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**ORIGINAL ARTICLE**

# Effects of lithium on extraction socket healing in rats assessed with micro-computed tomography

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**Abstract**

***Objective.*** Lithium is an activator of $\beta$-catenin signaling and $\beta$-catenin plays an important role in regulating bone formation and remodeling. The purpose of this study was to investigate the effects of lithium on bone repair in tooth extraction sockets in rats. ***Material and methods.*** Twenty male Wistar rats were subjected to maxillary left second molar extraction. The animals received a daily injection of lithium chloride (LiCl) or the same dose of sodium chloride (NaCl) starting 7 days before tooth extraction until sacrifice 14 days after extraction. Rats were randomly divided into: (1) a pre-treated group that received LiCl injection from 7 days before to 3 days after tooth extraction; (2) a post-treated group that received LiCl injection starting 4 days after tooth extraction; (3) a continuously treated group that received LiCl injection for the entire 21 days; and (4) a control group that received NaCl injection only. The volume of new bone and the bone density in the extraction socket were quantified by micro-computed tomography. ***Results.*** The percentage of new bone formation in the extraction socket was as follows: $63.2 \pm 13.4\%$ (pre-treated group), $53.9 \pm 9.8\%$ (post-treated), $23.8 \pm 8.0\%$ (continuously treated) and $37.5 \pm 4.2\%$ (control). The difference in percentage was statistically significant between each pair of groups. Pre- and post-treated groups also showed a significant increase in the density of new bone. ***Conclusions.*** Lithium enhances bone repair in extraction sockets when delivered before or after tooth extraction. Tooth extraction during lithium treatment may impair bone healing.

**Key Words:** *bone repair, bone mineral density, extraction socket, lithium, micro-computed tomography*

## Introduction

A tooth-extraction socket is a common defect in maxillofacial bones. Impaired repair of such bone loss often causes a functional deficit, poor aesthetics and difficulties in prosthetic restoration. Investigations of tooth extraction wound healing in rats have been used to understand the healing process in alveolar bone and provide clues to develop new strategies for bone reconstruction in the skull and face [1–4]. The initial phases of wound healing in extraction sockets include inflammation, coagulation and formation of granulation tissue [3]. The organization of coagulum with proliferative fibrous tissue is orchestrated with cell apoptosis and osteoclast activities [2,3]. With the migration of endothelial cells, pre-osteoblast cells and new bone matrix are formed from the fundus of the socket as early as 4 days after extraction [1,2]. New trabecular bone is gradually synthesized thereafter and the extraction socket may be filled by newly generated bone after 14 days [2,4]. This is a programmed cascade of proliferation, specification and maturation of the osteoblastic cell lineage, which is modulated by various intrinsic growth factors and cytokines. Consequently, any local or systemic drugs that are potent to regulate these signaling molecules may significantly affect bone repair after tooth extraction [2,4].

Lithium, a mood-stabilizing drug used for decades to treat bipolar and other psychiatric disorders, has been recently reported to increase bone mass and improve new bone regeneration [5–8]. Although the precise mechanism of its therapeutic effects remains unknown, lithium is well documented as an inhibitor of

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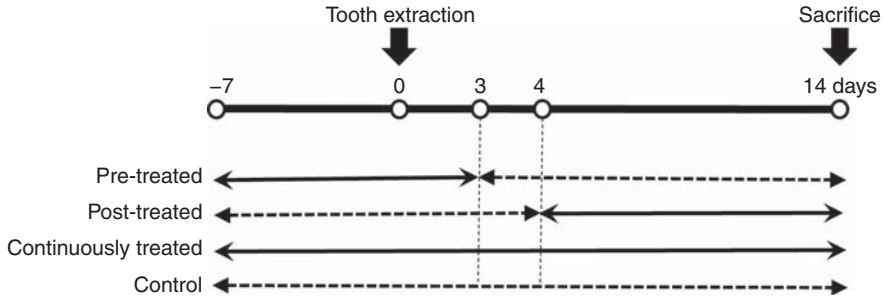


