New applications for star-shaped cationic polymers with magnetic core respective to purification and non-viral gene delivery

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The controlled and precise delivery of genes is an important step towards efficient gene therapy and genetic modifications of eukaryotic cells. Besides viruses, cationic polymers have been widely explored for that purpose. Gathering information about the intracellular fate of transfection complexes, notably identifying macromolecules (e.g., proteins) which interact with these complexes, will help to understand the intracellular transfection mechanisms. By using modern polymer chemistry, a precise tailoring of functional groups and topology of the polymers are available. Here, we present magnetic core-shell nanoparticles based on multi poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA)-based arms emanating from a common centre.

Cells containing the magnetic PDMAEMA can be separated via MACS (magnetic activated cell sorting) technique. This system was used to study the uptake of polymers in cell lines. One observed effect is that the amount of polymer, taken up by the cells, is not always associated with an efficient delivery of DNA to the nucleus as only a fraction of the “magnetic cells” expresses the transgene. Therefore, we conclude a strong influence of cytoplasmic events involved in the breakdown of the complex and playing a crucial role in efficient gene delivery. The magnetic PDMAEMA used in this study can also be applied for the identification of interacting proteins with the polyplexes. Based on this, a purification process of these proteins was established and will be presented here. Given the fact that the effects involved in transfection are not yet understood, the new tools and obtained results with magnetic PDMAEMA provide important information for the understanding and optimisation of efficient mammalian cell based production processes.

Aptamer-Quantum Dot Conjugates as sensitive Detection Molecules for a human Tumor Marker Protein

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The vascular endothelial growth factor A (VEGF-A) is a cytokine which stimulates the human vasculogenesis and angiogenesis in embryonic development as well as after injury. Beside its normal function VEGF-A also mediates the formation of blood vessels during tumor growth providing the oxygen supply of the proliferating tissue. This is induced by VEGF-A over-expression resulting in increased VEGF serum-concentration, a potential indicator for the presence of cancer. As the expected VEGF-A concentration in serum is in a low range of 200 - 1000 pg/mL a reliable quantification requires a highly sensitive detection platform. Further, blood serum contains a mixture of various different proteins; so additionally, the detection molecule has to bind VEGF-A exclusively. Aptamers fulfill these requirements as they bind to a single target with high affinity and specificity. Therefore, they are demonstrably well-suited for the VEGF-A detection.

In this work we conjugated VEGF-A-binding aptamers with quantum dots to obtain fluorescence-labeled detection molecules. We analyzed the binding affinity of labeled as well as unlabeled aptamers to VEGF via microscale thermophoresis measurement. Further, we established a microarray-based assay for VEGF detection and also used it for aptamer binding analysis.

The microscale thermophoresis measurement demonstrated a high binding affinity of two different DNA-aptamers towards VEGF-A indicated by K_D-values in a low nanomolar range. Further the K_D-values of labeled aptamers barely differed from those of unlabeled aptamers. Therefore, the aptamer folding seems to be nearly unaffected by quantum dot conjugation. Microarray analyses confirm these results and additionally demonstrate that using aptamer-quantum dot conjugates as detection molecules results in lower detection limits when compared to aptamer-Cy3 conjugates.

Aptamer-Quantum Dot Conjugates for Bioanalytical Applications

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Bioanalytical assays rely on specific recognition of molecules of interest and the sensitive detection of these events. Many assay systems use conjugated probes which combine the recognition and detection function like fluorescently labeled antibodies which are widely used in assay systems for ELISA, microarrays and flow cytometry. However, the use of antibodies labeled with organic dyes suffers from susceptibility of antibodies to thermal degradation resulting in low stability and short shelf lifes. Additionally most organic dyes have low photostability and quickly lose fluorescence intensity by light exposure.

Novel conjugates, made up of nucleic acid binding species, so called aptamers, and fluorescent nanoparticles, known as quantum dots could help to develop assays with high specifity and sensitivity that avoid the above mentioend problems.

In this study we showed the feasibility of conjugating aptamers with QDs for the specific detection of proteins in a microarray based assay. Intending to develop conjugates that finally will be useful in cytometric analysis, we chose the aptamer TD05 which is known to bind IgM on the surface of certain B-Lymphocytes with a low binding constant in the low nanomolar range.
Different conjugation experiments utilizing various nucleic acid concentrations were performed and the resulting conjugates monitored by agarose gel electrophoresis. Excessive DNA from the synthesis was quantified using a propidium iodide assay, enabling the estimation of aptamer load per quantum dot. The obtained conjugates were subsequently tested for their ability to bind the target protein IgM and distinguish between target and non-target proteins. The results indicate that aptamer-quantum dot conjugates can be used to replace and complement the commonly used antibody-dye probes in fluorescent based assays which suffer from low stability and photobleaching of these probes.

