DNA Ligands for Integrating Biointeractive Materials

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Statement of Purpose:

In the fields of drug delivery and tissue engineering, localization and integration of materials is highly desirable. Specifically and selectively targeting particular cell types or molecules in vivo is crucial for more efficient therapies. DNA aptamers are single stranded nucleic acids ranging from 30 to 70 nucleotides in length, whose three-dimensional structures bind to target molecules with high specificity and affinity. Unlike other functional nucleic acids, aptamers generally do not exert effects on the genetic level and leave protein function intact. Even though aptamers are larger than peptide antibodies, they have been shown to penetrate tissue and extracellular matrix (ECM). Given their unique properties, aptamers have high potential as ligands for localizing biomaterials in vivo. Currently, the method for developing high-affinity nucleic acids with high specificity for a particular target is systematic evolution of ligands by exponential enrichment (SELEX). In this study, SELEX was used to develop aptamers for Collagen Type I. These aptamers will be incorporated into polymeric materials to improve their adhesion into collagen-rich matrices.

Methods:

Aptamer Selection (SELEX): The DNA library obtained from Integrated DNA Technologies (IDT) was amplified via polymerase chain reaction (PCR). ssDNA was obtained by running the PCR product through an avidin-agarose bead column (Sigma). The ssDNA was then purified via polyacrylamide gel electrophoresis (PAGE) and quantified using UV-VIS. Once enough DNA was obtained, a negative control was carried out. 100 uL of the DNA sample at 0.5 mM was run through a Millipore nitrocellulose filter without protein (pore size of 0.45 um). The flow through was then passed through a Millipore filter bound with Collagen Type I. The desired products bound to the collagen-filter were eluted and purified. The product was then amplified via PCR to complete the first round of selection. This process is repeated for each round of selection, where the concentration of Collagen Type I on the positive filter is incrementally decreased per round to physiological concentration of approximately 1 nM.

Collagen Filter Fabrication: Type I Collagen solution (3 mg/mL, Vitrogen 100 Cohesion Technologies, in HCl, pH 2-3) was diluted to desired concentration in 1X phosphate buffered saline (PBS) solution and neutralized to a pH of 7 by adding 10% by volume 0.1 M NaOH. Collagen Type I was then polymerized to the filter by pipetting the neutralized solution onto the Millipore filters in a Petri dish, which was parafilmed, and then incubated at 37^{o}C for 1 hour. Prepared filters are stable and can be stored at room temperature for up to 6 weeks.

Aptamer Characterization: In order to obtain the sequence of the various aptamers obtained after several rounds of selection, cloning must be performed. The aptamer pool from the last round is ligated into linearized plasmid vectors, which is transformed into competent INVαF' e.coli. The e.coli is plated on LB agar plates containing 100 mg/mL ampicillin and spread with 40 uL of 40 mg/mL X-Gal. After 24 hours, individual white colonies are picked and grown overnight in 2-5 mL LB broth containing 100 mg/mL ampicillin. (Desired colonies exhibit resistance to ampicillin and disrupted expression of the *lacZ* gene due to successful insertion of aptamer sequence (~ 100 bp) within the *lacZ* reading frame of the plasmid vector). Qiagen's plasmid purification kit is used to lyse cells and purify aptamer DNA from culture. The DNA samples can then be sequenced, and data analyzed to obtain the aptamer sequence using DNA sequence analysis computer software. Scale up of aptamer synthesis can be carried out once the sequences of desired aptamers have been obtained.

Results / Discussion:

Following selection for aptamers against Collagen Type I at decreasing protein concentrations of 100 uM, 5 uM, and 21 nM, more selective and specific aptamer products have been identified. Aptamer products will be analyzed via adhesion and kinetic tests to obtain binding characteristics, selectivity, and specificity, and will be compared to undesired products. Characterized aptamers will then be covalently attached to biomaterials and in vitro experiments performed to further determine selectivity and specificity under more physiological conditions.

Conclusions:

Given their high selectivity and specificity, unique binding properties, and ability to penetrate tissue and ECM, DNA aptamers are attractive candidates as ligands for localizing biomaterials in vivo in drug delivery or tissue engineering applications.

References:

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