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Extracorporeal Shock Wave Therapy Induces Alveolar Bone Regeneration

ABSTRACT

Periodontal inflammation with alveolar bone resorption is a hallmark of periodontitis. We hypothesized that extracorporeal shock wave therapy (ESWT) could promote the regeneration of alveolar bone following *Porphyromonas gingivalis*-induced periodontitis in rats. Rats were infected with *P. gingivalis* for 10 wks, which caused alveolar bone resorption. The rats were then treated with a single episode of 100, 300, or 1000 impulses of shock wave on both cheeks at energy levels 0.1 mJ/mm^2. Alveolar bone levels were determined at 0, 3, 6, and 12 wks following ESWT and compared with those in untreated controls. Infected rats treated with 300 and 1000 impulses demonstrated significantly improved alveolar bone levels at 3 wks compared with untreated controls, and the improved levels remained for at least 6 wks in most rats. The results demonstrated effective regeneration of alveolar bone by ESWT and suggested that ESWT should be evaluated as an adjunct in the regeneration of periodontal tissues following periodontal disease. Abbreviations: ESWT, extracorporeal shock wave therapy; PCR, polymerase chain-reaction.

KEY WORDS: *Porphyromonas gingivalis*, Extracorporeal Shock Wave Therapy, periodontal disease, polymerase chain-reaction, alveolar bone regeneration.

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INTRODUCTION

Periodontitis is an immuno-inflammatory disease that leads to destruction of periodontal ligament and adjacent supporting alveolar bone, and is induced by pathogenic subgingival microbial biofilms containing several periodontal pathogens, including *P. gingivalis*. *P. gingivalis* is a major component of these biofilms in severe forms of human periodontal disease, and possesses multiple metabolic properties and likely virulence factors consistent with its pathogenic role in the disease (Kuramitsu, 2003; Holt and Ebersole, 2005). *P. gingivalis*, as an asaccharolytic micro-organism, elaborates several different proteases that have been suggested to contribute to bacterial virulence by *in vitro* studies (Gibson and Genco, 2001; Yagishita *et al.*, 2001; Chen *et al.*, 2002). Numerous studies have used both mouse and rat models to establish short-duration infections with periodontal micro-organisms that support their virulence potential (Baker *et al.*, 2000; Taubman *et al.*, 2005; Kesavalu *et al.*, 2006, 2007a). Generally, these studies have confirmed the potential of these pathogens to elicit inflammation and tissue destruction in the host (Madden and Caton, 1994).

Extracorporeal shock waves are high-energy acoustic waves generated under water with high-voltage explosion and vaporization. The application of shock wave therapy in humans has been primarily to disintegrate kidney stones, induce neovascularization to promote tissue regeneration, heal non-union long bone fractures, dissolve calcified tendonitis of the shoulder, and treat lateral epicondylitis of the elbow, proximal plantar fasciitis, avascular necrosis of the femoral head, and patellar tendonitis (Haupt, 1997; Schaden *et al.*, 2001; Wang, 2003; Trebinjac *et al.*, 2005; Kudo *et al.*, 2006). The exact biologic effects of shock wave therapy resulting in therapeutic outcomes for musculoskeletal disorders are not fully understood. Recently, it has been shown that shock waves stimulate the early expression of angiogenesis-related growth factors, including endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor (VEGF), and proliferating cell nuclear antigen (PCNA), contributing to induced vascularization and improving blood supply, with increased cell proliferation and tissue regeneration and repair (Wang *et al.*, 2002; Wang, 2003). Moreover, the induction of angiogenic biomolecules and clinical observations of vascularization kinetics indicate that shock wave therapy is dose-dependent and that symptoms improve over time (Wang *et al.*, 2002; Wang, 2003). Several *in vivo* studies confirmed a positive effect of shock wave treatment on fracture healing in rats, promotion of bony union, enhanced callous formation, and induction of cortical bone formation in dogs (Haupt *et al.*, 1992; Johannes *et al.*, 1994; Wang *et al.*, 2001).

