Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Nanotechnology in BioMedical Applications

Bruce A. Holm [a], Earl J. Bergey [a], Tapas De [a], David J. Rodman [a], Rakesh Kapoor [a], Laurent Levy [a], Christopher S. Friend [a] & Paras N. Prasad [a]

[a] School of Medicine and Biomedical Sciences and the Institute for Lasers, Photonics and Biophotonics, University at Buffalo State University of New York, Buffalo, New York, 14260-3000

Version of record first published: 29 Oct 2010

To cite this article: Bruce A. Holm, Earl J. Bergey, Tapas De, David J. Rodman, Rakesh Kapoor, Laurent Levy, Christopher S. Friend & Paras N. Prasad (2002): Nanotechnology in BioMedical Applications, Molecular Crystals and Liquid Crystals, 374:1, 589-598

To link to this article: http://dx.doi.org/10.1080/713738279

Mol. Cryst. Liq. Cryst., Vol. 374, pp. 589-598

1058-725X/02 $12.00 + .00

Nanotechnology in BioMedical Applications

BRUCE A. HOLM, EARL J. BERGEY, TAPAS DE, DAVID J. RODMAN, RAKESH KAPOOR, LAURENT LEVY, CHRISTOPHER S. FRIEND and PARAS N. PRASAD

School of Medicine and Biomedical Sciences and the Institute for Lasers, Photonics and Biophotonics University at Buffalo State University of New York, Buffalo, New York 14260-3000

School of Medicine and Biomedical Sciences and the Institute for Lasers, Photonics and Biophotonics University at Buffalo State University of New York , Buffalo, New York 14260-3000

Nanotechnology is rapidly expanding into the biomedical field. At the Institute for Lasers, Photonics and Biophotonics, research in nanotechnology has focused on the development of surface functionalized nanoparticles for diagnostics and targetted therapy. This technology provides a platform for the development of new imaging, diagnostic and therapeutic modalities. For bioimaging and diagnostics, nanoparticles are fabricated containing rare-earth ions which exhibit two-photon, anti-stokes luminescence by frequency up-converting infrared to visible light. We have successfully prepared the phosphor containing nanoparticles having a size ~25 nm with a silica shell around it which helps in aqueous dispersabilty, inhibits water quenching with nanophosphors and allows functionalization for covalent binding of bioprobes for targeting. The coupling of specific peptides, proteins or nucleic acid sequences to the silica shell will allow for the selective detection of biological entities and will have applications in bioimaging, flow cytometry, ELISA and DNA/RNA hybridization systems. Therapeutically, this nanoparticle platform was used to develop "nanoclinics" which can selectively target specific cancer cells. Our prototypic nanoclinic utilizes magnetocytolysis to effect distruction of luteinizing hormone-releasing hormone (LH-RH) receptor positive

cancers. Bioadhesive nanoparticles can also provide a novel mechanism for controlled drug delivery. Presently only 1-3 % of the topically administered Brimonidine penetrates the cornea and reaches intraocular tissues. A nanoparticle of the bioadhesive polymer, polyacrylic acid (PAA), incorporating the ocular drug, Brimonidine, has been prepared. This formulation would prolong drug contact in the precorneal area resulting in reduced drug cost to the patient.

Keywords – Nanoparticles; upconversion; drug delivery; PDT; cancer

INTRODUCTION

Nanomedical technology is opening new avenues in bioimaging, medical diagnostics and disease therapy. Examples of this technology include nanocrystal fluorophores for imaging, nanoscale biodevices as sensors and nanobubbles for drugs delivery [1-4] In cellular research, the PEBBLE nanotechnology of Kopelman and coworkers enables for the optical measurement of changes in intracellular calcium levels and pH [5,6]. Imaging in biological systems has become one of the most relied upon tools used by health care professionals in the diagnosis and treatment of human disease. The evolution of this technology from simple transillumination, to plain radiography, angiography, ultrasonography, computer assisted tomography, nuclear medicine, magnetic resonance imaging and finally digital signal processing of these data, have lead to revolutionary improvements in the quality of health care available in our society today. The quest now is for improved image resolution and resultant diagnostic abilities, less invasive and lower risk studies, use of non-ionizing photonic radiation, and decreased use of radioactive imaging.

