



OSSEOPULSE™  
CLINICAL AND SCIENTIFIC DOSSIER

January 2011

---

***PUBLISHED RESEARCH SUMMARIES***

---

***Accelerated Implant Stability After LED Photobiomodulation***

Brawn P, Kwong-Hing A, Boeriu S and Clokie CM, *J Dent Res* 87(Spec Iss B):2021, 2008

**Study:** This article examines the effect of OsseoPulse™ treatment on implant stability, as assessed by resonance frequency analysis. 35 patients had 63 dental implants placed. 23 patients were treated with the OsseoPulse™ for 21 days. All implants were tested for primary stability with an Osstell Mentor™ at the time of implant placement, 14, 30, 60 and 90 days.

**Conclusion:** Patients treated with the OsseoPulse™ demonstrated significantly ( $p < 0.05$ ) improved dental implant stability at day 14, day 30 and day 60. There was a 58% reduction in time required to achieve sufficient stability to load implants compared to controls. These results may suggest that OsseoPulse™ treatments may allow for more rapid integration of dental implants and earlier loading.

***Combined New Technologies to Improve Dental Implant Success and Qualitative Ultrasound Evaluation of NIR-LED Photobiomodulation.***

Bouquot JE, Brawn PR, Cline JC, Proceedings of Light-Activated Tissue Regeneration and Therapy Conference, *Springer Science & Business Media* 191-206: 2008

***Combined New Technologies to Improve Dental Implant Success -- Quantitative Ultrasound Evaluation of NIR-LED Photobiomodulation***

Bouquot J, Brawn P, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 2008; 106(3):e6

**Background:** Dental implants must be placed in healthy bone for successful osteointegration and stability. Low bone density (LBD) and ischemically damaged, desiccated bone both have a poor ability to remodel and are, therefore, contraindications for implants. Near-infrared light emitting diode (NIR-LED) therapy or photobiomodulation has been shown in cultured cells and animal models to stimulate bone healing and production.

**Study:** 68 patients received 12 weeks of OsseoPulse™ therapy to 294 sites of ischemic/low bone density. Bone density/hydration improved by 44%, and 42% of sites returned to completely normal bone.

**Conclusion:** OsseoPulse™ therapy seems to hold good potential for improving alveolar bone prior to implant placement.

***PUBLISHED RESEARCH SUMMARIES (CONTINUED)***

---

***Phototherapy promotes attachment and subsequent proliferation of human osteoblast-like cells***

Khadra M, Kasem N, and Brawn P, *J Dent Res* 87(Spec Iss B):3308, 2008

**Study:** The aim of the study was to investigate the effect of OsseoPulse™ treatment on attachment and proliferation of human osteoblast-like cells cultured on titanium implant material. Cells derived from human mandibular bone were exposed to OsseoPulse™ and then seeded onto titanium discs. Non-exposed cultures served as controls. Cell proliferation was determined after staining by counting cells under a light microscope. Furthermore, MTT analyses were also performed to determine cellular attachment and proliferation.

**Conclusion:** In this cellular model the attachment and proliferation of human osteoblast-like cells cultured on titanium implant material was significantly enhanced ( $p < 0.05$ ) after OsseoPulse™ treatment compared to controls.

***A histologic comparison of light emitting diode phototherapy-treated hydroxyapatite-grafted extraction sockets***

Brawn P, Kwong-Hing A, *Implant Dent* 2007; 16(2):204-11

**Case Study:** After bilateral extraction of periodontally involved lower molars an investigational OsseoPulse™ was used daily for 21 days on the treated side after grafting both sockets with Hydroxyapatite (HA) Osteograf LD300. Bone regeneration of the OsseoPulse™ treated and nontreated socket graft was compared. Histologic evaluations showed enhanced bone formation and faster particle resorption associated with the OsseoPulse™ treated socket graft compared with the untreated socket.

**Conclusion:** In this bilateral case study the accelerated bone healing in the OsseoPulse™ treated HA socket graft may provide faster implant placement compared to untreated treated socket grafts.

