Design of multifunctional nanoparticles for combined in-vivo imaging and advanced drug delivery

James F. Leary, Ph.D.
President and Founder, Aurora Life Technologies, LLC
Santa Fe Business Incubator
3900 Paseo del Sol
Santa Fe, New Mexico 87507

ABSTRACT

Design of multifunctional nanoparticles for multimodal in-vivo imaging and advanced targeting to diseased single cells for massive parallel processing nanomedicine approaches requires careful overall design and a multilayered approach. Initial core materials can include non-toxic metals which not only serve as an x-ray contrast agent for CAT scan imaging, but can contain T1 or T2 contrast agents for MRI imaging. One choice is superparamagnetic iron oxide NPs which also allow for convenient magnetic manipulation during manufacturing but also for re-positioning inside the body and for single cell hyperthermia therapies. To permit real-time fluorescence-guided surgery, fluorescence molecules can be included.

Advanced targeting can be achieved by attaching antibodies, peptides, aptamers, or other targeting molecules to the nanoparticle in a multilayered approach producing "programmable nanoparticles" whereby the "programming" means controlling a sequence of multi-step targeting methods. Addition of membrane permeating peptides can facilitate uptake by the cell. Addition of "stealth" molecules (e.g. PEG or chitosan) to the outer surfaces of the nanoparticles can permit greatly enhanced circulation times in-vivo which in turn lead to lower amounts of drug exposure to the patient which can reduce undesirable side effects. Nanoparticles with incomplete layers can be removed by affinity purification methods to minimize mistargeting events in-vivo.

Nanoscale imaging of these manufactured, multifunctional nanoparticles can be achieved either directly through super-resolution microscopy or indirectly through single nanoparticle zeta-sizing or x-ray correlation microscopy. Since these multifunctional nanoparticles are best analyzed by technologies permitting analysis in aqueous environments, super-resolution microscopy is, in most cases, the preferred method.

KEYWORDS: medical nanodevices; nanomedicine; nanomedical drug delivery systems;

1. INTRODUCTION

Nanomedicine is a fundamentally different approach to medicine and will eventually take its place as one of the revolutionary new medical technologies of the 21st century[1]. While conventional medicine is still at organ level, it should be understood that human disease is ultimately at single cell level. This means that human disease must be thought of in very different terms. Disease is ultimately defective molecular processes at single cell level. Hence a nanomedical approach to cell medicine must involve detecting and correcting, if possible, those defective molecular mechanisms. As with many new approaches initial hype is tempered with reality as there is a mixture of successful and unsuccessful attempts to achieve the promise of the new technology. This then inevitably leads to overly severe criticisms. A proper perspective takes into account both failures and successes. As I have written previously, I believe that the reality will eventually reach a more realistic level fulfilling the promise that nanomedicine deserves[2].

Advanced drug delivery is not just bringing the drug to the surface of the cell. It must also guide that drug to the appropriate subcellular organelle and/or three-dimensional location in the cell. Accomplishing that complex targeting requires an intelligent multistep targeting method. The drug dose at single cell level must be appropriate to affect a repair of that cell. This dictates some kind of drug delivery that involves biosensing and feedback control to have the proper dose per single cell. It is continuing to guide that drug to the proper subcellular organelle or location in the cell to affect a

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repair. The drug must be at the appropriate dose. If a single cell cannot be repaired to normal function, then it should be eliminated in a way that causes minimal damage to neighboring normal cells. In this paper I describe approaches my lab has taken over a number of years to solve these and other problems in the design and construction of advanced nanomedical systems. The design of nanomedical systems that can do advanced targeted drug delivery for therapeutics and simultaneously provide non-invasive, multimodal in-vivo imaging for diagnostics, requires a well thought out strategy involving a multilayered structure and core material appropriate for a variety of imaging modalities. Many of these general approaches are outlined in two of our review articles[3, 4].

2. SYSTEM DESIGN

The most fundamental design concept is to develop advanced targeting of drugs to diseased single cells and not to normal cells. Currently most drugs are untargeted, causing exposure of all cells to drugs and at sufficient concentrations to cause toxicity to multiple organs. Typically only 1-2 percent of most drugs are actually doing anything useful in the body in terms of reaching the desired diseased cells. Meanwhile the normal cells are exposed to these drugs leading to potentially dangerous side effects. First the nanomedical delivery system should not put into the human body any chemicals that are inherently toxic. This makes special demands on the chemistry used (e.g. avoid use of toxic solvents) in the manufacture of these nanomedical systems. Another general design goal should be to reduce the total amount of drug put into the body by at least 90 percent, to simultaneous prevent loss of this drug in the body by incorporating "stealth" techniques that prevent rejection of these drug molecules by the immune system, and to have a multi-step targeting system capable of making real-time decisions in the human body using nanomedical devices with multiple layers capable of producing a "programmable nanoparticles" using the chemistry in each of multiple layers. Finally the incorporation of nanomaterials for advanced in-vivo imaging is important for determining where these nanomedical systems are actually going inside the human body and for monitoring the efficacy of the particle drug for treating the disease.

