View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by University of Queensland eSpace

Invited Paper

Multifunctional nanoparticles for drug/gene delivery in nanomedicine

Mary-Margaret Seale[2], Dimitry Zemlyanov[4], Christy L. Cooper[1], Emily Haglund[2], Tarl W. Prow[5], Lisa M. Reece[1], James F. Leary[1 - 4]
[1] School of Veterinary Medicine - Department of Basic Medical Sciences,
[2]Weldon School of Biomedical Engineering, [3]Bindley Biosciences Center,
[4]Birck Nanotechnology Center,
Purdue University, West Lafayette, IN
[5] School of Medicine – Department of Ophthalmology,
Wilmer Ophthalmological Institute
Johns Hopkins University, Baltimore, MD

ABSTRACT

Multifunctional nanoparticles hold great promise for drug/gene delivery. Multilayered nanoparticles can act as nanomedical systems with on-board "molecular programming" to accomplish complex multi-step tasks. For example, the targeting process has only begun when the nanosystem has found the correct diseased cell of interest. Then it must pass the cell membrane and avoid enzymatic destruction within the endosomes of the cell. Since the nanosystem is only about one millionth the volume of a human cell, for it to have therapeutic efficacy with its contained package, it must deliver that drug or gene to the appropriate site within the living cell. The successive de-layering of these nanosystems in a controlled fashion allows the system to accomplish operations that would be difficult or impossible to do with even complex single molecules. In addition, portions of the nanosystem may be protected from premature degradation or mistargeting to non-diseased cells. All of these problems remain major obstacles to successful drug delivery with a minimum of deleterious side effects to the patient.

This paper describes some of the many components involved in the design of a general platform technology for nanomedical systems. The feasibility of most of these components has been demonstrated by our group and others. But the integration of these interacting sub-components remains a challenge. We highlight four components of this process as examples. Each subcomponent has its own sublevels of complexity. But good nanomedical systems have to be designed/engineered as a full nanomedical system, recognizing the need for the other components.

Keywords: nanomedicine; nanoparticles; drug delivery; gene delivery; therapeutic genes, SPIO

1. INTRODUCTION

1.1 General introduction:

Nanomedicine, still in its very early stages, involves the use of nanometer-sized drug delivery vehicles including micelles, dendrimers, and liposomes to treat and repair insult or injury inflicted upon living cells and tissues. These "nanoparticles" may be composed of biological and/or synthetic polymers, metals, lipids, peptides, or other materials. Nanoparticles can be used to deliver drugs and genes to specific locations within the human body to treat various cancers and other diseases more efficiently and with less side effects. For example, folic acid has been conjugated to poly (ethylene glycol) (PEG)-coated copolymer nanoparticles (Stella et al, 2000) , PAMAM dendritic polymers (Baker, James R et al, 2005), lipid-based nanoparticles (Low et al, 2004) and Quantum dots® (Chan, W. et al, 2002) to improve cell uptake of these therapeutic nanoparticles.

One drawback to using nanoparticles as drug delivery vehicles, is the inherent cytotoxicity of nanoparticle core materials. C60 ("Bucky balls") (Sayes, 2004) and cadmium selenide/zinc sulfide Quantum dots® (Kirchner, Parak, 2005) were shown to be cytotoxic in *in vitro* studies. Another drawback is the difficulty of targeting therapeutic nanoparticles to specific cell types and intracellular locations. We are interested in developing nanomedical systems for drug/gene delivery to cancer cells. Construction of multilayered dendritic nanoparticles for nanomedical systems could provide for more specific targeting of cancer therapeutics with reduced cytotoxicity. That part of our work will be the subject of a future paper dedicated to that part of the design of our nanomedical systems. We previously

Nanoscale Imaging, Spectroscopy, Sensing, and Actuation for Biomedical Applications IV,
edited by Alexander N. Cartwright, Dan V. Nicolau, Proceedings of SPIE Vol. 6447,
64470E, (2007) · 1605-7422/07/$18 · doi: 10.1117/12.701645

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 11/30/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

reported preliminary results in the development of an autonomous nanomedical system (Prow et al, 2004a; 2004b; 2005) developed in efforts to repair radiation-induced DNA damage to cells. This system utilized streptavidin-coated magnetic nanoparticles that were tethered to a biotin-labeled PCR product that encoded the EGFP sequence and subsequently coated with lipid. These NPs were characterized by measuring the zeta potential under different conditions of pH and ionic strength conditions at which particles were stable with little aggregation for up to five days. Fluorescence microscopy was also used as a characterization method; it was discovered that the EGFP-encoding gene, when tethered to nanoparticles, was statistically able to be expressed as the untethered, free EGFP-encoding gene (Prow et al., 2006a; 2006b).

