

# Titanium particles in the surrounding tissue of oral implants could promote a local aseptic inflammatory response resulting in marginal bone resorption of osseointegrated implants

## Macrophage polarization in aseptic bone resorption around dental implants induced by Ti particles in a murine model

Wang X, Li Y, Feng Y, Cheng H, Li D

### Background

Titanium particles/ions detected in peri-implant tissues - a potential etiologic factor for crestal bone loss around oral implants.

- Titanium particles could induce marginal bone resorption around dental implants independent of bacterial infection.
- Local adverse inflammation microenvironment leading to peri-implant bone resorption is promoted by macrophage recruitment and macrophage polarization toward a pro-inflammatory M1 phenotype with production of inflammatory mediators.

### Methods

- Sprague Dawley rats; 4 groups; titanium screw implanted in bilateral maxillary first molar area for 4 weeks for osseointegration; 20  $\mu$ g titanium particles in peri-implant tissue to induce aseptic foreign body reaction.
- Macrophages depleted by local injection of 100  $\mu$ L clodronate liposome (Ti + LipClod group).
- Titanium-injected rats treated with PBS (Ti + PBS) or empty liposome (Ti + Lip) and rats injected with PBS alone included as controls.
- Animals sacrificed at 8 weeks; samples collected; half analyzed radiologically to measure bone level change
- Macrophage markers (CD68, CCR7, CD163) also characterized by immunofluorescence to evaluate number, density, and phenotype distribution (CCR7+M1/CD163+M2).
- The rest of the samples used to determine the relative mRNA expression levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and RANKL with real-time PCR analysis.

### Results

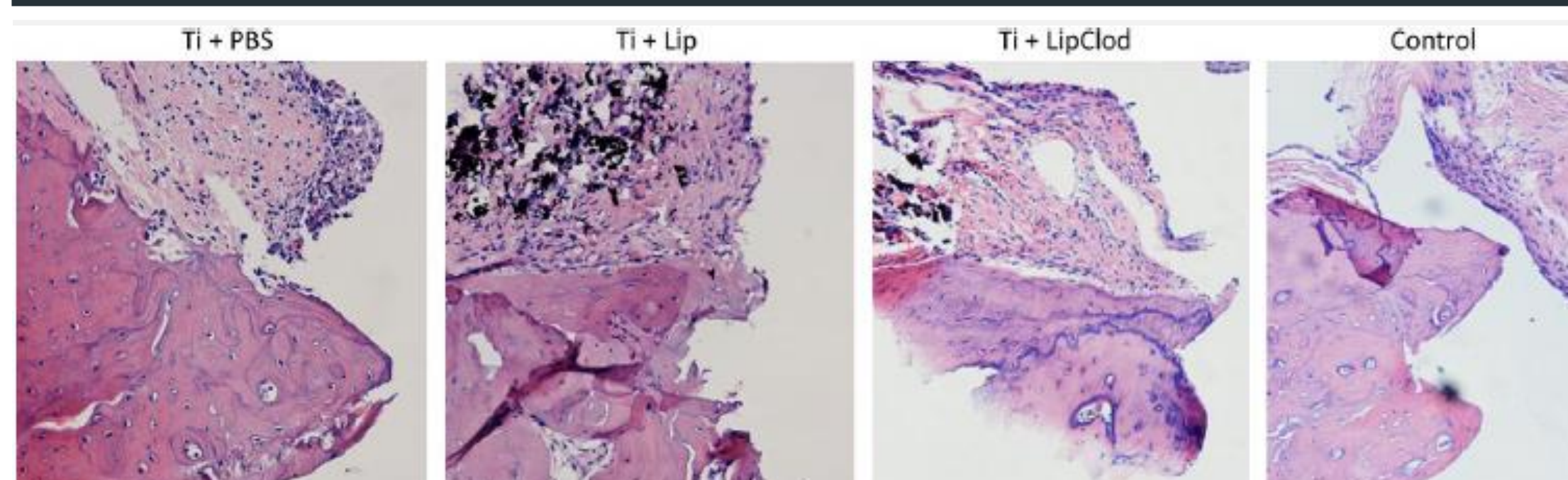


Figure. Representative images of hematoxylin and eosin stained sections 8 wk after treatment, 100 $\times$  magnification. Notice the presence of a dense mixed inflammatory cell infiltrate in the Ti + PBS and Ti + Lip groups as compared to the Control and Ti + LipClod groups