2.1 Need for safe biodegradability design

A fundamental design concept is to never put into the human body something toxic or that cannot be biodegraded to prevent toxicity. This means two things in terms of design and manufacture. Harsh chemical solvents should be avoided in the manufacture of these nanomedical systems because it is difficult or impossible to ever remove these solvents after manufacture. Second, a more subtle warning is to avoid using reagents and components that actually become toxic as they stay in the body long-term or break down into even more toxic chemical intermediates or final biodegraded products. This also includes the use of nanomaterials for multimodal, in-vivo imaging.

2.2 Need for advanced, multi-step targeting

While it may not seem intuitively obvious, advanced targeting, by necessity, involves a multi-step targeting process. First, the nanomedical system must make it successfully into the body through the blood, digestive system or skin, Then it must successful avoid clearance by the immune system through the process of opsonification and circulate long enough in the body so that only small amounts of drugs need be introduced into the body . Once the nanomedical system finds itself to the correct diseased cells it still has a complex, multi-step process to cross the cell membrane, avoid the natural single cell defense mechanisms for foreign molecules, and to target to the correct location within the single cell. It needs to be remembered that the nanomedical system is still tiny compared to the vast intracellular volume which is also filled with many subcompartments as well as biochemical pathways that can prevent delivery of the drug to the correct organelle.

2.3 Choice of targeting molecules (antibodies, peptides, aptamers) – advantages and disadvantages of each type of targeting molecule

Most people limit themselves to thinking only of antibodies as targeting molecules. This is a serious mistake for many reasons. First, antibodies are very large compared to nanomedical systems. Second, it is not possible to generate antibodies against every diseased cell receptor molecule by normal means because not all such receptor molecules are

antigenic in nature. Other alternatives to be considered are peptides and aptamers. Peptides are just much smaller subcomponents of proteins such as antibodies, and they can fold to recognize the shapes recognized by antibodies in a lock-key model without the much larger size of the antibodies. Peptides of any sequence can also be rapidly and easily manufactured. Peptides as short as six amino acids in length can not only target diseased cell surface receptors but can also perform multistep targeting within the cell[5]. Aptamers, which are DNA- or RNA-based, sequences, can also fold to perform this same diseased cell antigen recognition. Aptamers can be easily manufactures again with the exact sequence needed regardless of whether the diseased cell receptor is antigenic. If the correct sequence is not known it is possible to select the one that actually identifies the diseased cell receptor using combinatorial chemistry and high-throughput screening techniques we have reported previously[6].

2.4 Need for multilayered approach for "programmable nanoparticles" that go beyond multi-step targeting

While multistep targeting is essential, a good design should look beyond the targeting process and plan for how these nanomedical systems can perform advanced decision-making in the body. While it is unusual to think of nanomedical systems as capable of being "programmable", it should be remembered that "programming" is not just computer code. It is control of multiple processes in a multistep process that takes into account the immediate local microenvironments and can change how the nanomedical system performs based on what is sees biochemically in the microenvironment. Also it is possible for such a "programmable" nanomedical system to makes alternative decisions based on other factors such as pH, ionic strength, abundance or lack thereof of receptor molecules which may prevent it from falsely targeting to normal cells having similar receptors but also possessing molecules not found on the diseased cells. Sometimes the surface receptors of the normal and diseased cells may be similar but once the nanomedical system goes inside the cell it can strip off a layer and use its newest inside layer to see if the secondary target molecule is found within the cells. If not, the nanomedical system can biochemically "decide" to not go further, undergo planned self-destruction processes, and prevent harm to a normal cell. This means that we can create multilayers nanomedical system with a multistep decision process that can discover mistargeting problems and prevent injury to normal cells.

2.5 Novel ways to control the proper drug dose per diseased cell using the cell's own systems

Getting the proper dose of drug to single cells within the human body is a very difficult process. Bad design would be a system that is so toxic to a single targeted cell that mistargeting at the level of one mistargeting nanomedical system per cell would be lethal to that cell. If multiple nanomedical systems binding and entering and properly intracellular targeting inside single cells are needed that is a difficult proves to control and inherently involves a Poisson distribution of nanomedical systems bound to single cells that is difficult to both achieve and to control. Another totally different approach is to use the cell's own intracellular biochemical pathways, particularly those involving programmed cell death or apoptosis. The overall process is to use the nanomedical system inside the cell to trigger the programmed cell death process. Another big advantage of this approach is that the diseased cell does not die through necrosis (refs) during which the dying cell may release many of its intracellular molecules that can be toxic to neighboring cells. Instead, during apoptosis the cell breaks down all of these potentially harmful intracellular biochemicals before releasing them.