Selection and characterization of drug delivery vehicles is critical to the success of any nanomedicine-based research program. In this paper, we describe progress on the synthesis and also the characterization of iron oxide nanomedical systems, hopefully suitable in the future for *in vivo* human use. While this subject area is vast and this paper is brief, we highlight four areas of importance to successful design of nanomedical systems: (1) general design criteria for multilayered, multifunctional nanomedical systems, (2) multi-step targeting strategies, (3) strategies for achieving proper drug/therapeutic gene dosage at the single cell level, and (4) gene tethering to achieve efficient expression of therapeutic genes within individual living cells.

1.2 Designing programmable nanomedical systems:

We have previously described some of the design criteria and methods for construction of multilayered, multifunctional nanoparticles for which the systematic step-by-step de-layering can constitute a form of "molecular programming" through the use of layer-by-layer (LBL) disassembly. The LBL strategy of these chemical structures in each layer will disassemble when they encounter the molecules in the cell that they are designed to detect. We start by building these nanomedical systems on a core particle, typically about 40 nm in diameter, composed of ferromagnetic materials that are relatively non-toxic and suitable for *in vivo* human use. Therapeutic genes are then "tethered" to the surface of these cores in a manner such that the DNA is free to interact with its eventual target and have high rates of gene expression (Prow et al, 2006a; 2006b). The next functional layer includes intracellular targeting molecule(s) since a human cell can be more than one million times the volume of nanoparticles (NPs), so it is important to bring the nanoparticles (NP) close to its intended site of action. Lastly, the outermost layer of the nanomedical system contains the cell surface targeting molecules designed to help the NP bind to the cell of interest and to try to avoid binding to other cell types. These targeting molecules can be antibodies, peptides, aptamers and other molecules. This latter aspect is particularly important if the nanomedical system is to provide improvement over current therapies which can cause damage to "bystander", non-targeted cells. Sometimes the outermost two layers of the nanomedical system can be accomplished with a single molecule, e.g. a single peptide sequence that might not only bind to the cell surface but also pull the nanoparticles through the cell membrane. We are using such dual-purpose peptides for other nanomedical applications. The resultant nanomedical system appears conceptually as seen in **Figure 1**:

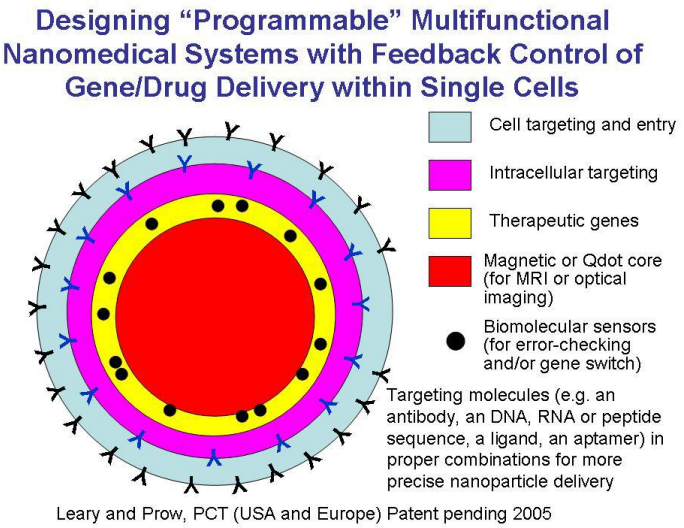

Figure 1: A multilayered nanoparticle system contains targeting, biosensing and drug delivery molecules that are released a layer at a time. This produces a smart nanoparticle system that results in "molecular programming", an ordered series of events, for drug/gene delivery. Biosensing molecules allow the feedback-controlled release of drugs, or expression of therapeutic gene sequences, at the individual cell level.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 11/30/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

1.3 The importance of designing a multi-step targeting process:

It is important to understand that a successful nanomedical system requires a "multi-step targeting " scheme (**Figure 2**), since delivering a nanomedical system to the correct cell, while itself a challenge for *in vivo* situations where there is inherent targeting to rare frequency cell subpopulations, is still only the first step of a more complex, multi-step targeting process required to deliver the nanomedical system to the correct internal location of the desired cell which is perhaps more than a million times the volume of the nanomedical system itself.

