**Anti-inflammatory biocompatible dexamethasone-loaded porous microparticles for acute lung injury**

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**Statement of Purpose:** Reactive oxygen species (ROS) are a collective term of very small reactive molecules that include a variety of free oxygen radicals (superoxide anion, nitrite and hydroxy radicals) and oxygen derivatives such as hydrogen peroxide ($H_2O_2$), peroxy-nitrite and ozone. ROS are an essential physiological regulator and serve as an important biological messenger in cell signal transduction cascades. However, the over-expression of ROS leads to oxidative stress, resulting in significant damage to cell structure. Vanillyl alcohol (VA) is the active ingredient in Gastrodia elata blume and mitigates the increasing of ROS level. Although it has potent anti-inflammatory activities, the use of VA in clinical applications is limited by poor stability and lack of specificity toward injured tissue. In this study, We have developed fully biodegradable and antioxidant vanillyl alcohol-incorporated copolyoxalte (PVAX) that chemi-cally incorporates VA and peroxalte linkage in its backbone. We incorporated Dexamethasone (DEX) with porous microparticles. DEX is widely used to treat inflammatory disease such as acute lung injury. In inflammational environments, DEX-loaded porous PVAX microparticles showed that it reacted with $H_2O_2$ and hydrolyically degrade, leading to $H_2O_2$scavenging and release of therapeutic VA and DEX.

**Methods:** PVAX was synthesized. In brief, 1-4-cyclo-hexanedimethanol and 4-hydroxy-3-metoxy benzyl alcohol were dissolved in 10 mL of dry tetrahydrofuran (THF), under nitrogen, to which triethylamine was added dropwise at 4°C. Oxalyl chloride in 20mL of dry THF was added to the mixture dropwise at 4°C. The reaction was kept under nitrogen atmosphere at room temperature for 6h and polymer obtained through the extraction using dichloromethane and isolation by precipitating to cold hexane. DEX Porous PVAX micro-particles were prepared by water-in-oil-in-water double emulsion-solvent evaporation method using ammonium bicarbonate as a progen. The morphology and size of DEX-loaded porous PVAX microparticles were observed by a scanning electron microscopy (SEM) with accelerating voltage of 10Kv. MTT assay was performed to evaluate the cytotoxicity of PVAX microparticles on RAW264.7 cells. Also the H2O2 scavenging activity of PVAX microparticles were determined using Amplex Red assay according to the manufacturer's protocol. Animal studies were performed. Female BLAB/c mice were challenged with an intratracheal instillation of LPS. The challenge of LPS were injected with the Intraperitoneal instillation 1 h after the saline or particles challenge. Naïve control mice (without LPS) were injected with saline. The mouse TNF-α in lung tissues were measured by by RT-PCR. 24 h after administration, the animals were sacrificed. The lung tissues were stained with hematoxylin and eosine (H&E), TUNEL.

**Results:** As shown in figure1, PVAX was synthesized from a simple one-step reation of oxalyl chloride.

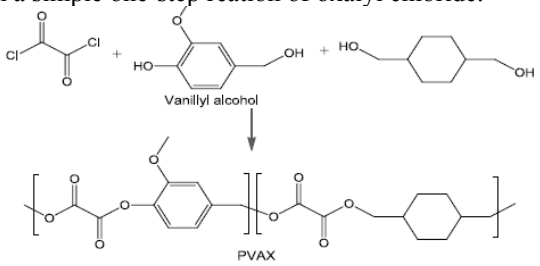


**Figure 1**. The synthetic route of vanilyl alcohol incorporateed copolyoxalate (PVAX).

DEX-loaded porous PVAX microparticles was prepared by double emulsion. PVAX microparticles were round sphere and found to have many surface pore. It has a mean diameter of 5~10μm. DEX-loaded porous PVAX microparticles appeared highly scavenging activity. This is explained peroxalate linkages in PVAX microparticles reacted to $H_2O_2$.

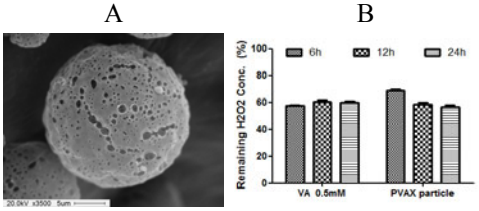


**Figure 2**. (a)Representive SEM image.
(b) Scavenging of $H_2O_2$ by porous PVAX microparticles.

Cell biocompatibility of PVAX microparticles represented low cytotoxicity such as PLGA microparticles We inquired the ability of DEX-loaded porous PVAX microparticles to limit LPS-mediated oxidative stress in lung tissues in mice, mearsured by RT-PCR. As a result, TNF-α expression of DEX-loaded porous PVAX micro-particles were smaller than only LPS administration mice. It can be suggest that DEX-loaded porous PVAX microparticles are suitable for pulmonary drug delivery. .

**Conclusions:** We synthesized PVAX which was designed to react with $H_2O_2$ and release antioxidant and anti-inflammatory vanillyl alcohol during its hydrolytic degradation. Dexamethasone-loaded porous PVAX microparticles included peroxalate linkage scavenged $H_2O_2$ and inhibited the generation of TNF-α. We anticipate that the administration of DEX-loaded PVAX porous microparticles are useful in the treatment of pulmonary inflammatory diseases.