***PUBLISHED RESEARCH SUMMARIES (CONTINUED)***

---

***Resonance frequency analysis of orthodontic miniscrews subjected to light-emitting diode photobiomodulation therapy***

Uysal T, Ekizer A, Akcay H, Etoz O, Guray E. Eur J Orthod. 2010 Dec 27

The aim of this prospective experimental study was to evaluate the effect of light-emitting diode (LED) photobiomodulation therapy (LPT) on the stability of immediately loaded miniscrews under different force levels, as assessed by resonance frequency analysis (RFA). Sixty titanium orthodontic miniscrews with a length of 8 mm and a diameter of 1.4 mm were implanted into cortical bone by closed flap technique in each proximal tibia of 15 New Zealand white adult male rabbits (n = 30). The animals were randomly divided into irradiated and control groups under different force levels (0, 150, and 300 cN). OsseoPulse® LED device (Biolux Research Ltd.) 618 nm wavelength and 20 mW/cm<sup>2</sup> output power irradiation (20 minutes/day) was applied to the miniscrews for 10 days. The RFA records were performed at miniscrew insertion session (T1) and 21 days after surgery (T2). Wilcoxon and Mann-Whitney U-tests were used for statistical evaluation at P < 0.005 level. It was found that initial primer stability of all miniscrews was similar in all groups at the start of the experimental procedure. Statistically significant differences were found for changes in implant stability quotient (ISQ) values between LED-photobiomodulated group and the control (0 cN, P = 0.001; 150 cN, P < 0.001; and 300 cN, P < 0.001). Significant increase was found in ISQ values of LPT applied miniscrews under 0 cN (+11.63 ISQ), 150 cN (+10.50 ISQ), and 300 cN (+7.00 ISQ) force during observation period. By the increase of force levels, it was determined that ISQ values decreased in non-irradiated control miniscrews. Within the limits of this in vivo study, the present RFA findings suggest that LPT might have a favourable effect on healing and attachment of titanium orthodontic miniscrews.

**ONGOING STUDIES**

---

***Implant stability study***

In progress, started Oct. 2009

Cam Clokie, DDS OMS, principal investigator  
University of Toronto, approved by University of Toronto IRB  
Investigators: private practices in Canada and University of Toronto residents

Goal: Evaluate effect of 10-day treatment protocol  
Goal: Evaluate effect of pre-treatment (day of surgery, prior to surgery)  
Goal: Evaluate effect on analgesics (reduction of swelling, pain, need for pain medications)

Use commercially available OsseoPulse™ devices  
Measurement: implant stability out over 90 days, at 0, 15, 30, 60, and 90 days.  
Objective measurement of implant stability with Osstell device

***Animal study***

Scheduled to start Jan./Feb. 2009

Cam Clokie, DDS OMS, principal investigator  
University of Toronto, approved by University of Toronto IRB  
Investigators: University of Toronto residents

Goal: Evaluate effect of OsseoPulse™ treatment on bone regeneration in cranial vault defects and around Titanium implants versus control.  
Goal: Evaluate effect of Bone Morphogenix Protein combined with OsseoPulse™ treatment in bone regeneration.

Rat animal study, mini-implants.  
Use same LED technology from OsseoPulse™ product  
Measurements: physical measurement of defect size, micro-CT, radiography, and histological.

**ONGOING STUDIES (CONTINUED)**

---

**Case studies**

In progress

Private practices in Canada

Investigators: private practices in Canada, various dentists, luminaries

Goal: Document interesting and challenging implantology and periodontic cases where regeneration is a challenge or higher risk.

Evaluate commercially available OsseoPulse™ device with various combinations of grafting and membrane materials, different surgical procedures.

**SUPPLEMENTARY LITERATURE AND ABSTRACTS - PHOTOBIOMODULATION**

---

**Low-Level Laser Therapy for Implants Without Initial Stability**

Campanha BP, Gallina C, Geremia T, Drumond Loro RC, Valiati R, Hubler R, Gerhardt de Oliveira M

*Photomedicine and Laser Surgery* 2009 00(00):1-5

**Objective:** This study evaluated the effect of low-level infrared laser on removal torque values of implants with poor initial stability inserted in rabbit tibias.

**Background Data:** It is important to analyze the effects of laser radiation on bone repair when low-quality bone and implants with poor initial stability are used.

