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Effect of gelatin sponge with colloid silver on bone healing in infected cranial defects

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ABSTRACT

Oral infectious diseases may lead to bone loss, which makes it difficult to achieve satisfactory restoration. The rise of multidrug resistant bacteria has put forward severe challenges to the use of antibiotics. Silver (Ag) has long been known as a strong antibacterial agent. In clinic, gelatin sponge with colloid silver is used to reduce tooth extraction complication. To investigate how this material affect infected bone defects, methicillin-resistant *Staphylococcus aureus* (MRSA) infected 3-mm-diameter cranial defects were created in adult female Sprague-Dawley rats. One week after infection, the defects were debrided of all nonviable tissue and then implanted with gelatin sponge with colloid silver (gelatin/Ag group) or gelatin alone (gelatin group). At 2 and 3 days after debridement, significantly lower mRNA expression levels of *IL-6* and *TNF-α* and lower plate colony count value were detected in gelatin/Ag group than control. Micro-CT analysis showed a significant increase of newly formed bone volume fraction (BV/TV) in gelatin/Ag treated defects. The HE stained cranium sections also showed a faster rate of defect closure in gelatin/Ag group than control. These findings demonstrated that gelatin sponge with colloid silver can effectively reduce the infection caused by MRSA in cranial defects and accelerate bone healing process.

1. Introduction

Oral infectious diseases, such as periodontitis, peri-implantitis, dry socket and osteomyelitis of the jaws, often manifest as pain, bleeding and pus, and subsequently lead to bone loss. Local treatment of infection and promoting bone healing are of great significance for further oral restoration.

Management of these infections often requires multiple staged surgeries and the use of antibiotics as a supportive therapy for eradication [1,2]. However, in recent years, the rise of multidrug resistant bacteria has put forward severe challenges to the use of antibiotics [3,4]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common pathogens of oral infection. Facing the urgent need for the development of novel therapeutic agents, silver (Ag), which has long been known as a strong antibacterial agent of a wide range of microbes [5–8], has attracted much attention [9–11]. Many medical instruments and products, such as venal catheters [12,13], wound and burn bandages are silver-coated [14–18]. Both in vitro [19] and in vivo [20] experiments confirmed its good antibacterial activity. And its antibacterial effect was further demonstrated as causing irreversible damage on bacterial cells [21–23].

In recent years, gelatin is widely used for tissue engineering. It is obtained by a controlled hydrolysis of collagen, which is a major component of skin, bones and connective tissue. It exhibits excellent qualities such as biocompatibility, biodegradability [24], low antigenicity [25–29], and is more economical than collagen. Gelatin's easily modified ability also makes it a good material for drug delivery [30,31]. A wide variety of gelatin-based composites have been developed for tissue engineering, such as gelatin-siloxane hybrids [32], β-TCP/chitosan/gelatin [33], chitosan-gelatin-alginate-hydroxyapatite [34], gelatin-chitosan-nanobioglass 3D porous scaffold [35] and hybrid macroporous gelatin/bioactive-glass/nanosilver scaffolds [36].

In oral treatment, gelatin sponge with colloidal silver has been used in clinic to prevent the complication of tooth extraction [37]. Its porous structure can promote blood clotting and stabilize blood coagulation. Along its absorption, it continuously releases silver ions against almost all microbes found in the oral environment even antibiotic resistance bacteria.

Existing researches worked mostly on the antibacterial effect of Ag, but few of these studies focused on how it affect infected wound healing. The purpose of this study was to investigate the effect of gelatin sponge with colloid silver on bone healing in infected cranial defects.

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2. Materials and methods

2.1. Characterization of gelatin sponge

The characterization of functional groups on the gelatin sponge (with or without Ag) were performed by Fourier-transform infrared spectroscopy (FTIR) on a Varian 680-IR spectrometer (Agilent Tech, USA). The FTIR spectra were recorded using 16 scans/min, with a resolution of 1 cm^{-1} in the 400–4000 cm^{-1} wave number region.

X-ray photoelectron spectroscopy (XPS) measurement was done using DAR400-XM 1000 (OMICRON Nanotechnologies, Germany) equipped with dual Al/Mg anodes as the X-ray source. The Al anode was used to attain the survey and elemental spectra. All spectra were calibrated using *C*1 s peak at 284.5 eV to exclude the charging effect on the sample.

2.2. Animals

10-week-old female Sprague-Dawley rats weighing 250–300 g were obtained from Experimental Animal Center of Sichuan University. All the experiments performed were approved by the Subcommittee on Research and Animal Care (SRAC) of Sichuan University. The experimental procedures were in accordance with the Care and Use of Laboratory Animals published in the National Institute of Health Guide (1996). All efforts were made to minimize animal suffering. These animals were kept in standard conditions (22 ± 2 °C; 50–70% relative humidity) and a 12 h light–dark cycle (lights on at 08:30 a.m.).

