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Controlled nanoparticle release from a hydrogel by DNA-mediated particle disaggregation

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ABSTRACT

For many pharmaceutical applications, it is important that different drugs are present in the human body at distinct time points. Typically, this is achieved by a sequential administration of different therapeutic agents. A much easier alternative would be to develop a drug delivery system containing a whole set of medically active compounds which are liberated in an orchestrated and controlled manner. Yet, such a controlled, sequential release of drugs from a carrier system that can be used in a physiological situation is difficult to achieve. Here, we combine two molecular mechanisms, i.e. a build-up of osmotic pressure by the depletion of a control molecule and triggered disaggregation of nanoparticle clusters by synthetic DNA sequences. With this approach, we gain spatio-temporal control over the release of molecules and nanoparticles from a gel environment. The strategy presented here has strong implications for developing complex drug delivery systems for wound healing applications or for the sustained release of pharmaceuticals from a drug-loaded gel and will lower the need for multiple drug administrations.

1. Introduction

To function smoothly, the human body relies on the spatio-temporal interplay of countless molecules. One example of a complex biological process requiring the orchestrated action of multiple molecules is wound healing: Here, the regeneration of lost tissue is more successful if growth factors are released sequentially within narrow time windows [1]. After an injury, a finely tuned and well-timed healing cascade takes place: Different kinds of cytokines are needed at certain points in time, including the platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β) and vascular endothelial cell growth factor (VEGF). PDGF initiates the chemotaxis of a variety of cell types such as neutrophils, macrophages, smooth muscle cells and fibroblasts – a process that is critically needed to initiate wound healing. TGF-β is also required during the early stage of a healing cascade, as it attracts macrophages and stimulates them to secrete additional cytokines [2–4]. VEGF, in contrast, is the most important cytokine of the angiogenic cascade, which takes place at a later stage of wound healing [5,6]. This example involving only three different cytokines already illustrates the complexity of the wound healing process. It also shows that these three molecules need to be available at different time points to ensure that the process correctly runs its course.

To support the natural wound healing abilities of the human body after injuries or to compensate for a pathological defect in the wound healing cascade of certain patients, medical products loaded with a set of therapeutic agents can be used. Typically, a gel enriched with molecules beneficial for the wound healing process is applied to the damaged tissue area [7–10]. In many cases, it is necessary to replace such wound gels several times during the healing process or to apply different liquids or gel formulations – each containing another set of bioactive molecules [11,12]. Even though certain medical treatments are more efficient when drugs are administered sequentially [13], this is not easily possible when repetitive access to the tissue area is restricted, e.g. for internal wounds after surgery. Here, all relevant molecules and pharmaceuticals are typically applied at the same time [14,15] – although their individual function is required at different time points. In addition to wound healing, also pharmaceutical applications in cancer therapy could benefit from a precisely controlled release of multiple drugs from a gel. Here, e.g. pre-treatment with a first drug can be used to sensitize the tumor environment to a second chemotherapeutic agent, thus enhancing the delivery efficiency of this second drug into the tumor [16,17]. Such a sequential combination therapy can also reduce the potential risk of toxicity or unwanted pharmacological interaction – phenomena that need to be considered when a combination of drugs is simultaneously applied during a cancer treatment even though the individual therapeutic agents are only needed at distinct time points of the therapy [18].

One existing strategy for establishing control over the release kinetics of therapeutic agents from gels targets the chemical composition of these gels to tune their pore size [19,20], or the binding affinity of the drug to the gel constituents [20]. However, this strategy requires a

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tailored gel matrix for each drug. Examples for such a tailored drug/gel release system include the release of lidocaine from poloxamer 407 or sodiumcarboxymethyl cellulose gels [21], or the release of doxorubicin from acrylate-based hydrogels [22]. Another approach makes use of the liberation of molecules from nanoparticles (NPs); here, the type and architecture of the NP determines the release kinetics of the encapsulated drug [23]. When liposomes are used as drug carriers, variations in the lipid composition result in different release kinetics [24]. Inorganic NPs, on the other hand, such as mesoporous silica nanoparticles (MSNs) and titanium dioxide (TiO_2) particles can be generated with a uniform porous structure, a high surface area, tunable pore sizes and well-defined surface properties [25–29]. When such porous NPs are loaded with drugs, they release their cargo by diffusion. Surface modifications of porous NPs such as amination make use of electrostatic interactions to achieve high loading rates and sustained release of (negatively) charged drugs like Ibuprofen and Aspirin [30,31]. A different drug release mechanism requires polymeric NPs and combines the diffusive release of cargo molecules from the NPs with NP degradation [32]. Examples for polymers used for such polymeric NPs include chitosan [33,34], dextran [35,36] and poly(methyl methacrylate) [37].

For medical applications such as wound treatment, NPs are typically incorporated into hydrogels. This makes it possible to retard the release of the incorporated drugs by two mechanisms: release from the NP and subsequent release from the gel [38,39]. Yet, whereas this combined strategy is sufficient to achieve prolonged drug release, always the same molecule is liberated. Therefore, drug co-delivery systems have been developed which make use of encapsulating multiple therapeutic agents into NPs or embedding multiple NP species into a hydrogels [40,41].

