New Applications of Nanopartcles in Cardiovascular Imaging

Rakesh R Sharma*
*FAMU-FSU College of Engineering, Tallahassee FL 32310

Presenter: Professor Ching Jen Chen*

ABSTRACT

Nanotechnology involves working at the atomic, molecular, and macromolecular levels including imaging. Recently, four areas have emerged in cardiovascular imaging: 1.Targeted therapeutics to deliver cardioprotective drugs where they are needed; 2. Myocardial tissue engineering to replace defective valves, damaged heart muscle, clogged blood vessels; 3. Molecular imaging using "smart" imaging agents in targeted therapeutics and imaging; 4.Biosensors and myocardial diagnostics. Several approaches of nanoparticles i.e. dendrimers, liposomes, polymer delivery molecules, cantilevers, nanoscaffolds, nanofibers are potential candidates in cardiac visualization. The extracellular matrix plays significant role by chemokines, cytokines and growth factors. The limitations of these emerging techniques and a new possibility of MRI visualization of mice cardiac atheroma by superparamagnetic iron-oxide gadolinium-apoferritin (SPIOA), myoglobin (SPIOM), for targeted functional and molecular imaging of atherosclerosis are main focus of this paper. These emerging techniques provide opportunity of tracking functional and structural changes in myocardium and heart tissue.

Keywords: **MRI, molecular imaging, contrast agents, atherosclerosis, thrombosis, mice**

1 INTRODUCTION

Nanoparticles are very small sized molecules in the range of 10-100 nm. These particles penetrate across the cells as payload delivery system, biomarker, targeted path tracking biosensors. In cardiovascular imaging, these have significant role for imaging and drug delivery to identify thrombi, clots, apoptosis-linked gene expressed can be visualized by nanoparticles. They have major cardiovascular applications in: 1. Targeted therapeutics: delivering drugs where they are needed; 2. Tissue engineering: building new tissues to replace defective valves, damaged heart muscle, clogged blood vessels, and so forth; 3. Molecular imaging: using "smart" imaging agents that identify disease more specifically; and 4. Biosensors and diagnostics: improved diagnostic devices for the lab, and implantable sensors to detect problems inside the heart and vessels.

From the standpoint of capitalizing the nanoparticles, different applications of their use in industry and clinics are reviewed.

1.1 Targeted therapeutics and molecular imaging

Nanoparticles with a payload, either an imaging or a drug or a combination of both, can be used for delivery. The nanoparticle also has a targeting system. Typically, they act as a molecular zip code, for example, an antibody that binds to specific cells in the body. The targeting system allows for the precise delivery of the drug payload in the body. The drug is encapsulated in nanospheres and nanocapsules inside a shell such as polymeric micelles have a hydrophobic core and hydrophilic tails. Liposomes are lipid micelles and can contain a drug or imaging agent inside and antibody on the outside with polyethylene glycol coat to avoid immune system. It functions for targeting. Dendrimers are highly branched polymer particles do perform delivery, typically known as "quantum dots." These quantum dots have fluorescent properties useful for fluorescent imaging for cell tracking to assess the efficiency of heart transplantation. Recently, doxorubicin bound NK911 nanoparticles (core-shell nanoparticles of PEG-based block copolymer encapsulating doxorubicin) were reported to inhibit restenosis [1]. The nanoparticles easily permeate blood vessels and vascular injury sustains hyperpermeability. As a result nanoparticles help in preventing neointima formation and maintaining luminal area. Other benefit of nanoparticle is to decrease the toxicity of doxorubicin. Other example of targeted therapeutics is action on bacterial bioforms in the lung around myocardium. These can be targeted with inhalation of antimicrobial drugs coated with nanoparticles. Stronger or poorly soluble drugs can be encapsulated to deliver at target with controlled release. We used taxotere for cancer treatment and presumed it for prevention of stent restenosis. It was poorly soluble in solvent but its encapsulation in nanoparticles enhanced the solubility up to 40 mg/ml of the drug and allowed for sustained release over several days to monitor its chemosensitivity effect [2]. Other advancement is microlevel imaging of cells. The in vivo cell tracking by nanoparticles is restricted by poor sensitivity of imaging methods. However, the superparamagnetic iron oxide nanoparticle (CLIO-HD) can enter into lymphocytes through Tat peptide. These labeled T-lymphocytes can be detected with MRI upto the level of 10 microns. The treatment of myocardial infarction can be evaluated and optimized by tracking of labeled bone marrow cells. Recently, author proposed the possibility of nanoparticles can bind with inflammatory molecules that recognize alveolar inflammation and detect the bacteria or viruses. These nanoparticles can act as targeted imaging probes that are activated at disease sites. In cardiovascular imaging, these imaging probes can be useful. These probes get activated by enzymes responsible for remodeling of the heart (heart failure) or arteries (aneurysms).

