Recognition, Neutralization, and Clearance of Target Peptides in the Bloodstream of Living Mice by Molecularly Imprinted Polymer Nanoparticles: A Plastic Antibody

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Recognition, neutralization and clearance of target peptides in the blood stream of living mice by molecular imprinted polymer nanoparticles: a plastic antibody

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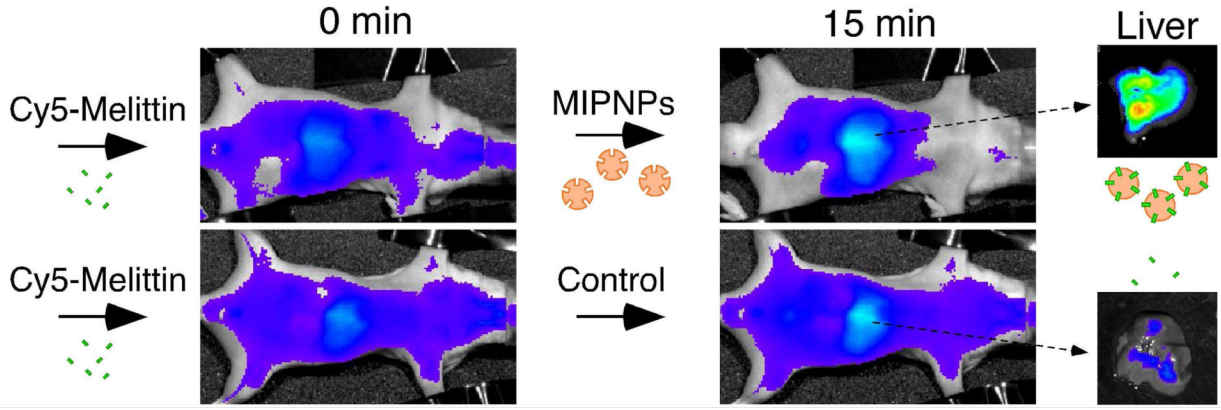
Abstract



We report that simple, synthetic organic polymer nanoparticles (NPs) can capture and clear a target peptide toxin in the bloodstream of living mice. The protein-size polymer nanoparticles, with a binding affinity and selectivity comparable to natural antibodies, were prepared by combining a functional monomer optimization strategy with molecular imprinting nanoparticle synthesis. As a result of binding and removal of melittin by NPs *in vivo*, mortality and peripheral toxic symptoms of melittin were significantly diminished. *In vivo* imaging of the polymer nanoparticles or “plastic antibodies” establishes the NPs accelerate clearance of the peptide from blood where they accumulate in the liver. Coupled with their biocompatibility and nontoxic characteristics, plastic antibodies offer potential for neutralizing a wide range of biomacromolecules *in vivo*.

In nature, antibodies recognize target molecules by a combination of multiple weak electrostatic, hydrophobic and hydrogen bonding interactions between complementary three-dimensional surfaces. To mimic these interactions, nanoparticles (NPs) with affinity for a target peptide or protein have been synthesized by optimizing the composition and ratio of functional groups that make up the NPs.1,2 However, the specificity and affinity of the random

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Supporting Information Available: Experimental procedures and supporting data. This material is available free of charge via the Internet at http://pubs.acs.org.

copolymers are not as effective as antibodies. It is known however that by polymerizing monomers in the presence of the molecular target or a fragment thereof, collective weak interactions between monomers and target during polymerization results in formation of populations of complementary binding sites in the resulting polymer.3 This molecular imprinting (MIP) approach has been extended from bulk materials to nanofibers4, nanoparticles5 and dendrimers6. The majority of the imprinting effort has targeted small organic molecules but more recently, biologically relevant molecules that include peptides7 and proteins[8] have appeared.

We have developed methods for synthesizing protein-size polymer particles with a binding affinity and selectivity comparable to natural antibodies by combining molecular imprinting nanoparticle synthesis with a functional monomer optimization strategy (Figure 1).9 The first stage of this process involves screening small libraries of NPs that span a compositional space chosen for its complementarity to the biological target.[2] The affinity of each NP to the biological target is evaluated and the composition of subsequent NP generations is adjusted to enhance specificity. At the final stage the optimized combination and ratio of functional monomers are polymerized in the presence of the imprinting biological target (peptide or epitope).[9] Following extensive dialysis, polymer NPs exhibit binding affinity, selectivity and particle size comparable to natural antibodies *in vitro*.