UPCONVERTING NANOPARTICLES AND IMAGING

Nanotechnology will bring us next generation of materials and devices for the new millennium. Nanosize control of the local structure provides an opportunity to manipulate the local excitation dynamics to judiciously enhance a desired photonic function. We have developed multiphasic nanocomposite materials consisting of many phases separated only on nanoscale, much smaller than the wavelength of light. The bulk material is, therefore, of high optical quality and yet each

nanoscale phase can provide a specific function, thus bringing multifunctionality.

Nanoparticles composed of rare earth elements have been prepared from functionalized surfactants by dissolving appropriate amounts of the dried functionalized surfactant in isooctane [7]. Particles of varying sizes can be synthesized by altering the water to surfactant ratio, known as the W_o. Encapsulation and functionization for ligand coupling of nanophosphors with SiO_2 was accomplished by adding sodium silicate to form a silica shell around the nanoparticles. The spacer molecules were attached by reacting with the silica surface with 3-(triethoxylsilanylpropyl-carbamoyl)-butyric acid, 3-aminopropyltrimethoxysilane or trimethoxysilyl propyl urea. The targeting ligand was coupled to the COOH groups. The same procedure is followed to synthesize Er/Yb co-doped Y_2O_3 and Tm/Yb co-doped Y_2O_3. The upconverted emission from the particles for Er/Yb co-doped Y_2O_3 is red (640 nm), green for Er-doped Y_2O_3 (550 nm) and blue for Tm/Yb co-doped Y_2O_3 (480). Currently, a 3:1 ratio of Yb to Tm or Er give optimal spectral emission. (Er - erbium, Yb - ytterbium, Tm - thulium, Y - yttrium, Y_2O_3 - yttrium oxide).

Initial studies utilizing these nanophosphors as biological reporters are illustrated in Figure 1. To demonstrate the potential of these nanophosphors, a human oral epthelial cell line, KB was incubated with silica coated upconverting nanoparticles and visualized using a BioRad Confocal microscope (model 1024) equipped with 1 watt fiber coupled diode laser emitting at 980 nm. However, any microscope can be modified to visualize these upconverting nanoparticles. As can be seen in Figure 1B, excitation of these nanoparticles bound to living cells results in no visible autofluoresence. The result is an extremely high signal to noise ratio. This would allow for the use of this technology in detection of low numbers of biological entities on the surface of cells. The selective targeting of upconverting nanoparticles can be used to detect cell surface receptors/antigens. Avidin-labeled nanoparticles can be utilized to detect biotin-labeled reagents such as antibodies to known receptors. Attachment of ligands to nanoparticles can specfically detect active receptors. In tissue sections, visualization of cytoskeleton structures, internal organelles can be accomplished with biotinylated antibodies and phalloidin-biotin utilizing avidin coupled to nanoparticles. Nuclear staining can accomplished using trimethoxysilyl propyl urea treated nanoparticles. Multiplexing of colors can be accomplished using different targeting systems with different emission spectra.

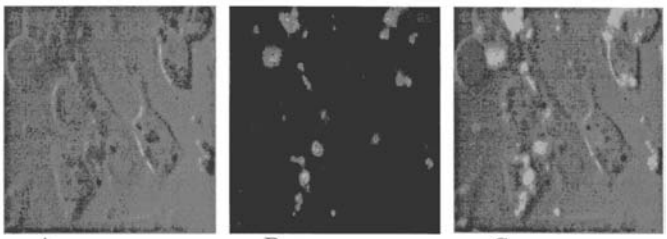

FIGURE 1: Oral epithelial carcincoma cells (KB) were incubated with nanoparticles consisting of Er-doped Y_2O_3 nanophosphors in silica shell. Plate A represents the light transmission image of the KB cells. Plate B is the fluorescent emission after excitation with 980 nm. Plate C is the composite of Plates A and B. All images collected on a Nikon Diaphot inverted microscope converted to excite the nanophosphors with a 980 diode laser.

For example, avidin labeled Er:Y_2O_3 (green) nanoparticles can be used in conjunction with Protein A labeled Er/Yb:Y_2O_3 (red) nanoparticles and urea functionalized Tm/Yb:Y_2O_3 (blue) nanoparticles. In this manner, ability to visualize and collect data on one wavelength scanning can be assessed using cells and tissues employing a wide variety of instrumentation (ie. confocal and standard microscopy, flow cytometry, and ELISA plate readers) commonly used in studying biological events. These instruments can be modified to enable them to utilized these nanophosphors.