Figure 1. The groups and timeline of this study. Twenty rats that underwent maxillary left second molar extraction were sacrificed on day 14. There were four groups of animals ($n = 5$ for each): (1) the pre-treated group received LiCl injections from 7 days before to 3 days after tooth extraction; (2) the post-treated group received LiCl injections starting 4 days after tooth extraction; (3) the continuously treated group received LiCl injections for the entire 21 days; (4) the control group received NaCl injections only. The solid line denotes LiCl injections; the dashed line denotes NaCl injections.

glycogen synthase kinase-3 (GSK-3) [9]. GSK-3 is a serine/threonine protein kinase and one of its downstream targets is Wnt/$\beta$-catenin signaling [10]. GSK-3 is normally active and it phosphorylates $\beta$-catenin, leading to its ubiquitin-dependent degradation. GSK-3 is inhibited when Wnt glycoproteins bind to the transmembrane receptors of frizzled proteins and low-density lipoprotein receptor-related protein 5/6 (LRP5/6) [11]. The resulting signaling increases the amount of $\beta$-catenin in the cell, which in turn translocates to the nucleus and initiates transcription from Wnt target genes [11]. Lithium increases $\beta$-catenin levels by inhibiting GSK-3 and thus has been frequently used to experimentally activate Wnt/$\beta$-catenin signaling [6–9].

Lithium induces alkaline phosphatase expression in C3H10T1/2 pluripotent stem cells and promotes osteocalcin mRNA expression and bone formation in calvarial osteoblast cultures [12,13]. In mice, lithium administration stimulates Wnt/$\beta$-catenin-mediated transcription in bone, leading to an increase in bone mass and bone formation [8]. It has been pointed out that lithium plays different roles in regulating the proliferation, differentiation and function of osteoblasts during bone regeneration [7]. During fracture repair in mice, healing is enhanced when lithium is delivered in later phases of repair, after multi-potential mesenchymal cells have become committed to the osteoblast lineage [7]. On the other hand, early lithium treatment appears to inhibit the repair process at the fracture site because proliferation of mesenchymal cells increases but their osteogenic differentiation is blocked [7]. It is, thus, reasonable to hypothesize that lithium could also promote bone repair in tooth-extraction sockets if delivered on a suitable schedule.

In recent years, high-resolution micro-computed tomography (micro-CT) has been widely used to study bone mass and bone morphology, including healing of alveolar bone defects and tooth-extraction sockets [4,14]. Compared with traditional histological measurement, micro-CT scanning is non-destructive; it is able to provide 3D morphologic measurements and allows for computer-aided reconstruction. Furthermore, bone tissue mineralization can be quantified [15]. Thus, the purpose of our current study was to evaluate the effects of different lithium delivery schedules on extraction-socket healing by micro-CT evaluation.

## Material and methods

### *Tooth extraction and lithium administration*

Twenty male Wistar rats (body weight 120–130 g) were subjected to maxillary left second molar extraction and sacrificed 14 days later. Care was taken to minimize surgical injury during tooth removal. Rats were randomly divided into four treatment groups ($n = 5$ each) and given daily intraperitoneal injections of lithium chloride (LiCl, 2.5 mEq/kg body weight; Sigma, St. Louis, MO) or the same dose of sodium chloride (NaCl) based on the following regimens: (1) a pre-treated group that received LiCl injections from 7 days before to 3 days after tooth extraction; (2) a post-treated group that received LiCl injections starting 4 days after tooth extraction; (3) a continuously treated group that received LiCl injections starting 7 days before tooth extraction for a entire 21 days; and (4) a control group that received NaCl injections only (Figure 1). The LiCl dosing schedule was chosen to yield a mean plasma lithium concentration of 0.6 mM [16]. To reduce the potential of lithium toxicity, normal saline was substituted for drinking water every other day for the LiCl-treated rats [16]. No antibiotic or other medication was used. The experiment was approved by Shanghai Jiao Tong University (Ethics Approval No. SCXK 2008-0016).