Several *in vitro* studies have shown that shock waves had a bactericidal effect on *Streptococcus aureus, Streptococcus epidermidis, Pseudomonas aeruginosa,* and a MRSA 27065 strain (von Eiff *et al.*, 2000; Gerdesmeyer *et al.*, 2005), with decreases of over 3-logs for certain species. Gerdesmeyer *et al.* (2005) evaluated the bactericidal activity on *S. aureus* as a function of

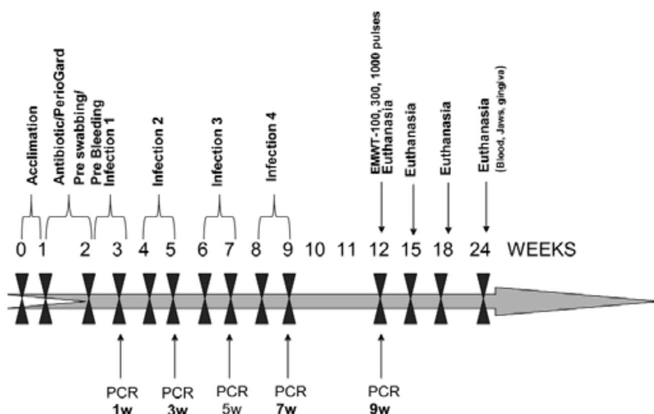

Figure 1. Schematic diagram illustrating experimental design, *P. gingivalis* infections, plaque sample collection, PCR analysis, ESWT administration, euthanasia, and alveolar bone collection. (For details, see "Materials & Methods".) Rats were killed at 0, 3, 6, and 12 wks post-ESWT application.

energy flux density (EFD) and impulse number, to determine optimal *in vivo* conditions.

While many studies have emphasized the beneficial effects of shock wave therapy in musculoskeletal tissue and bone regeneration, no reports to date have determined its effect on the healing of periodontal tissues and alveolar bone resorption that results from the chronic inflammation of periodontitis. The hypothesis tested in this investigation was that extracorporeal shock wave therapy could promote the healing of periodontal tissues following the induction of periodontitis by *P. gingivalis* infection in a rat model of periodontitis.

MATERIALS & METHODS

Bacteria

P. gingivalis strain 381 was used in this study, and cultured and maintained for the animal infections as described previously (Kesavalu *et al.*, 2006, 2007a,b).

Rats

Female Sprague-Dawley rats (8-9 wks old, Harlan, Indianapolis, IN, USA) were maintained under micro-isolator conditions, fed standard powdered chow (Teklad Global 18% protein rodent diet 2918, Harlan Teklad, Madison, WI, USA), and H_2O *ad libitum*, and were kept at 25°C with alternating 12-hour periods of light and dark. All procedures were performed in accordance with the approved guidelines set forth by the Institutional Animal Care and Use Committee at the University of Kentucky. Following acclimation (1 wk), rats were randomized into 5 groups [*P. gingivalis*-infected (Gr I: n = 24); *P. gingivalis*-infected and ESWT-treated (100 impulses) (Gr II; n = 18); *P. gingivalis*-infected and ESWT-treated (300 impulses) (Gr III: n = 18); *P. gingivalis*-infected and ESWT-treated (1000 impulses) (Gr IV; n = 18); and uninfected/ untreated controls (Gr V: n = 18)]. Six infected rats were killed at 10 wks post-infection and served as the baseline for alveolar bone resorption compared with uninfected rats. At 3, 6, and 12 wks after ESWT treatment, 6 rats from each group were killed and their alveolar bone levels determined. Food consumption was monitored, and all rats were weighed weekly until termination of the protocol to ensure that the ESWT treatment did not adversely affect their overall health.

Bacterial Infection

All rats were administered kanamycin (20 mg) and ampicillin (20 mg) daily for 4 days in the drinking water, and the oral cavity was swabbed with 0.12% chlorhexidine gluconate (PerioGard: Procter & Gamble, Cincinnati, OH, USA) mouthrinse, to suppress the oral microbiota (Kesavalu *et al.*, 2006, 2007a,b). *P. gingivalis* cells at 2 x 10^{10} *per* mL were mixed with equal amounts of sterile 2% carboxymethylcellulose (CMC; Sigma Chemical Co., St. Louis, MO, USA) and used to infect rats orally within 15 min of removal from the anaerobic environment.