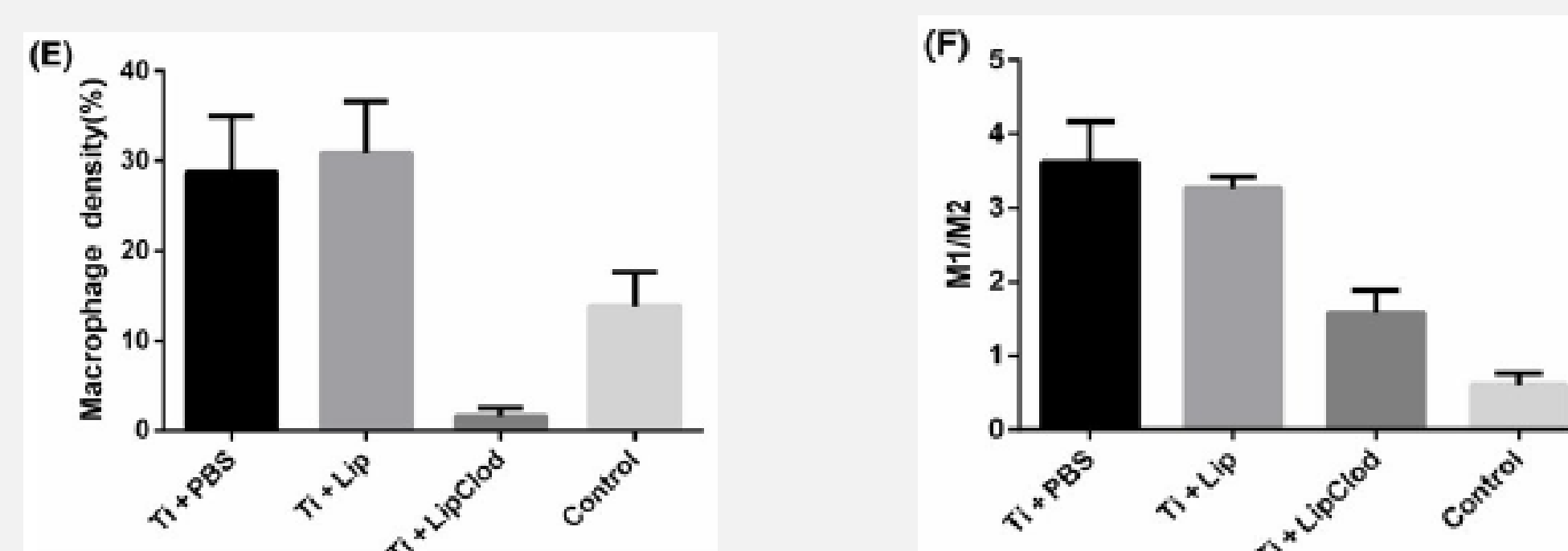


Figure. Quantification of total CD68 + macrophages in 3 randomly selected HPFs of four groups (left), quantification of the M1/M2 ratio in different groups (right)

### Conclusion

Titanium particles had a negative effect on peri-implant tissue by activating macrophages which induced an M1 macrophage phenotype promoting local secretion of inflammatory cytokines. It was found that clodronate liposome treatment attenuated the severity of inflammation and bone loss by depletion of macrophages.

Table. Experimental Groups

Group	Treatment
Ti + PBS	20 mg Ti particles + 100 $\mu$ L PBS
Ti + Lip	20 mg Ti particles + empty liposome (100 $\mu$ L, i.v. every 3 d)
Ti + LipClod	20 mg Ti particles + clodronate liposome (initial dose at 100 $\mu$ L, followed by 100 $\mu$ L every 3 d, i.v.)
Control	100 $\mu$ L PBS alone