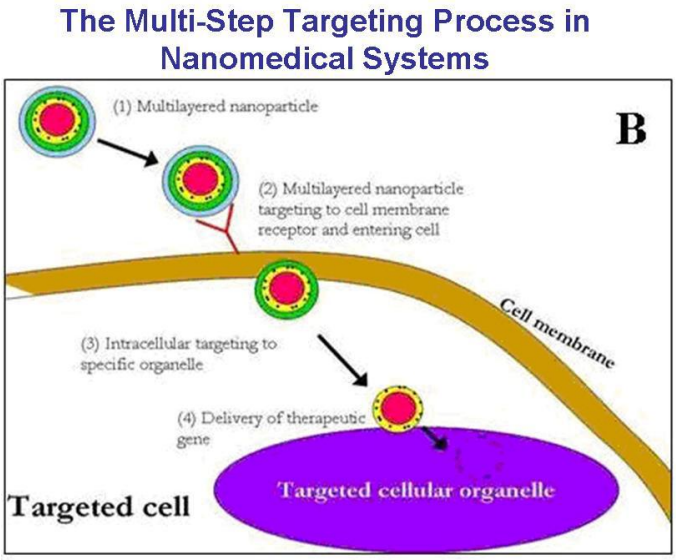

Figure 2: General sequence of at least four steps for nanomedical systems (NMS) interacting with the targeted cell of interest:
(**1**) nanoparticles in the extracellular environment, (**2**) NMS attachment to the cell membrane and its proper entry, (**3**) intracellular targeting to desired site of action, and (**4**) delivery of drugs or production of therapeutic genes at the desired site.

1.4 Dealing with proper dosage at the single-cell level using the "nanofactory" approach:

Even after achieving successful targeting strategies to potentially rare diseased cells *in vivo*, a remaining major challenge to nanomedicine is delivering the proper dosage of drug or therapeutic gene to each diseased cell. Since it will be very difficult to control the number of nanoparticles delivered to each cell, an alternative strategy has been devised. Instead of delivering the therapeutic gene itself to the cell, we deliver a *template* of the gene that can then be manufactured *in situ* within a living cell. This manufacturing process can be further controlled in a feedback loop by having sensing regions upstream of the promoter switch causing the promoter to drive the therapeutic gene expression in response to biosensed molecules that are associated with the diseased state. The therapeutic gene will then be manufactured only when it senses these "diseased state" molecules. Otherwise, it will remain off or turn off and be dormant until needed. The process can continue on and off as required over time for regenerative medicine to control the cell's activities, or the process can be done once and then allow the manufacturing template to be degraded. This "nanomanufacturing" process uses the host cell machinery to manufacture the therapeutic genes and uses molecules already present within the cell as raw materials. The general concept is described in **Figure 3**:

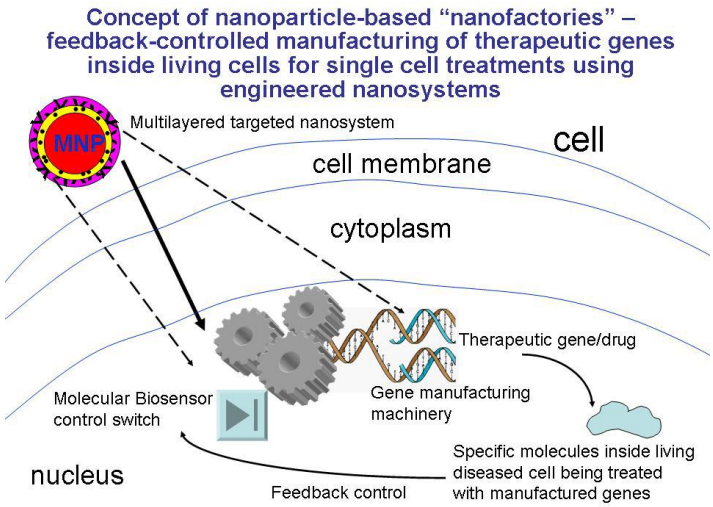

Figure 3: Schematic showing how multilayered nanomedical systems can deliver a therapeutic gene manufacturing template, driven by an upstream promoter sequence, which also acts as an ON/OFF molecular biosensor switch depending on whether therapeutic gene targets are present in sufficient quantities to turn the switch on.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 11/30/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

1.4 Gene tethering for efficient therapeutic gene expression:

Nanoparticles tethered to DNA can be efficient delivery vehicles for delivery of therapeutic and biological sensing genes. These biologically functional nanostructures can be used for very diverse biological systems. There are several unique benefits from using such a nanomaterial when compared to traditional non-viral gene delivery systems, e.g. DNA condensing agents or liposomes. One of the primary benefits to using this system is that the genetic material can be replaced or altered in a matter of hours through PCR. This type of flexibility is not possible with other systems, especially viral agents which take days to weeks to produce and purify. Additionally, the superparamagnetic properties of the core iron oxide nanocrystals aid in the purification of the nanoparticles. The DNA tethered nanoparticles are not capable of integrating into the genome which does happen when using DNA condensing agents and liposomes. Finally, these nanoparticles provide a platform technology that can be easily manipulated, swapping in or out specific molecules for different applications, resulting in more sophisticated nano-devices that have multi-functionality.