NANOTECHNOLOGY AND DRUG DELIVERY

Nanotechnology provides another dimension to drug delivery systems. Targeted and more efficient delivery of molecules for therapeutic applications is a priority for pharmaceutical industry. Effective strategies should reduce the required dose, increase safety and improve efficacy by focusing molecules at the desired site of action. Mucosal routes of drug delivery offer a number of logistical and biological advantages. Mucoadhesion is an important feature of topical sustained-release dosage forms, which may increase the duration or

intensity of contact between drug molecules and the epithelium and thus providing some control over the site and duration of drug release [8-10]. However, the use of these sites requires restricting conditions where the mucosal layer is neither disrupted nor irritated by the agent. For ocular drug delivery, one of the major problems is to provide and maintain an adequate concentration of the drug in the precorneal area over a duration of up to several hours. Topical drop-wise administration of ophthalmic drugs in aqueous solutions usually results in extensive drug loss, mainly due to tear fluid and eye-lid dynamics which cause rapid removal of the solution from the eye [11]. Only 1-3 % of the administered dose penetrates the cornea and reaches intraocular tissues [12].

Nanoparticles also have the capacity to increase the ocular therapeutic effect of associated drugs [13,14]. Research at the Institute has investigated the behavior of polyacrylic acid (PAA) nanoparticles loading for the β_2-adrenoceptor agonist and an anti-glaucoma agent, brimonidine, as well as the *in vitro* release of the drug have been investigated. Polyacrylic acid nanoparticles were prepared using a reverse microemulsion polymerization technique and were found to be ~ 50 nm in size. The loading of drug increases almost linearly as the concentration of the drug in the loading solution increases. The loading efficiency is between 80-85% (w/w).

The *in vitro* kinetic release profiles of brimonidine from the particles were compared with the dialysis of a simple brimonidine solution as a control, dialysing against either deionized water or phosphate buffered saline (pH = 7.4). The results demonstrated that the release of brimonidine into either water or phosphate buffered saline was considerably slower from the PAA nanoparticles than from a free brimonidine. Dialysis against phosphate buffered saline resulted in faster release of brimonidine from the nanoparticle solution. This observation supports the hypothesis that the drug cation may be binding with the anionic carboxylate groups of the particles when the particles are suspended in deionized water.

In an *in vitro* preliminary biocompatibility evaluation of PAA nanoparticle hyrogels with Caco-2 cells (adenocarcinoma). Monolayers of differentiated Caco-2 cells show morphological and biochemical similarity to normal intestinal enterocyte. They develop effective tight junctions [15,16] and often serve as a model to study absorption of drugs [17]. Experiments into the release of brimonidine across a Caco-2 cell monolayer grown on Anopore membrane demonstrated that release of brimonidine from the PAA nanoparticles formulation is slower than that from alphagan formulation presently used in ocular therapy. As

PAA is bioadhesive in nature, brimonidine associated with PAA nanoparticle remains attached to the cell surface for an extended period of time and thus show slower release of the drug.

Confocal laser scanning microscopy was used to study the interaction of fluorescently labelled PAA nanoparticles with Caco-2. Figure 2 shows Caco-2 cells where the nanoparticles were bound. The surface of the cell cluster (Figure 3A) and the intercellular spaces (Figure 2b-f) were significantly stained suggesting entry of the particles through the paracellular spaces. From this location, slow release of the absorbed drug would be most effective.