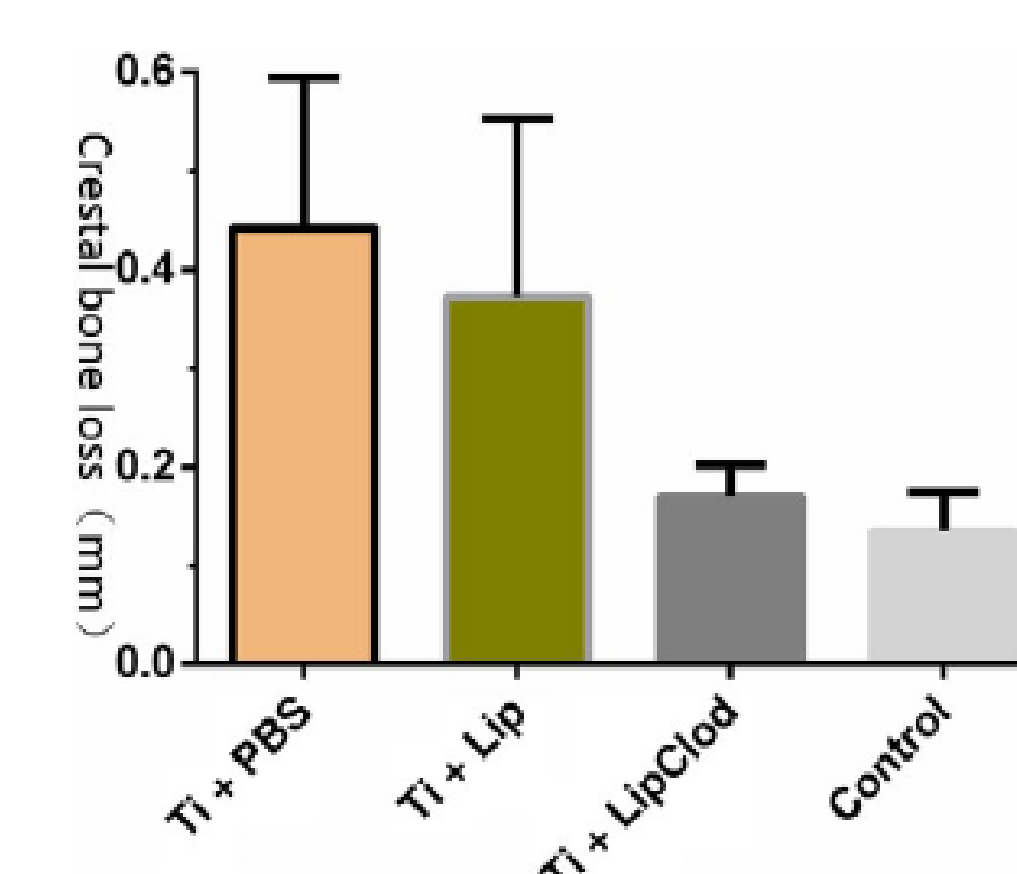


Figure. Average peri-implant crestal bone height changes as measured by the distance of the implant head to the alveolar bone crest on the mesial, distal, buccal, and palatal aspect of the fixtures

