

COMPARATIVE STUDY OF THE EFFECTS OF LOW-LEVEL LASER AND LOW-INTENSITY ULTRASOUND ASSOCIATED WITH BIOSILICATE® ON THE PROCESS OF BONE REPAIR IN THE RAT TIBIA

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ABSTRACT

Objective: Verify the effects of the association between Biosilicate® and ultrasound and, Biosilicate® and laser in bone consolidation process of rats, through the biomechanical and histological analysis. **Methods:** Forty male rats were used. The animals were randomized into four groups (n=10): control group fracture no treated (CGF); group treated with Biosilicate® (BG); group treated with Biosilicate® and laser (BLG); group treated with Biosilicate® and ultrasound (BUG). **Results:** The biomechanical analysis showed no significant difference among any groups after 14 days post-surgery. In the morphometric analysis, the control group showed moderate presence of new formed bone tissue inside the defects areas and the

Biosilicate® group showed similar results. Despite those facts, the biomaterial osteogenic potential was demonstrated by the great amount of cells and bone tissue around the particles. Curiously, the Biosilicate® plus laser or ultrasound groups showed lower amounts of bone tissue deposition when compared with control fracture and Biosilicate® groups. **Conclusion:** The data from this study can conclude that Biosilicate® was able to accelerate and optimized the bone consolidation, through the modulation of the inflammatory process and the stimulation of new bone formation. However, when resources were associated, there are no positive results.

Keywords - Bone Tissue; ultrasound; Laser; Biocompatible Materials; Rats

INTRODUCTION

Bone repair is a highly complex regenerative process that includes interactions between a series of biological events, such as active gene synthesis and the action of a large number of cells and proteins, which will give rise to restoration of the integrity of the bone tissue⁽¹⁾. However, over the course of this process, changes culminating in regenerative deficiencies and consequent delayed consolidation and even bone nonunion may occur. It has been estimated that in the United States, out of the 6.2 million fractures that occur every year, around 10% evolve to non-consolidation and pseudarthrosis⁽¹⁾.

Within this context, various biophysical and bio-

chemical advances have been studied in an attempt to minimize the bone consolidation time and diminish the chances of possible complications stemming from the abnormal regeneration process⁽²⁾. Among the topics studied have been the effects of treatments such as application of morphogenetic bone proteins, bioactive materials, use of low-intensity ultrasound (US) and use of low-level laser therapy (LLLT)⁽³⁾.

Bioactive materials or biomaterials are defined as materials that are capable of producing a specific biological response at the interface between the material and the tissue, thus forming a bond between them, without being toxic or promoting immunological respon-

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ses^(4,5). In the 1990s, our research group developed a highly bioactive glass ceramic with high-performance mechanical properties, named Biosilicate®⁽⁶⁾. Some studies have been demonstrating the osteogenic potential of Biosilicate®, both *in vitro* and *in vivo*^(5,7).

In addition, LLLT and US have also been highlighted through their osteogenic potential. The effects from this therapy have been reported by several authors. Renno et al⁽⁸⁾ and Stein et al⁽⁹⁾ showed that there was a significant increase in osteoblast proliferation after irradiation with laser at 830 nm and 20 J/cm². Moreover, the laser seemed to accelerate the fracture repair process and caused increases in callus volume and bone mineral density⁽¹⁰⁾. Pinheiro et al⁽¹¹⁾ showed that laser (830 nm, 40 mW, 4.8 J/cm²) was capable of increasing the quantity of mineralized bone tissue in fractures induced in the femurs of rats. US was found to have the effects of increasing cell proliferation and accelerating bone consolidation after a fracture, and increasing the mechanical strength of the bone callus in rats and rabbits^(12,13). Takikawa et al⁽¹²⁾ observed in an experimental study that, after six weeks of US treatment, it had significantly increased the consolidation rate in fractures with bone nonunion, compared with the control group. The same results were found by Sun et al⁽¹⁴⁾ and Lirani et al⁽¹⁵⁾.

As stated above, biomaterials, US and LLLT have been emerging as promising alternatives for treating bone fractures. These resources not only present great osteogenic potential but also constitute noninvasive treatment methods and present relatively low cost. Studies investigating the effects from associating these resources in the consolidation process are scarce in the literature. Within this context, the present study had the aim of ascertaining the effects from associating Biosilicate® with US and LLLT in the bone consolidation process in rats, from biomechanical and histological analyses.

METHODOLOGY

Forty male Wistar rats weighing between 280 and 320 g were used in this study. The animals were kept under controlled environmental conditions (dark/light cycle of 12 hours each, cleaned environment, temperature of 24 ± 2 °C and adequate ventilation), where they received ordinary feed and water *ad libitum*. This study was approved by the Ethics Committee for Animal Experimentation of the Federal University of São

Carlos (Opinion Report 023/2006).

The animals were randomly distributed into four groups (n = 10): control group with fracture (FG): the rats in this group were subjected to a fracture but did not receive any treatment; Biosilicate® group (BG): the rats were subjected to a fracture and were treated with Biosilicate®; Biosilicate® + laser group (BLG): the rats were subjected to a fracture and were treated with an association of Biosilicate® + laser; Biosilicate® + US (BUSG): the rats were subjected to a fracture and were treated with an association of Biosilicate® + US.

To produce the bone defects, the rats were anesthetized in accordance with their body weight, using a mixture of ketamine and xylazine (80/10 mg/kg). After anesthesia, shaving and asepsis, an incision was made in both tibias. With the aid of a mini-drill fitted with a milling disc of 2.0 mm in diameter, irrigated with physiological serum, bone defects were made in the middle third of the tibia (10 mm below the knee joint). The procedure was completed by suturing the muscle and skin using monofilament 4.0 nylon thread, with a distance of 1 cm between the stitches, and cleaning the site. The animals continued to be provided with free access to water and food until the time of sacrifice.

TREATMENTS

Biomaterial

The bioactive material used was Biosilicate®. This is a highly bioactive glass ceramic composed of the chemical elements silicon, oxygen, sodium, calcium and phosphorus (Si, O, Na, Ca and P), which are released into solution in the form of Si(OH)_4 , Na^+ , Ca^{2+} and PO_4^{2-} . Biosilicate® was used in this study in granulated form, with grain size of approximately 180-212 μm . This was introduced into the defect by means of an appropriate spatula, just after the defect was made, and the circular fracture was completely filled. The details of the composition of Biosilicate® and the thermal treatment are described in the patent WO 2004/074199.

Low-level laser

The device used was a portable DMC laser: THERALASE version 24, class 3B, Ga-Al-As diode, with a wavelength of 830 nm, continuous emission,

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