2. MATERIALS AND METHODS

2.1 Construction of ferric oxide nanoparticles:

Iron oxide nanoparticles were synthesized via thermal co-precipitation of ferrous and ferric chlorides in ammonium hydroxide. The black precipitate produced was washed with water and ethanol and vacuum dried to yield a black powder. The size distribution of these particles, as determined by TEM, was 8-31nm (data not shown). Because these nanoparticles contained no functional groups for subsequently attaching biomolecules, the particles were functionalized by coating with (3-aminopropyl)trimethoxysilane (APTMS), the amino groups of which are free to bind various biomolecules, including peptides and oligonucleotides,

APTMS-coated iron oxide nanoparticles were synthesized by placing 1.5g of the iron oxide nanoparticles synthesized as described above into a three-neck flask with a solution of 10% (v/v) APTMS in ultrapure water, heating to 110^{o}C for three hours and allowing the solution to reflux under nitrogen atmosphere while vigorously stirring throughout the reaction. At the end of the reaction, the product was collected by using 0.5 Tesla field strength magnet and washed twice with ultrapure water and once with ethanol. The product was dried overnight at 70^{o}C in a vacuum oven until a black powder formed that could be stored at room temperature until ready for use.

2.2 X-ray Photoelectron Spectroscopy

To more fully characterize the coated and uncoated iron oxide nanoparticles, x-ray photoelectron spectroscopy (XPS) (Kratos Ultra DLD spectrometer) was used. XPS data were obtained using monochromatic Al Kα radiation ($h\nu$ = 1486.58 eV). The survey and narrow-region spectra were collected at a fixed analyzer pass energy of 160 and 20 eV, respectively. The atomic concentrations of the chemical elements in the near-surface region were calculated after the subtraction of a Shirley-type background, taking into account the corresponding Scofield atomic sensitivity factors. The binding energy (BE) values referred to the electron energy levels of the elements (Fermi levels) and were calibrated using the following standard procedure: the Cu $2p_{3/2}$ peak and Au $4f_{7/2}$ were set at BE of 932.67 eV and 83.98 eV, respectively; the Ag 3d peak was measured at 368.26 eV. Typical resolution was measured at 0.9-1.1 eV as full width at half maximum of the deconvoluted C 1s peaks. Since samples were non-conductive, a standard Kratos charge neutralizer was used. The energy scale was corrected using the C 1s line to be set at 284.8 eV.

XPS utilizes a photo-ionization process to study the composition and electronic state of the surface region of a sample. Thus, the photon is absorbed by an atom causing the emission of a core level electron. The kinetic energy, E_K, of the emitted photoelectrons can be measured by an electron energy analyzer. The binding energy, E_B, can be calculated using the equation:

$$E_B = h\lambda - E_K - \varphi_{spect}\,. \tag{1}$$

where $h\lambda$ is photon energy and φ_{spect} is a spectrometer work function.

Every element is characterized by a unique set of electron levels and, therefore, every element exhibits a unique set of photoemission peaks. The peak intensity is proportional to the elemental concentration. Since photoelectrons can typically escape from only the topmost 5 nm of the sample surface, XPS is surface sensitive in that region. The element sensitivity is about 0.5 atomic percent.

In the sample preparation for XPS analysis, approximately 50 mg of nanoparticles were dispersed in 500μL of ultrapure water and sonicated for 15 minutes at room temperature. Dispersed nanoparticles were applied to clean 10mm^2 gold-coated glass microscope slides and dried on a slide warmer at 37°C overnight. Analyses of the NP is shown in **Figures 4** and **5**.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 11/30/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

2.3 TUNEL Assay

The potential cytotoxicity of ferromagnetic nanoparticles was examined using a commercially available assay for late apoptosis (Invitrogen, Inc. Carlsbad , CA APO-BrdU TUNEL Assay Kit). In late apoptosis, the cell self-degrades its DNA into intranucleosomal 200 base pair fragments (leading to the "DNA ladder" originally observed in gel electrophoresis). A single cell "TUNEL" (Terminal Deoxynucleotide Transferase dUTP Nick End Labeling) assay equivalent utilizes the 3'-hydroxyl ends occurring during the DNA fragmentation process. These breaks serve as starting points for terminal deoxynucleotidyl transferase (TdT) which adds deoxynucleotides that contain an analog, 5-bromo-2'-deoxyuridine 5'-triphosphate (BrdUTP) to label the break sites. The BrdU labeled DNA fragments are then detected with a fluorescent Alexa-488 labeled monoclonal antibody. Cells prepared and labeled according to the TUNEL kit assay procedures were then examined by flow cytometry to determine the percentage of cells that had been induced into apoptosis and compared to cells which had not been treated with ferromagnetic nanoparticles (negative control) (See **Figure 6**).