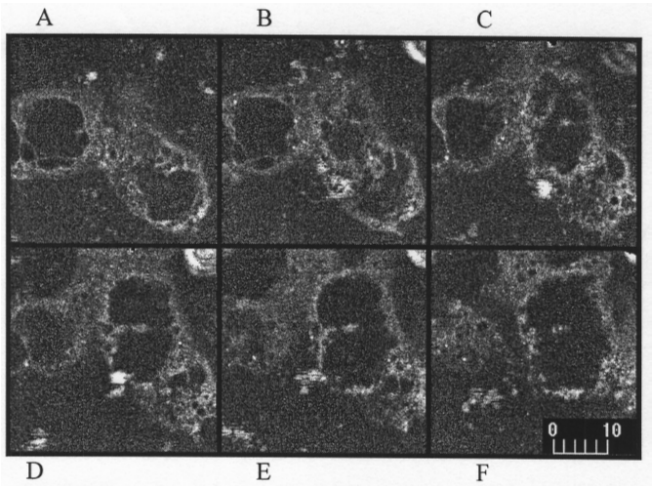

FIGURE 2: Caco-2 cells were incubated with 0.45 mg/ml AF-240 conjugated PAA nanoparticles for 30 mins. and then observed by a two-photon laser scanning microscope. A series of six horizontal optical cross-sections at successively higher focal levels is shown (step size 2 μm). Due to the presence of the dye conjuagted particles in the intercellular spaces, the shape of the cells becomes visible (scale bar 10 μm).

NANOTECHNOLOGY AND CANCER THERAPY

Nanotechnology will open up new therapeutic approaches in the treatment of human diseases and notably into the therapeutics of human cancer. At the Institute, integration of the ferrofluid, nanotechnology and

peptide hormone targeting has resulted in the fabrication of multifunctional nanoparticles, defined as "nanoclinics". One example of a therapeutic nanoclinic is a multilayered nanosized structure consisting of an iron oxide core, a two-photon optical probe, and a silica shell with luteinizing hormone-releasing hormone (LH-RH) covalently coupled to the surface of the shell. This protocol can produce nanoparticles with a tunable size from 5 to 40 nm in diameter. They are small enough (20 nm) to be able to diffuse into the tissue and to enter the cells (by endocytotic processes) and are large enough to respond to the applied magnetic field at 37°C. High resolution transmission electron microscopy shows that the structure of these nanoparticles is composed of the crystalline core corresponding to Fe_2O_3 and one amorphous silica layer (bubble). The same crystalline/amorphous structure was obtained by electron diffraction of the particle and also confirmed by X-ray diffraction. The selective interaction and internalization of these nanoparticles with cells was visualized using two photon laser scanning microscopy allowing for real time observation of uptake of nanoparticles (Figure 3.)

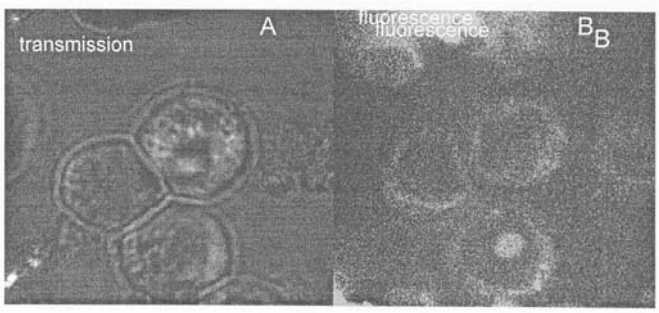

FIGURE 3 - Scanning microscopy showing uptake of nanoclinics by MCF-7 cells (breast carcinoma, LH-RH receptor positive). The particles are incubated with adherent cells and imaged using using a two photon laser scanning microscope. A: Transmission; B: Fluorescence

Two different types of particles were used in this study, LH-RH-positive (surface coupled) and LH-RH-negative (spacer arm only). A suspension of functionalized nanoclinics was added to adherent breast

carcinoma cells (MCF-7, LH-RH receptor positive) and the uptake was observed using laser scanning microscopy. The time dependent uptake of the LH-RH positive nanoparticles by LH-RH receptor bearing cells has been identified. A similar accumulation was not observed in LH-RH-negative nanoparticles studies or LH-RH positive nanoclinic incubated with receptor negative cells (UCI-107, uterine cancer cell line). Magnetocytolysis of the nanoclinic loaded cells was effected by their exposure to a 5 tesla DC magnetic field. No significant lytic activity could be obtained with receptor negative cells lines or nanoparticles not possessing the appropriate ligand when exposed to the magnetic field.

NANOTECHNOLOGY AND PHOTODYNAMIC THERAPY

The coupling of photonics and medical therapy is best demonstrated with photodynamic therapy (PDT). PDT treatment of premalignant and malignant tissue is a relatively new technique for treating cancers [18-20]. Visible light photosensitizers have been developed which accumulate in tissues after intravenous injection [21] When exposed to light with the proper wavelength, the sensitizer produces an activated oxygen species, singlet oxygen, which oxidizes critical elements of the cells. This is also dependent on the tissue the light has to penetrate to get to the photosensitizer. Currently, the only FDA approved photosensitizer, porfrimer sodium, for example, has peak absorption in the range of 405 nm (blue-violet), and a lower absorption peak at 630 nm (red) [22]. However, because the longer wavelength penetrates tissue deeper, a red wavelength is used, usually delivered from a laser.

