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● *Original Contribution*

EFFECT OF LOW-INTENSITY PULSED ULTRASOUND ON A RAT MODEL OF DENTIN–DENTAL PULP INJURY AND REPAIR

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Abstract—This study investigated histopathologic changes in dental pulp after treatment with low-intensity pulsed ultrasound (LIPUS). Fifty rats were randomly divided into an experimental group (n = 25) and a blank control group (n = 25). In the experimental group, a cavity was prepared in the bilateral maxillary first molars. The upper right first molars were stimulated with LIPUS (30 mW/cm^2, 1.5 MHz) for 20 min/d. The cavities prepared in the left teeth were used as experimental controls (*i.e.,* no LIPUS). Five rats in each group were sacrificed at days 1, 3, 5, 7 and 14. Inflammatory response was visible at different time points after cavity preparation, peaking at day 3, after which it gradually weakened. More reparative dentin was found on the LIPUS treatment side. transforming growth factor-β1 expression increased after treatment, peaking at day 5 and returning to normal at day 14 on both sides, but was stronger with LIPUS treatment. SMAD2 and SMAD3 expressions in the dental pulp gradually increased after cavity preparation, especially in the experimental group. LIPUS promoted the repair of dentin–pulp complex injury, to a certain extent and should be investigated further as a potential therapy. (E-mail: zhizhoucq@sina.com) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Low-intensity pulsed ultrasound, Dentin–pulp complex, transforming growth factor-β1, SMAD2, SMAD3.**

INTRODUCTION

The dentin and dental pulp arise from the dental papilla, which are formed during embryonic development and participate in tooth activities together as the dentin–pulp complex (Ţuculină et al. 2013). Dentin includes primary dentin, physiologic secondary dentin and tertiary dentin. Tertiary dentin is dentin formed in pulp cavities after external stimulation. Smith et al. (1995) classified tertiary dentin at different stimulus intensities into reactionary dentin and reparative dentin. Reactionary dentin is formed by surviving primary odontoblasts in response to weak stimuli, whereas reparative dentin is formed by dental pulp cells after the death of primary odontoblasts from strong stimuli. Morphologic examination of reactionary and reparative dentin revealed differences in the continuities of small tubes on the surface of the dentin; however, it is difficult to distinguish reactionary dentin from reparative dentin. Chen and Fan (2000) established animal models of reparative dentin in rat molars to observe the histomorphology. Apparent reparative dentin was visible at days 15 and 30 after stimulation, by which time the primary odontoblasts had disappeared and irregular odontoblast-like cells had formed. The authors presumed that large amounts of reparative dentin are formed under pathologic conditions and suggested that reparative dentin corresponds to tertiary dentin (Chen and Fan 2000).

The function of tertiary dentin is to protect dental pulp by forming a thick barrier, which also provides a hard tissue foundation for dental restoration. Its formation can be regulated by many signaling molecules and pathways in the dentin–pulp complex, especially the transforming growth factor (TGF)-β family member TGF-β1 (Li et al. 2011). TGF-β1, a widely derived multifunctional growth factor, has been shown to regulate the differentiation of odontoblasts from dental pulp cells and the formation of reparative dentin in the repair of dentin pulp injury; however, it is also the most important

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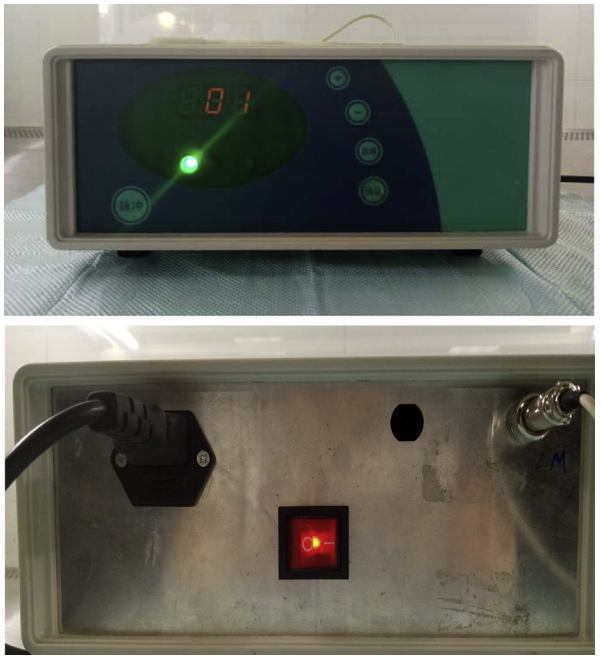