Isolation, Characterization, Expansion, and Application Strategies of Human Mesenchymal Stem Cells

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Mesenchymal stem cells (MSC) have proven to offer great promise for cell-based therapies and tissue engineering applications. MSC are capable for extensive self-renewal and display a multilineage differentiation potential. Furthermore, MSC were shown to exhibit immunomodulatory properties and display supportive functions through paracrine effects.

For therapeutic purposes and other tissue regeneration approaches a high number of cells is required. We therefore used bioreactors in order to maximize ex vivo expansion of MSC. The integration of sensors for online monitoring of various parameters (e.g. pH, pO_2, pCO_2) ensured cultivation under well controlled and reproducible conditions. Cell expansion in a rotating bed bioreactor provided a high number of MSC. The use of micro carriers for MSC cultivation was shown to be a suitable and effective method to expand adherent cells under stirred dynamic or flow conditions.

Beside their application for cell therapy purposes, MSC might be a powerful tool in tissue reconstruction. In our studies we focus on the guided differentiation of MSC from adipose tissue or umbilical cord matrix towards the osteogenic lineage. MSC were seeded on 3D ceramic matrices and cultivated in perfusion bioreactors under well controlled conditions. Furthermore a stimulus by fluid flow was applied on the cells, which has been shown to promote the osteogenic differentiation.

3D cultivation of MSC without the need for a 3D matrix was achieved using hanging drop techniques as well as non-adhesive silicone-coated surfaces to form cell spheroids.

In summary, we demonstrated the numerous opportunities for MSC applications in cell therapy, tissue engineering and toxicology.

Erforschung neuartiger Polymere für den nicht-viralen Gentransfer

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Die Grundlage der Genveränderung von Säugetierzellen ("Transfektion") ist das Einbringen von Transgenen in die Zelle. Dieses beinhaltet zum einen die erfolgreiche Aufnahme der pDNA in die Zelle, sowie den Transport ins Innere des Nukleus‘. Für den nicht-viralen Gentransfer werden kationische Polymere als Transportsystem verwendet. Ihre Transfektionseffizienz und Biokompatibilität ist besonders von der polymeren Chemie und Struktur abhängig. Die jüngste Forschung zeigt, dass nicht lineare Polymerstrukturen im Vergleich zu Polymeren gleicher Größe einen effektiveren Transfektionsvektor darstellen (e.g., Synatschke *et al.*, 2011). Im Besonderen zeigen aus PDMAEMA aufgebaute, sternförmige Nanopartikel, die entweder um einen festen anorganischen Kern ($PDMAEMA_{230/20}$), oder um einen „flüssigen“ Polybutadien Kern ($B_{290}D_{245}$) synthetisiert sind ein großes Potential zur Transfektion von primären und differenzierten Zellen ebenso wie einen effektiveren pDNA Transport in herkömmliche Zelllinien im Vergleich zu PEI. Unabhängig von der Beschaffenheit des Kerns resultiert eine erfolgreiche Transfektion aus einem geringeren Molekulargewicht und einer sternförmigen Struktur mit möglichst vielen Armen. Daraus ergeben sich weitere Möglichkeiten für die Entwicklung verbesserter Genvektoren, im speziellen für Primärzellen [Schallon *et al.*, 2012]. Allerdings ist bis jetzt der verantwortliche Mechanismus für das außerordentlich gute Transfektionspotential der sternförmigen, auf PDMAEMA basierenden Nanopartikel unbekannt. Wechselwirkungen zwischen positiv geladenen Polyplexen und Oberflächenproteinen der Zellmembran werden gewöhnlich als Grundlage einer erfolgreichen Aufnahme in die Zelle postuliert. Aus diesem Grund präsentieren wir eine systematische Analyse der physikalisch-chemischen Eigenschaften der Polyplexe (z.B. Nettoladung, Größenverteilung, ...) im Bezug auf den Erfolg der Transfektion. Außerdem wird der Einfluss der Kulturführung (adhärent/ in Suspension) ebenso wie die Anwesenheit von Oberflächenproteinen während der Transfektion diskutiert.

Synatschke CV, Schallon A, Jérôme V, Freitag R, Müller AHE. Influence of Polymer Architecture and Molecular Weight of Poly(2-(Dimethylamino)ethyl Methacrylate) Polycations on Transfection Efficiency and Cell Viability in Gene Delivery. *Biomacromolecules.* 2011**;** 12: 4247-4255.

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