TARGETED POLY(PROPYLENE SULFIDE) NANOPARTICLES FOR INTRA-ARTICULAR DRUG DELIVERY

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Statement of Purpose:
Clinical entities which cause early cartilage degradation are increasingly identified. Despite advances in surgical treatment, the fate of the joint depends on the condition of the articular cartilage, for which no established therapy exists to date. Exploitation of potential molecular targets is complicated by the low bioavailability of therapeutic molecules in the joint. While oral administration is associated with systemic side effects, direct intra-articular injection results in a rapid clearance out of the joint and therefore is not suitable to improve the bioavailability of therapeutic agents in the cartilage matrix. In the present work we present a novel nanoparticle-based drug delivery system which exhibits specific active targeting functionality for the cartilage extra-cellular matrix. The nanoparticles are immobilized in the tissue which results in a continuous release of therapeutic agents into the adjacent cartilage matrix and back into the joint cavity.

Methods: *Phage display*: A peptide-on-phage display library (fUSE5/6-mer) with a complexity of $6.4x10^7$ has been screened against bovine cartilage grafts to find a novel targeting peptide for the cartilage matrix. Five cycles of biopanning have been carried out with increasing stringency of binding conditions in each cycle. Negative screenings were done against the articular cartilage surface and synovial fluid. The sequence of the displayed peptide was obtained by DNA sequencing after cycle 5 of retained and amplified phage clones. Binding assays have been carried out in vitro against bovine cartilage in physiological conditions (37°C, with and without synovial fluid) to probe specificity and competetive binding with 10^8 TU/ml of each phage clone. Titers were determined as transducing units per ml (TU/ml) and binding in % of control. The selected peptide and its mismatch with a scrambled sequence were synthesized using standard Fmoc-chemistry with a cysteine at the C-terminus.
Nanoparticle synthesis: The targeting peptide was conjugated via the thiol group of the cysteine by Michael-type addition to Pluronic F-127 which was previously functionalized with vinyl sulfone. PPS nanoparticles were prepared as described before by our group[1] in an inverse emulsion polymerization with conjugated (10%) and non-conjugated (90%) Pluronic F-127 as the emulsifier, such that an average size of 38nm was obtained as measured by dynamic light scattering. After polymerization of the PPS core (pentaerythritol tetrathioester as initiator and propylen sulfide monomer), the Pluronic with the conjugated targeting peptide remains displayed on the particle surface, thereby forming a surface-functionalized nanoparticle.
In vivo experiments: A preliminary in-vivo study has been done to investigate the targeting of articular cartilage. Functionalized and non-functionalized nanoparticles were labelled with fluoresceine-iodoacetamide and injected into knee joints of mice (n=3/group) eight weeks of age. The animals were sacrificed after 24hrs and cryosections obtained. The density of fluorescently labelled nanoparticles per cartilage volume was assessed on a Zeiss LSM510 meta confocal laser scanning microscope using emission fingerprinting and analysed using ImageJ, 10 z-planes in 10 regions of each joint.

Results/Discussion: DNA sequencing revealed three putative peptide sequences after 5 cycles. All of these sequences have been shown to be specific to cartilage versus synovial membrane by two orders of magnitude. A competetive binding assay between the three phage clones and the original library retained only two phage clones. The free peptide of the clone with the highest titer was subjected to a competetive binding assay against the corresponding phage clone, which resulted in an IC_{50} of the free peptide of about 200nM, suggesting a fairly high affinity to its target. PPS Nanoparticles with surface functionalisation of 10% were obtained at a size of 38nm and non-conjugated ones at 36nm. Similarly, the surface-functionalized nanoparticle was subjected to a competetive binding assay against the corresponding phage clone and compared to the free peptide and its mismatch. The conjugated nanoparticles at a concentration of 2.5% w/v exhibited similar binding as the free peptide at a concentration of 10μM (10.4±6% vs.13.9±2.8% of control), whereas the mismatch peptide did not bind competetively and thus did not reduce the phage titer (92±12%). Preliminary in vivo results indicate a targeting effect of the conjugated nanoparticles after 24hrs with 29.0±1.5%/volume of non-conjugated (non-targeted) vs. 83.8±4.0%/volume in the conjugated (targeted) group.

Conclusions: We have characterized a novel targeting peptide and showed its specificity as well as its competetive binding to articular cartilage under physiological conditions. PPS nanoparticles at 2.5% w/v with the targeting peptide resulted in similar binding at 10% surface functionalisation as the free peptide at 10μM. Moreover, preliminary in vivo results indicate favorable accumulation of the targeted/conjugated nanoparticles in the cartilage matrix. Current work is investigating the long-term intra-articular retention *in vivo* of targeted nanoparticles vs. non-targeted nanoparticles and nanoparticles which have a scrambled peptide on their surface.