2.6 Advantages of incorporating nanomaterials in an integrated, multimodal in-vivo imaging design

It is important to build into the design of these nanomedical systems a way of tracking where they go within the human body and also to provide a way of providing information as to the efficacy of the treatment process. One way to do this is to build into the design nanomaterials that serve as in-vivo imaging contrast agents. Such nanomaterials can be multimodal in nature, simultaneously serving not only X-ray and magnetic resonance imaging contrast agents but become of the targeting process itself.

Many researchers are attracted to gold nanomaterials because they provide a simple core to build the rest of the multiple layers of the overall nanomedical system. Certainly gold provides an easy to manufacture process as well as very simple surface chemistry to easily attach biomolecules by the gold-cysteine process. While such gold-core based nanoparticles can be visualized by plasmon resonance imaging that is only useful if the body is made open either by prior surgery or accessible by endoscopy. For more general, much deeper imaging, it is better to use nanomaterials that are either X-ray contrast agents or magnetic imaging contrast agents. While the first step biochemistry is harder using superparamagnetic

iron oxide nanomaterials, such a n approach has several advantages. First, it makes the manufacture and purification of nanomedical system easier by making the nanosystems separable using a simple magnetic rather than an ultracentrifugation process. Second, such nanomedical systems can be actually concentrated initially within the human body using magnetic field to help aid in the overall targeting process and even help remove such nanomedical system mistargeted to the wrong parts of the body.

3. EXPERIMENTAL RESULTS

3.1 Production of safe, biodegradable nanoparticles

Since the ultimate design should at the very least "do no harm" these nanomedical systems should only include materials of very low toxicity and preferably be biodegradable. If non-biodegradable materials are to be used then there should be design for easy elimination of these nanomedical systems after either successful drug delivery or by assisted elimination of these systems from the body for mistargeted nanomedical systems.

Successful strategies take into account the interactions between core design, targeting strategies and the goal of non-invasive imaging, as shown in **Figure 1**.

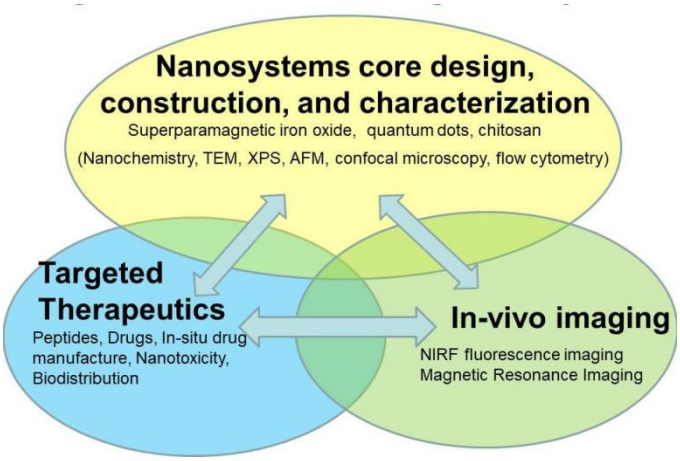

Figure 1: The design variables from three different areas are interactive. The ultimate design must be envisioned in the beginning so that each of the multistep process is done in proper order and in a way that one layer does not preclude a subsequent layer.

One good nanomaterial as a choice for the core material is superparamagnetic iron oxide. Iron oxide is easily degradable by the body. It superparamagnetic quality allows it to be initially concentrated in specific areas of the body by external magnetic field. Superparamagnetic also means that it is only temporarily magnetized when in the presence of a magnetic field and then readily dispersible to prevent formation of embolisms in the body which could lead to serious consequences. Additionally iron oxide is an X-ray contrast agent for conventional X-ray and computerized axial tomography (CAT) non-invasive in-vivo imaging. This material also is a T2 contrast agent for magnetic resonance imaging (MRI). Lastly, magnetic fields, rather than ultracentrifugation, can be used to harvest and wash these nanomedical systems as they are manufactured. A disadvantage is that the initial surface chemistry is a little more complicated. That is why many researchers use gold nanoparticles which have simple surface chemistry. Gold nanoparticles do have interesting and useful spectral properties including plasmon resonance. A summary of various types of nanomaterial cores is given in **Table 1**.

Table 1: Summary of Core Materials

Types of Core Materials and their detection

Core material	Detection
Iron oxide	x-ray, MRI, add fluorescent probe
C60 and carbon nanotubes	add fluorescent probe
Gold	surface plasmon resonance
Silver	surface plasmon resonance
Silica	add fluorescent probe
Quantum dots	intrinsic long-lifetime fluorescence
"Next generation"quantum dots	intrinsic fluorescence
Hybrid materials	mixture of detectable properties

3.2 Construction of a multilayered multistep targeting system

A multilayered approach to targeting leads to the construction of "programmable nanoparticles" [5-7]. "Programming" is not limited to computer software. Rather it means ways to control a multistep process in a controlled fashion. The goal of this approach is to use each layer of the nanomedical system to accomplish one or more tasks in the multistep process of targeted drug delivery. Layers can be peeled back by a variety of factors including heat, pH, ionic strength, and activation upon binding to a specific targeted molecule, as well as use of external forces to activate these systems at a specific location and time. How a multilayered approach leads to multistep targeting becomes obvious in **Figure 2**.