Fig. 1. Ultrasound therapy device.

factor involved in promoting of fiber formation (Rutherford et al. 1994; Smith et al. 1995; Tziafas and Papadimitriou, 1998). These findings indicate that TGF-β1 promotes tissue repair and wound healing while stimulating the formation of fibronectin and collagen, which leads to the occurrence of fiber adhesions (Ruch et al. 1995). Its specific performance indicates that high levels of TGF-β1 could be produced by autocrine and paracrine signaling in the initial stages of cavity repair to lessen dental pulp inflammation. Over a prolonged period, abundant levels of TGF-β1 could promote the increased migration and proliferation of odontoblasts, the proliferation and differentiation of mesenchymal stem cells into odontoblasts, the deposition of reparative

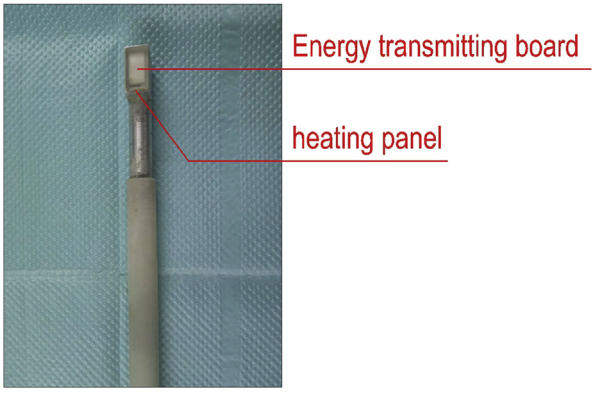

Fig. 2. Ultrasound transducer.

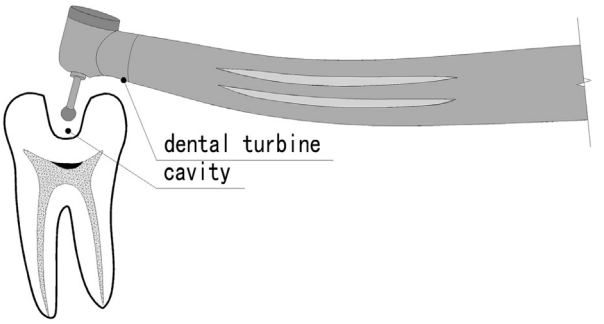

Fig. 3. Establishment of the rat model of dentin-dental pulp injury and repair.

dentin in exposed parts and the protection of injured pulp tissue (Ruch et al. 1995).

SMADs are the recently discovered intracellular signal transduction molecules of the TGF-β superfamily. The SMAD pathway is also the most important pathway involved in TGF-1 signal transduction (Henderson and Andrew, 1998; Zhang et al. 1996). SMAD2 and its homologous molecule SMAD3 are members of the SMAD family of proteins. SMAD2 and SMAD3 are receptor-regulated SMADs and specific intracellular signal transduction molecules of TGF-β1, which mediate the interaction of TGF-β1 and its target genes (Dennler et al. 2002; Massagué et al. 1994). However, there are few studies addressing the expression, localization and effects of SMAD2 and SMAD3 in human dental pulp tissue. Studies have shown that SMAD2 and SMAD3, as a specific intracellular signal transduction molecules in TGF-β1 downstream signaling, can transmit TGF-β1 signals from the cytoplasm to the nucleus, thereby

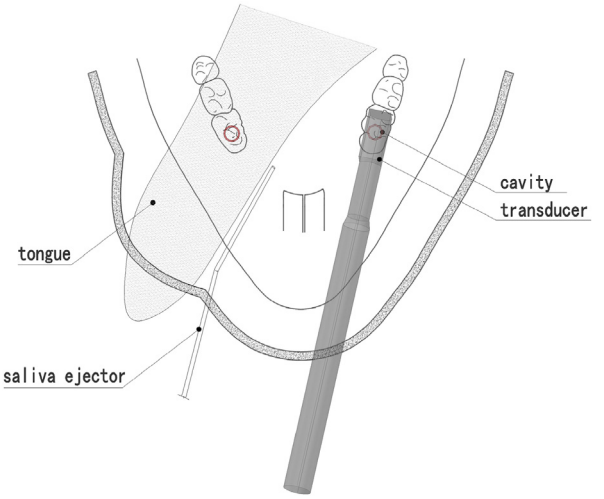

Fig. 4. Low-intensity pulsed ultrasound treatment: frontal view. *Red circle* shows the cavity prepared in "the bilateral maxillary first molars" and the anticipated acoustic field of LIPUS irradiation.