# Bioimaging and Pulmonary Applications of Flt1 peptide – Hyaluronate Conjugate Nanoparticles

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**Statement of Purpose:** Asthma is one of the chronic inflammatory immune diseases with reversible airway obstruction and airway hyper-responsiveness. Although symptoms of asthma can usually be relieved with corticosteroids for eosinophilic inflammation, the poor therapeutic effect on a neutrophilic subtype of asthma prohibits the full recovery of asthma patients [1]. In this work, dexamethasone (Dexa) was loaded in Flt1 peptide – hyaluronate (HA) conjugate nanoparticles (NPs) for the treatment of both eosinophilic and neutrophilic pulmonary inflammation. VEGF receptor 1 (VEGFR1, Flt1) is important for Th1 and Th17 cell responses to inhaled allergens in neutrophilic asthma model mice [2]. In addition, HA can prolong the residence time of NPs and remain close to the main absorption sites in the deep lung due to its mucoadhesive property [3]. We evaluated the pulmonary delivery characteristics and therapeutic effect of the NPs on asthma with *ex vivo* bioimaging study.

**Methods:** Flt1 peptide – HA conjugates were synthesized using benzotriazol-1-yloxy-tris(dimethylamino)phosphon--ium hexafluorophosphate (BOP) chemistry. After confocal imaging for *in vitro* receptor-mediated endocytosis, biodistribution of the HiLyte 647-Labeled NPs encapsulating CFSE instilled via orotracheal route was investigated by *ex vivo* fluorescence analysis. Dexa loaded Flt1 peptide – HA NPs were prepared by ultrasonication followed by dialysis against distilled water. The formation of NPs was assessed by transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential analysis, and high performance liquid chromatography (HPLC). The therapeutic efficacy of Dexa/Flt1 peptide – HA NPs has been evaluated and discussed in model mice of eosinophilic asthma induced by allergens plus aluminum hydroxide (Alum) and neutrophilic asthma induced by LPS-containing allergens.

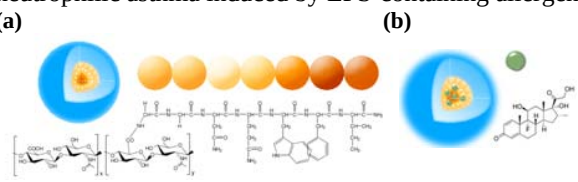


**Fig. 1**. Schematic illustration of (a) self-assembled Flt1 peptide – HA NP and its encapsulation of dexamethasone.

**Results:** The successful conjugation of Flt1 peptide to HA was confirmed by $^{1}$H NMR and GPC analyses as we reported elsewhere [4]. *In vitro* confocal imaging after treatment of the NPs in A549 cells showed efficient internalization of Flt1 peptide – HA nanoparticles into lung epithelial cells by HA-receptor mediated endocytosis (data are not shown). Also, *ex vivo* imaging for the biodistribution in ICR mice revealed long-term retention of Flt1 peptide – HA NPs in deep lung tissues possibly due to mucoadhesive property of HA (Fig. 2).

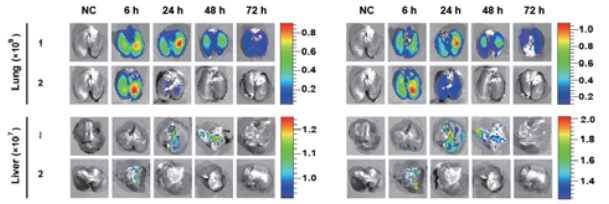


**Fig. 2**. Biodistribution of CFSE/Flt1 peptide – HA – HiLyte 647 NPs (row 1) and dye mixtures (row 2) under (a) a red filter for HiLyte 647 and (b) a green filter for CFSE after orotracheal instillation.

On the basis of bioimaging results for pulmonary drug delivery applications, we prepared Dexa/Flt1 peptide – HA NPs. TEM images confirmed the formation of nanoparticles and encapsulated amount of Dexa was quantified by HPLC analysis (Fig. 3). According to the bronchoalveolar lavage (BAL) cellularity and histological analysis (histology data are not shown), Dexa/Flt1 peptide – HA NPs showed remarkable therapeutic effect in both eosinophilic and neutrophilic asthma model mice (Fig. 4).

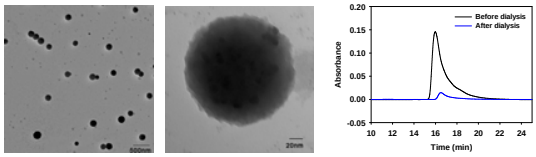


**Fig. 3**. (a) TEM images and (b) HPLC chromatograms of Dexa/Flt1 peptide – HA NPs.

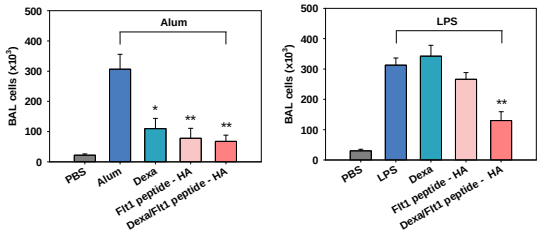


**Fig. 4**. BAL cellularity after treatment of the NPs in (a) eosinophilic and (b) neutrophilic asthma model.

**Conclusions:** We successfully developed Flt1 peptide – HA NPs for pulmonary drug delivery applications. The NPs were effectively delivered and up-taken to the lung tissues by HA receptor mediated endocytosis with a prolonged residence time. Furthermore, Dexa/Flt1 peptide – HA NP showed remarkable therapeutic effects on both eosinophilic and neutrophilic subtypes of asthma.

## References

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