An exploratory study of gold wire implantation at acupoints to accelerate ulnar fracture healing in rats

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Abstract By causing long-term stimulation, gold wire implantation at acupoints has been used empirically to prolong the effects of acupuncture. This study shows that subcutaneous gold wire implantation at acupoints has long-term effects on bone regeneration in the rat ulna bone defect model.

Keywords Gold wire · Ulna · Healing

Introduction An ancient form of traditional acupuncture is to insert solid needles simply into the skin at specific points on the body. To potentiate the effects of simple acupuncture treatment, various stimulation methods, such as electroacupuncture, ultrasound acupuncture, laser acupuncture, aqua-acupuncture, and implantation at acupoints, have been increasingly developed by combining new medical techniques over the last half-century [1]. Of the above methods, implantation at acupoints has been used empirically for the sustained stimulation of acupoints in chronic diseases [2–4]. Various materials can be implanted, the commonest being gold wire, surgical suture materials, surgical staples, and stainless-steel wire [1, 3].

Although its mechanism requires clarification, gold implantation has been used empirically as an alternative therapy for chronic disorders related to bone, like hip dysplasia, degenerative joint disease, osteochondritis, osteochondritis dessicans, bone fracture, and ventral spondylosis [3, 5, 6]. Previous clinical studies support the long-term effects of gold implantation, although it is still controversial [5, 7–10]. Jaeger et al. [10] reported that the pain-relieving effect of gold bead implantation in dogs with hip dysplasia continued throughout the 2-year follow-up period. Goiz-Marquez et al. [9] reported that 9 of 15 dogs (60%) had at least a 50% reduction in seizures frequency during the 15 weeks after gold wire implantation at acupoints. In contrast, a controlled clinical study by Hielm-Bjorkman et al. [8] suggested that, as compared with a control group needled subcutaneously, gold wire implantation at acupoints used to treat hip dysplasia in dogs had no significant effects in improving clinical symptoms in the treated group. Although some clinical studies showed that gold implantation has prolonged effects in chronic diseases, there is no laboratory evidence to show whether implantation at acupoints has long-term effects in chronic disease.

We used rat ulna osteotomy model to determine whether gold wire implantation at acupoints has long-term effects;...
the effect of gold wire implantation on bone healing was evaluated up to 8 weeks after implantation.

Materials and methods

Male Sprague–Dawley rats weighing approximately 250 g were used. The animals were kept in a restricted access room with controlled temperature (23°C) and light/dark cycle (12/12 h). The rats were housed in individual cages. Food and water were provided ad libitum. The experimental procedures were carried out according to the animal care guidelines of the NIH and the Kyung Hee University Institutional Animal Care and Use Committee.

Segmental ulnar defect

The experimental bone defect was performed as described previously [11]. In brief, the rats were premedicated by gentamycin (3 mg/kg, s.c.) and anaesthetized by intraperitoneal injection with mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). The right forelimb was clipped and scrubbed. A 2.5-cm-long skin incision was made along the caudal surface of the antebrachium, and the shaft of the ulna was exposed. To create a bone defect 3 mm wide, a fine bone cutter was used to sever the ulna transversely at the border between its proximal and second quarters. The skin incision was closed routinely. Gold wire was then implanted into the right forearm in gold implantation group.

Gold wire implantation

The rats were randomized into control (n = 14) and gold implantation groups (n = 10). The gold wire (Hanglim, Korea) was in the form of 0.15-mm-diameter 24-carat gold wire 2.5 cm in length. Using a 24-gauge hypodermic needle, the gold wire was introduced subcutaneously along the Small Intestine Meridian from SI8 acupoint (Xiao Hai) to SI6 acupoint (Yang Lao). The wire was left in the tissues as the hypodermic needle was withdrawn (Fig. 2c). The ends of the gold wire protruding through the skin were cut off and cleaned. The control group received only the surgery to create the bone defect, without gold wire implantation.