**Materials and Methods:** Thirty male white New Zealand rabbits (*Oryctolagus Cuniculus*) about 2mo old and weighing 1.5–2.0kg were used. Machined implants with poor initial stability were inserted in the tibia of each animal. Animals were randomly divided into two groups: laser irradiated and laser nonirradiated. Each group was further divided into three subgroups, according to the day the animals were killed: 15, 30, or 45d. Torque values were measured with an axial digital torquemeter that applied counter-torque. The Student's t-test was used to calculate means and standard deviations for the comparisons between laser and control groups.

**Results:** A significant increase ( $p=0.050$ ) in removal torque values was found in the group of laser-irradiated implants at 15 and 30d when compared with the control groups. At 45d, no significant differences were found.

**Conclusion:** In this study, low-level laser therapy promoted the osseointegration of implants with poor initial stability, particularly in the initial stages of bone healing.

**SUPPLEMENTARY LITERATURE AND ABSTRACTS – PHOTOBIOMODULATION (CONTINUED)**

---

***The effects of low level laser irradiation on osteoblastic cells.***

Coombe AR, Ho CT, Darendeliler MA, Hunter N, Philips JR, Chapple CC, Yum LW. *Clin Orthod Res.* 2001 Feb;4(1):3-14.

**Abstract:** Low level laser therapy has been used in treating many conditions with reports of multiple clinical effects including promotion of healing of both hard and soft tissue lesions. Low level laser therapy as a treatment modality remains controversial, however. The effects of wavelength, beam type, energy output, energy level, energy intensity, and exposure regime of low level laser therapy remain unexplained. Moreover, no specific therapeutic window for dosimetry and mechanism of action has been determined at the level of individual cell types. The aim of this study was to investigate the effects of low level laser irradiation on the human osteosarcoma cell line, SAOS-2. The cells were irradiated as a single or daily dose for up to 10 days with a GaAlAs continuous wave diode laser (830 nm, net output of 90 mW, energy levels of 0.3, 0.5, 1, 2, and 4 Joules). Cell viability was not affected by laser irradiation, with the viability being greater than 90% for all experimental groups. Cellular proliferation or activation was not found to be significantly affected by any of the energy levels and varying exposure regimes investigated. Low level laser irradiation did result in a heat shock response at an energy level of 2 J. No significant early or late effects of laser irradiation on protein expression and alkaline phosphatase activity were found. Investigation of intracellular calcium concentration revealed a tendency of a transient positive change after irradiation. Low level laser irradiation was unable to stimulate the osteosarcoma cells utilized for this research at a gross cell population level. The heat shock response and increased intracellular calcium indicate that the cells do respond to low level laser irradiation. Further research is required, utilizing different cell and animal models, to more specifically determine the effects of low level laser irradiation at a cellular level. These effects should be more thoroughly investigated before low level laser therapy can be considered as a potential accelerator stimulus for orthodontic tooth movement.

**SUPPLEMENTARY LITERATURE AND ABSTRACTS - CARDIOLOGY**

---

***Low Level Laser Irradiation Precondition to Create Friendly Milieu of Infarcted Myocardium and Enhance Early Survival of Transplanted Bone Marrow Cells***

Zhang H, Hou, JF, Wang W, Wei YJ, Hu S *J of Cell and Mol Med* 2009 Sep 1.