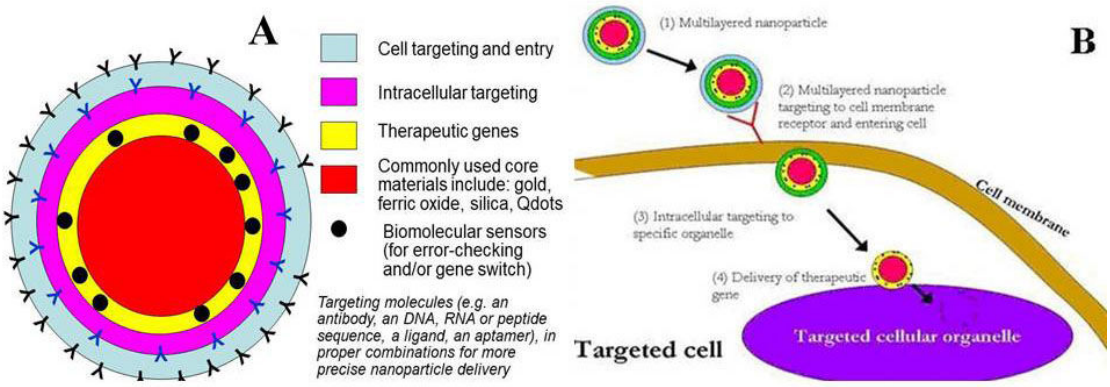

Figure 2: A multistep targeting strategy dictates a multilayered construction design. Each layer is responsible for one or more steps in the targeting process.

3.3 Choice of targeting molecules

While antibodies can certainly be used to target these nanomedical systems, it should be remembered that antibodies are relatively large entities in the nanoscale world. Since nanomedical systems are quite small (typically 50 – 150 nm in diameter), the surface area is quite limited making attachment of many antibodies a challenge. Fab and Fab2 fragments of antibodies are perhaps a better choice not only due to their smaller size but also due to their absence of the FC portion of the antibody molecule which can lead to non-specific binding by that Fc part of the molecule.

Other types of targeting molecules should be seriously considered. Relatively small peptides can have shape recognition properties for targeting. Additionally these peptides may also have membrane permeating properties. For example, we found one 6-amino acid peptide sequence that had shape recognition for a biomarker found on human breast cancer cells. It also had membrane permeating properties as well as intracellular targeting[5] as shown in **Figure 3**. When one looks at more sensitive single cell toxicity assays such as use of dihydroethidium, as shown in Figure 3D, one sees that quantum dots can produce oxidative stress damage in single cells[8].

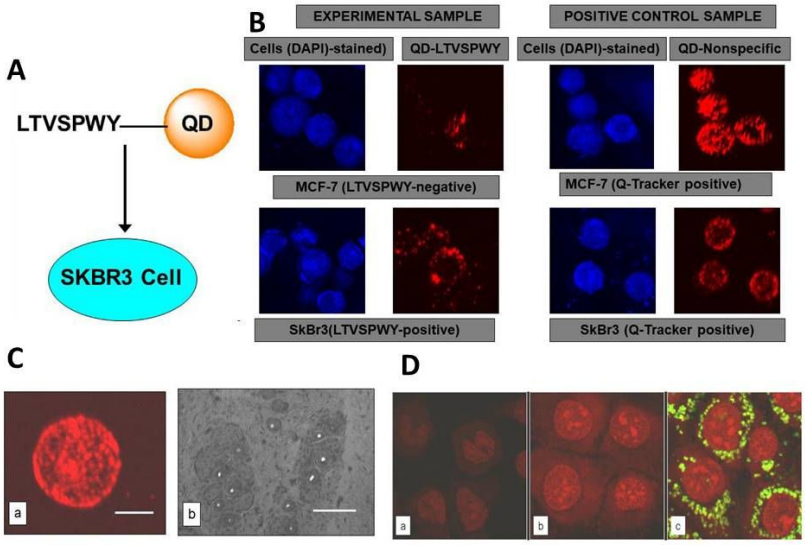

Figure 3: The 7 amino acid sequence shown in Figure A will not only guide a quantum dot nanoparticle to a human SKBr3 breast cancer cell, but also pull it through the cell membrane.

Aptamers made from DNA and RNA precursors can not only can have targeting properties but also have useful properties due to their PCR amplification properties and hybridization properties. We have previously shown that aptamer targeting molecules can be rapidly generated and screened using combinatorial chemistry and high-speed flow cytometry/cell sorting methods[9-11]. Aptamers can be generated to recognize virtually any epitope regardless of whether it is immunogenic enough to produce antibodies.

