Multimodal Molecular Imaging Agents for In Vivo pH Profiling

Donald D. Nolting

Institute of Imaging Science (VUIIS), Department of Radiology & Radiological Sciences, Vanderbilt University School of Medicine, Nashville Tennessee, 37232

INTRODUCTION

♦Tumor cell metabolism, glucose and oxygen consumption rates, cell proliferation and viability, tumor cell migration and immune cell response may be influenced by pH levels in the extracellular milieu[1]. Currently, there are few, if any, noninvasive methods able to characterize the discernable intra- and inter-tumor variations in interstitial pH and pO_2.

♦Techniques such as electrode measurements, NMR, PET, biomarker measurements, and implantable microchambers either do not provide sufficient temporal or spatial resolution, are invasive, or cannot be combined for simultaneous measurements[1].

♦Development of imaging agents capable of enhancing contrast across multiple imaging modalities combines the strengths of each modality.

♦We are developing a library of multimodal optical and MR imaging agents using PAMAM dendrimers, which can be decorated with pH responsive dyes and macrocyclic Gadolinium (Gd) complexes[2].

♦Our multimodal compounds will include agents capable of profiling pH gradients (optical) within the context of vascular perfusion (MRI).

♦The PAMAM dendrimer is the central core and is currently being employed to yield molecular imaging agents capable of quantitatively reporting naturally occurring pH gradients by optical and MR imaging arising in cancerous tissues in live animals.

MATERIALS AND METHODS

♦A multimodal imaging agent is being synthesized (**Figure 1**) utilizing a generation 2 PAMAM (G2) dendrimer coupled to *p*-SCN-Bn-DTPA and SNARF®-4F.

♦The G2 dendrimer has 16 terminal amines which can be used as attachment points for functional groups. Using standard coupling chemistry, *p*-SCN-Bn-DTPA was attached to the dendrimer by means of an isothiocyanate bridge.

♦Gd was chelated by DTPA via addition of Gd as GdCl3•6H2O. Excess Gd was removed by dialyzing against distilled deionized water. The G2GdDTPA was obtained as a fluffy, white solid after lyophilizing for 24 hours.

♦Biological phantoms were prepared by adding 28.2 mL of 10x Tris-buffered saline to 250 mL deionized water. The solution was heated to 40 - 50 ºC, 30 g of NF grade gelatin was added. 3.3 g of bovine hemoglobin and 15 mL of Intralipid 20% were dissolved in the solution at 35 ºC. Solution was titrated with NaOH to the intended pH and SNARF®-4F added.

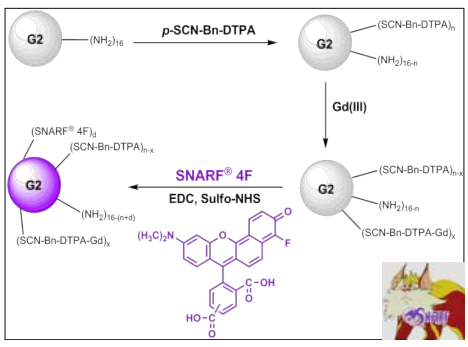

Figure 1: Synthesis of multimodal dendrimer complex.