PCR Analysis

Rats were orally infected with approximately 10^{10} *P. gingivalis* for 4 consecutive days on 4 alternate wks during the study period (Fig. 1). In total, 4 post-infection microbial samples were collected from all *P. gingivalis*-infected rats, by means of sterile cotton swabs at 1, 5, 7, and 9 wks post-infection. The swabs were suspended in 300 μL of Tris-EDTA (TE) buffer. The oral microbial samples were boiled in TE buffer with an equal volume of 1 N NaOH, and DNA was dissolved in 40 μL of sterile distilled water (Kesavalu *et al.*, 2006, 2007a,b). Polymerase chain-reaction (PCR) was carried out in 50 μL volume with 10 pM of primer, and 1 mM of each deoxynucleotide triphosphate,**(AQ)** and 1.5 mM $MgCl_2$. A two-unit quantity of *Taq* DNA polymerase (Invitrogen, Life Technologies, Carlsbad, CA, USA) in the manufacturer's buffer was used with a GeneAmp PCR System. The PCR oligonucleotide species-specific primer used 16S rDNA for *P. gingivalis*: (forward) 5′ GGT AAG TCA GCG GTG AAA CC 3′ and (reverse) 5′ ACG TCA TCC ACA CCT TCC TC 3′. After denaturation at 94°C for 5 min, 35 PCR cycles were performed, with each cycle consisting of 30 sec of denaturation at 94°C, 30 sec of annealing at 55°C, 60 sec of polymerization at 72°C, and final extension at 72°C for 7 min. PCR products were analyzed by 1.5% agarose gel electrophoresis and stained with ethidium bromide for detection of a 601-bp product.

ESWT Administration

Extracorporeal shock waves were generated with a DermaGold® (MTS, Konstanz, Germany). Rats were anesthetized with isoflurane inhalation anesthesia and shaved on both cheeks. A thin film of ultrasound gel was applied, and unfocused shock waves were applied equally on both cheeks in a single session. After 10 wks of infection and induction of periodontitis, rats were treated with a single application of 100, 300, or 1000 impulses of unfocused ESWT at EFD 0.1 mJ/mm^2. The pulse rate applied was 5 pulses *per* sec. Rats were then killed at 0 (baseline), 3, 6, and 12 wks post-ESWT for evaluation of the potential effects of ESWT on the healing of periodontal tissues. Skulls were removed, autoclaved, and de-fleshed, and maxillae and mandibles were hemi-sected for radiographic evaluation of alveolar bone resorption.

Radiographic Assessment of Alveolar Bone Resorption

Hemi-sected maxillae and mandibles were trimmed to reduce the bucco-lingual dimensions to allow the teeth to be close to the radiographic film. Each jaw was secured, by means of rope wax, to Kodak Ultra Speed size 2 films (Kodak, Rochester, NY, USA), and a Planmeca Prostyle Intra x-ray unit (Planmeca, Roselle, IL, USA) was placed at a right angle to the film. Each jaw was radiographed with an exposure time of 0.05 sec at a setting of 70 KvP and 8 mA. Radiographs were analyzed for alveolar bone height as the primary