**Abstract:** We hypothesized that low-level laser irradiation (LLLI) precondition prior to cell transplantation might remodel the hostile milieu of infarcted myocardium and subsequently enhance early survival and therapeutic potential of implanted bone-marrow mesenchymal stem cells (BMSCs). Therefore, in this study we wanted to address: (1) whether LLLI pretreatment change the local cardiac micro-environment after MI; and (2) whether the LLLI preconditions enhance early cell survival and thus improve therapeutic angiogenesis and heart function. Myocardial infarction was induced by left anterior descending artery ligation in female rats. A 635 nm, 5 mW diode laser was performed with energy density of 0.96 J/cm<sup>2</sup> for 150 seconds for the purpose of myocardial precondition. Three weeks later, qualified rats were randomly received with LLLI precondition (n=26) or without LLLI precondition (n=27) for LLLI precondition study. Rats received thoracotomy without coronary ligation was served as sham group (n=24). For the following cell survival study, rats were randomly received serum-free culture media injection (n=8), LLLI precondition and culture media injection (n=8), 2 millions male BMSCs transplantation without LLLI pretreatment (n=26) and 2 millions male BMSCs transplantation with LLLI precondition (n=25). Vascular endothelial growth factor (VEGF), glucose-regulated protein 78 (GRP78), superoxide dismutase (SOD) and malondialdehyde (MDA) in the infarcted myocardium were evaluated by Western blotting, real-time polymerase chain reaction (real-time PCR) and colorimetry, respectively, at 1 hour, 1 day and 1 week after laser irradiation. Cell survival was assayed with quantitative real-time PCR to identify Y chromosome gene and apoptosis was assayed with TUNEL staining. Capillary density, myogenic differentiation and left ventricular function were tested by immunohistochemistry and echocardiography, respectively, at 1 week. After LLLI precondition, increased VEGF and GRP78 expression, as well as the enhanced SOD activity and inhibited MDA production, was observed. Compared with BMSCs transplantation and culture media injection group, although there was no difference in the improved heart function and myogenic differentiation, LLLI precondition significantly enhanced early cell survival rate by 2-fold, decreased the apoptotic percentage of implanted BMSCs in infarcted myocardium and thus increased the number of newly formed capillaries. Taking together, LLLI precondition could be a novel non-invasive approach for intraoperative cell transplantation to enhance cell early survival and therapeutic potential.

**SUPPLEMENTARY LITERATURE AND ABSTRACTS – ANALGESIA**

---

***Placebo-controlled randomized clinical trial of the effect two different low-level laser therapies (LLLТ)—intraoral and extraoral—on trismus and facial swelling following surgical extraction of the lower third molar.***

Aras MH, Güngörmüş M, *Lasers Med Sci.* 2009 May 31.

**Abstract:** The purpose of this study is to compare the effects of extraoral and intraoral low-level laser therapies (LLLТ) on postoperative trismus and oedema following the removal of mandibular third molars. Forty-eight patients who were to undergo surgical removal of their lower third molars were studied. Patients were randomly allocated to one of three groups: extraoral LLLТ, intraoral LLLТ, or placebo. In the study, a Ga-Al-As diode laser device with a continuous wavelength of 808 nm was used, and the laser therapy was applied by using a 1 x 3-cm handpiece. The flat-top laser beam profile was used in this therapy. For both of the LLLТ groups, laser energy was applied at 100 mW (0.1 W) for a total of 120 s (0.1 W x 120 s = 12 J). Patients in the extraoral-LLLТ group (n = 16) received 12-J (4 J/cm<sup>2</sup>) low-level laser irradiation, and the laser was applied at the insertion point of the masseter muscle immediately after the operation. Patients in the intraoral-LLLТ group (n = 16) received 12-J (4 J/cm<sup>2</sup>) low-level laser irradiation intraorally at the operation site 1 cm from the target tissue. In the placebo group (n = 16), the handpiece was inserted intraorally at the operation site and then was touched extraorally to the masseter muscle for 1 min at each site (120 s total), but the laser was not activated. The size of the interincisal opening and facial swelling were evaluated on the second and seventh postoperative days. At the second postoperative day, trismus (29.0 +/- 7.6 mm [p = 0.010]) and swelling (105.3 +/- 5.0 mm [p = 0.047]) in the extraoral-LLLТ group were significantly less than in the placebo group (trismus: 21.1 +/- 7.6 mm, swelling: 109.1 +/- 4.4 mm). Trismus (39.6 +/- 9.0 mm [p = 0.002]) in the extraoral-LLLТ group at the seventh postoperative day was also significantly less than in the placebo group (29.0 +/- 6.2 mm). However, at the seventh postoperative day in the intraoral-LLLТ group, only trismus (35.6 +/- 8.5 [p = 0.002]) was significantly less than in the placebo group (29.0 +/- 6.2 mm). This study demonstrates that extraoral LLLТ is more effective than intraoral LLLТ for the reduction of postoperative trismus and swelling after extraction of the lower third molar.