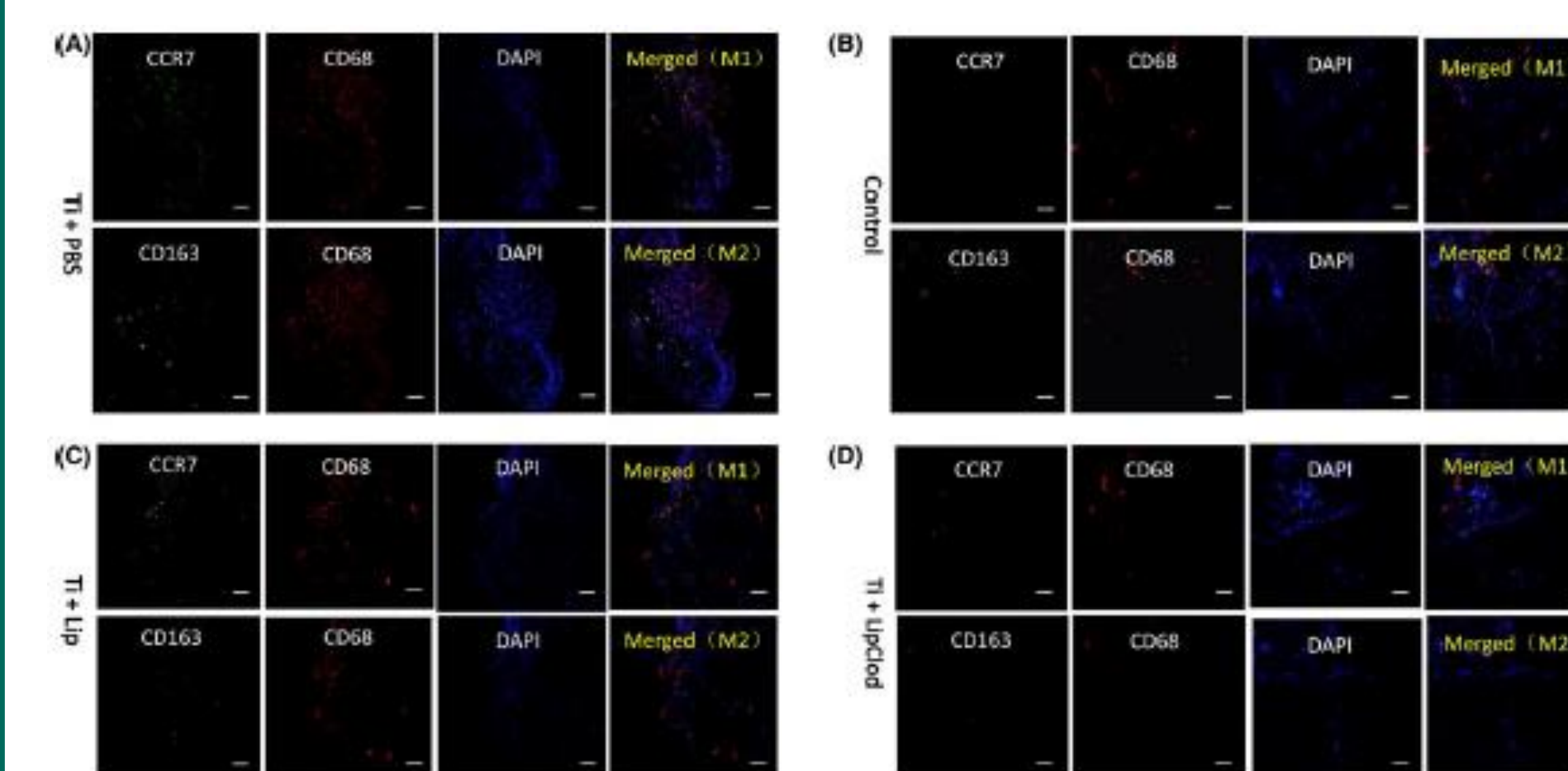


Figure. Altered M1 and M2 macrophage infiltrations around tissues bordering the bone resorption areas of implants. A and B, immunofluorescent images showed an increased infiltration of CD68 + CCR7 + M1 macrophages and a decreased infiltration of CD163 + M2 macrophages in the Ti + PBS and Ti + Lip groups. (n = 4). Scale bars, 50  $\mu$ m. C, macrophages infiltrated in the Ti + LipClod group was decreased and presented as M1 phenotype. D, CD163 + M2 macrophages

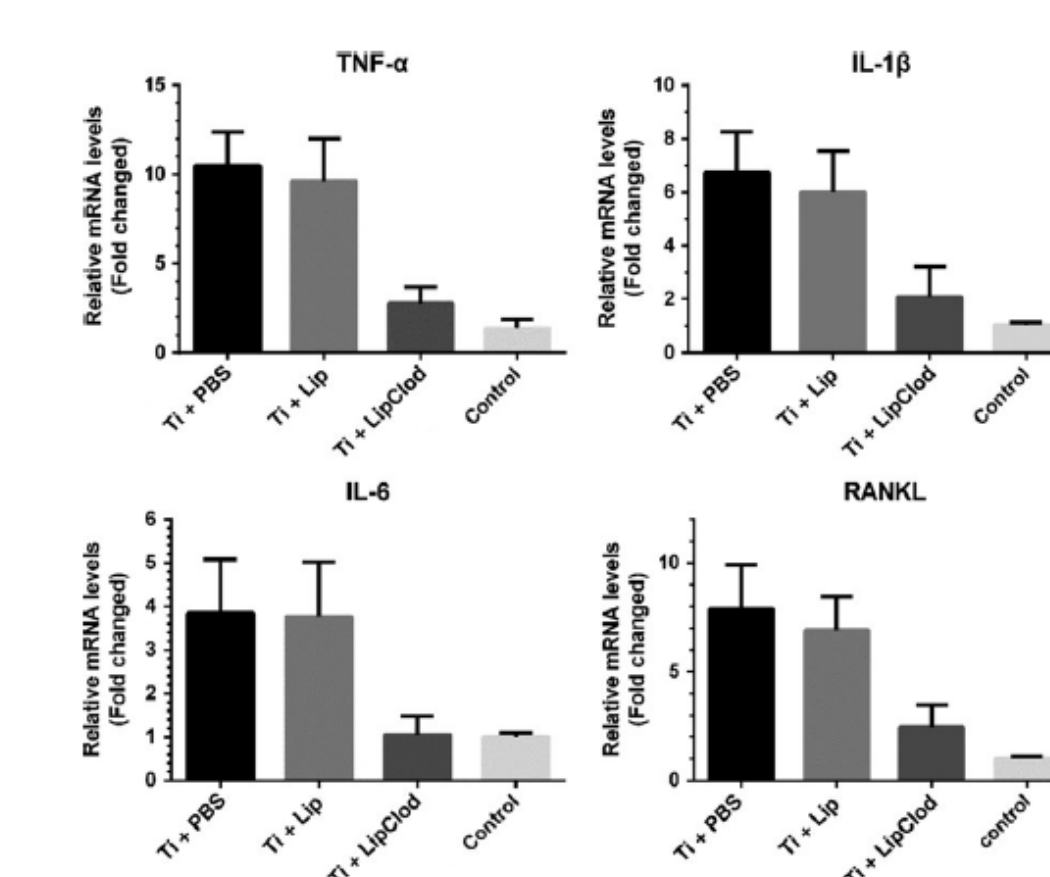
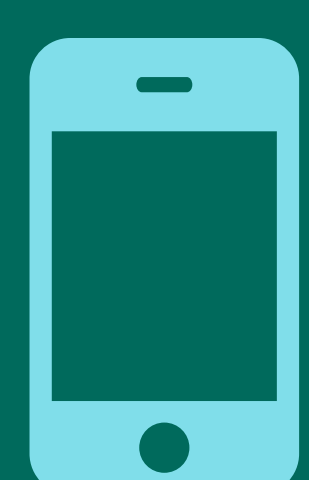
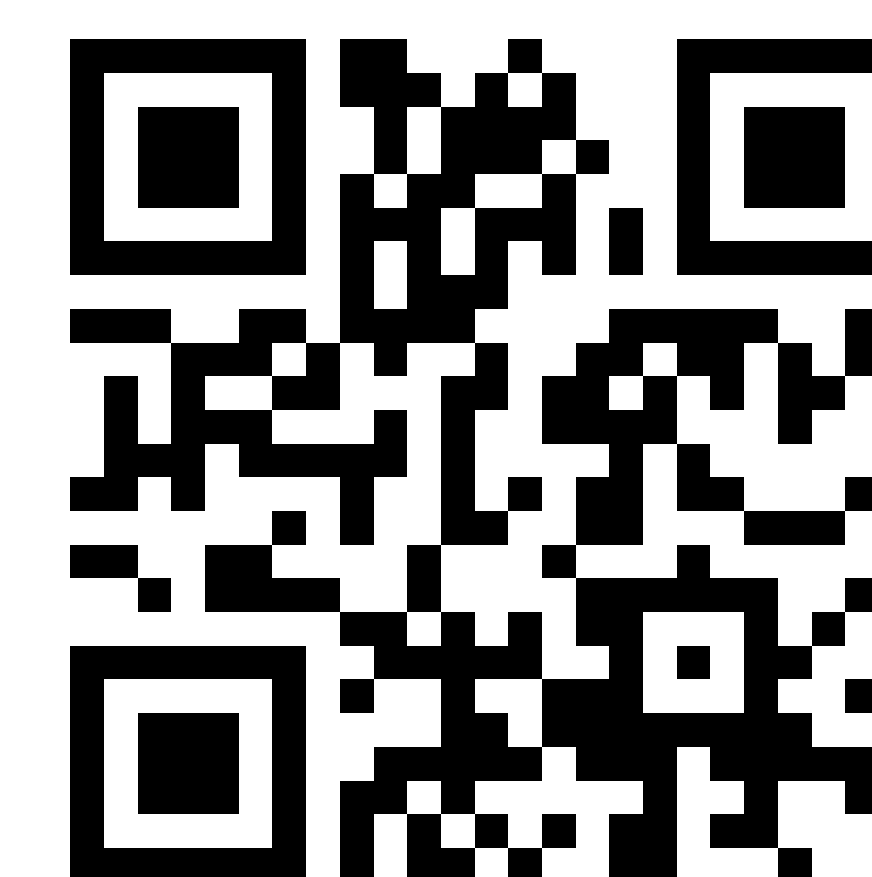