2.4 Gene tethering for efficient therapeutic gene expression:

The efficient gene transfer with DNA-tethered magnetic nanoparticles began with the amplification of the genetic construct (CMV-EGFP-pA) by PCR using a biotin labeled primer and a non-biotin labeled primer. The resulting biotin labeled PCR product was then coupled to a streptavidin-labeled superparamagnetic nanoparticle (SPIO) using the non-covalent biotin-streptavidin linkage. These nanoparticles were then purified via a magnetic column and incubated with a lipid to enhance cell penetration. Once the incubation was completed, the nanoparticles were incubated with cultured cells. Within 1-1.5 hours the nanoparticles entered the cells and within 16-24 hours reporter proteins were detected by EGFP fluorescence with blue Hoechst 33342 counterstained nuclei. The DNA tethered nanoparticles appear to remain intact for at least 14 days (**Figure 7**).

3. DATA AND RESULTS

3.1 XPS analysis of ferric oxide nanoparticle surfaces:

A typical survey XPS spectrum obtained from the iron oxide nanoparticles is shown in **Figure 4**. As can be seen, iron, fluorine, oxygen, nitrogen, gold, carbon and chlorine were detected. The atomic percentage of each element at the nanoparticle surface was calculated in CasaXPS software based on the area under the peak as shown in **Table 1**. The gold signal was coming from the substrate and therefore was not included in the calculation.

Figure 5 shows the survey spectrum obtained from APTMS-coated nanoparticles. XPS confirms iron oxide particle coating with APTMS. First, notice that the nitrogen concentration is higher for APTMS-coated nanoparticles than for uncoated nanoparticles which can be attributed and assigned to the amino group contribution. Second, APTMS nanoparticles contain silicon which was expected with this coating. Finally, the carbon content increased for the coated NP, whereas iron concentration shows a decreased content. Taken together, this points to the coating of the iron oxide nanoparticles by the carbon-containing (APTMS) layer. Oxygen signals also followed the trend of iron, supporting the hypothesis that iron oxide nanoparticle cores are shielded with APTMS.

Sample	C 1s %	Cl 2p %	F 1s %	Fe 2p %	N 1s %	O 1s %	Si 2p %
uncoated sample #1	13.66	4.88	0.74	25.09	3.29	52.33	0.00
uncoated sample #2	12.18	2.63	1.05	27.80	1.39	54.95	0.00
APTMS coated sample #1	24.76	3.51	0.34	11.94	5.37	46.75	7.33
APTMS coated sample #2	27.88	3.49	0.00	10.86	5.37	45.32	7.08

Table 1. Calculated atomic percentages for uncoated and APTMS-coated iron oxide nanoparticles.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 11/30/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

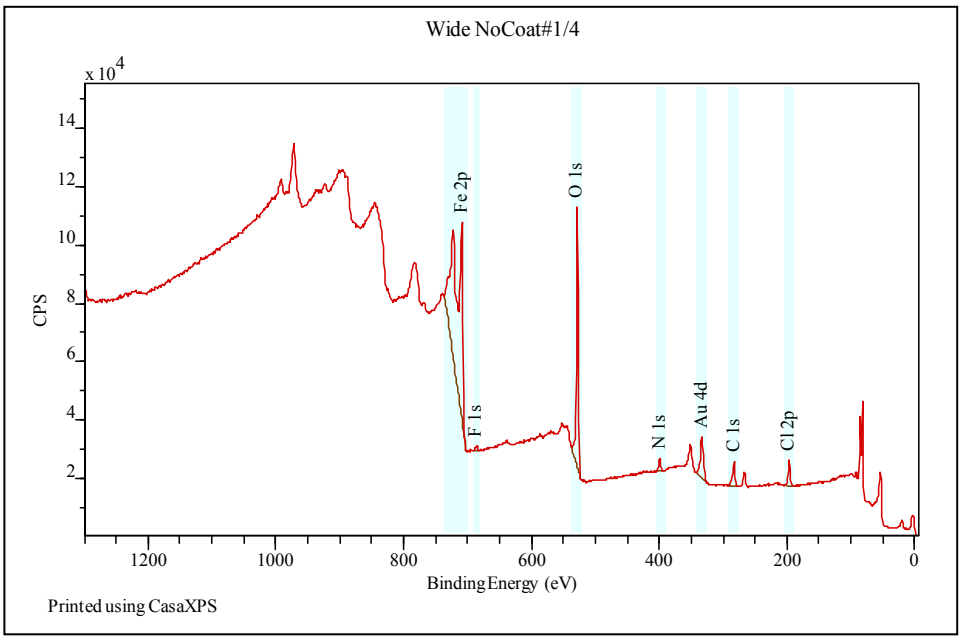

Figure 4: Survey spectrum obtained from non-coated iron oxide particle samples. The selected regions are characteristic core-level peaks for the elements containing in the sample (one peak per element).