1.2 Role in Myocardial Tissue Engineering

Extracellular signaling includes extracellular matrix, soluble signals, growth factors, cytokines, chemokines, and cell-to-cell contact affecting signal transduction pathways that regulate transcription and gene expression, tissue formation, tissue homeostasis, or tissue regeneration. Injectable self-assembling short peptides into nanofibers can be injected into myocardium to form cell-friendly micro-environments primarily endothelial cells and sarcomeric actins with slower increase in myocytes. This shows potential for tissue damage recovery in myocardial infarction. Engineering growth factors and other signals into the matrix show the potential for promoting the differentiation of the myocytes. The matrix also contains smooth muscle cells forming vessel-like structures, so there may be the potential for vascularization of the tissue.

1.3 Diagnostics by cantilever sensors

In myocardial infarction, each nanocantilever can be functionalized individually with a different antibody to detect a specific protein. So, binding of an antigen to the nanocantilever causes bending of the cantilever, this in turns deflects a laser beam and thereby provides quantitative measurements. The cantilevers can functionalize plasma antibodies to troponin and myoglobin (indicative of MI). The cantilever array can show very rapid quantitative detection of changes in the DNA sequences, multiplex protein concentration or virus infection in plasma by cantilever array coated with antibodies.

1.4 Transferrin, troponins and myoglobins

Transferrin molecules can be attached to each iron oxide particle with chemical linkers, increasing the particle's affinity for the transferrin receptor. The addition of amine groups during dextran cross-linking makes antibodies, snippets of DNA, and other compounds. This advance has opened up entirely new avenues of research. It can functionalize plasma antibodies to troponin and myoglobin (indicative of MI). Author achieved resolution up to 100 microns using myoglobin conjugates as shown in Figure 1.

1.5 Lipid coated Perfluorocarbons

Perfluorocarbon emulsion coated with a layer of lipid can home antibodies, peptides or short protein fragments and cell surface receptors more than 200 to 300 of each nanoparticle. Up to 90,000 molecules of gadolinium-DTPA can detect of the targeted cell. The perfluorocarbon nanoparticles are useful in cardiovascular imaging. The particles may be able to identify atherosclerotic condition such as fibrin in clots. A protein alpha v beta 3-integrin can detect the immature blood vessels that characterize angiogenesis. A perfluorocarbon nanoparticle can be labeled with a peptidomimetic targeted to alpha v beta 3-integrin. Combined fibrin with alpha 5 beta 3-integrin targeting can indicate inflammation, rupture.

1.6 "Smart Contrast Agents

"Smart" agents remain undetected and cage gadolinium as delivery molecule shielded with water protons. These move freely and reach to the target site where an enzyme or cellular ion releases the gadolinium. The interaction of water and gadolinium results in changes in T1 relaxation hence useful for MRI.

For example, the chelated gadolinium caged by a galactopyranose molecule is opened by β-galactosidase enzyme. Similarly, matrix metalloproteinases, kinases, β-galactosidase and intracellular calcium ions also release gadolinium indicating changes in cell signaling, regulation, and metabolism. Once the molecule changes shape, water can come in contact with gadolinium, with a detectable reduction in T1 relaxation.