Figure. The mRNA expression levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and RANKL were presented as fold changes using real-time PCR



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# Macrophage polarization in aseptic bone resorption around dental implants induced by Ti particles in a murine model

Xin Wang<sup>1,2</sup>, Yu Li<sup>2</sup>, Yuan Feng<sup>1</sup>, Haode Cheng<sup>1</sup>, Dehua Li<sup>1</sup>

<sup>1</sup>State Key Laboratory of Military Stomatology & National Clinical Research Center for Oral Diseases & Shaanxi Engineering Research Center for Dental Materials and Advanced Manufacture, Department of Oral Implants, School of Stomatology, The Fourth Military Medical University, Xi'an, China, <sup>2</sup>Department of Stomatology, the 901th Hospital, Hefei, China

## Question

To investigate the effects of titanium particles-induced foreign body reaction on the crestal bone level alterations of submerged implants and analyze the local immune response changes represented by macrophage phenotype transformation in a murine model.

## Background

Titanium particles/ions detected in peri-implant tissues - a potential etiologic factor for crestal bone loss around oral implants.

➤ Titanium particles could induce marginal bone resorption around dental implants independent of bacterial infection.

➤ Local adverse inflammation microenvironment leading to peri-implant bone resorption is promoted by macrophage recruitment and macrophage polarization toward a pro-inflammatory M1 phenotype with production of inflammatory mediators.

## Methods

- Sprague Dawley rats; 4 groups; titanium screw implanted in bilateral maxillary first molar area for 4 weeks for osseointegration; 20  $\mu$ g titanium particles in peri-implant tissue to induce aseptic foreign body reaction.
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- Macrophage markers (CD68, CCR7, CD163) also characterized by immunofluorescence to evaluate number, density, and phenotype distribution (CCR7+M1/CD163+M2).
- The rest of the samples used to determine the relative mRNA expression levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and RANKL with real-time PCR analysis.