Although molecular recognition by imprinted materials has been extensively studied in controlled settings, little is reported about their application in the bloodstream of living animals. [10] It is well known that the performance (affinity, specificity and function) of synthetic materials when introduced into a complex biological milieu can be profoundly compromised. Introduction of foreign substances including synthetic NPs into the bloodstream results in the immediate formation of a “corona” of proteins on the surface that can alter and/or suppress the intended function of the NP.[11] Further complications can arise fron an immunogenic response to the foreign material.12 In this study, we describe the *in vivo* application of molecularly imprinted polymer NPs with designed affinity for melittin (K_{dapp} < nM, Figure 1, Supporting Information), a cytolytic peptide that is the principle component of bee venom.[9,13]

Prior to the evaluation of NP efficacy *in vivo* we tested their biocompatibility. The optimized melittin imprinted NPs (MIPNPs)[9] were found to be non-toxic to cultured cells in vitro (fibrosarcoma cells) within the tested concentration (3 to 3,000 μg ml^{-1}, Supporting Figure 2). The MIPNPs (30 mg kg-1) were then injected intravenously into the bloodstream of mice. Over a period of 2 weeks, there was no significant difference in body weight between groups administered NPs and control mice. Furthermore, no detectable toxicity was observed histopathologically in tissue samples from the liver, lung and kidney 2 weeks after injection (Supporting Figure 3).

At a high dose, melittin induces cell lyses *in vivo*, which eventually results in death due to renal failure or cardiac complications.[14] The ability of MIPNPs to neutralize melittin’s toxicity was tested *in vivo* by systemic administration following injection of the toxin. Mice were injected intravenously with melittin followed by intravascular injection of MIPNPs or NIPNPs (polymer NPs with the identical composition but synthesized in the absence of the imprint molecule melittin9). The controls did not receive the injection of MIPNPs or NIPNPs. A 100 percent mortality rate was observed in mice that were intravenously administered melittin at a dose of 4.5 mg kg^{-1} (Figure 2a). Upon intravenous infusion of MIPNPs (30 mg kg^{-1}) 20 second after 4.5 mg kg^{-1} of melittin, a significant decrease in mortality was observed (p = 0.030). In contrast, NIPNPs did not significantly neutralize melittin *in vivo* (p = 0.207). This indicates that while in the bloodstream, imprinted NPs recognized the specific toxin melittin and neutralized its activity. In addition to the reduced mortality, peritoneal inflammation (p =

0.004, Figure 2b and Supporting Figure 4) and weight loss (p = 0.005, Figure 2c) caused by melittin were also significantly alleviated by systemic administration of MIPNPs.

To observe distribution of melittin and NPs in mice, melittin was labeled with the fluorescent dye (Cy-5) at the ε amine of an additional lysine on the *N*-terminal and MIPNPs were labeled with radioisotope (^{14}C) or the fluorescent dye (fluorescein) by co-polymerization MIPNPs with ^{14}C enriched acrylamide [1-^{14}C] (5 mol%) or fluorescein *o*-acrylate (1 mol%). *In vivo* fluorescent imaging of Cy5-melittin revealed that the biodistribution of melittin was significantly altered by post administration of MIPNPs in living mice–the fluorescent intensity of Cy5-melittin diminished immediately after administration of MIPNPs (Figure 3a). *Ex vivo* results showed that Cy5-melittin accumulated in the liver with a dose dependence on the amount of MIPNPs administrated (Figure 3b,c). Radioactivity analysis of each organ also showed that the NPs accumulated mainly in the liver (Figure 3d). Furthermore, fluorescent images of histological sections of a liver observed by confocal microscope showed that both MIPNPs (labeled by fluorescein) and Cy5-melittin were captured together in the same cells (macrophages) 10 min after injection of melittin and the NPs (Figure 3e).