Radiographic and histological examinations

The repair of bone defects was evaluated by radiography and histology. To measure the extent of bone regeneration in the fractured site, radiographs of the right antebrachium of each rat were obtained under inhalation anaesthesia with oxygen and isoflurane from 2 weeks after segmental ulnar defect and thereafter weekly (up to 8 weeks). Then, the width of bone defect on X-ray film was measured using a dial calliper (Ozaki MGF, Japan). Bone regeneration in each rat was calculated as follows: bone regeneration (%) = (bone defect width at 2 weeks after surgery-bone defect width weekly)/(bone defect width at 2 weeks after surgery) × 100. In this study, the bone defect width was measured from 2 weeks after surgery, due to technical difficulties from obscure bone fracture line on radiographs by marked oedema and haematoma at ulna fracture sites during the first week after surgery.

After the last radiographic examination (8 weeks), all rats were sacrificed with an overdose of pentobarbital for histological examination. Bone segments were extracted and inspected carefully under low power microscopy, as a union (afferent bone bridging), partial union (failure primarily along original fracture but with some bone bridging evident), or non-union (no evidence of bone bridging). All bones were fixed immediately in 4% buffered paraformaldehyde overnight at 4°C. Sections were taken out by paraffin wax buried into blocks following 20% ethylene diamine tetraacetate (EDTA) decalcification. The specimens were stained with haematoxylin–eosin (HE) and examined under a light microscope. Images of fractured sites were taken from three randomly selected sections from each animal with a Zeiss Axoplan microscope (Carl Zeiss MicroImaging, Hamburg, Germany) and the extent of new bone formation in fractured site was measured using the image program.

Statistical analysis

All values are expressed as mean ± SE. Statistical significance between two groups was evaluated using Student’s t test. P values < 0.05 were regarded as significant [12].

Results

Figure 1 shows the effects of subcutaneous gold wire implantation at acupoints SI6–SI8 on bone regeneration in segmental ulnar defect. In both gold implantation and control groups, the radiographic evidence of new bone formation was apparent from 4 weeks and the bone defect width was decreased gradually and consistently, indicating gradual bone regeneration. From 4 weeks after gold implantation, the gold-implanted group showed significantly faster bone regeneration than the control group and this significant bone regeneration lasted up to 8 weeks after gold implantation (Fig. 1; P < 0.05). At 8 weeks after surgery, the bone regeneration of the gold-implanted group reached a value of 73.3 ± 14.5% of initial bone defect width (2 weeks post-surgery) while that of control group was 23.7 ± 3.4%.
Eight weeks after bone defect surgery, the healing osteotomy site was assessed under low power microscopy (10×), as a union, partial union, or non-union. The implanted group (n=10) showed 5 complete unions (50%), 1 partial union (10%), and 4 non-unions (40%). On the other hand, the control group (n=14) showed 0 complete unions (0%), 1 partial union (7%) and 13 non-unions (93%). The incidence of complete/partial union in the gold implantation group was much higher than that in the control group (60:7%). Figure 2 shows representative radiographs of non-union cases of control and implanted rats 8 weeks after bone defect surgery. Although non-union at the fractured site in both rats was observed, there is a more marked increase in new bone formation in the rat with gold implantation (Fig. 2c), compared to the control rats (Fig. 2b). To confirm the extent of bone formation, the osteotomy sites of all rats were examined histologically and the non-union cases in both groups were compared especially carefully (Fig. 3a, b). Compared with the control rats, the implanted rats showed more extensive accumulation of new bone on the fracture line. When quantified, the extent of new bone formation from the fractured line in the implanted group showed significant increases, compared to that of the control group (1.09 ± 0.22 vs 0.35 ± 0.05 mm; P < 0.05; Fig 3c).

Discussion

This study explored the effects of subcutaneous gold wire implantation at acupoints SI6 through SI8 on bone healing in rats. Compared to control rats, implanted rats showed significant increases in new bone formation on radiographic and histological examination. On post-mortem examination, 50% of the implanted rats showed complete unions, while there was no complete union in the control rats. This study suggests that gold wire implantation at acupoints had long-term effects on healing of ulnar defect in rats.