## Macrophage polarization has a marked impact on peri-implant bone loss caused by titanium particles

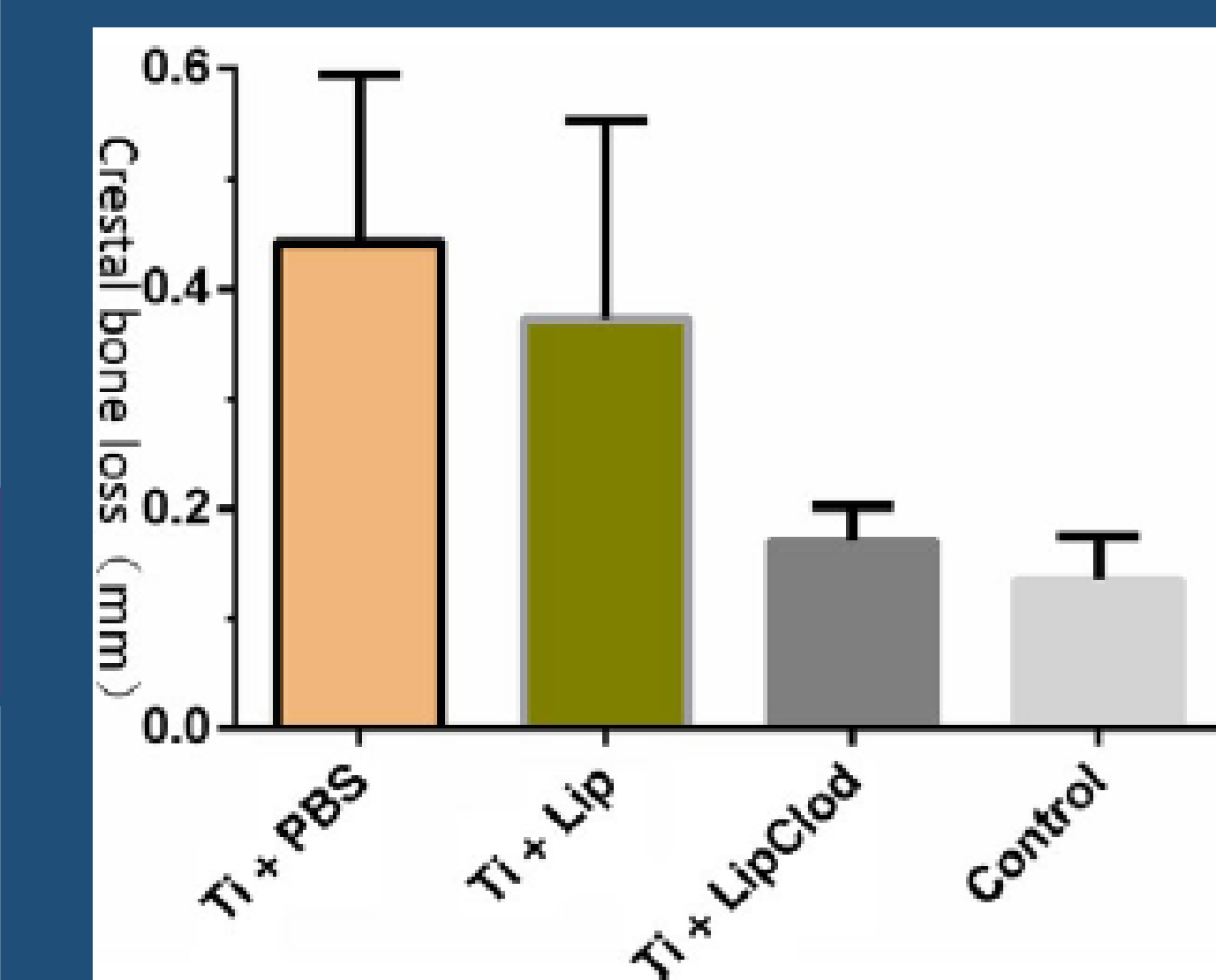


Figure 1. Average peri-implant crestal bone height changes as measured by the distance of the implant head to the alveolar bone crest on the mesial, distal, buccal, and palatal aspect of the fixtures