### *Micro-computed tomography (micro-CT)*

The maxillary bones were collected and fixed in 4% paraformaldehyde at 4°C before scanning with a desktop micro-CT system (eXplore Locus SPmicro

Table I. CT values for different tissues.

| Tissue | Minimum (HU) | Maximum (HU) |
|---|---|---|
| Alveolar bone | 656 | 3071 |
| Soft tissues | –224 | 1636 |
| Tooth | 2300 | 3071 |

CT, GE Healthcare Technologies, Milwaukee, WI). Samples were fixed in a sample holder in such a position that the midpalatal suture was parallel to the X-ray source. The area scanned began at the mesial surfaces of the bilateral maxillary first molars and ended at the distal surfaces of the third molars. Images were acquired at 80 kVp, 80 μA, 2000 ms integration time and 16 μm voxel size. A calibration phantom provided by the manufacturer was included in each scan for CT value correction and converting CT values into mineral density values. This phantom contained air, water, fat and two kinds of hydroxyapatite with different but known densities. The captured images were exported as DICOMs format and loaded in Mimics 10.01 software (Materialise, Leuven, Belgium) to perform bone morphometric analysis.

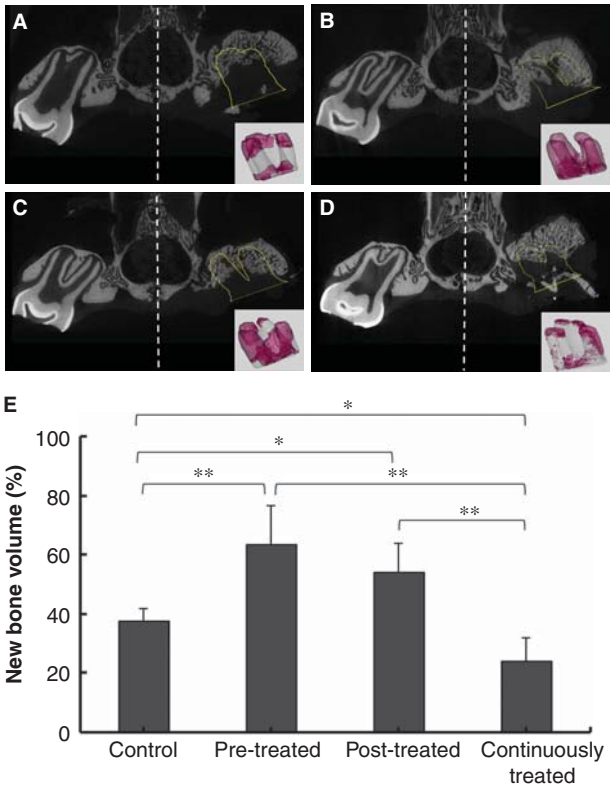


Figure 2. The coronal plane of the maxillary right second molar and the left extraction socket in the control group (A), pre-treated group (B), post-treated group (C) and continuously treated group (D). The extraction socket of left molar (yellow outline) was reconstructed by mirroring the alveolar socket of the right molar to the left side according to the mid-sagittal plane of the maxilla (dashed vertical line). 3D-reconstruction images of the extraction socket (silvery white) and new bone (fuchsia) are shown in the lower-right corner of each panel. (E) Quantitative analysis shows the percentage of new bone in the extraction socket among the various groups. Values are the mean ± SD ($n$ = 5). Significant differences between each pair of groups are marked with asterisks (* $p < 0.05$, ** $p < 0.01$).