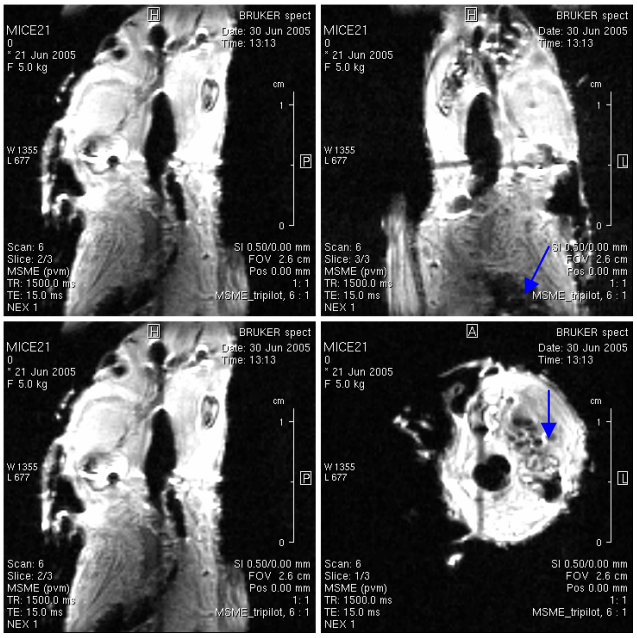

Figure 1: A mice was injected with gadolinium bound MION-myoglobin particles (courtesy of Dr Yousef Haik) through tail in vien and imaged by MRI at 500 MHz. The vascular regions showed clear wall and lumen areas as shown by arrows. Multislice-multiecho(Bruker Biospin at TE=15, TR=1500 ms gave proton density images with spatial resolution of 100 microns..

2. NEWER APPROACHES OF MOLECULAR IMAGING APPLICATIONS

2.1 Fibronectins and adhesion molecules

Selective targeting of atheromas in mice can be done using the human antibody to the ED-B domain of fibronectin. It may set the stage for antibody-based molecular imaging of

atherosclerotic plaques in the intact organism [3]. Adhesion molecules ICAM and VCAM immunoactive molecules have shown specific role in prediction of endothelial injury.

2.2 Possible Applications

Several current approaches can be useful to researchers or utilizable. They are grouped as following:

2.2.1. The specific and sensitive site-targeted contrast agents act as epitopes. Membrane phosphatidyl serine epitopes are exposed during apoptosis[4].

2.2.2. Transfection events by imaging proteins that are expressed after reporter gene transcription i.e. thymidine kinase genes as reporter construct in association with a therapeutic gene by phosphorylating certain exogenously supplied radiolabeled probes (or substrates) that are then trapped inside of cells where they can be imaged[5].

2.2.3. Biocompatible nanotechnologies provide targeted contrast agents with long circulating half-life (hours), selective binding to epitopes of interest, low background signal and prominent contrast-to-noise enhancement, acceptable toxicity profile, ease of production and clinical use, applicability with standard commercially available imaging modalities, and promise for adjunctive therapeutic delivery in drug and/or gene delivery nanosystems.

2.2.4. The contrast mechanism of nanoparticles (liposomes or emulsions)[6-8], dendrimers[9,10], viral constructs[11], buckeyballs[12], or various polymers[13,14] can hold paramagnetic or superparamagnetic metals, optically active compounds (eg, fluorescent molecules), or radionuclides. These can be detected with standard imaging equipment i.e. liquid perfluorocarbon nanoparticles [6,15,16],