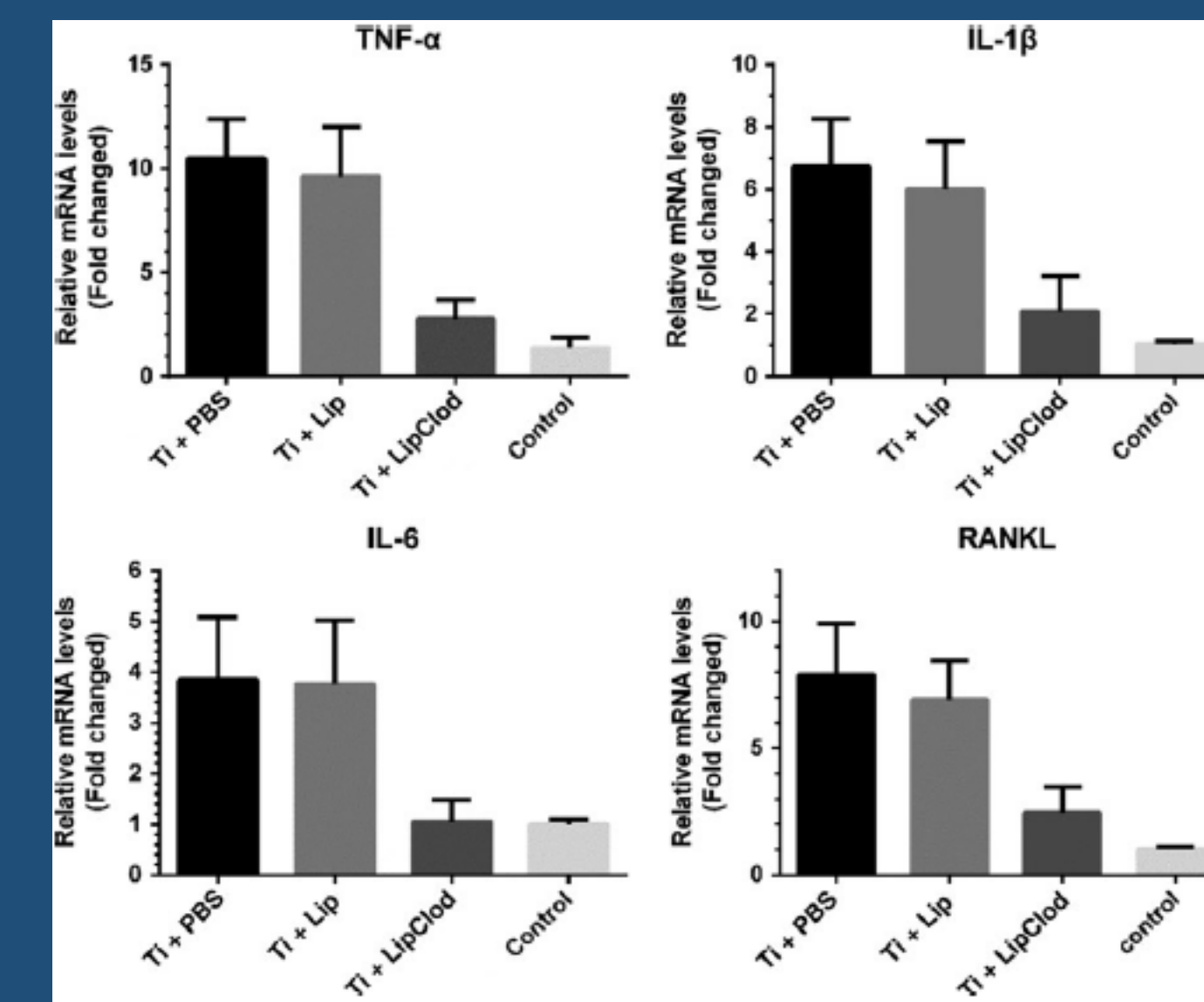


Figure 4. The mRNA expression levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and RANKL were presented as fold changes using real-time PCR

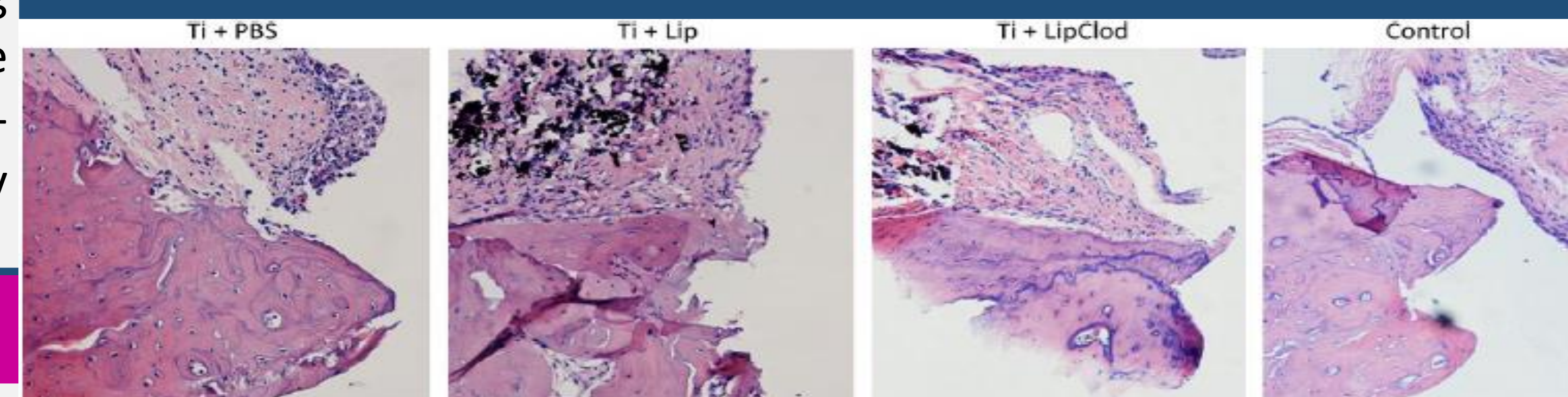


Figure 2. Representative images of hematoxylin and eosin stained sections 8 wk after treatment, 100 $\times$  magnification. Notice the presence of a dense mixed inflammatory cell infiltrate in the Ti + PBS and Ti + Lip groups as compared to the Control and Ti + LipClod groups