### *Determination of new bone formation in the extraction socket*

The extraction socket of the left second molar was reconstructed by mirroring the alveolar socket of the right second molar to the left side, assuming that the sockets on both sides were identical. A 3D model of the roots and the periodontal ligament of the maxillary right-second molar was first created based on automatic segmentation of different CT values (Table I), Boolean operation and manually tracing the upper limits by lines passing through the buccal and palatal alveolar crests in the coronal plane. This 3D model was then mirrored to the left side along the middle sagittal plane of the maxilla, regarding it as the original extraction socket (Figure 2). Afterwards, bone tissue between the maxillary left first and third molars was reconstructed by auto segmentation. Finally, the intersection of the reconstructed extraction socket and the bone tissue was calculated by the software and considered as new bone in the extraction socket (Figure 2). New bone volume was determined as a percentage of new bone to the total volume of the reconstructed extraction socket.

### *Determination of bone mineral density (BMD)*

BMD in the mesial palatal root of the extraction site and the alveolar bone of the non-extraction side was measured. The average CT value of the mesial palatal root in the extraction site was measured from the bottom at intervals of 10 images in a horizontal plane. In the non-extraction side, a cylindrical region of interest with 0.1 $mm^2$ cross-sectional area was selected in the palatal alveolar crest from the mesial surface of the third molar to the distal surface of the first molar. The average CT value of the region of interest was measured at intervals of 10 images. A regression equation was calculated using the phantom to convert CT numbers into BMD values [17]. The regression equation obtained was: $y = 0.2917x + 0.7494$, where $x$ is average CT number and $y$ is BMD.

### *Statistical analysis*

All measurements were repeated 4 weeks later by the same observer. Statistical differences were analyzed using one-way ANOVA with SAS software 9.2 (SAS Institute, Inc., Cary, NC). A $p$-value below 0.05 was considered statistically significant.

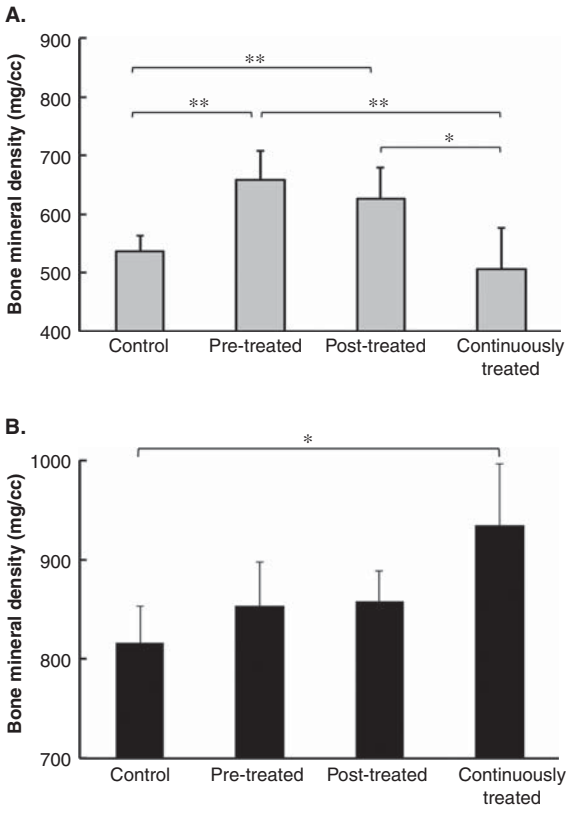


Figure 3. Bone mineral density (BMD) in the extraction socket (A) and alveolar bone of the non-extraction side (B) in the pre-treated, post-treated, continuously treated and control groups. Values are the mean ± SD ($n = 5$). Significant differences between each pair of groups are marked with asterisks (* $p < 0.05$, ** $p < 0.01$).

## Results

All animals tolerated the tooth extraction and no inflammation was observed in the extraction site. The average weight of the animals in each group decreased slightly on day 1 after tooth extraction and recovered by day 2. There were no significant differences in mean body weight among the groups during the course of the experiment.

The percentage of new bone formation in the extraction socket was 63.2 ± 13.4% in the pre-treated group, 53.9 ± 9.8% in the post-treated group, 23.8 ± 8.0% in the continuously treated group and 37.5 ± 4.2% in the control group (Figures 2A–D). The difference in percentage was statistically significant between each pair of groups (Figure 2E).

