Cellular responses to particles of calcium polyphosphate composite scaffolds both in vitro and in vivo

[1]Song, W, [1]Jawad, A, [2]Markel, D, [2]Jackson, N, and [1,2]Ren, W.*
[1]Wayne State University Biomedical Engineering, Detroit, [2]Department of Orthopaedics, Providence Hospital and Medical Centers, Southfield, MI

INTRODUCTION:

Wear particles generated from implants are phagocytosed by tissue macrophages, leading to their activation. These activated macrophages play a critical role in the pathology of aseptic loosenging. Calcium polyphosphate (CPP) bioceramic scaffolds are biocompatible and biodegradable. It is unclear whether wear particles generated from CPP scaffolds initiate the similar tissue inflammation as observed in various types of wear particles (metal, cement or polyethylene). The purpose of this study was to determine the cellular response to CPP particles both in vitro and in vivo using a mouse air pouch model developed in our laboratory.

MATERIALS AND METHODS:

Particles preparation: Crystalized CPP (CCPP) particles were synthesized as described elsewhere[1]. Amorphous CPP –CCPP-Tetracalcium phosphate (ACPP-CCPP-TTCP) particles were prepared via a self-setting approach. In brief, ACPP colloids were mixed with CCPP in 1:1 ratio (w/w).TTCP powder was added to the mixture to initiate the setting of the paste. After curing for overnight, the hardening ceramics was ground to pre-designated size. All particles size: 2~3 μm.

In vitro study Murine macrophage RAW264.7 cells were cultured at 1×10^5 /well in 12-well plate for 24h, and then 100 μL of particle suspension (10 μg/mL) were added, respectively. Cells treated with PMMA (10 μg/mL) were included as control. The production of nitrite (NO_2^-) was measured by determining NO_2^- in the culture supernatants using the colorimetric Griess reaction. 5 μg/mL of LPS was added to cell culture medium followed by the particle treatment. Cell toxicity was determined by measuring both the release of LDH from medium and cell lysate.

In vivo study: A mouse air pouch model was used to examine the tissue response to injected particles (10 mg/mL) in 0.5 mL PBS. Pouch tissue were harvested after two weeks and conducted to histology analysis.

RESULTS:

Fig.1 shows CCPP and ACPP-CCPP-TTCP particles induced no inflammation in mouse pouch tissue compared to control group. Quantitative histology analysis showed no significant difference in pouch membrane thickness and infiltrated cells density compared to the control.

Fig.2 shows semi-quantitative immunostaining for F4/80 and TNF-α, mouse tissue macrophage-specific markers presents positive staining in UHMWPE-stimulated pouches, compared with PBS control pouch membranes. However, both staining was significantly diminished in CCPP and ACPP-CCPP-TTCP particles treated tissue.

Fig.3 shows that PMMA particles significantly increase the cellular apoptosis and NO_2^- production, whereas CCPP and ACPP-CCPP-TTCP particles were not.

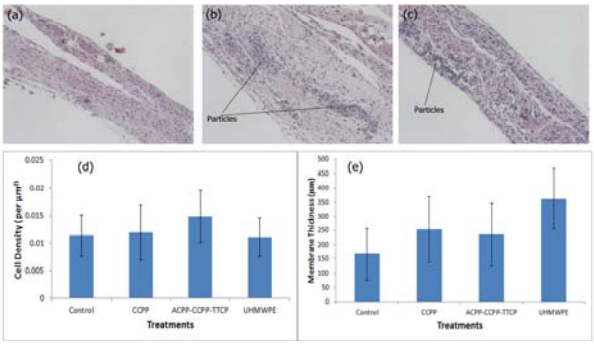

Fig.1 Representative pouch tissues histology of microscopic appearance. All the main micrographs are tissue sections stained with hematoxylin and eosin (H&E). (Original magnification, x100.) Treatments: (a) Control (PBS); (b) CPP; (c) ACPP-CCPP-TTCP.

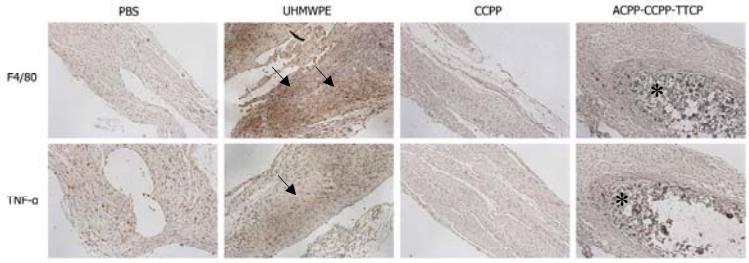

Fig.2 F4/80 and TNF-α expression in mouse pouch membranes. Upper Panel: F4/80 and Lower panel: TNF- α expression in mouse pouch membranes. Arrow indicates positive staining and star indicates particles.

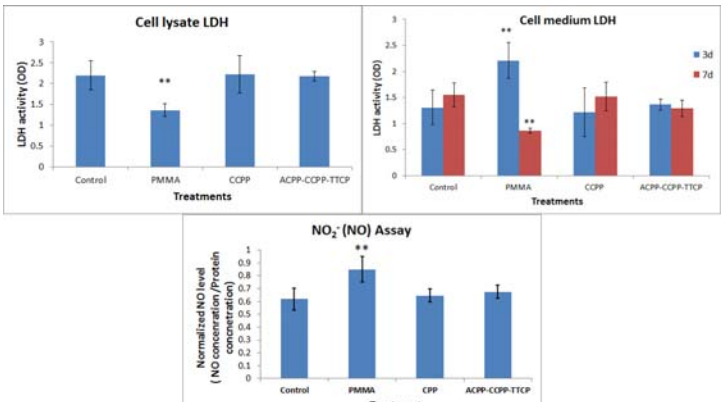

Fig.3 RAW cells LDH activity level in Cell lysate and medium and NO_2^- production in vitro. n=6, $^{**}p<0.005$.

CONCLUSION:

In this study, we demonstrated that CCPP and ACPP-CCPP-TTCP particles in similar size with PMMA and UHMWPE significantly reduced tissue inflammation both in vitro and in vivo.

REFERENCE

1. Song W, Tian M, Chen F, Tian Y, Wan C, Yu X. J Biomed Mater Res B Appl Biomater 2009; 89B(2):430-438