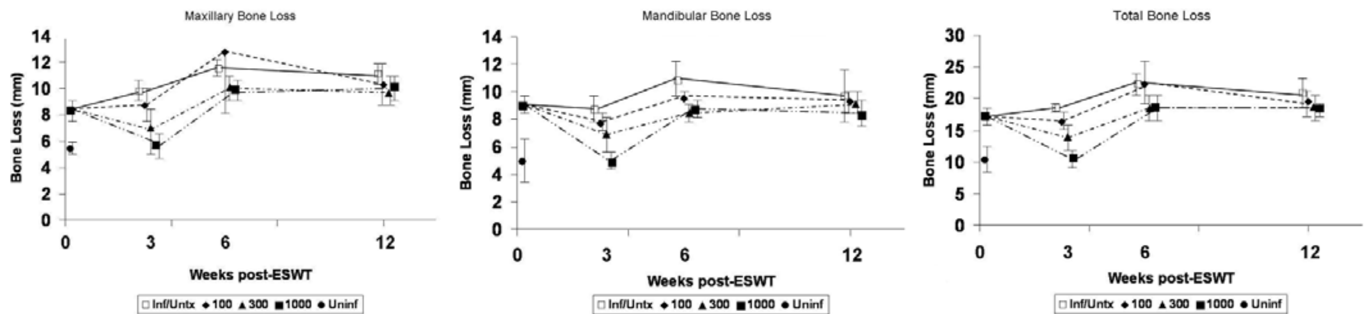

Figure 2. Effect of ESWT on *P. gingivalis*-induced maxillary, mandibular, and total alveolar bone resorption in rats. Each point represents the mean alveolar bone resorption in mm, as a summation of 2 sites *per* tooth and 3 teeth in each quadrant (n = 6 rats *per* group/*per* timepoint; four timepoints = 0, 3, 6, and 12 wks). The vertical brackets denote 1 standard deviation from the mean. 'Inf' denotes infected and untreated control rats, and 'Uninf' denotes uninfected control rats.

outcome parameter of the study. We used radiographs projected at a 5x size to obtain linear measures from the cemento-enamel junction to the bone height at mesial and distal interproximal surfaces (2 sites *per* tooth) of each of the 2 molars and 1 premolar in each quadrant (Reed and Polson, 1984; Kesavalu *et al.*, 2006, 2007a). So that comparability of the results could be ensured, the measures were determined by two investigators blinded as to the group designation, evaluating a given radiograph blindly, and two replicate evaluations were done. Approximately 5-10% of variability was observed between investigators doing the readings and routinely calibrated with a set of standard radiographs from the rats. The summation of bone resorption in mm was tabulated and analyzed for intra- and inter-group differences.

Statistical Analysis

Descriptive evaluation of the alveolar bone data is presented as mean + SD. Statistically significant differences between the groups were determined by an ANOVA and the Holm-Sidak *post hoc* multiple-comparisons test (SigmaStat 3.0, SYSTAT Software Inc., Chicago, IL, USA) for normally distributed data. Data determined to be non-normal were analyzed by Kruskal-Wallis ANOVA on ranks, and multiple comparisons were adjusted by Dunn's method (SigmaStat 3.0).

RESULTS

PCR evaluation of the oral microbial samples collected at 4 timepoints demonstrated that 85-100% of rats (Gr I, 20/24; Gr II, 18/18; Gr III, 17/18; Gr IV, 16/18; Gr V, 0/24) were infected with *P. gingivalis* during the experimental periodontal disease period. None of the uninfected control rats was positive for *P. gingivalis* infection during the study period. The *P. gingivalis*-infected and uninfected control rats were normal and healthy following the single application of ESWT on both their cheeks. The body weights of rats following ESWT application were unchanged in both *P. gingivalis*-infected and uninfected control rats (data not shown).

P. gingivalis infection resulted in significantly increased maxillary, mandibular, and total alveolar bone resorption at 10-18 wks post-infection compared with uninfected control rats (Fig. 2). There was an overall loss in bone height, with associated circumferential angular defects. All measures were taken to the most coronal level of the supporting level of alveolar bone on the mesial and distal surfaces of the study teeth. In contrast, 3 wks following ESWT treatment at 300 and 1000 impulses in infected animals (Figs. 2, 3), maxillary, mandibular, and total alveolar bone levels were significantly improved compared with those in untreated and infected controls (Fig. 3, Table). Furthermore, ESWT administration at 300 and 1000 impulses continued to demonstrate a significant improvement in alveolar bone height at 6 and 12 wks post-treatment that was most notable in the maxillary bone (Figs. 2, 3). A summary assessment of a single application of ESWT, showing a dose-response effect for increased pulse number over time for maxillary, mandibular, and total alveolar bone, is given in the Table.

DISCUSSION

This study demonstrated our ability to colonize rats orally with *P. gingivalis* for an extended interval, to quantify the resulting alveolar bone resorption from this oral infection, and to determine the effect of ESWT on the regeneration of alveolar