In each group, the BMD value of the new bone in the extraction socket was lower than that measured for the alveolar bone in the non-extraction side (Figure 3). In the extraction sockets, the BMD value for each of the pre- and post-treated groups was significantly higher than for the control group ($p < 0.01$, Figure 3A). However, there was no significant difference between the continuously treated group and the control group. With respect to alveolar bone of the non-extraction side, the BMD value was significantly higher in the continuously treated group compared with the control group ($p < 0.05$), whereas there were no differences between the other groups (Figure 3B).

## Discussion

Considering the importance of Wnt/$\beta$-catenin signaling for the control of bone growth and repair, the present study sought to investigate whether treatment of rats with lithium interferes with new bone formation using as an experimental model the alveolar bone healing after tooth extraction. With the aid of high-resolution micro-CT scanning and 3D computer reconstruction, we showed here that lithium intake increased bone formation and bone mass in a time-dependent manner.

In the control group without any lithium treatment, the amount of new bone formation on day 14 was 37.5% of the extraction socket on average (Figures 2A and E). In a previous micro-CT study, Hikita et al. [4] reported that the percentage of new bone volume in the extraction socket 14 days after tooth extraction was 70.88%. This is mainly due to a different determination of the volume of the tooth-extraction socket. We found that it was difficult to differentiate the newly formed bone from the alveolar bone using CT values (Figure 2). This is probably because sufficient bone remodeling took place by 14 days after tooth extraction. In contrast, the alveolar socket of the non-extraction molar can be easily outlined by the apparent differences in the CT values among the bone, periodontal ligament and tooth (Table I). We thus reconstructed the extraction socket by mirroring the alveolar socket of the non-extraction side, which is more precise and reliable. In addition, the upper limits of the tooth-extraction socket determined by lines passing through the alveolar crest and septum at 14 days after tooth extraction in Hikita's report differed from those in our current study, which were equivalent to the alveolar crests immediately after tooth extraction. In the early stage of wound healing after tooth extraction, obvious bone resorption in the alveolar crest and septum occurs [18]. Thus, the lower percentage of new bone recorded in our study could also be a result of over-estimating the extraction-socket volume.

When rats were treated with lithium daily starting from 7 days before tooth extraction and ending 14 days after tooth extraction (the continuously treated group), the new bone formation was significantly reduced (Figures 2D and E). These results are consistent with earlier findings that activation of Wnt/$\beta$-catenin signaling increases the number of the osteoprogenitors but suppresses their osteogenic differentiation [6,19]. Likewise, continuous treatment with lithium activates $\beta$-catenin, similar to the mutation resulting in constitutively active $\beta$-catenin, which leads to a block of osteoblast differentiation and a delay in bone-fracture healing [7]. However, once

these undifferentiated cells have become committed to the osteoblast lineage, the elevated β-catenin signaling promotes osteoblastic differentiation and function, leading to enhanced osteogenesis [7]. In extraction sockets, osteoblastic cells are observed as early as 4 days after tooth extraction [1]. As a result, when lithium was given after osteoblast differentiation was initiated, as in our post-treated group, an increase in the amount of new bone was observed (Figures 2C and E).

An interesting finding of our study is that the new bone formation was also enhanced in the pre-treated group, in which lithium was given from 7 days before tooth extraction to 3 days after surgery (Figures 2B and E). Although no significant difference was found between the pre- and post-treated groups, the mean value of the amount of new bone formation was even larger in the pre-treated group than in the post-treated animals (Figure 2E). Lithium treatment elevates β-catenin levels and promotes the proliferation of undifferentiated mesenchymal cells during the early stage of bone regeneration [6,7]. These proliferating cells are candidate osteoprogenitors, which will differentiate to osteoblasts and build new bone [6]. It is important to note that the osteoprogenitors in extraction sockets are actively proliferating on day 3 after tooth removal [3]. Thus, it is conceivable that prior treatment with lithium until day 3 after tooth extraction led to an increase in the population of proliferating osteoprogenitors in the extraction sockets. Unlike in the continuously treated group, the osteoprogenitors in the pre-treated extraction sockets normally differentiated into osteoblasts in the following days because lithium had been withdrawn. If more osteoblasts are recruited in the sockets, more new bone will be formed. Thus, our data suggest that both pre-treatment and post-treatment with lithium enhance new bone formation in extraction socket healing, through different mechanisms. Although the precise mechanisms need to be clarified, our recent report and others suggest that Wnt/β-catenin signaling is responsible for the lithium effects on bone formation and repair [6–8].