## **SUPPORTING LITERATURE**

---

**Phototherapy enhances bone regeneration in direct sinus lifts**, Kwong-Hing and Brawn, poster at *BIOS 2007*

**Effects of laser therapy on attachment, proliferation and differentiation of human osteoblast-like cells cultured on titanium implant material**, Khadra et al, *Biomaterials 2005*; 26: 3503-3509

**Low-Level laser therapy stimulates bone-implant integration: an experimental study in rabbits**, Khadra et al, *Clin. Oral Impl. Res.*, 2004

**Determining optimal dose of light emitting diode phototherapy for proliferation of human oral fibroblasts**. Khandra and Brawn

**Absorption measurements of cell monolayers relevant to mechanisms of laser phototherapy: reduction or oxidation of cytochrome c oxidase under laser radiation at 632.8nm**. Karu T, Pyatibrat L, Kolyakov SF and Afanasyeva NI: *Photomedicine and Laser Surgery*, 2008 26:593-599

**Resonance frequency analysis of implants subjected to immediate or early functional occlusal loading. Successful vs. failing implants**. Glauser R, Sennerby L, Meredith N, Rée A, Lundgren AK, Gottlow J, Hämmerle CHF: *Clin. Oral Impl.*, 2004 15:428-434

**Effect of low-level laser therapy on bone repair: Histological study in rats**. Pretel H, Lizarelli RFZ, and Ramalho LTO: *Lasers Surg. Med.*, 2007 39:788-796

**Effect of low-level laser treatment after installation of dental titanium implant-immunohistochemical study of RANKL, RANK, OPG: an experimental study in rats**. Kim Y-D, Kim S-S, Hwang D-S, Kim S-G, Kwon Y-H, Shin S-H, Kim U-K, Kim J-R, and Chung I-K: *Lasers Surg. Med.*, 2007 1-10

**Mitochondrial signal transduction in accelerated wound and retinal healing by near-infrared light therapy**. Eells JT, Wong-Riley MTT, VerHoeve J, Henry M, Buchman EV, Kane MP, Gould LJ, Das R, Jett M, Hodson BD, Margolis D, Whelan HT: *Mitochondrion*, 2006 4:559-567

**Near infrared light protects cardiomyocytes from hypoxia and reoxygenation injury by a nitric oxide dependant mechanism**. Zhang R, et al: *J Mol & Cell Card.*, 2009 46:4-14

**Pretreatment with near-infrared light via light-emitting diode provides added benefit against rotenone- and MPP+ -induced neurotoxicity**. Ying R, Kiang HL, Whelan HT, Ealls JT, Wing-Riley MTT: *Brain Reseach*, 2008 1243:167-173

**Power games**. Lane N: *Nature*, 2006 443: 901-903

**SUPPORTING LITERATURE (CONTINUED)**

---

**Irradiation effect of low-energy laser on alveolar bone after tooth extraction.** Takeda Y: Experimental study in rats. *Int J Oral Maxillofac Surg* 1988, 17:388-391.

**The effects of the helium-neon laser on postsurgical discomfort: a pilot study.** Clokie C, Bentley KC, Head TW: *J Can Dent Assoc* 1991, 57:584-586.

**Enhancement of angiogenesis in regenerating gastrocnemius muscle of the toad (*Bufo viridis*) by low-energy laser irradiation.** Bibikova A, Belkin V, Oron U: *Anat Embryol (Berl)* 1994, 190: 597-602.

**Effect of low-energy laser (He-Ne) irradiation on the process of bone repair in the rat tibia.** Barushka O, Yaakobi T, Oron U: *Bone* 1995, 16:47-55.

**Promotion of bone repair in the cortical bone of the tibia in rats by low energy laser (He-Ne) irradiation.** Yaakobi T, Maltz L, Oron U: *Calcif Tissue Int* 1996, 59:297-300.

**Laser modulation of angiogenic factor production by T-lymphocytes.** Agaiby AD, Ghali LR, Wilson R, Dyson M: *Lasers Surg Med* 2000, 26:357-363.

**Low-energy laser irradiation stimulates bone nodule formation at early stages of cell culture in rat calvarial cells.** Ozawa Y, Shimizu N, Kariya G, Abiko Y: *Bone* 1998, 22:347-354.

**Effects of near-infrared low-level laser irradiation on microcirculation.** Maegawa Y, Itoh T, Hosokawa T, Yaegashi K, et al: *Lasers Surg Med* 2000, 27:427-437.

**In vitro effects of low-level laser irradiation at 660 nm on peripheral blood lymphocytes.** Stadler I, Evans R, Kolb B, Naim JO, et al: *Lasers Surg Med* 2000, 27:255-261.

