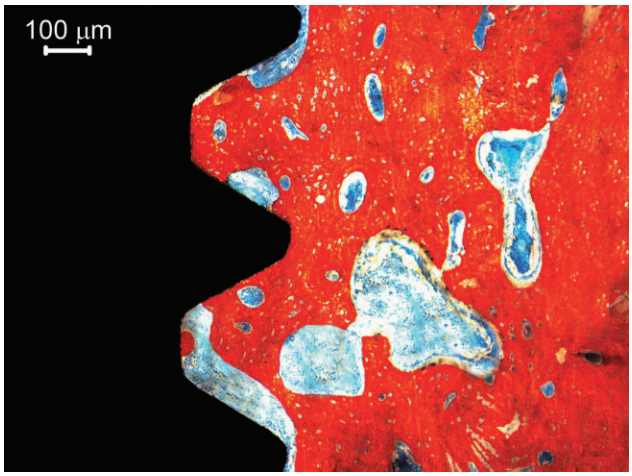

Figure 6 Photomicrography at 10× resolution. Histological image of the LPP group at 12 weeks.

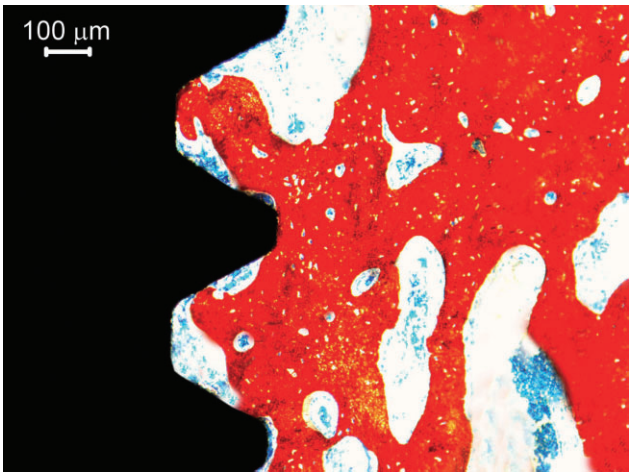

Figure 8 Photomicrography at 10× resolution. Histological image of the coagulum group at 12 weeks.

healing time ($p \geq .05$). At 12 weeks, the Cg group showed more bone formation (BAR) compared with the BG group ($p \leq .05$). However, LPP treatment did not show different values of bone formation compared with Cg and BG. Cg and LPP groups showed higher BAR values at the 12-week period ($p \leq .05$) compared with the 4-week period. When the BG group was evaluated, difference in bone formation during time of healing was not observed ($p \geq .05$) (Figure 10).

ISQ final stability values at 12 weeks were statistically higher than final stability values measured at 4 weeks after implant installation, and both were higher than the primary stability measurement. Primary stability was similar in all implants ($p \geq .05$). Improved osseointegration was observed over time for all biomaterials evaluated (Figure 11).

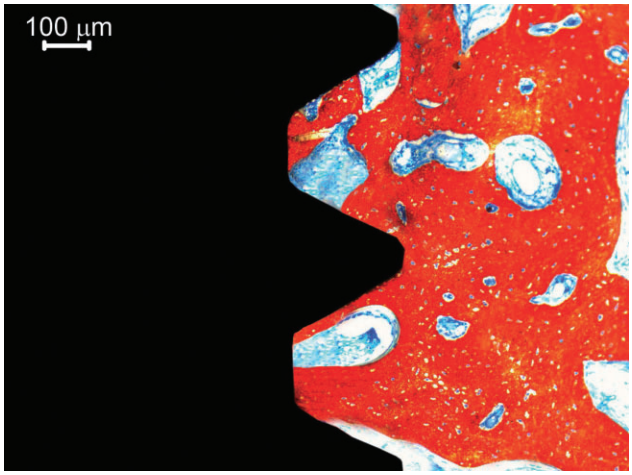

Figure 7 Photomicrography at 10× resolution. Histological image of the bone graft group at 12 weeks.

DISCUSSION

The search for alternate biomaterials to substitute autogenous bone in areas with insufficient amount of bone intended for implant placement is still a challenge.