INITIAL RESULTS

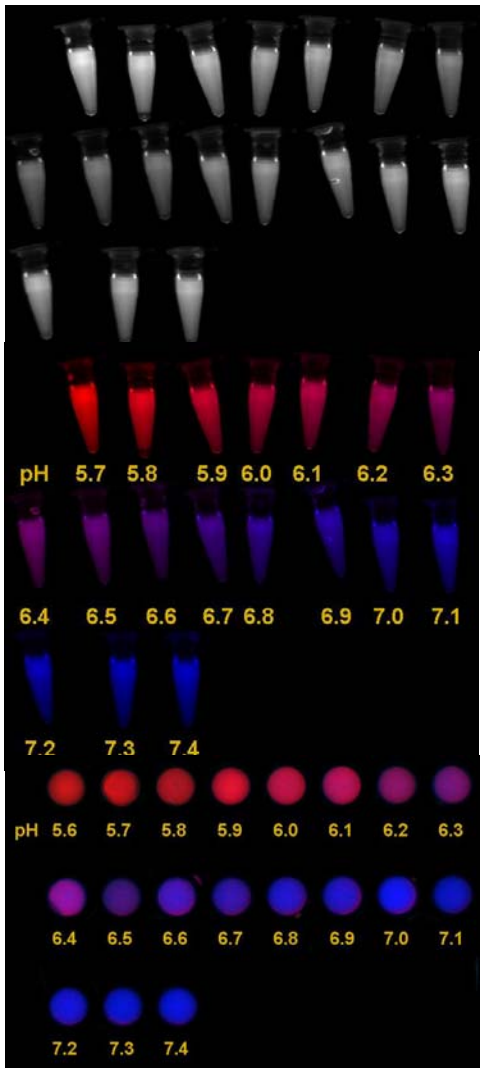

Figure 2: MaestroQ images of SNARF®-4F in water (Eppendorf tubes) and in biological phantoms (well plate).

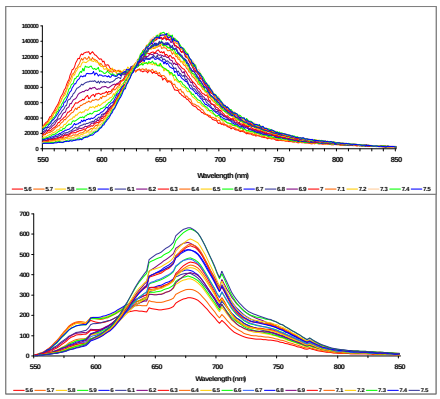

Figure 3: Fluorimeter spectra (top, 523 nm excitation) of SNARF®-4F in buffered solutions and MaestroQ spectra (bottom, 523 nm excitation) of SNARF®-4F in biological phantoms.

DISCUSSION

♦Elemental analysis of the G2Gd dendrimer confirmed the successful attachment of 13 DTPA ligands, of which 10 chelated Gd. MR data at 21 MHz on the G2Gd conjugate displayed very good relaxivity[3], ~14 $mM^{-1}s^{-1}$.

♦SNARF®-4F is currently being coupled to the G2GdDTPA through the remaining terminal amine groups on the dendrimer utilizing the peptide coupling reagents O-(N-Succinimidyl)-1,1,3,3-tetramethyl uranium tetrafluoroborate (TSTU) or 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC).

♦We have observed pH-dependent changes in UV-Vis and fluorescence spectra for SNARF®-4F. pH values from 5.6 to 7.5 can be determined utilizing a combination of optical imaging and spectroscopy techniques (**Figures 2 & 3**).

♦Differences between the spectra of SNARF®-4F from fluorimeter data and Maestro-imaged phantoms can be explained by hemoglobin absorption. Hemoglobin has an extremely high absorbance at wavelengths below 630 nm, as well as several sharp peaks and valleys in absorbance that affect the obtained spectrum.

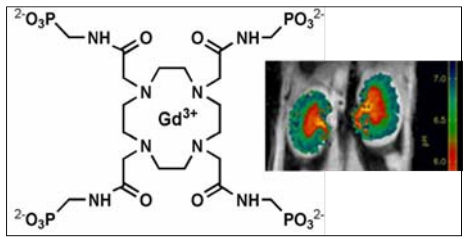

Figure 4: pH sensitive chelating ligand and resulting pH map of kidneys[4].

CONCLUSIONS & FUTURE WORK

♦G2GdSNARF®-4F can sense pH changes of ≙ 0.1 pH units in the physiological pH range.

♦SNARF®-4F can be seen in biological phantoms in the presence of hemoglobin.

♦Replace DTPA with a pH sensitive ligand similar to that in **Figure 4**. Perform same experiments with multimodal dendrimer complex.

♦Perform in vivo studies in a mouse model of cancer.

♦Our ongoing efforts include the synthesis of additional complexes with alternative dyes (e.g., cyanine dyes and NIR quantum dots) with suitable spectroscopic properties for deep tissue imaging.

ACKNOWLEDGMENTS & REFERENCES

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Vanderbilt University Institute of Imaging Science

VUIIS Building, Medical Center North
1161 21st Ave, South
Nashville, TN 37232-2310

Tel: (615) 322-8359
Fax: (615) 322-0734
URL: www.vuiis.vanderbilt.edu