Although implantation at acupoints has been used empirically to induce prolonged mechanical stimulation of acupoints in chronic diseases [3–5, 10], there is no published evidence until now to support whether one treatment by implantation at acupoints has long-term effects in chronic disease. Our exploratory findings provide evidence that gold implantation at acupoints has long-term effects on healing of segmental ulna defect through 8 weeks after one implantation. However, many questions remain to be solved. One of the important questions is how gold implantation at acupoints accelerates bone healing in rats. Here, we speculate on two possibilities which will be focused on in our further studies on acupuncture effects by gold wire and direct current effect.

One possibility is that the long-term mechanical stimulation by gold wire at acupoints SI6–SI8 by gold wire helps to accelerate healing in ulna bone defect. Acupuncture is
believed to be able to stimulate bone healing using points that tonify and move Qi along the meridians that pass through the affected area [13]. Previous studies have shown the beneficial effects of acupuncture on bone healing [14–16]. In dogs with experimental radial defect, Sharifi et al. [14] showed that acupuncture treatment of 14 days, 10 min/day has positive and stimulatory effects on formation of callus, trabecule, and fibrocartilage, compared to a control group. Acupuncture has been reported to increase bone weights, bone strength, trabecular volume, and bone formation rate in osteopenic ovariectomized rats [15–18]. And it has also been reported that acupuncture at acupoints has stronger effects on bone healing than at non-acupoints, indicating point-specificity on bone healing [17]. In our present study, the gold wire was introduced subcutaneously along the Small Intestine Meridian from SI8 (Xiao Hai) to SI6 (Yang Lao). Acupoints from SI6 to SI8 have been used clinically to eliminate symptoms such as pain and swelling in the arm and elbow [2, 13]. The present results, that the stimulation of acupoints SI6–SI8 accelerates bone healing in rats, support previous reports concerning the beneficial effects of acupuncture on bone healing [17].

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To confirm how gold implantation at acupoints enhances the bone healing in rats, further studies will be required concerning the effects of simple needling, gold wire itself or non-specific stimulation of other materials on bone healing, analysis of local release of neurotransmitters, or the point-specific effect of site of gold wire implantation by comparing implanting to the other areas, which is an important subject in our future studies on the mechanism(s) involved.

Another possibility is that gold wire acts as a low current cathode to stimulate osteogenesis electrically. Becker [22] noted differences in the “current of injury” between regenerating and non-regenerating types of amphibians and that partial limb regeneration can be induced in the latter type by simulating the “current of injury” of the regenerating form. He reported partial limb regeneration in rats due to manipulation of electrical currents at the amputated limb. Cathodes from gold wire, stainless-steel, cobalt-chromium, and titanium are known to stimulate osteogenesis over electric current ranges of 0.075–20 μA [23, 24]. Previous studies suggest that these low currents produce electrochemically-mediated micro-environmental changes (increased hydroxyl ion concentration and decreased oxygen tension) near the cathode, which consequently trigger osteogenesis [24, 25]. Low currents of 1.5–9.6 μA were measured after trauma to healthy animal skin [26]. These current intensities after trauma are within a current range (0.075–20 μA) to electrically stimulate osteogenesis. In this study, subcutaneous gold wire near ulna defect may contribute to deliver the ionic currents from skin trauma to the fractured ulna, which may consequently stimulate healing of ulnar defect. It should be noted that our results are inconclusive in the clinical applications of gold wire implantation for bone defect or non-union in humans. Further studies are needed on the safety and optimum amounts of gold wire or the effect of implanting the other wire which produces similar mechanical stimulation, with different electrical effect.

![Image](https://example.com/image.png)

**Fig. 3** Representative histological pictures of the fractured area in non-union rats 8 weeks after bone defect (a, b) and comparison of new bone formation (c). More extensive accumulation of new bone occurred on the fracture line (arrow) in the gold-implanted rat (b), compared with that of the control rat (a). c shows significant increases in extents of new bone formation in the gold-implantation group (n = 10) compared to the control group (n = 14). *P < 0.05.
In conclusion, this study shows that subcutaneous gold wire implantation from SI6 through SI8 enhanced fracture healing through 8 weeks in rat ulna bone defect model.

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