2.2.5. The contrast agent can be monoclonal antibodies or their fragments, peptides, small molecule peptidomimetics, or haptamers. Dissociation constants in the nanomolar range, Multivalent binding enhance avidity and reduce "off-rates" to permit imaging at convenient times after delivery of the agent. Polyvalent binding is possible with the use of more than one ligand type per carrier, or with mixtures of ligand-carrier constructs directed at different targets. MRI and ultrasound use paramagnetic polymerized liposomes bearing antibody ligands to neovascular integrins alpha 5 beta 3 [7] Similarly, liquid perfluorocarbon nanoparticle emulsions targeted to alpha 5 beta 3 integrins permit fibrin visualization in thrombi[17] and carotid plaques ex vivo[18]. The maximum T1 per molecule binding site for complex of paramagnetic perflurocarbon agents can give sensitivity in the picomolar range of concentrations[18]. Even single cells may be imaged with such agents[19]. However, epitopes such as fibrin in thrombi can be used in targeting.

2.2.6. ultrasmall iron oxide imaging agents have potential for delineation of early atherosclerosis in experimental models by uptake of ultra-small particles of iron oxide in plaque macrophages[20].

2.2.7. "smart" agents carry gadolinium at pathologic states, or by the protein products (enzymes) of reporter genes after therapeutic transfection[21]. Cleavage of active sites on these agents exposes sequestered gadolinium atoms to free water and facilitates rapid water exchange to produce an effect on local proton relaxation, in turn enhancing image contrast.

2.2.8. Targeted perfluorocarbon nanoparticles are useful for ultrasound applications and fibrin thrombi in vivo by 2 orders of magnitude or more[6,22,23]. Additionally, targeting to vascular epitopes such as tissue factor, whose expression is induced in smooth muscle cells in vivo after angioplasty, is possible because these particles can penetrate through microfissures into the vascular media[15,24]. Reflective liposomes have also been used to specifically target endothelial integrins[8]. Other microscale systems (stabilized perfluorocarbon gas microbubbles) have been used for molecular ultrasound imaging of thrombi[25] but generally these are restricted to the vasculature in view of their size (5 μm) and their susceptibility to destruction with clinical ultrasound imaging intensities.

3 APPLICATIONS IN CARDIOVASCULAR THERAPY

3.1 The emerging use is the ability to incorporate drugs or genes into detectable site-targeted nanosystems as drug delivery and dosing. Payloads of therapeutic agents, such as genes or radionuclides, can be complexed to the carriers themselves. Drugs in the lipid coatings get trapped within the carriers themselves. In the case of ultrasound, methods for gene delivery with synthetic vectors such as microbubbles rely on mechanical stimulation of microbubbles that operate like miniature gene guns[26]. Stabilized gaseous microbubble contrast agents (5 nm in diameter) have demonstrated potential for use as transfection agents by incorporating DNA directly into the bubble shell or interior[27,28].

3.2. Focused ultrasound destroys the bubbles and releases genes at selected sites. The opportunity to confirm drug delivery and dose by imaging represents a novel feature for nanosystems that provide controlled drug release. Fortunately, the imaging signals produced by the carriers themselves, along with knowledge of the amount of the specific drug contained per particle, will allow estimation of tissue drug levels. In the case of paramagnetic nanoparticles, a range of perfluorocarbon components can be detected and quantified by NMR spectral analysis to permit quantitative differentiation among a variety of simultaneously targeted molecules[19]. Recently, targeted "quantum dot" nanosystems might be useful in imaging[29].

Finally, major focus is to utilize: 1. The molecular imaging and the development of biocompatible nanotechnologies. 2. Complete characterization of the human genome and the subsequent emergence of proteomics, 3. useful molecular targets.

4 CONCLUSION

Nanotechnology has enormous potential for diagnosing and treating heart. The major cardiovascular available applications are monocrystalline iron oxide nanoparticles, or MIONs wrapped in dextran, cross-linked iron oxide nanoparticles, or CLIOs, transferrin receptors. CLIOs have been attached to multiple sets of DNA building blocks, or oligonucleotides, messenger RNA-targeted CLIOs. Smart imaging agents, delivery systems, ferritin, integrins in targeted imaging of arteries and heart are in development stage.