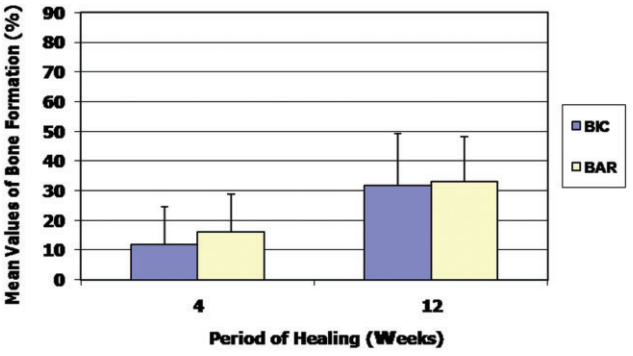

Figure 9 Diagram of bone formation (bone-implant contact [BIC] and BAR) comparisons between different periods of healing.

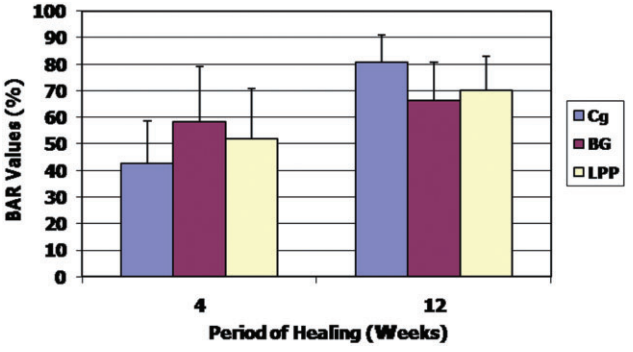

Figure 10 Diagram of BAR comparisons among the different treatments considering the period of healing. BG = bone graft; Cg = coagulum; LPP = latex angiogenic proteins pool.

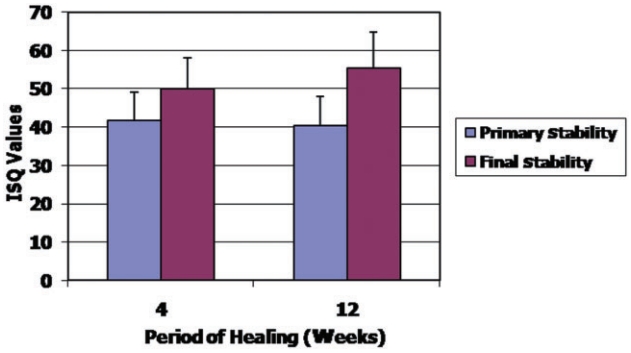

Figure 11 Diagram of comparisons between primary stability and final stability (implant stability quotient [ISQ] values) considering the period of healing.

Several current materials have been used in reconstructive surgeries, such as membranes for guided bone regeneration (GBR), autogenous bone, aloplastics, allogeneic or xenogeneic bones, and growth factors and their multiple clinical combinations. Considering the importance of angiogenesis in the osteogenic process[11–21] and the favorable outcomes of previous studies in animals[2,4,5,8,9] and patients[1,3,6,7] using latex biomaterials, an experimental model in vivo was designed to evaluate the osteogenic process around dental implants in poor conditions of osseointegration. Experimental models used to compare different biomaterials regarding osteogenesis and osseointegration such as in the present research were previously described elsewhere.[29–34]

In the present study, a time-depending bone formation was observed, that is, higher BAR and BIC values were obtained at 12 weeks compared with 4 weeks, regardless of the biomaterial used. The implant stability assessment was corroborated by the histometric evaluation, as all treatments led to higher implant stability values as compared with primary stability values. These findings are in agreement with previous studies.[35,36]

A significant increase in values for BAR from 4 to 12 postoperative weeks was observed for Cg and LPP treatments, while the BG group showed no variation. However, the data on BAR revealed that the BG group presented higher BAR values compared with Cg at 4 weeks. All together, these findings suggest that filling the defect with autogenous bone lead to a faster bone deposition up to 4 weeks, as the BG can provide for bone cells and nutrients, and act as a scaffold for new bone formation as described in a recent review on bone substitutes.[24] However, stabilization of bone formation over time could be also related to corticocancellous microarchitecture of BG used in this study, as the cancellous portion of the graft is likely to be submitted to a more intense remodeling as time progresses, similarly as described for onlay grafts in reconstructive surgeries.[26,37–41] The histological evidence of bone remodeling was indicated by the presence of bone particle resorption as early as 4 weeks to support such statement.