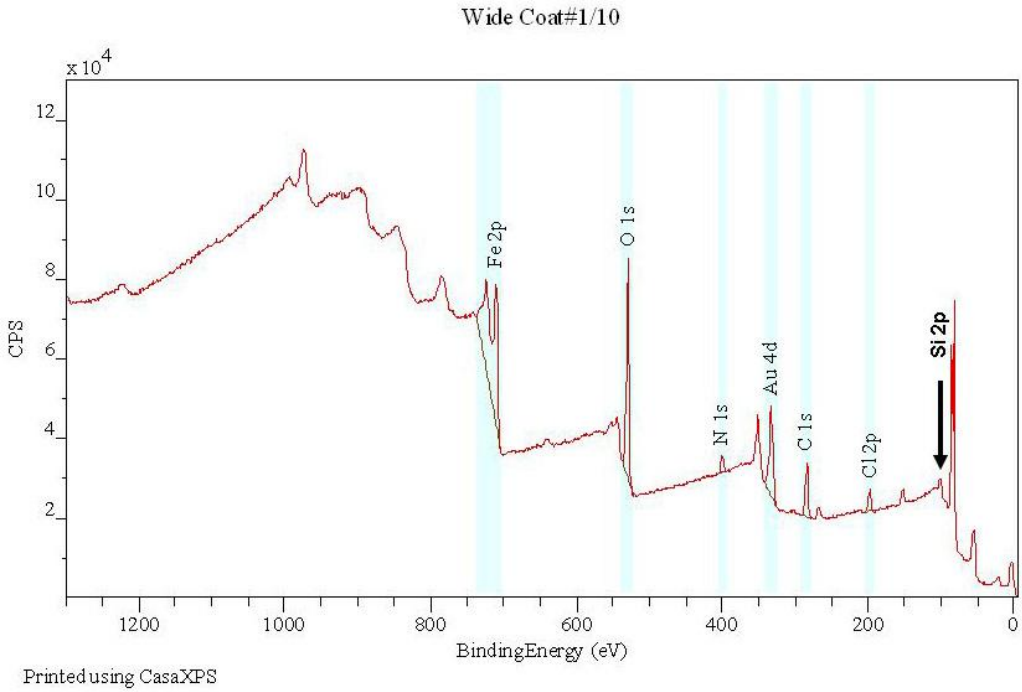

Figure 5: Survey spectrum obtained from APTMS-coated iron oxide particle samples. The selected regions are characteristic core-level peaks for the elements contained in the sample (one peak per element). The Si 2p line, while small, confirms the presence of the APTMS coating on the NP.

3.2 TUNEL assays of potential nanoparticle cytotoxicity:

While this assay, described early in Methods, only constitutes one measure of cytotoxicity, namely late stage apoptosis wherein DNA strand breaks are occurring, results showed very low amounts of induced apoptosis as seen in **Figure 6**. A number of other single cell assays for early apoptosis (e.g. Annexin V) and cell viability by dye exclusion (e.g. trypan blue, or propidium iodide), can be used as simple measures. More sophisticated tests of distributions in the gene expression profile and metabolic function of the cell can be accomplished with gene or protein arrays, the subject of other studies in our laboratory. Here we present one commonly used single cell assay of late apoptosis (TUNEL assay) as a reminder that all nanomedical systems must evaluate the potential nanotoxicity of not only the on-board drug or gene, but also the potential toxicity of the nanodelivery vehicle itself.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 11/30/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

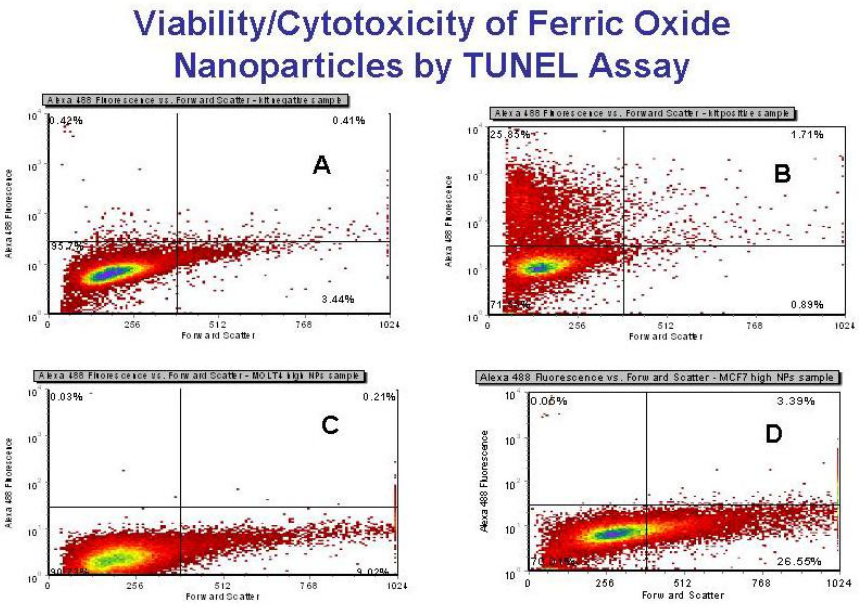

Figure 6: Flow cytometry cell density plots of Alexa 488 fluorescence versus forward light scatter for the following cell samples (**A**) negative control fixed lymphoma cell line, (**B**) positive control fixed lymphoma cell line, (**C**) post-fixed MOLT-4 cells exposed to 0.5 mg/mL nanoparticles, and (**D**) post-fixed MCF-7 cells exposed to 0.5 mg/mL nanoparticles.