We next evaluated bone mineral density (BMD) to identify the quality of the new bone formed after lithium treatment by measuring the CT values. In a previous study, lithium was reported to decrease BMD associated with suppression of osteoid formation [20]. In that research, the circulating lithium level was 1.4 mM, which was at the higher end of the therapeutic range for treatment of bipolar disorders in humans (0.6–1.5 mM) [21]. The dosing schedule used in our present study was previously demonstrated to result in a mean plasma lithium concentration of 0.6 mM, which activates β-catenin signaling and enhances new bone regeneration in rats [6,16]. In extraction sockets, BMD in the pre- and post-treated groups was significantly higher, suggesting that lithium enhances both the quality and quantity of new bone (Figure 3A). In the continuously treated group, no difference in BMD was found compared to the control group, probably because new bone formation was impaired. Importantly, a significant increase in BMD was also detected in alveolar bone of the non-extraction side in the continuously treated group (Figure 3B). This suggests that lithium can increase bone density in those places in which bone formation or remodeling is actively taking place. The mean BMD values may not have increased significantly in the pre- and post-treated alveolar bones relative to the control group because the lithium treatment period was shorter than in the continuously treated group (Figure 3B). Supporting findings show that 4 weeks of oral lithium intake increases bone volume and BMD in tibias of LRP5 mutant mice as a result of Wnt/β-catenin pathway activation [8]. Osteoblasts lacking the β-catenin gene exhibit impaired maturation and mineralization, whereas constitutive activation of β-catenin in mutant mice results in dramatically increased bone deposition and a disappearance of osteoclasts [22]. Thus, the increase in BMD by lithium treatment is probably due to an increase in osteoblast function or a decrease in osteoclastogenesis. Similarly, patients on maintenance therapy with lithium have a higher BMD in the lumbar spine, femoral neck and trochanter [23]. These patients may have lower rates of bone resorption because they have lower serum total ALP (alkaline phosphatase), lower serum osteocalcin or reduced urinary calcium excretion [23,24]. Whether these changes in metabolic parameters upon lithium treatment are mediated by Wnt/β-catenin signaling or by other mechanisms needs further investigation.

Craniofacial bone loss caused by trauma, tumor or congenial defects, or after various surgical procedures, is a major concern for both patients and clinicians. One of the goals of bone-defect therapy is to accelerate healing. Our current study demonstrates that lithium treatment before or after tooth extraction increases new bone volume during extraction-socket healing, suggesting that lithium could be a pharmaceutical aid for improving bone repair. More importantly, lithium also increases the BMD of the new bone. The enhancement of both bone volume and density will be most beneficial in clinical events such as new bone regeneration after rapid palatal expansion and bone grafts in alveolar clefts, where bone resorption and relapse frequently occur [6,25]. On the other hand, our results also show that bone healing is greatly retarded in sockets continuously treated with lithium. Thus, special care must be taken when jaw surgeries such as tooth extraction are performed in patients receiving lithium therapy for bipolar disorder. Although the evidence available to date is encouraging, long-term and high-dose lithium intake can have adverse effects, including vomiting, drowsiness,

decreased thyroid function and impaired memory [5]. Thus, the risks and benefits of lithium as a therapeutic aid for inducing bone formation and repair await comprehensive long-term animal studies and human trials.

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