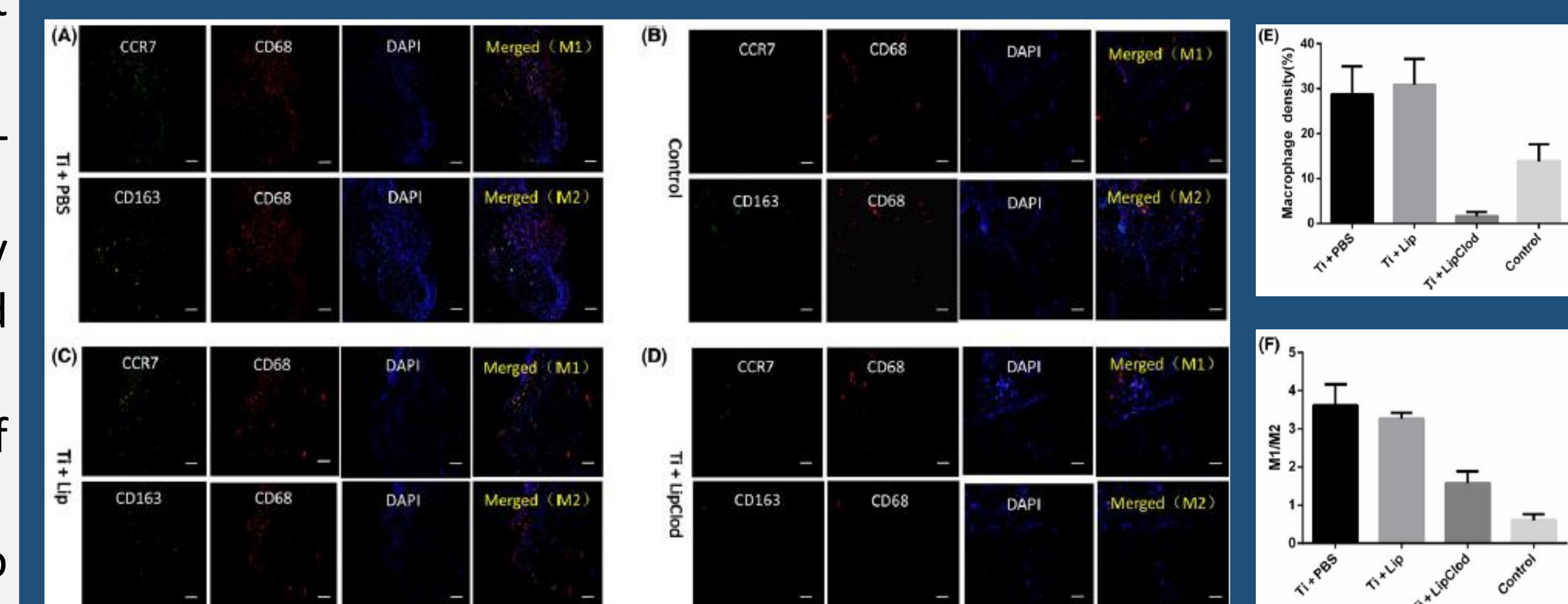


Figure 3. Altered M1 and M2 macrophage infiltrations. A and B, immunofluorescent images showed increased infiltration of CD68 + CCR7 + M1 macrophages and decreased infiltration of CD163 + M2 macrophages in the Ti + PBS and Ti + Lip groups. (n = 4). Scale bars, 50  $\mu$ m. C, macrophages infiltrated in the Ti + LipClod group was decreased and presented as M1 phenotype. D, CD163 + M2 macrophages E, quantification of total CD68 + macrophages in 3 randomly selected HPFs of four groups. F, quantification of the M1/M2 ratio in different groups

## Results

- No significant difference was found on average peri-implant bone loss between Ti + LipClod group and Control group. (Figure 1).
- Histological observation revealed that bone absorption was found around the neck of implants in the Ti + PBS group and the Ti + Lip group (Figure 2).
- No bacterial contamination found in titanium-injected areas, and the implant survival rate was 100% with no implant loss; macrophage density ( $1.64 \pm 0.86\%$ ) infiltrated into peri-implant tissue and bone loss ( $0.17 \pm 0.03$  mm) around implant decreased significantly in the Ti + LipClod group.
- Immunofluorescence - More macrophage infiltrated into peri-implant tissue in the Ti + PBS and Ti + Lip groups, predominantly with M1 phenotype. Macrophage density was lower and M2 phenotype was dominant in the Control group.
- Macrophage density was significantly reduced and the M1 type macrophages were slightly more than M2 type in the Ti + LipClod group (Figure 3).
- TNF- $\alpha$ , IL-1 $\beta$ , IL6, and RANKL mRNA expression increased significantly in the Ti + PBS and Ti + Lip groups compared with Control and Ti + LipClod groups (Figure 4).

## Conclusion

Titanium particles had a negative effect on peri-implant tissue by activating macrophages which induced an M1 macrophage phenotype promoting local secretion of inflammatory cytokines. It was found that clodronate liposome treatment attenuated the severity of inflammation and bone loss by depletion of macrophages.