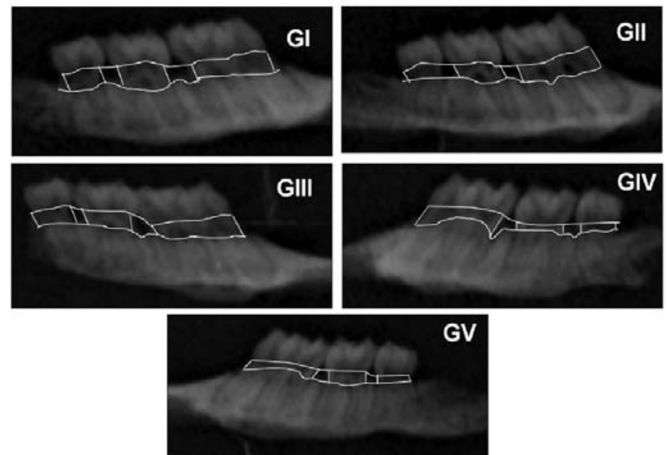

Figure 3. Representative digital radiographic images of *P. gingivalis*-infected (Gr I: left maxilla), infected, and ESWT-treated rats at 100 impulses (Gr II: left maxilla), 300 impulses (Gr III: left maxilla), and 1000 impulses (Gr IV: right maxilla), and control uninfected and untreated (Gr V: right maxilla) of rat jaws (n = 6 rats *per* group/*per* timepoint; four timepoints = 0 (baseline), 3, 6, or 12 wks post-ESWT). The horizontal lines denote the bone level and CEJ (cemento-enamel junction) level on the radiographs. The vertical lines denote examples of the 6 sites in each quadrant where interproximal vertical alveolar bone measures were obtained.

Table. Statistical Evaluation of ESWT Effects on Alveolar Bone Levels Related to Numbers of Impulses and Times of Evaluation

Comparisons		Weeks post-ESWT, P =		
No. of Impulses	No. of Impulses	3	6	12
Maxillary				
0 (untreated) Gr V	100 Gr II	0.128*	NS	NS
0 (untreated) Gr V	300 Gr III	**0.0021**	**0.006**	**0.0084**
0 (untreated) Gr V	1000 Gr IV	**<0.001**	**0.0028**	NS
100	300	0.053	NS	NS
100	1000	**=0.0018**	NS	NS
300	1000	NS	NS	NS
Mandibular				
0	100	0.095	NS	NS
0	300	**0.0173**	**0.0328**	NS
0	1000	**<0.001**	**0.0265**	0.1187
100	300	NS	NS	NS
100	1000	**0.0001**	NS	NS
300	1000	**0.0033**	NS	NS
TOTAL				
0	100	**0.0386**	NS	NS
0	300	**0.0007**	**0.0031**	0.1149
0	1000	**<0.001**	**0.0026**	**0.0261**
100	300	0.059	NS	NS
100	1000	**0.001**	NS	NS
300	1000	**0.0076**	NS	NS

* Values indicate *P*-value for comparison. Bold values indicate significant difference. NS denotes not significant. 0 denotes animals were not treated (Group V) with extracorporeal shock waves. Comparisons were made between untreated group (Gr V) *vs.* treated groups at 100 (Gr II), 300 (Gr III), and 1000 (Gr IV) impulses in maxillary, mandibular, and total alveolar bone levels. Also comparisons were made between 100 inpulses *vs.* 300 & 1000 impulses and 300 *vs.* 1000 impulses in maxillary, mandibular, and total alveolar bone levels.

bone levels in this model. The literature is replete with evidence supporting ESWT as a non-operative treatment in humans for urolithiasis (Chaussy and Wilbert, 1997), bone pseudoarthrosis (Valchanou and Michailov, 1991), and chronic tendopathies. Molecularly, ESWT has been shown to regulate/activate several genes, *e.g.*, TGF-β1, IGF-1, and BMP-2, linked to bone formation in rats with collagenase-induced Achilles tendonitis and segmental femoral defects (Chen *et al.*, 2003, 2004). It has been demonstrated that expression patterns of select collagen genes, as well as osteocalcin (OC) and osteopontin (OPN), during shockwave-induced osteogenesis, are similar to those of periosteal hard callus formation during fracture healing (Takahashi *et al.*, 2004). Furthermore, ESWT significantly increases NOS, OC, and TGF-β1 production in human osteoblast-like cells, indicating osteoblast differentiation and consequent bone matrix deposition (Martini *et al.*, 2003). Thus, it might be expected that both resident cells and infiltrating inflammatory/immune cells in gingival and periodontal tissues might also reflect functional alterations from ESWT.