**Impact of low level laser irradiation on infarct size in the rat following myocardial infarction.** Ad N, Oron U: *Int J Cardiol* 2001, 80:109-116.

**Comparison of the low level laser therapy effects on cultured human gingival fibroblast proliferation using different irradiance and same fluence.** Almeida-Lopes L, Rigau J, Zangaro RA, Guidugli-Neto J, et al: *Lasers Surg Med* 2001, 29:179-184.

**Improvement of macromolecular clearance via lymph flow in hamster gingiva by low-power carbon dioxide laser-irradiation.** Shimotoyodome A, Okajima M, Kobayashi H, Tokimitsu I, et al: *Lasers Surg Med* 2001, 29:442-447

**Effect of NASA light-emitting diode irradiation on wound healing.** Whelan HT, Smits RL Jr, Buchman EV, Whelan NT, et al: *J Clin Laser Med Surg* 2001, 19:305-314.

**Laser stimulation on bone defect healing: an in vitro study.** Guzzardella GA, Fini M, Torricelli P, Giavaresi G, et al: *Lasers Med Surg* 2002, 17:216-230.

**SUPPORTING LITERATURE (CONTINUED)**

---

**Histology study of the Effect of Laser Therapy on Bone Repair.** Blaya DS, Guimaraes MB, Pozza DH, Weber JBB, do Oliviera MG, *J Cont. Dent. Pract.* 2008 Sept.1, 9(6):1-8.

**Low level 809-nm diode laser-induced in vitro stimulation of the proliferation of human gingival fibroblasts.** Kreisler M, Christoffers AB, Al-Haj H, Willershausen B, et al: *Lasers Surg Med* 2002, 30:365-369.

**Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts.** Pereira AN, Eduardo Cde P, Matson E, Marques MM: *Lasers Surg Med* 2002, 31:263-267.

**Computerized morphometric assessment of the effect of low-level laser therapy on bone repair: an experimental animal study.** Silva Junior AN, Pinheiro AL, Oliveira MG, Weismann R, et al: *J Clin Laser Med Surg* 2002, 20:83-87.

**Effect of low-power GaAlAs laser (660 nm) on bone structure and cell activity: an experimental animal study.** Nicola RA, Jorgetti V, Rigau J, Pacheco MT, et al: *Lasers Med Sci* 2003, 18:89-94.

**High-intensity pulsed laser irradiation accelerates bone formation in metaphyseal trabecular bone in rat femur.** Ninomiya T, Miyamoto Y, Ito T, Yamashita A, et al: *Bone Miner Metab* 2003, 21:67-73.

**Effect of 830-nm laser light on the repair of bone defects grafted with inorganic bovine bone and decalcified cortical osseous membrane.** Pinheiro AL, Limeira Junior Fde A, Gerbi ME, Ramalho LM, et al: *J Clin Laser Med Surg* 2003, 21:301-306.

**Direct stimulatory effect of low-intensity 670 nm laser irradiation on human endothelial cell proliferation.** Schindl A, Merwald H, Schindl L, Kaun C, Wojta J: *Br. J Dermatol* 2003, 148:224-336.

**Therapeutic photobiomodulation for methanol-induced retinal toxicity.** Eells JT, Henry MM, Summerfelt P, Wong-Riley MT, Buchmann EV, Kane M, Whelan NT, Whelan HT. *Proc Natl Acad Sci U S A.* 2003 Mar 18;100 (6):3439-44

**Increased fibroblast proliferation induced by light emitting diode and low power laser irradiation.** Vinck EM, Cagnie BJ, Cornelissen MJ, Declercq HA, Cambier DC. *Lasers Med Sci.* 2003; 18 (2):95-9.

**Light-emitting diode treatment reverses the effect of TTX on cytochrome oxidase in neurons.** Wong-Riley MT, Bai X, Buchmann E, Whelan HT. *Neuroreport.* 2001 Oct 8; 12 (14):3033-7.

**Effect of NASA light-emitting diode irradiation on molecular changes for wound healing in diabetic mice.** Whelan HT, Buchmann EV, Dhokalia A, Kane MP, Whelan NT, Wong-Riley MT, Eells JT, Gould LJ, Hammamieh R, Das R, Jett M. *J Clin Laser Med Surg.* 2003 Apr; 21 (2):67-74.