It is worth noting that DNA sequences in aptamers can also information coded into each nanoparticle permitting the use of multiple simultaneous experiments and controls in the same animals. It is difficult to find nanoparticles in large areas of tissues. However a "nanobarcoding" concept can allow for in-situ PCR amplification of amplicon sequences within single cells and permit their ready detection by imaging[12-14], as shown in **Figure 4**.

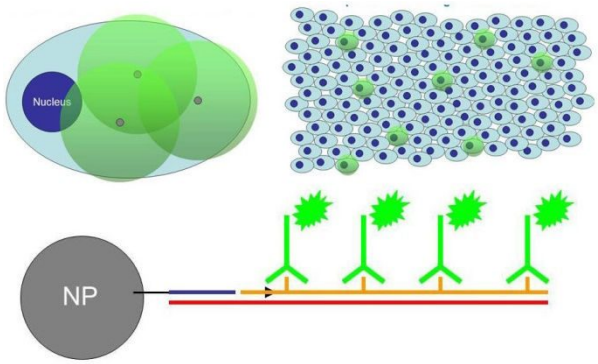

Figure 4: If a nucleotide sequence is properly tethered to a nanoparticle then that sequence can be amplified by PCR to produce fluorescent amplicons that can partially leak out of the cell to form a larger area fluorescent spot that can be easily detected in an imaging system so that cells containing these nanoparticles can be rapidly located and subsequently analyzed.

3.4 Drug dosing approaches

Single cell drug delivery in nanomedicine requires some re-thinking about drug dosing. If the aim is to deliver drugs exclusively, or at least preferentially, to single cells then one needs to think about how best to eliminate diseased cells and not cause injury to healthy cells. Violent destruction of diseased cells can lead to release of toxic molecules such as hydrogen peroxide normally housed safely within vacuoles within the cells. Controlling the dose to single cells by nanomedical systems leads to the question of how many nanomedical systems it should take to eliminate a diseased cell. The more nanomedical systems required the more chance for mistargeting and side-effect problems. Ideally one would hope to use a single nanomedical system to trigger the cell's own apoptosis (programmed cell death) process, whereby the cell breaks itself down and eliminates most of the harmful molecules so that the health of nearby normal cells are not jeopardized.

One can also manufacture drugs within a diseased single cell using a feedback loop controlled drug that produces just the right amount of drug to accomplish the task. Nanomedicine using a biosensor-controlled feedback system can give controlled drug delivery at the precise dose. Most drug delivery currently an exponential of bi-exponential decay process with approximately 2 percent effective uptake by diseased cells and massive over-exposure of normal cells leading to unpleasant side effects in the patient. Such untargeted drug delivery is costly and can be counter-productive. Untargeted drug delivery is not only wasteful but when the drug dose is far above the optimal dose it can actually cause harm, whereas when it is far below the optimal dose it may have no therapeutic effect. Its exponential decay structure insures that it will be present and useful for only a very short period before it is eliminate d from the body. A good targeted delivery system should see to reduce the amount of drug required for effective treatment by more than 90 percent. More modern "time release" delivery systems use coatings to get a saw-toothed type decay structure which is closer to the optimal amount. However, biosensor feedback control system in nanomedical systems can be designed to release much closer to the optimal drug release rate and minimize over-exposure harm of nearby normal cells. Concepts of drug dosing are shown in **Figure 5**.

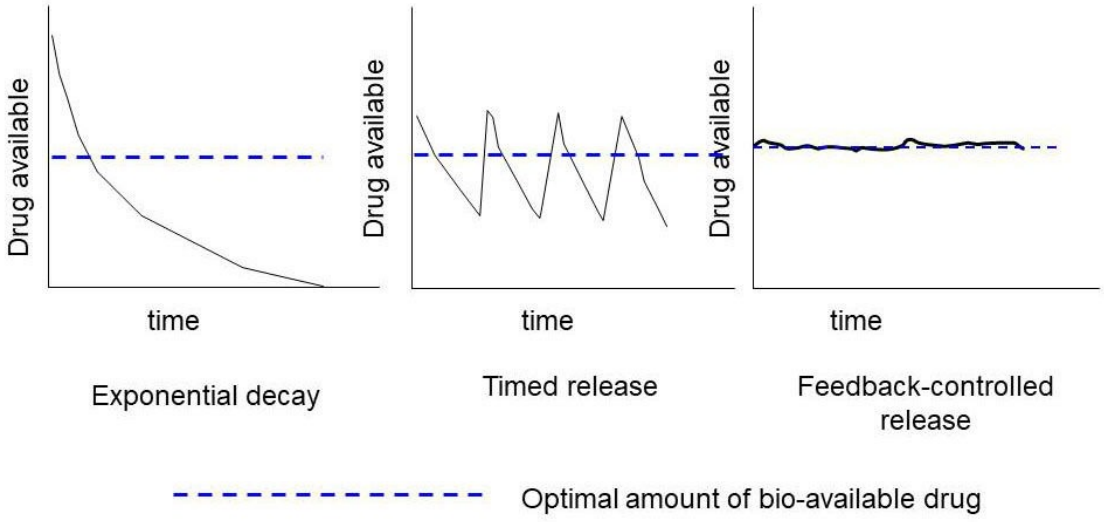

Figure 5: While the saw-toothed decay of timed release is more effective than conventional exponential decay it is still far from the optimal dosing that can be achieved by feedback controlled drug release that can be built into nanomedical systems.

