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Enhanced immunostimulatory effects of DNA-encapsulated peptide hydrogels

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ABSTRACT

DNA that encodes tumor-specific antigens represents potential immunostimulatory agents. However, rapid enzymatic degradation and fragmentation of DNA during administration can result in limited vector expression and, consequently, poor efficacy. These challenges have necessitated the use of novel strategies for DNA delivery. Herein, we study the ability of cationic self-assembling peptide hydrogels to encapsulate plasmid DNA, and enhance its immunostimulatory potential *in vivo*. The effect of network charge on the gel's ability to retain the DNA was assessed employing three gel-forming peptides that vary systematically in formal charge. The peptide HLT2, having a formal charge of +5 at neutral pH, was optimal in encapsulating microgram quantities of DNA with little effect on its rheological properties, allowing its effective syringe delivery *in vivo*. The plasmid, DNA(TA), encapsulated within these gels encodes for a melanoma-specific gp100 antigen fused to the alarmin protein adjuvant HMGN1. Implantation of DNA(TA)-loaded HLT2 gels into mice resulted in an acute inflammatory response with the presence of polymorphonuclear cells, which was followed by infiltrating macrophages. These cellular infiltrates aid in the processing of encapsulated DNA, promoting increased lymphoproliferation and producing an enhanced immune response mediated by CD4+/IFNγ+ expressing Th1 cells, and complemented by the formation of gp100-specific antibodies.

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1. Introduction

DNA constructs, including plasmids and oligonucleotides, have been explored as potential therapeutics with applications in immunotherapy, vaccine platforms and gene therapy [1,2]. As an immunostimulatory agent, DNA vectors that encode for antigenic protein are capable of inducing antigen-specific antibodies through humoral mechanisms, and eliciting T cell responses, following their expression in cells [3,4]. Consequently, upon antigen expression, plasmids can serve as both effective vaccines and therapeutics, with potential to treat a variety of diseases [1,2]. However, systemic administration of naked DNA, even at high doses, often results in poor efficacy due to its susceptibility to enzymatic degradation, as well as DNA fragmentation as a consequence of shear stress applied during injection [5,6]. To enhance the efficacy of delivered DNA, and overcome these issues, techniques including “gene gun” administration [7], electroporation [2,6], and nanoparticle delivery [8,9], are currently being explored. While these methods offer some advantages, a significant disadvantage is that the delivered DNA does not stay localized to the tissue to which it is applied. Consequently, the vector can be rapidly cleared *in vivo* before sufficient expression of the antigen.

Alternatively, implantable soft hydrogel materials have the potential to encapsulate large quantities of DNA, and show prolonged retention of the vector at the site of injection, increasing the probability of a more sustained expression and, subsequently, an enhanced immune response [10–12]. In addition, the initial foreign body response, which is elicited by nearly every type of implanted material, results in significant inflammatory cell infiltration [13]. This creates a large local concentration of cells that can internalize and express the DNA. Expression by the host of cellular responders can provide a depot of antigenic protein for infiltrating antigen-presenting cells (APCs), such as macrophages, which are capable

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of processing the expressed protein and facilitating an adaptive immune response. Since the soft material is capable of being bioresorbed, the acute/chronic inflammation associated with the foreign body response will give way to subsequent tissue repair and remodeling. Such a material represents a traceless delivery vehicle for implanted DNA. The choice of gel composition to facilitate this approach is critical. Candidate materials are those that can directly encapsulate plasmid DNA and allow for its subsequent facile implantation *in vivo*. The gel must be capable of retaining the DNA locally within its network over a time period commensurate with the inflammatory response, while allowing for cellular uptake of the construct and its effective expression. Herein, we employ a class of hydrogels formed from self-assembling peptides to directly encapsulate and deliver plasmid DNA, Fig. 1A. We assessed the bioresportion profile of these DNA-loaded gels, and determine their immunostimulatory potential *in vivo*.

Table 1 shows the sequence of the parent gel-forming peptide, MAX1, which is comprised of two amphiphilic β-strands containing alternating hydrophobic and hydrophilic residues (lysine and valine) flanking a tetrapeptide type II′ β-turn [14–16]. In low temperature, low ionic strength aqueous solutions at pH 7.4, the peptide is freely soluble and remains unfolded due to side-chain electrostatic repulsions. A sol–gel phase transition can be triggered by increasing the ionic strength of the solution and warming to 37 °C. Increasing the ionic strength screens the lysine-borne charge of the peptide, and increasing the solution temperature facilitates the desolvation of hydrophobic residues. These environmental changes trigger the folding and assembly of the peptide into a nanofibrillar hydrogel network [16–20]. Importantly, the exterior of each fibril comprising the gel displays cationic lysine residues allowing for direct encapsulation of the anionic DNA when present during triggered hydrogelation.

Table 1
Sequence and formal charge of peptides utilized to prepare DNA-loaded hydrogels. Underlined residues represent design changes relative to the MAX1 sequence.

