Reduced inflammatory responses of Poly(lactide-co-glycolide) by incorporating hydroxybenzyl alcohol releasing copolyoxalate

Dongwon Lee, Yujeong Lee, Seounyeoung Jeong, Donghyun Hong, Gilson Khang
Dept. of BIN Fusion Technology and Dept. of Polymer·Nano Science and Technology, Chonbuk National University, Korea

Statement of Purpose: *p*-Hydroxybenzyl alcohol (HBA) is one of phenolic compounds in herbal agents and plays a pivotal role in protection against oxidative damage-related diseases due to anti-inflammatory effects. We have developed fully biodegradable HBA-incorporated copolyoxalate (HPOX), in which HBA was chemically incorporated into its backbone. HPOX readily degraded hydrolytically to release HBA which exerts potent antioxidant and anti-inflammatory activities. PLGA has been widely used for tissue engineering application, in various formations because of its biodegradation and biocompatibility. However, its applications have been impeded by the acidic degradation products which can lead to reduction in pH values in the vicinity of the scaffolds and cause inflammation. We hypothesized that blending PLGA with antioxidant and anti-inflammatory HPOX would reduce inflammatory responses caused by PLGA implants *in vivo*. Here, we report the physico-chemical properties and inflammatory responses of PLGA/HPOX blend *in vitro* as well as *in vivo*.

Materials and Methods: HPOX was prepared as previously reported. Blend films were prepared using a solvent evaporation method. PLGA (210 mg) was dissolved in 5 mL of DCM (dichloromethane) to which 90 mg of HPOX and 5 mg of Poloxamer were added. The solution was thoroughly mixed and poured into a glass dish (30 mm in diameter), followed by drying at room temperature to cast a film. Films were further dried in a vacuum oven for 1 week and sterilized using 70% ethanol for 2 h. The mechanical properties of PLGA/HPOX blend films were determined using a tensile tester (LRX plus, Lloyd Instrument, UK) according to D412 specifications. The release kinetics of HBA from PLGA/HPOX blend films was studied using a high performance liquid chromatography system (Futecs, Korea), equipped with a P1000 solvent pump unit and an UV1000 UV–visible detector operating at 270 nm. 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) assay was performed to test the cytotoxicity of PLGA/HPOX blend films using RAW 264.7 macrophage cells. ELISA (Enzyme-Linked Immunosorbent assay) was performed to evaluate the level of inflammatory responses and analyze the concentration of inflammation-mediating cytokine TNF-α. The level of intracellular reactive oxygen species (ROS) in macrophages cultured on PLGA/HPOX blends was measured using a non-fluorescent probe DCFH-DA (dichlorodihydrofluorescein diacetate). In order to study the inflammatory responses *in vivo*, PLGA/HPOX blend films were implanted into a rat for 2 weeks and tissues surrounding the implants were extracted and observed by immunohistological staining (H&E and ED-1).

Results: PLGA/HPOX showed a less tensile strength than PLGA films, but they exhibited mechanical strength suitable for cell culture and tissue engineering applications. HPOX in the blend films degraded hydrolytically and HBA was released completely within 7 days under physiological conditions. The incorporation of HPOX into PLGA showed no adverse effects on cell attachment, adhesion and proliferation. PLGA/HPOX blend films elicited a significantly lower level of TNF-α and ROS generation compared to pure PLGA films. In addition, PLGA/HPOX blend films exhibited a less inflammatory cell accumulation than pure PLGA films because of anti-inflammatory HBA released.

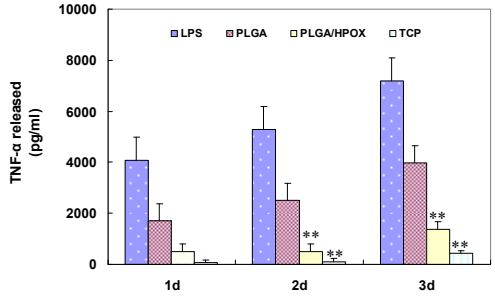

Fig. 1. TNF-α production by macrophages cultured on PLGA films or PLGA/HPOX blend films (n=3, ** $p<0.01$, relative to PLGA).

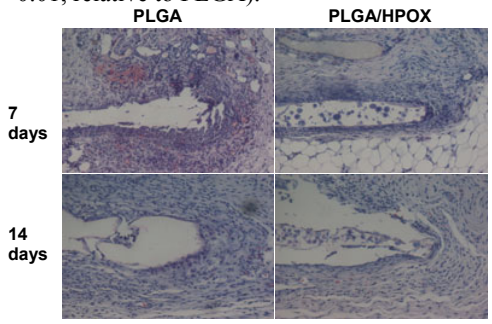

Fig. 2. H&E staining of tissues surrounding PLGA and PLGA/HPOX blend implants.

Conclusions: Anti-inflammatory HBA was released from the PLGA/HPOX blend during hydrolytic degradation. PLGA/HPOX blend implant showed the very few accumulated inflammatory cells and very thin fibrous capsule at the interface with tissues, demonstrating remarkably reduced inflammatory responses. PLGA/HPOX blends appear to be a promising approach for tissue engineering applications due to excellent biocompatibility and anti-inflammatory activities.

References:

1. H. Park et al, *Biomacromolecules*, 2010, 11, 2103-2108.
2. E. Lim, *J. Pharma. Pharmacol*, 2007, 59, 1235-1240.