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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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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 copolymers are not as high as those of antibodies. It is known, however, that when monomers are polymerized in the presence of the molecular target or a fragment thereof, collective weak interactions between the monomers and the target during polymerization result in the formation of populations of complementary binding sites in the resulting polymer.[3] This molecular imprinting (MIP) approach has been extended from bulk materials to nanofibers,[4] nanoparticles,[5] and dendrimers.[6] The majority of the imprinting effort has targeted small organic molecules, but more recently, biologically relevant molecules, including peptides[7] and proteins,[8] have been employed.

We have developed methods for synthesizing protein-sized polymer particles with a binding affinity and selectivity comparable to those of natural antibodies by combining MIP 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 for the biological target is evaluated, and the composition of subsequent NP generations is adjusted to enhance the specificity. In 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, the polymer NPs exhibit a binding affinity, selectivity, and particle size comparable to those of natural antibodies in vitro.

Although molecular recognition by imprinted materials has been extensively studied in controlled settings, little has been 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 can be profoundly compromised upon introduction into a complex biological milieu. 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 NPs.[11] Further complications can arise from 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 (K_{dapp} < 1 nM) for melittin (Figure 1a), a cytolytic peptide that is the principal component of bee venom.[9,13]

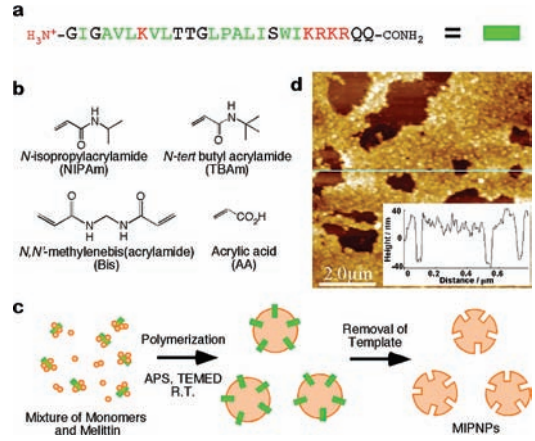

Figure 1. Preparation and characterization of polymer NPs. (a) Amino acid sequence of the target peptide, melittin. Hydrophobic, positively charged, and hydrophilic residues are rendered in green, red, and black, respectively. (b) Monomers used for NP synthesis. (c) Schematic of the preparation of MIPNPs. (d) Solution-phase AFM images of MIPNPs. A height profile of the cross section (sky-blue line) is shown in the inset.

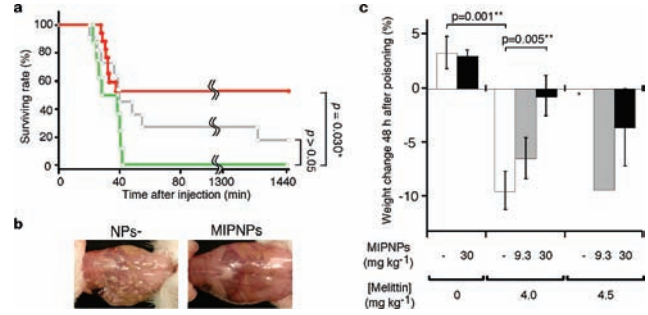

Figure 2. Neutralization of melittin toxicity by NPs. (a) Survival rates of mice over a 24 h period after intravenous injection of 4.5 mg kg^{-1} melittin (green); 30 mg kg^{-1} MIPNPs (red) or NIPNPs (gray) was systemically administered via a tail vain 20 s after melittin injection. Values of p were calculated using the Willcoxon test. (b) Macroscopic pathology of peritoneal inflammation of mice injected with melittin (4.0 mg kg^{-1}) followed with (left) nothing or (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^{-1}) followed with (white) 0, (gray) 9.3, or (black) 30 mg kg^{-1} MIPNPs (48 h after melittin injection). * indicates that no animal was alive. The data represent the mean ± standard error of the mean.

Prior to the evaluation of NP efficacy in vivo, we tested the biocompatibility of the NPs. The optimized melittin-imprinted NPs (MIPNPs)[9] were found to be nontoxic to cultured cells (fibrosarcoma cells) in vitro over the tested concentration range (3−3000 μg mL^{-1}; Supporting

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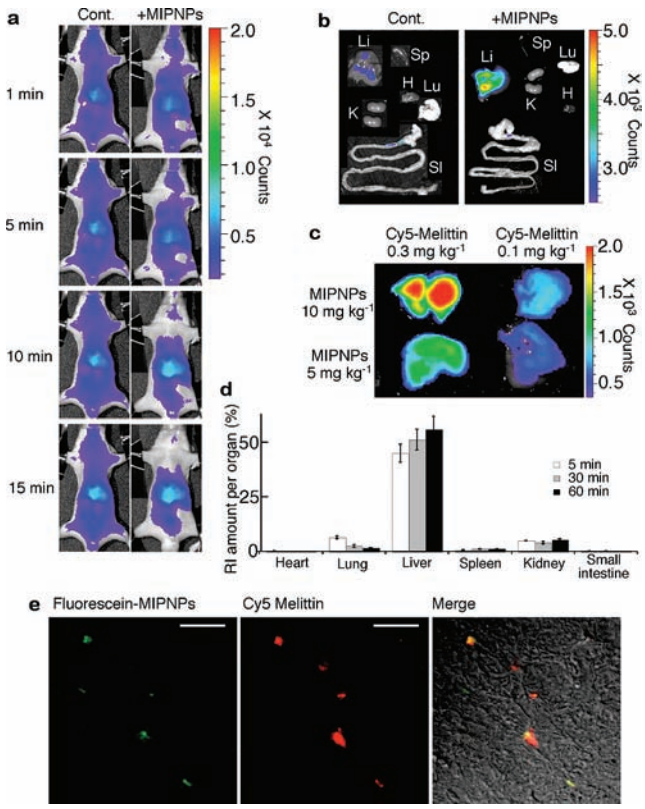

Figure 3. Biodistribution of melittin and NPs. (a) Fluorescent images of Cy5-melittin after intravenous injection of Cy5-melittin (1 mg kg^{-1}); 27 mg kg^{-1} MIPNPs was injected 20 s after the injection of melittin (right). (b) Fluorescent ex vivo images of Cy5-melittin (0.3 mg kg^{-1}, 10 min after injection) of mice followed with (left) nothing and (right) 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}; MIPNPs, 10 mg kg^{-1}). Green, Cy5-melittin; red, fluoroscein-MIPNPs. Scale bars: 25 μm.

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, or kidney 2 weeks after injection (Supporting Figure 3).

At a high dose, melittin induces cell lysis 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 and then intravenously with MIPNPs or NIPNPs (polymer NPs with the identical composition but synthesized in the absence of the imprint molecule melittin[9]). The controls did not receive the injection of MIPNPs or NIPNPs. A 100% 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 s 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 the distribution of melittin and NPs in mice, melittin was labeled with a fluorescent dye (Cy5) at the ε amine of an additional lysine on the N-terminal and MIPNPs were labeled with a radioisotope (^{14}C) or a fluorescent dye (fluorescein) by copolymerization with 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 postadministration 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 administered (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 microscopy showed that both MIPNPs (labeled with 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 were 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 due to melittin were significantly diminished (Supporting Figure 5). These results establish for the first time that a simple, nonbiological synthetic nanoparticle with antibody-like affinity and selectivity (i.e., a plastic antibody) can effectively function in the bloodstream of living animals.

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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.

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