As complex as those release approaches already are, they still share a key disadvantage: drug release can be prolonged but is immediately initiated for all molecules at the same time, i.e. after the gel sample is prepared. Yet, there is a clear need for devising a control mechanism which allows for coordinating the release of the different incorporated drugs, e.g. liberating a second drug only when a another one has already left the gel. The key idea pursued here is to achieve such control by making use of artificial DNA-sequences to trap drug carriers in a gel and liberate the very same drug carriers from the gel in a controlled way. When their length is short, synthetic DNA sequences are easy to generate, programmable, allow for tuning the interaction strength between two DNA strands by varying the sequences of the constructs [42], and they can serve as a tool to control nanoparticle aggregate formation [43,44].

The DNA-based strategy introduced here enables the controlled release of NPs from a hydrogel and is based on a NP disaggregation process, which separates individual NPs from a NP cluster. As this NP cluster is stabilized by DNA-mediated cross-links, NP disaggregation can be achieved by binding of complementary DNA strands. Those complementary DNA sequences, in turn, are liberated from liposome particles, which have been embedded into the gel as well. DNA release is triggered by a build-up of osmotic pressure inside the gel after diffusive depletion of a small control molecule from the gel. Furthermore, control over the kinetics of the release process is achieved by retarding the diffusive spreading of the disaggregation-inducing DNA strands through the gel.

2. Materials and methods

2.1. Polynucleotide design

We designed cross-linker DNA (crDNA) sequences with self-complementary regions such that the constructs can form cross-links between gold nanoparticles (Au-NPs, 5 nm in diameter, PDI ≤ 0.2, stock solution of $5.5 * 10^{13}$ Au-NP/mL stabilized in a citrate buffer, Sigma-Aldrich) onto which they are bound (Fig. 1a). For simplicity, only a single crDNA cross-link is depicted in Fig. 1a. In the experiments discussed in our manuscript, each gold NP is likely to carry several crDNA sequences on its surface and thus can form multiple cross-links with other gold NPs. As a consequence of this cross-linking process, the Au-NPs are supposed to build large aggregates (Fig. 2a) which are then trapped in the gel due to geometric constraints. Au-NP disaggregation is supposed to occur only in the presence of a suitable displacement DNA (dDNA) with a higher affinity to the crDNA.

To enable covalent binding of the crDNA to the surface of Au-NPs, we integrated a thiol-C6 capped poly(A)-tail at the 5′-end of the sequence. The self-complementary region of the crDNA was chosen such that it had a melting temperature T_m above 37 °C so that the construct allows for the formation of stable cross-linked Au-NP aggregates at temperatures ≤ T_m. In contrast, the dDNA sequence was designed to exhibit a higher affinity to the crDNA than two crDNA molecules have to each other. Thus, the crDNA/dDNA complex had a much higher (predicted) melting temperature than the crDNA/crDNA complex. As a control molecule, a polynucleotide sequence (coDNA) with the same number of nucleotides as the dDNA was chosen but designed such that it had only a negligible binding affinity to the crDNA sequence.

Two sets of polynucleotides pairs (crDNA and dDNA) with different T_m values and different sequences were designed as described, tested with the software OligoAnalyzer 3.1 [45] (parameters: target type, DNA; oligo concentration: 0.25 μM; Na^+ concentration: 150 mM; Mg^{2+} concentration: 5 mM; deoxynucleoside triphosphate (dNTPs) concentration: 0 mM) to ensure that they fit the above mentioned requirements and then obtained from Integrated DNA Technologies (IDT, München, Germany). The detailed sequences and calculated melting temperatures of those constructs are listed in Table 1.

Here, the self-complementary regions of the respective crDNA sequences responsible for establishing cross-links are highlighted in red, and the melting temperatures given in the figure describe the stability of the formed crDNA/crDNA or dDNA/crDNA complex. The NUPACK web application was used to calculate the minimum free energy (MFE) structures of the designed DNA sequences. The free energy of a secondary structure was calculated using nearest-neighbor empirical parameters as outlined in ref. [46] for DNA at 37 °C in the presence of 150 mM Na^+ and 5 mM Mg^{2+}.

2.2. PAGE analysis of DNA-hybridization efficiency

To verify that our designed polynucleotide constructs only efficiently hybridize when DNA constructs with complementary sequences are mixed, we performed an analysis by polyacrylamide gel electrophoresis (PAGE). In our constructs, a high degree of complementarity is only expected for sequence combinations with a high calculated T_m (i.e. only for matching crDNA/dDNA sequences). As a control, synthetic DNA sequences (coDNA) with a length identical to the crDNA molecules but with a different sequence (and thus very low calculated T_m) was used. DNA sequences were mixed in 1:1 ratios at a concentration of 1 nmol each and were incubated at room temperature (RT) for 2 h in presence of 500 mM dithiothreitol (DTT) and 0.5 mM tris-(2-carboxyethyl)-phosphin hydrochlorid (TCEP, Carl Roth, Karlsruhe, Germany) to prevent the formation of S-S-bonds. Subsequently, 6× sample loading buffer (Sigma-Aldrich, Schnelldorf, Germany) was added, and the samples were loaded into Mini-PROTEAN TBE Precast Gels (BIO-RAD, Munich, Germany). Electrophoresis was performed at 100 V in 0.5× Tris-Borat-EDTA (TBE) buffer (pH 8.0) containing 5 mM DTT, and pictures were recorded on a Molecular Imager Gel Doc XR System (BIO-RAD) after the gels were stained at RT for 1 h with SYBR Green I solution (Sigma-Aldrich) in 0.5× TBE buffer.

2.3. Gold nanoparticle functionalization and aggregate formation

Polyvalent DNA-functionalized gold nanoparticles were generated by coating colloidal gold with a monolayer of DNA. This coating approach makes use of the strong interaction between gold and thiols, the latter of which have been integrated at the terminus of the synthetic