Analysis of the data demonstrates that a single application of ESWT appeared to stimulate re-establishment of more normal bone levels within 3 wks post-treatment that would have been lost during the initial 10 wks of *P. gingivalis* infection (*i.e.*, untreated, infected rats). It remains to be determined how rapidly the effect can be visualized, but future studies could estimate the potential for "regeneration" of the resorbed bone at 1-2 wks after ESWT administration. Moreover, the experimental design demonstrated a dose-response effect, showing greater bone height (*e.g.*, regeneration) with increasing pulse number. The effects of ESWT on maxillary, mandibular, and total bone regeneration were clearly and significantly evident at 3 wks for all ESWT groups and for all pulse numbers after the single application of ESWT. There was a general tendency for this regenerative effect on bone levels to last for at least 6 wks after the single ESWT application of 300 and 1000 impulses, although the effect was not sustained at 100 impulses for bone levels at 6 and 12 wks. It is also clear, however, that the effects on bone destruction of oral infection with *P. gingivalis* continued for the 12 wks post-ESWT, with many of the rats showing bone levels at 12 wks post-ESWT that were not significantly greater than levels in untreated rats. This suggests that *P. gingivalis* infection in the rats persisted past the 12-week timepoint and continued to promote inflammation and bone resorption that ultimately overcame the beneficial effects of the single treatment with ESWT. The kinetics of the response to ESWT suggests that additional applications would likely enhance the alveolar bone regeneration lost during periodontal infections. This would be consistent with the beneficial effects of ESWT on tissue regeneration seen in previous human studies. The ability to identify various therapeutic modalities that could regenerate periodontal tissues lost through destructive periodontitis would be an important contribution to the repertoire of therapies used to manage chronic periodontal disease. This report provides initial data suggesting that ESWT may be of use in the management of periodontal bone loss. Further studies are required to determine the effects of ESWT on mechanisms of alveolar bone regeneration, on critical biomolecules in periodontal tissues, and on outcomes when used as an adjunct to conventional periodontal treatment.

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REFERENCES

Baker PJ, Dixon M, Roopenian DC (2000). Genetic control of susceptibility to *Porphyromonas gingivalis*-induced alveolar bone loss in mice. *Infect Immun* 68:5864-5868.

Chaussy C, Wilbert DM (1997). [Extracorporeal shockwave lithotripsy today—an assessment of current status]. *Urologe A* 36:194-199 (article in German).**(AQ)**

Chen YJ, Kuo YR, Yang KD, Wang CJ, Huang HC, Wang FS (2003). Shock wave application enhances pertussis toxin protein-sensitive bone formation of segmental femoral defect in rats. *J Bone Miner Res* 18:2169-2179.

Chen YJ, Wang CJ, Yang KD, Kuo YR, Huang HC, Huang YT, *et al.* (2004). Extracorporeal shock waves promote healing of collagenase-

induced Achilles tendinitis and increase TGF-beta1 and IGF-I expression. *J Orthop Res* 22:854-861.

Chen YY, Cross KJ, Paolini RA, Fielding JE, Slakeski N, Reynolds EC (2002). CPG70 is a novel basic metallocarboxypeptidase with C-terminal polycystic kidney disease domains from *Porphyromonas gingivalis*. *J Biol Chem* 277:23433-23440.

Gerdesmeyer L, von Eiff C, Horn C, Henne M, Roessner M, Diehl P, *et al.* (2005). Antibacterial effects of extracorporeal shock waves. *Ultrasound Med Biol* 31:115-119.

Gibson FC 3rd, Genco CA (2001). Prevention of *Porphyromonas gingivalis*-induced oral bone loss following immunization with gingipain R1. *Infect Immun* 69:7959-7963.

Haupt G (1997). Use of extracorporeal shock waves in the treatment of pseudarthrosis, tendinopathy and other orthopedic diseases. *J Urol* 158:4-11.

Haupt G, Haupt A, Ekkernkamp A, Gerety B, Chvapil M (1992). Influence of shock waves on fracture healing. *Urology* 39:529-532.

Holt SC, Ebersole JL (2005). *Porphyromonas gingivalis*, *Treponema denticola,* and *Tannerella forsythia*: the "red complex", a prototype polybacterial pathogenic consortium in periodontitis. *Periodontol 2000* 38:72-122.