3.3 Efficient expression of therapeutic genes using gene tethering:

As described in Section 1.4, efficient expression of therapeutic genes with nanomedical systems requires that the genes be properly tethered on the surface of superparamagnetic iron oxide (SPIO) nanoparticles permitting as much full expression as possible. As a test case, we substituted an EGFP (Enhanced Green Fluorescent Protein) reporter gene for the therapeutic gene and used an upstream CMV (Cytomegalovirus) promoter to provide constitutive expression of the EGFP gene sequence. The nanoparticles were subsequently coated with a Lipofectamine 2000 (Invitrogen, Inc.) lipid coating for biocompatibility and for providing efficient transfection of the nanosystem into living cells. Subsequent expression of EGFP was measured and found to be expressed at similar levels to EGFP not attached to nanoparticles, but directly transfected into these cells as shown in **Figure 7**:

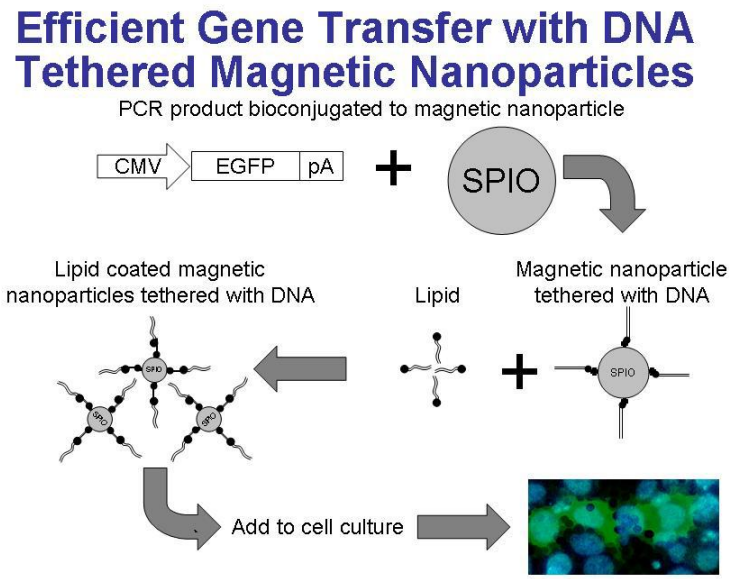

Figure 7: Schematic of experiment showing that EGFP reporter genes tethered to SPIO nanoparticles gave efficient expression of EGFP under these conditions.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 11/30/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

4. CONCLUSIONS

Nanomedical systems for drug/gene delivery will become increasingly important in the years ahead. Pharmaceutical companies could have many drugs that would be much safer, with greatly reduced adverse drug side effects, if encapsulated within nanodelivery systems carefully targeted to diseased cells. That technology is already in use with liposome delivery systems. But multilayered, multifunctional nanomedical systems can deliver these drugs with much greater specificity. "Biological therapies" involving the use of transiently expressing therapeutic genes inside individual living cells, raises the future possibility of not just killing diseased cells, the object of most nanodelivery systems currently being designed and used, but the repair of diseased cells to either a normal state or at least a less dangerous diseased state (e.g. arresting cancer cells at the benign stage and not allowing them to turn on the genes necessary for successful metastasis). While this is still at the speculative stage, it should indeed be possible to achieve this goal with at least moderate degrees of success. If this becomes possible and practical, the implications for medicine and human health are immense. Much earlier detection and treatment of many human diseases might prevent the expression of traditional systems, and tissue/organ damage, associated with those diseases, leading to potential long-term management of these diseases.

5. ACKNOWLEDGMENTS

This work was supported by grants from NASA-Ames (BAA N01-CO-17014-32 and current NASA subcontract from UTMB), the Purdue Cancer Center, and the Purdue Oncological Sciences Center. Work was performed at the XPS facility at the Birck Nanotechnology Center, the Molecular Cytometry Facility, and at the PUCL (Purdue University Cytometry Laboratory) of the Bindley Biosciences Center, at Discovery Park at Purdue University. Flow cytometry data was acquired in the PUCL - Purdue Cancer Center Analytical Cytometry Laboratories supported by the Cancer Center NCI core grant # NIH NCI-2P30CA23168.

6. REFERENCES

Arends, MJ, Morris, RG, Wyllie, AH. "Apoptosis. The role of the endonuclease." Am J Pathol 136, 593-608 (1990)

Bortner, CD, Oldenburg, NBE, Cidlowski, JA The role of DNA fragmentation in apoptosis Trends in Cell Biology 5: 21-26 (1995).