Gene delivery is very similar to drug delivery and can also be used to introduce genes to be expressed in the cytoplasm of single cells under biosensor feedback control as we have previously reported[15-19], as shown in **Figure 6**.

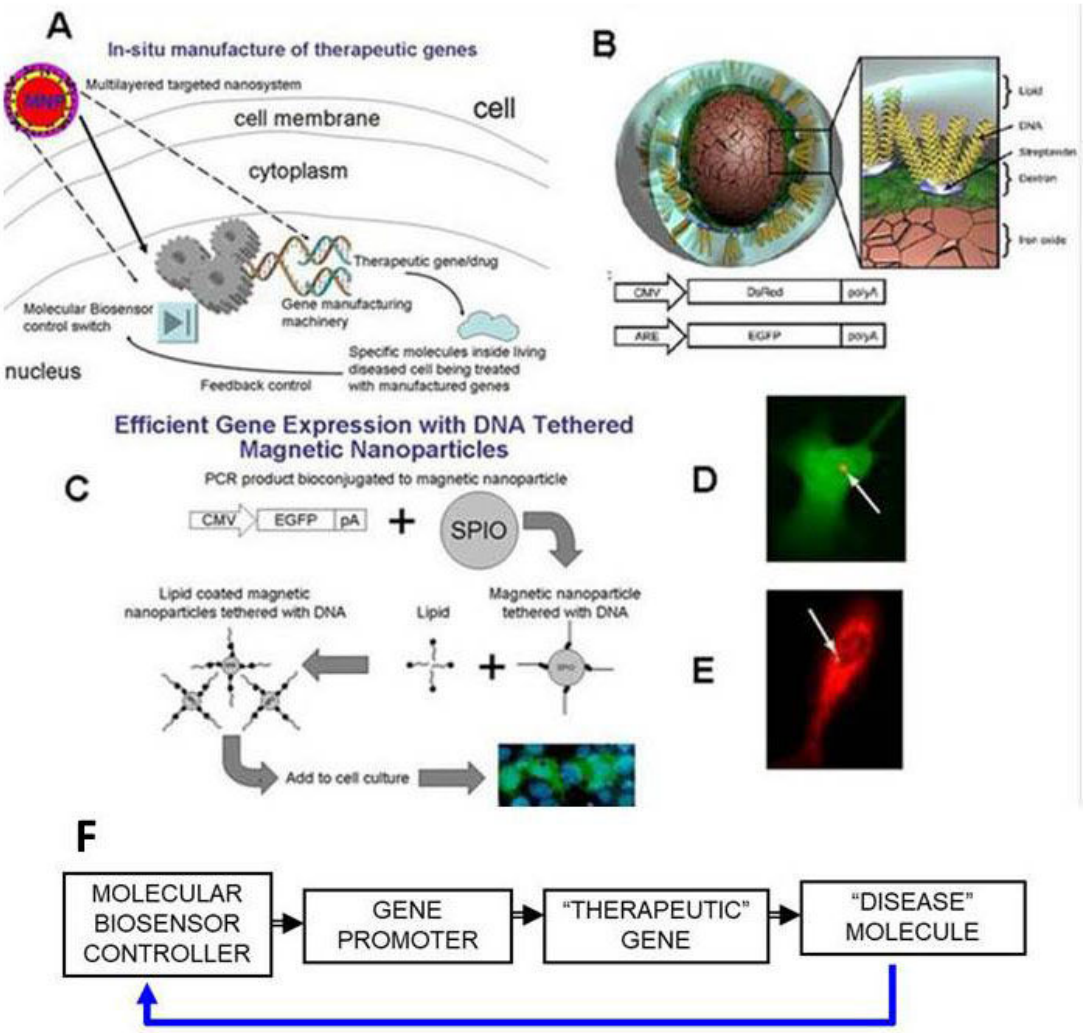

Figure 6: (A) The nanoparticle delivery system delivers the therapeutic gene template which uses the host cell machinery and local materials to manufacture therapeutic gene sequences that are expressed under biosensor-controlled delivery, **(B)** The layered anatomy of a lipid coated DNA tethered nanoparticle. **(C)** Lipid-coated nanocrystal transfected human retinal epithelium cells. Cells were cultured with lipid-coated nanocrystals tethered to either **(D)** EGFP (green) or **(E)** DsRed (red) for 48 h or 10 days, respectively to simultaneously visualize nanocrystals and tethered fluorescent gene expression. The nanocrystals are marked by white arrows and nanocrystal aggregate is marked with a white arrowhead. The general concept is to use feedback loop control system consisting of a molecular biosensor controller upstream from a gene promoter to produce a potentially therapeutic gene in the proper dose for treating disease at single cell level.