With the exception of retinal disease, PDT is mainly limited to skin and subcutaneous malignancies due to the limited penetration of the wavelength of light used to activate the sensitizer. This limitation has been overcome through the development of new photodynamic agents that absorb at longer wavelengths of light and through the coupling of the currently approved photofrin with longer wavelength fluorophores. At the Institute, current technology in fluorophore development has been utilized that could extend the efficacy of photodynamic therapy. The successful use of two-photon absorbing dyes to activate a photodynamic agent to treat cancer has already been reported [23]. Upconverting nanoparticles could provide novel

mechanism to activate photosensitizing agents thereby increasing the penetration depth of the therapeutic action of PDT.

ACKNOWLEDGEMENTS

We thank Dr. Jeffery Kingsbury, Dr. Haridas Pudavar, and Mr. Leighton Stein for their valuable help in preparation of this manuscript. This work is supported by the School of Medicine and BioMedical Sciences of the State University of New York at Buffalo, National Science Foundation grant #0075867 and the Chemistry and Life Sciences Diretorate of the Air Force Office of Scientific Research (grant #F496200010064).

REFERENCES

[1] J. Kreuter, J. Anat. Chem **189,** 503-515, (1996)
[2] J. Kreuter, J. Pharma. Biotech. ,**6** 463-472, (1995)
[3] B.R. Rosen, T.J. Brady, **17**, S36-42, (1993)
[4] A.G. Tibbe, et al., Nature Biotech. **17**, 1210-1213, (1999)
[5] Clark, H.A., Kopelman, R., Tjalkens, R., & Philbert, M.A. Anal. Chem. **71**, 4837-4843, (1999)
[6] H.A. Clark, et al. Sensors and Actuators B **51**, 12-16, (1998)
[7] R. Kapoor, C.S Friend, A. Biswas, and P.N. Prasad, Optics Letters, **25**, 338-340, (2000).
[8] N.A. Peppas, J. J. Sahlin, Biomaterials **17**, 1533-1561, (1996)
[9] A. Ahuja, R. K. Khar, J. Ali, Drug Dev. Ind. Pharm **23,** 489-515, (1997)
[10] K.M. Tur, H. S. Chang, Int. J. Pharm. **1,** 61-74, (1998)
[11] V.H. L. Lee, J. R. Robinson, J. Pharm. Sci. **68**, 673-684, (1979)
[12] T.F. Patton, J. R. Robinson, J. Pharm. Sci. **65,** 1295-1301, (1976)
[13] A. Zimmer, et al., Pharm. Res. **11,** 1435-1442, (1994)
[14] C. Losa et al., J. Pharm. Pharmacol. **43,** 548-552, (1991)
[15] P. Artursson, CRC Crit. Rev. Ther. Drug Carrier Syst. **8,** 305-330, (1991)
[16] I.J. Hidalgo, T. J. Raub, R.T. Borchardt, Gastroenterology, **96,** 736-749, (1989)
[17] P. Artursson, J. Karlsson, Biochem. Biophys. Res. Comm., **175,** 880-885, (1991)

[18] J.S. McCaughan, Jr., Drugs and Aging. **15**, 49-68, (1999)
[19] T.J. Dougherty, et al., J. Nat. Can. Inst. **90,** 889-905, (1998).
[20] J. Kingsbury, W. Cecere, T. Mang, C. Liebow, J. Oral Maxil. Surg. **55,** 376-381, (1997).
[21] J. Webber, M. Herman, D. Kessel, D. Fromm, Ann. of Surg. **230,** 12-23 (1999).
[22] S.W. Taber, W.C. Buschmeyer 3rd, V.H. Fingar, T.J. Wieman, Surg. **126,** 730-733, (1999).
[23] J.D. Bhawalkar, N. D. Kumar, C-F. Zhao, P. N. Prasad, J. Clin. Laser Med. & Surg. **15,** 201-204, (1997).