Peptide	Sequence	Net charge
MAX1	VKVKVKVKV^{D}PPTKVKVKVKV-NH_2	+9
MAX8	VKVKVKVKV^{D}PPTKV<u>E</u>VKVKV-NH_2	+7
HLT2	V<u>L</u><u>T</u>KVK<u>T</u>KV^{D}PPTKV<u>E</u>VKV<u>L</u>V-NH_2	+5

The primary sequence of MAX1 contains eight lysine residues, and along with its N-terminal amino group, is characterized by a formal charge of +9 at neutral pH. This electropositive character, although needed to sequester and retain the DNA in the network during delivery and during the initial foreign body response, may also inhibit dissociation of the peptide from the DNA, which ultimately must occur for the proper gene expression of the antigenic protein. In fact, earlier studies investigating the mass transport properties of similar hairpin peptide gels with respect to protein delivery showed that network charge significantly impacts the retention of protein within the gel [21,22]. To determine if network electrostatics play an important role in the encapsulation and delivery of highly charged DNA, we also studied the activity of two additional peptides that differ in their overall charge state. The peptide MAX8 incorporates a glutamate residue at position 15 instead of lysine reducing its formal charge to +7 [23]. The third peptide, HLT2 contains the same glutamate, but also replaces two additional lysine residues at positions 2 and 19 with leucine affording a peptide having a +5 formal charge [24]. HLT2 also contains two threonine residues, replacing two of MAX1's valine residues, to increase its hydrophilicity. The electropositive character of these three hydrogel networks vary systematically, which may influence not only their DNA binding and delivery aptitude, but also their bioresorptive properties.

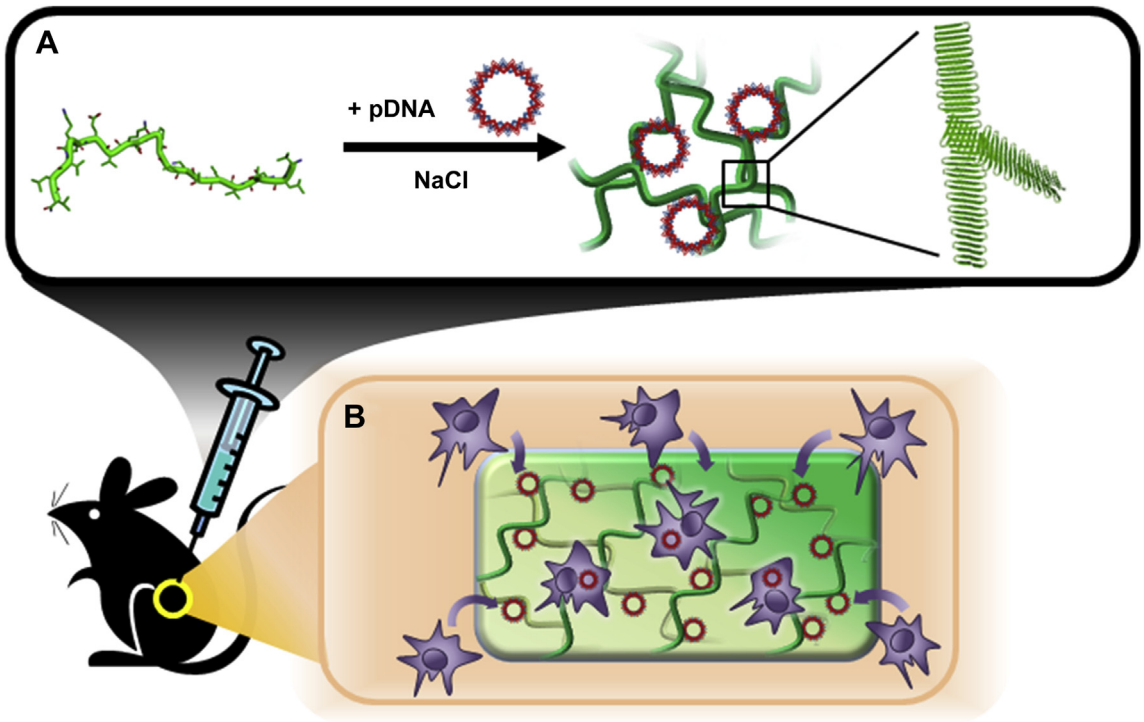

Fig. 1. DNA encapsulation into peptide hydrogels and the proposed delivery of the vector to infiltrating cells following subcutaneous injection. (**A**) Direct encapsulation of plasmid DNA into hydrogel networks composed of self-assembled β-hairpin peptide fibrils. (**B**) Shear-thin injection of DNA-loaded gels into the flanks of mice and subsequent infiltration of inflammatory cells. Internalization, transcription and translation of the encapsulated pDNA by various cell types produce the adjuvant-antigen fusion protein HMGN1-gp100 to be taken up by antigen-presenting cells.