3.6 Multimodal imaging designs

Proper choice of nanomaterials for a nanoparticle core, allows for the possibility of one or more multimodal imaging modalities. Our use of superparamagnetic iron oxide cores allowed for X-ray and magnetic resonance imaging[20-23]. Attachment of fluorescent molecules allows for confocal and super-resolution imaging at single cells level. Although electron microscopy can be used, its requirement for vacuum conditions and cell fixation tend to introduce artifacts since the cells are no longer in a natural water environment. Super-resolution imaging allows for imaging of single cells and nanoparticles under biological conditions. Magnetic cores also allow for targeting and therapeutic actions (e.g. hyperthermia at single cell level) as shown in **Figure 7**.

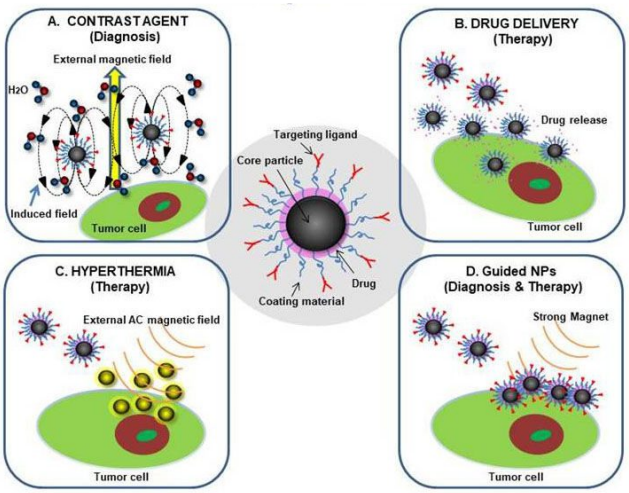

Figure 7: **(A)** Magnetic nanoparticles as MRI contrast agents, **(B)** External magnetic fields can be used to release drugs within nanomedical systems in the body, **(C)** Applying an oscillating magnetic field to magnetic nanoparticles can produce heat which provides for hyperthermic therapy at the single cell level, **(D)** External magnetic fields can help concentrate magnetic nanoparticles within the body.

4. CONCLUSIONS

Proper design of smart targeted nanoparticles for advanced drug delivery should follow an intelligent inside-to-outside multilayered construction. All materials should be biodegradable and avoid the use of harsh organic solvents during the manufacturing process. The choice of nanomaterials for the core layer dictates both the multimodal imaging capability as well as the initial bonding chemistry required for addition of subsequent layers. Drug molecules can be added in multiple steps depending on the pre-existing chemistry to allow for appropriate targeted release inside single cells. Intracellular targeting should precede drug delivery because it is important to release and target these drugs to the proper organelle or location within the single cell. Outermost covering layers should include stealth molecules that help the nanoparticle escape elimination by the body's immune system. Molecules should also be included in the outer layers to permit drug uptake wither through receptor internalization or by use of membrane-permeating peptides. The outermost layer should contain molecules which target diseased cells at the single cell level.

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REFERENCES

[1] Leary, J.F., "Nanotechnology: What is it and why is small so big," Canadian Journal of Ophthalmology 45(5), 449-456 (2010).
[2] Leary, J.F., "Nanomedicine – Reality will trump hype!," J Nanomedicine Biotherapeutic Discovery 4, e125 (2013).
[3] Seale, M-M, Leary, J.F., "Nanobiosystems," WIREs (Wiley Interdisciplinary Reviews) Nanomed Nanobiotechnol 1, 553–567 (2009).
[4] Key, J., Leary, J.F., "Review: Nanoparticles for multimodal imaging in nanomedicine," International Journal of Nanomedicine 9, 711–726 (2014).