From the preceding results, we conclude that imprinted polymer nanoparticles efficiently capture the cytotoxic peptide melittin in the bloodstream. The strong and specific affinity of the imprinted NPs enabled the rapid sequestration of the target peptide in the biological milieu. The melittin·MIPNP complexes are then cleared from the blood by the mononuclear phagocytic system in the liver.[11] As a result of binding and removal of melittin by MIPNPs *in vivo*, mortality and peripheral toxic symptoms of melittin were significantly diminished (Supporting Figure 5). These results establish for the first time that a simple, non-biological synthetic nanoparticle with antibody-like affinity and selectivity; a plastic antibody, can effectively function in the bloodstream of living animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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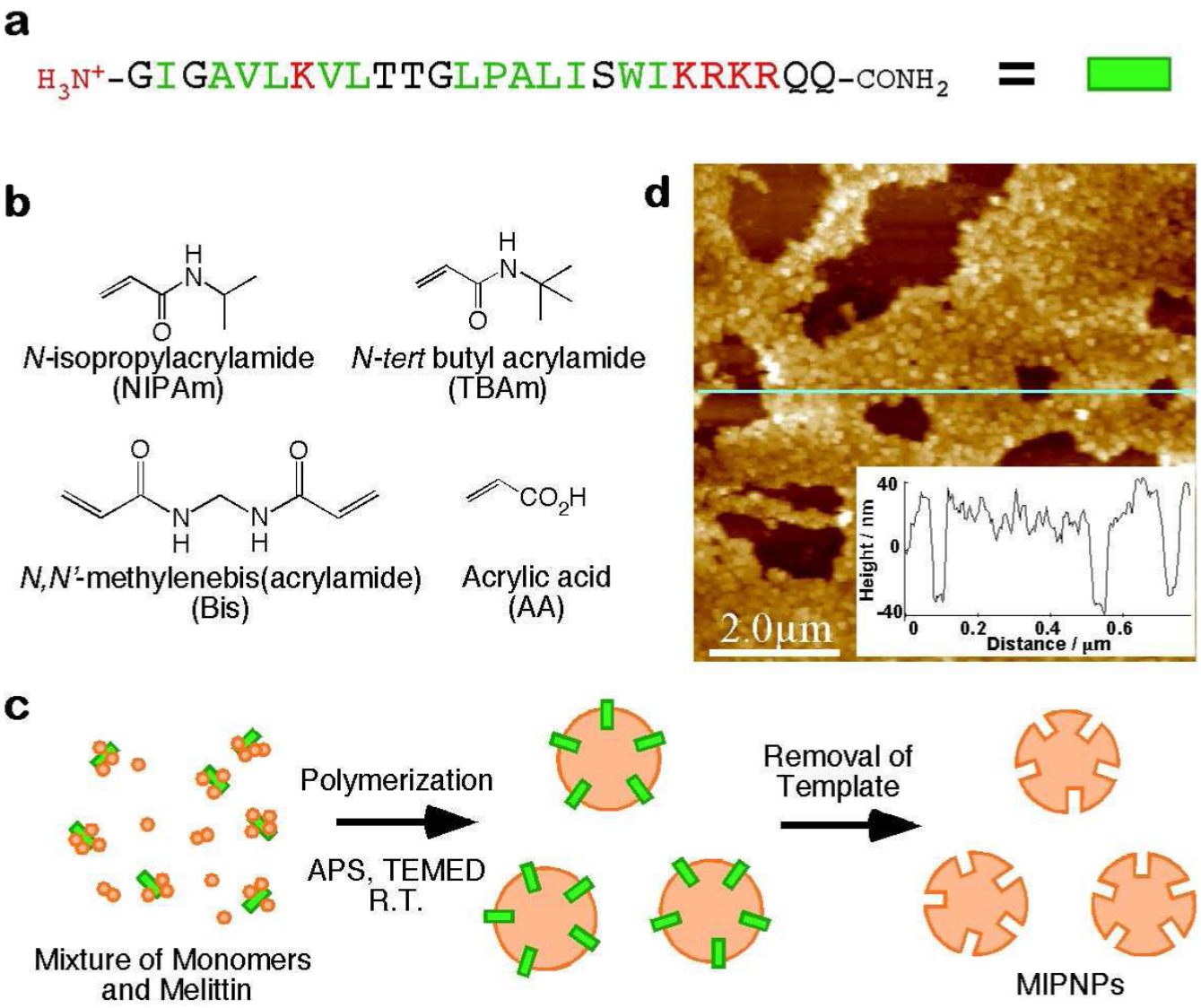

Figure 1.

Preparation and characterization of polymer NPs. **a.** Amino acid sequence of target peptide Melittin. Hydrophobic, positive charged and hydrophilic residues are rendered in green, red and black respectively. **b.** Monomers used for NP synthesis. **c.** Schematic of the preparation of MIPNPs. **d.** Solution phase AFM images of MIPNPs. A height profile of cross-section (sky blue line) is shown in insert.