REFERENCES

1. Uwatoku T, Shimokawa H, Abe K, Matsumoto Y, Hattori T, Oi K, Matsuda T, Kataoka K, Takeshita A. Circ Res. 2003 Apr 18;92(7):e62-9.

2. Sharma R, Kline RP, Wu EX, Katz JK. Cancer Cell Int. 2005 Aug 3;5:26.

3. Christian M. Matter, Pia K. Schuler, Patrizia Alessi, Patricia Meier, Romeo Ricci, Dongming Zhang, Cornelia Halin, Patrizia Castellani, Luciano Zardi, Christoph K. Hofer, Matteo Montani, Dario Neri, and Thomas F. Lüscher Circ. Res. 95: 1225 -1233.

4. Blankenberg FG, Strauss HW. *Cardiol Clin.* 2001; 19: 165–172.

5. Bengel FM, Anton M, Avril N*Circulation.* 2000; 102: 948–950.

6. Lanza GM, Wallace KD, Scott MJ *Circulation.* 1996; 95: 3334–3340.

7. Sipkins DA, Cheresh DA, Kazemi MR *Nat Med.* 1998; 4: 623–626.

8. Demos S, Alkan-Onyuksel H, Kane B *J Am Coll Cardiol.* 1999; 33: 867–875.

9. Wiener C, Brechbiel MW, Brothers H, et al. *Magn Reson Med.* 1994; 31: 1–8.

10. Bryant LHJ, Brechbiel MW, Wu C, et al. *J Magn Reson Med.* 1999; 9: 348–352.

11. Bulte JW, Zywicke H, Frank JA, et al. *Molecular Imaging.* 2001; 1: 225.

12. Noon WH, Kong Y, Ma J. *Proc Natl Acad Sci U S A.* 2002; 99: 6466–6470.

13. Roessler BJ, Bielinska AU, Janczak K, et al. *Biochem Biophys Res Commun.* 2001; 283: 124–129.

14. Ma Q, Remsen EE, Kowalewski T, et al. *J Am Chem Soc.* 2001; 123: 4627–4628.

15. Lanza GM, Abendschein DR, Hall CH, et al. *J Am Soc Echocardiogr.* 2000; 13: 608–614.

16. Yu X, Song S-K, Chen J, et al. *Magn Reson Med.* 2000; 44: 867–872.

17. Anderson SA, Rader RK, Westlin WF, et al. *Magn Reson Med.* 2000; 44: 433–439.

18. Flacke S, Fischer S, Scott MJ, et al. *Circulation.* 2001; 104: 1280–1285.

19. Lanza GM, Yu X, Winter PM, et al. *Circulation.* 2002; 106: 2842–2847.

20. Ruehm SG, Corot C, Vogt P, et al. *Circulation.* 2001; 103: 415–422.

21. Louie AY, Huber MM, Ahrens ET, et al. *Nat Biotechnol.* 2000; 18: 321–325.

22. Bulte JW, Douglas T, Witwer B, et al. *Nat Biotechnol.* 2001; 19: 1141–1147.

23. Lanza GM, Trousil RL, Wallace KD, et al. *J Acoust Soc Am.* 1998; 104: 3665–3672.

24. Lanza GM, Abendschein DR, Hall CH, et al. *Invest Radiol.* 2000; 35: 227–234.

25. Takeuchi M, Ogunyankin K, Pandian N, et al. *J Am Soc Echocardiogr.* 1999; 12: 1015–1021.

26. Song J, Chappell JC, Qi M, et al. *J Am Coll Cardiol.* 2002; 39: 726–731.

27. Unger EC, McCreery TP, Sweitzer RH. *Invest Radiol.* 1997; 32: 723–727.

28. Shohet R, Chen S, Zhou YT, et al. Echocardiographic destruction of albumin microbubbles directs gene delivery to the myocardium. *Circulation.* 2000; 101: 2554–2556.

29. Åkerman ME, Chan WCW, Laakkonen P, et al. *Proc Nat Acad Sci U S A.* 2002; 99: 12617–12621.