2.3. Bacteria preparation

MRSA (ATCC 43300) was purchased from the American Type Culture Collection (ATCC) (Manassas, VA). MRSA was grown overnight in brain heart infusion broth at 37 °C with shaking at 200 rpm. The numbers of colony-forming units (CFUs) of the inoculum were determined using turbidimetry, then the MRSA solution was diluted with sterile saline to 1×10^7 CFUs.

2.4. Creation of cranial defects and bacteria inoculation

The infected cranial defects were created according to our previous work [38]. Rats were anesthetized by an intraperitoneal injection of ketamine (75.0 mg/kg) (Ketaset; Aveco, Fort Dodge, IA, USA)/dexmedetomidine (0.25 mg/kg). After shaving and disinfection, the scalp was incised along the midline. The skin and periosteum was reflected laterally to the muscle attachment. Full-thickness cranial defects were created bilaterally between the coronal suture and lambdoid suture using a trephine (3 mm in diameter). At the same time, normal saline irrigation was used to prevent heat damage. The defect edge was examined to confirm no residual bone left. Then gelatin sponges (without Ag) were wetted by 0.1 mL MRSA solution or saline (used as control) and inserted into the defects. The periosteum was sutured with 5–0 polyglactin-910 suture to fix the sponges. The skin was approximated along the midline and closed with 3–0 polyglactin-910 suture.

2.5. Debridement and material implantation

One week after MRSA inoculation, the cranium were re-exposed through the same midline scalp incision. The wound was treated with debridement and thorough irrigation with saline. The defects were filled with gelatin sponge with colloid silver (Gelatamp, Roeko, Coltene whaledent, Langenau, Germany) (gelatin/Ag group). The control defects were implanted with gelatin sponge (Gelfoam, Pfizer, New York, USA) (Gelatin group). The skin was approximated along the midline and closed with 3–0 polyglactin-910 suture.

2.6. Plate colony count

2 and 3 days after material implantation, rats were sacrificed. The granulation tissue in the defect was obtained using a curette. The surface moisture was wiped off using filter paper and the tissue was weighted. Then the tissue was homogenized and diluted by 20 mL saline. 100 μL of each sample was separately inoculated on sterile plates (35 mm in diameter) containing appropriate amount of plate count agar. The samples were stored at 37 °C in incubator for 24 h. Then the colony-forming units grown were counted.

2.7. Quantitative real-time PCR

Total RNA from the granulation tissue was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. For qRT-PCR, cDNA was prepared using QuantiTec reverse transcription kit (Qiagen, Valencia, CA) and analyzed with SYBR GreenMaster Mix (SABiosciences, Valencia, CA) in the iCycler (Bio-Rad, Hercules, CA) using specific primers designed for each targeted gene (Table 1) [39]. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method by normalizing with *β-actin* gene expression, and presented as fold increase relative to control.

F-forward; R-reverse.

2.8. Micro-CT analysis

The craniums were harvested 2 and 4 weeks after debridement, then immediately fixed in 10% buffered formalin overnight and stored in 70% ethanol at 4 °C. Micro-computed tomography (Micro-CT) analysis with three-dimensional reconstructions were performed as previous described [40,41] using Skyscan 1176μCT imaging system (Bruker, Kontich, Belgium) at a spatial resolution of 18 μm (1 mm aluminum filter, 100 kV, 100 μA). The data were analyzed with NRecon 1.6 and CTAn 1.8 to determine the degree of ectopic bone formation in healed area. Three-dimensional (3D) images of the samples were also reconstructed using CTvox (SkyScan).

2.9. Histology

The specimens were then decalcified with 17% EDTA for 8 weeks and processed for paraffin embedding. The specimens were sectioned in the coronal plane to a thickness of 5 μm and stained with HE staining. The sections were observed by Nikon Eclipse 80i microscope (Nikon, Yokohama, Japan) and digital images were generated using NIS-Elements software.

2.10. Statistical analysis

All values were expressed as mean ± SD. Statistically significant differences were evaluated by Student's *t*-test. A *p* value of less than 0.05 was considered to be statistically significant.

Table 1
Primers designed for each target gene.

Target gene	Direction	Primer sequence (5′-3′)
TNF-α	F	5′-GGCAATGGCATGGATCTCA-3′
	R	5′-ATGGCAAATCGGCTGACGG-3′
IL-6	F	5′ACTTCCAGCCAGTGCCTTCT-3′
	R	5′-GGTCTGTTGTGGGGTGTATCCT-3′
β-actin	F	5′-ACGGTCAGGTCATCACTATCG-3′
	R	5′-GGCATAGAGGTCTTTACGGATG-3′