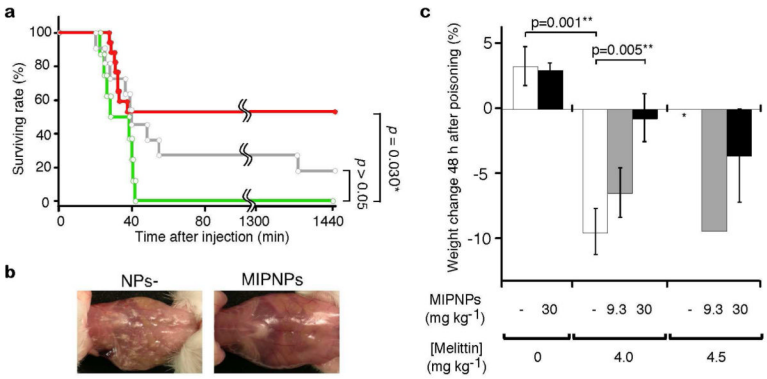

Figure 2.

Neutralization of melittin toxicity by NPs. **a.** Survival rates of mice over a 24 h period after intravenous injection of 4.5 mg kg^{-1} melittin (green). 30 mg kg^{-1} of MIPNPs (red), NIPNPs (gray) was systemically administrated via a tail vain 20 seconds after melittin injection. *P* values are calculated by the Willcoxon test. **b.** Macroscopic pathology of peritoneal inflammation of mice injected with melittin (4.0 mg kg^{-1}) followed without (**left**) or with (**right**) MIPNPs (30 mg kg^{-1}). **c.** Body weight change of mice injected with melittin (right two columns; 0 mg kg^{-1}, center three columns 4.0 mg kg^{-1}, right three columns 4.5 mg kg) followed with 0 mg kg^{-1} (white), 9.3 mg kg^{-1} (gray) or 30 mg kg^{-1} (black) MIPNPs (48 hours after melittin injection). * No animal was alive. The data represent the mean ± SEM.

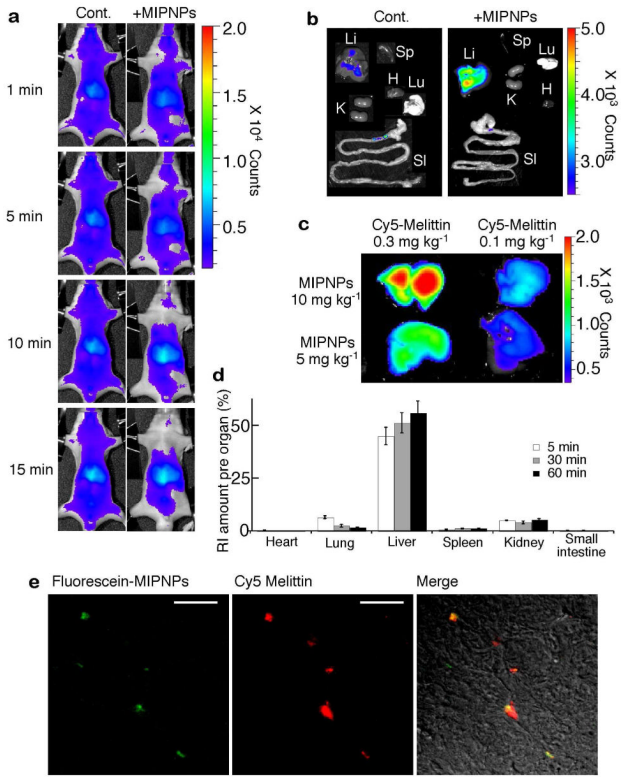

Figure 3.

Biodistribution of melittin and NPs. **a.** Fluorescent images of Cy5-melittin after intravenous injection of Cy5-melittin (1 mg kg^{-1}). 27 mg kg^{-1} of MIPNPs was injected 20 sec after the injection of melittin (**right**). **b.** Fluorescent *ex vivo* images of Cy5-melittin (0.3 mg kg^{-1}, 10 min after injection) of mice followed with and without 10 mg kg^{-1} MIPNPs. Li, Sp, SI, K, H and Lu indicate liver, spleen, small intestine, kidney, heart and lung respectively. **c.** Fluorescent images of Cy5-melittin (70 min after injection) in livers from mice with various doses of Cy5-melittin and MIPNPs. **d.** Biodistribution of ^{14}C-labeled NPs in a mouse (n = 5 or 4, 10 mg kg^{-1}). **e.** Fluorescence histology images of the liver shown in **c** (Cy5-melittin 0.3 mg kg^{-1} and 10 mg kg^{-1} of MIPNPs). Green; Cy5-melittin, red; fluoroscein-MIPNPs. The scale bars; 25 μm.