Chan, WCW, Maxwell, DJ, Gao, X, Bailey, RE, Han, M, Nie, S Luminescent Quantum Dots for Multiplexed Biological Detection and Imaging, Current Opinion in Biotechnology, 13:40 (2002)

Choi, Y, Mecke, A, Orr, BG, Holl MM, Banaszak, M, Baker, JR, DNA-Directed Synthesis of Generation 7 and 5 PAMAM Dendrimer Nanoclusters Nano Letters, 4:391 (2004)

Clark, I. B., E. G. Hanania, et al. (2006). "Optoinjection for efficient targeted delivery of a broad range of compounds and macromolecules into diverse cell types." J Biomed Opt **11**(1): 014034.

Cohen, G. (1997). "Caspases: the executioners of apoptosis." Biochemical Journal **326**(1): 1-16.

De Smedt, S. C., J. Demeester, et al. (2000). "Cationic polymer based gene delivery systems." Pharm Res **17**(2): 113-26.

Guchelaar, H., A. Vermes, et al. (1997). "Apoptosis: molecular mechanisms and implications for cancer chemotherapy." Pharmacy World and Science **19**(3): 119-125.

Kirchner, C , Liedl, T, Kudera, S, Pellegrino, T, Munoz J, Almudena; G, HE. ; Sto1lzle, S ; Fertig, N.; Parak, WJ "Cytotoxicity of Colloidal CdSe and CdSe/ZnS Nanoparticles." Nano Letters, 5:331-338 (2005)

Potten, C. and J. Wilson (2004). Apoptosis: the life and death of cells. Cambridge, UK, Cambridge University Press.

Prow,T.W., Salazar, J.H., Rose, W.A., Smith, J.N., Reece, L.M., Fontenot, A.A., Wang, N.A., Lloyd, R.S., Leary, J.F. (2004a). "Nanomedicine – nanoparticles, molecular biosensors and targeted gene/drug delivery for combined single-cell diagnostics and therapeutics" Proc. of SPIE 5318: 1-11.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 11/30/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx

Prow, T.W., Kotov, N.A., Lvov, Y.M., Rijnbrand, R., Leary, J.F. (2004b). “Nanoparticles, Molecular Biosensors, and Multispectral Confocal Microscopy” Journal of Molecular Histology, Vol. 35, No.6, pp. 555-564.

Prow, T.W., Rose, W.A., Wang, N., Reece, L.M., Lvov, Y., Leary, J.F. (2005). "Biosensor-Controlled Gene Therapy/Drug Delivery with Nanoparticles for Nanomedicine" Proc. of SPIE 5692: 199 – 208.

Prow, T.W., Smith, J.N., Grebe, R., Salazar, J.H., Wang, N., Kotov, N., Lutty, G., Leary, J.F. (2006a). "Construction, Gene Delivery, and Expression of DNA Tethered Nanoparticles" Molecular Vision 12: 606-615.

Prow, T.W., Grebe, R., Merges, C., Smith, J.N., McLeod, D.S., Leary, J.F., Gerard A. Lutty, G.A. (2006b). "Novel therapeutic gene regulation by genetic biosensor tethered to magnetic nanoparticles for the detection and treatment of retinopathy of prematurity" Molecular Vision 12: 616-625.

Sayes, CM, Fortner, JD., Guo, W , Lyon, D, Boyd, AM, Ausman, KD, Tao, YJ, Sitharaman, Balaji, Wilson, LJ, Hughes, JB, West,| JL, Colvin, VL. The Differential Cytotoxicity of Water-Soluble Fullerenes Nano Letters, 4:1881 (2004)

Shadidi, M. and M. Sioud (2003). "Identification of novel carrier peptides for the specific delivery of therapeutics into cancer cells." Faseb J **17**(2): 256-8.

Stella, B; Silvia, A; Peracchia, MT; Desmaele, D; Hoebeke, J; Renoir, M; De'Angelo, J;Cattel, L; Couvreur, P. "Design of Folic Acid-Conjugated Nanoparticles for Drug Targeting Journal of Pharmaceutical Sciences, 89:1452 (2000)

Turk MJ.;Waters, DJ.;Low, PS., Folate-Conjugated Liposomes Preferentially Target Macrophages Associated with Ovarian Carcinoma Cancer Letters, 213: 165 (2004)

Zhang, J., M. Xu. "Apoptotic DNA fragmentation and tissue homeostasis." Trends Cell Biology **12**(2):84-89. (2002)

Zhao, M., D. A. Beauregard, et al. (2001). "Non-invasive detection of apoptosis using magnetic resonance imaging and a targeted contrast agent." Nat Med **7**(11): 1241-4.

Downloaded From: http://proceedings.spiedigitallibrary.org/ on 11/30/2015 Terms of Use: http://spiedigitallibrary.org/ss/TermsOfUse.aspx