At the 12-week period, the use of Cg alone resulted in bone formation (BAR values) similar to LPP and higher than BG. The favorable results obtained with the use of Cg confirm that the dimension of the circumferential bone defects performed in this study was not critical.[37,42–49] The thrombogenic property of titanium surface seems to be an important factor for successful bone formation around dental implants, specially when Cg was the treatment of choice to fill bone defects in reconstructive dental surgery.[22,23] Interestingly, the present study suggests that thrombogenic property of implant surface affected positively the BAR values, but not the BIC values. A recent experimental research in primates[50] and previous clinical trials[22,51] have demonstrated bone formation when blood alone was allowed to contact the implant surface during sinus membrane elevation using a simultaneous implant installation procedure. The outcomes of the Cg group observed in the present study confirm the properties of implant surface treatments on bone tissue regeneration.[23,33,34]

The LPP group demonstrated an increased bone formation over time, when the BAR parameter was evaluated. Furthermore, bone formation promoted by LPP was similar to that obtained for BG at 4 weeks and for Cg at 12 weeks. These results are supported by the histometric and histologic parameters of bone repair in rats' dental socket treated with granules form of latex-derived angiogenic proteins and without treatment.[9] The authors reported that the latex-derived biomaterial stimulated angiogenesis and bone deposition after 1 week of alveolar healing, while the bone volume in experimental and control animals equalized after 3 weeks and 6 weeks. Contrary to the latter study, the LPP delivered similar amounts of bone formation at both 4 weeks and 12 weeks following implantation as compared with Cg and BG. This discrepancy may have resulted from differences between latex protein vehicles

used in each study. In the former investigation, the latex-derived proteins were applied in a biopolymer formulation that may have allowed prompt release of the active proteins into the rat socket. The fact that in the present study the biomaterial was embedded in gel, its diffusion into the bone defect was somewhat hindered, especially in the early stages following implantation.

The active proteins identified in latex are thought to constitute a conglomerate of growth factors, considering that LPP has been demonstrated to increase vascular permeability, cellular proliferation, and angiogenesis; promote fibroplasia; and stimulate epithelization, that is, critical steps of tissue repair.[10] This study showed that LPP applied in circumferential bone gaps around dental implants was associated with bone formation similar to the BG and Cg groups, exhibiting potential for an osteogenic biomaterial. At least partially, these effects may be explained by the angiogenic properties derived from latex proteins. However, further biochemical, in vitro bone essays and in vivo testing studies are required to confirm this hypothesis. The fact that LPP can be obtained through low-cost manufacture when compared with other biomaterials used for the same purpose, as well as the scientific evidence that the use of LPP does not cause any harm to hosting tissues, it might be advantageous over routine treatments.

CONCLUSIONS

Based on methodological limitations, the outcomes of the present study indicate the following: (1) Cg alone delivers higher amount of bone in the defect as compared with BG at 12 weeks; (2) compared with Cg and BG, the treatment with LPP exhibits no advantage in terms of osteogenic potential in this experimental model; (3) the implant stability is not affected by the treatments employed in this study.

ACKNOWLEDGMENTS

This investigation was fully supported by The State of São Paulo Research Foundation (FAPESP, Brazil), grant 2006/04359-0 awarded to Dr. Samuel P. Xavier. Maya Fernanda Manfrin Arnez was the recipient of the fellowship from FAPESP (2006/58059-7). Tatiana Ramirez Cunha was the recipient of the scholarship from The National Council for Scientific and Technological Development (CNPq, Brazil) (2007.1.381.58.4). The authors thank Mr. Sebastião Carlos Bianco for his technical assistance in histological processing, and Dr. Paulo Tambasco de Oliveira for his relevant comments during histological analysis.

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