[5] Haglund, E.M., Seale-Goldsmith, M-M, Dhawan, D., Stewart, J., Ramos-Vara, J., Cooper, C.L., Reece, L.M., Husk, T., Bergstrom, D., Knapp, D., Leary, J.F., "Peptide targeting of quantum dots to human breast cancer cells," Proc. SPIE 6866, 1–8 (2008).
[6] Seale, M., Haglund, E., Cooper, C.L., Reece, L.M., Leary, J.F., "Design of programmable multilayered nanoparticles with in situ manufacture of therapeutic genes for nanomedicine," Proc. SPIE 6430, 1-7 (2007).
[7] Seale, M-M., Zemlyanov, D., Haglund, E., Prow, T.W., Cooper, C.L., Reece, L.M., Leary, J.F., "Multi-functional nanoparticles for drug/gene delivery in nanomedicine," Proc. SPIE 6447, 1–9 (2007).
[8] Eustaquio, T., Leary, J.F., "Single-cell Nanotoxicity Assays of Superparamagnetic iron oxide nanoparticles (SPIONs)," Nanotoxicity, Editor: Joshua J. Reineke, Humana Press (ISBN: 9781627030014), (2012).
[9] Yang, X., Bassett, S.E., Li, X., Luxon, B.A., Herzog, N.K., Shope, R.E., Aronson, J., Prow, T.W., Leary, J.F., Kirby, R., Ellington, A.D., Gorenstein, D.G., "Construction and selection of bead bound combinatorial oligonucleoside phosphorothioate and phosphorodithioate aptamer libraries designed for rapid PCR-based sequencing," Nucleic Acids Research 30, 1-8 (2003).
[10] Yang, X., Li, X., Prow, T.W. Reece, L.M. ,Bassett, S.E., Luxon, B.A., Herzog, N.K., Aronson, J., Shope, R.E., Leary, J.F., Gorenstein, D.G., "Immunofluorescence Assay and Flow Cytometric Selection of Bead Bound Aptamers," Nucleic Acids Research 31(10), 1-8 (2003).
[11] Leary, J.F., Reece, L.M., Yang, X., Gorenstein, D.G., "High-Throughput Flow Cytometric Screening of Combinatorial Chemistry Bead Libraries for Proteomics and Drug Discovery," Proc. SPIE 5692, 216–223 (2005).
[12] Eustaquio, T., Leary, J.F., "Nanobarcoding: a novel method of single nanoparticle detection in cells and tissues for nanomedical biodistribution studies" Proc. SPIE 8099, 1-13 (2011).
[13] Eustaquio, T., Cooper, C.L., Leary, J.F., "Single-cell imaging detection of nanobarcoded nanoparticle biodistributions in tissues for nanomedicine," Proc. SPIE 7910, 1-11 (2011).
[14] Eustaquio, T., Leary, J.F., "Nanobarcoding: detecting nanoparticles in biological samples using in situ polymerase chain reaction," International Journal of Nanomedicine 7, 5625–5639 (2012).
[15] Prow, T.W., Kotov, N.A., Lvov, Y.M., Rijnbrand, R., Leary, J.F., "Nanoparticles, Molecular Biosensors, and Multispectral Confocal Microscopy," Journal of Molecular Histology 35(6), 555-564 (2004).
[16] 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., "Nanomedicine – nanoparticles, molecular biosensors and targeted gene/drug delivery for combined single-cell diagnostics and therapeutics," Proc. SPIE 5318, 1-11 (2004).
[17] Prow, T.W., Rose, W.A., Wang, N.A., Reece, L.M., Lvov, Y., Leary, J.F., "Biosensor-controlled Gene Therapy/Drug Delivery with nanoparticles for Nanomedicine," Proc. SPIE 5692, 199-208 (2005).
[18] Prow, T.W., Smith, J.N., Grebe, R., Salazar, J.H., Wang, N., Kotov, N., Lutty, G., Leary, J.F., "Construction, Gene Delivery, and Expression of DNA Tethered Nanoparticles," Molecular Vision 12, 606-615 (2006).
[19] Prow, T.W., Grebe, R., Merges, C., Smith, J.N., McLeod, D.S., Leary, J.F., Gerard A. Lutty, G.A., "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 (2006).
[20] Key, J., Cooper, C., Kim, A.Y., Dhawan, D., Knapp, D.W., Kim, K., Park, J.H., Choi, K., Kwon, I.C., Park, K., Leary, J.F., "In Vivo nirf and mr dual modality imaging using glycolchitosan nanoparticles," J Controlled Release 163, 249-255 (2012).
[21] Key, J., Kim, K., Dhawan, D., Knapp, D.W., Kwon, I.C., Choi, K., Leary, J.F., "Dual-modality *in vivo* imaging for MRI detection of tumors and NIRF-guided surgery using multi-component nanoparticles" Proc. SPIE 7908, 1-8 (2011).
[22] Key, J., Dhawan, D., Knapp, D.K., Kim, K., Kwon, I.C., Choi, K., Leary, J.F., "Multimodal in-vivo MRI and NIRF imaging of bladder tumors using peptide conjugated glycol chitosan nanoparticles," Proc. SPIE 8225, 1-8 (2012).
[23] Key, J., Dhawan, D., Knapp, D.K., Kim, K., Kwon, I.C., Choi, K., Leary, J.F., "Design of peptide-conjugated glycol chitosan nanoparticles for near-infrared fluorescent (NIRF) in vivo imaging of bladder tumors," Proc. SPIE 8233, 1-10 (2012).