Johannes EJ, Kaulesar Sukul DM, Matura E (1994). High-energy shock waves for the treatment of nonunions: an experiment on dogs. *J Surg Res* 57:246-252.

Kesavalu L, Vasudevan B, Raghu B, Browning E, Dawson D, Novak JM, *et al.* (2006). Omega-3 fatty acid effect on alveolar bone loss in rats. *J Dent Res* 85:648-652.

Kesavalu L, Sathishkumar S, Bakthavatchalu V, Matthews C, Dawson D, Steffen M, *et al.* (2007a). Rat model of polymicrobial infection, immunity, and alveolar bone resorption in periodontal disease. *Infect Immun* 75:1704-1712.

Kesavalu L, Vasudevan B, Rahman MM, Su J, Raghu B, Dawson D, *et al.* (2007b). Omega-3 fatty acid regulates inflammatory cytokine/mediator messenger mRNA expression in *Porphyromonas gingivalis*-induced experimental periodontal disease. *Oral Microbiol Immunol* 22:232-239.

Kudo P, Dainty K, Clarfield M, Coughlin L, Lavoie P, Lebrun C (2006). Randomized, placebo-controlled, double-blind clinical trial evaluating the treatment of plantar fasciitis with an extracorporeal shockwave therapy (ESWT) device: a North American confirmatory study. *J Orthop Res* 24:115-123.

Kuramitsu HK (2003). Molecular genetic analysis of the virulence of oral bacterial pathogens: an historical perspective. *Crit Rev Oral Biol Med* 14:331-344.

Madden TE, Caton JG (1994). Animal models for periodontal disease. *Methods Enzymol* 235:106-119.

Martini L, Giavaresi G, Fini M, Torricelli P, de Pretto M, Schaden W, *et al.* (2003). Effect of extracorporeal shock wave therapy on osteoblastlike cells. *Clin Orthop Relat Res* 413:269-280.

Reed BE, Polson AM (1984). Relationships between bitewing and periapical radiographs in assessing crestal alveolar bone levels. *J Periodontol* 55:22-27.

Schaden W, Fischer A, Sailler A (2001). Extracorporeal shock wave therapy of nonunion or delayed osseous union. *Clin Orthop Relat Res* 387:90-94.

Takahashi K, Yamazaki M, Saisu T, Nakajima A, Shimizu S, Mitsuhashi S, *et al.* (2004). Gene expression for extracellular matrix proteins in shockwave-induced osteogenesis in rats. *Calcif Tissue Int* 74:187-193.

Taubman MA, Valverde P, Han X, Kawai T (2005). Immune response: the key to bone resorption in periodontal disease. *J Periodontol* 76(11 Suppl):2033S-2041S.

Trebinjac S, Mujic-Skikic E, Ninkovic M, Karaikovic E (2005). Extracorporeal shock wave therapy in orthopaedic diseases. *Bosn J Basic Med Sci* 5(2):27-32.

Valchanou VD, Michailov P (1991). High energy shock waves in the treatment of delayed and nonunion of fractures. *Int Orthop* 15:181-184.

von Eiff C, Overbeck J, Haupt G, Herrmann M, Winckler S, Richter KD, *et al.* (2000). Bactericidal effect of extracorporeal shock waves on *Staphylococcus aureus*. *J Med Microbiol* 49:709-712.

Wang CJ (2003). An overview of shock wave therapy in musculoskeletal disorders. *Chang Gung Med J* 26:220-232.

Wang CJ, Huang HY, Chen HH, Pai CH, Yang KD (2001). Effect of shock wave therapy on acute fractures of the tibia: a study in a dog model. *Clin Orthop Relat Res* 387:112-118.

Wang FS, Yang KD, Chen RF, Wang CJ, Sheen-Chen SM (2002). Extracorporeal shock wave promotes growth and differentiation of bone-marrow stromal cells towards osteoprogenitors associated with induction of TGF-beta1. *J Bone Joint Surg Br* 84:457-461.

Yagishita H, Kumagai Y, Konishi K, Takahashi Y, Aoba T, Yoshikawa M (2001). Histopathological studies on virulence of dipeptidyl aminopeptidase IV (DPPIV) of *Porphyromonas gingivalis* in a mouse abscess model: use of a DPPIV-deficient mutant. *Infect Immun* 69:7159-7161.