Clinically Relevant CNT Dispersions with Exceptionally High Dielectric Properties for Microwave Theranostic Applications

Shawn X. Xie, Fuqiang Gao, Student Member, IEEE, Sunny C. Patel, John H. Booske, Fellow, IEEE, Susan C. Hagness, Fellow, IEEE, and Balaji Sitharaman

Abstract—We present a formulation for achieving stable high-concentration (up to 20 mg/ml) aqueous dispersions of carbon nanotubes (CNTs) with exceptionally high microwave-frequency (0.5-6 GHz) dielectric properties. The formulation involves functionalizing CVD-synthesized CNTs via sonication in nitric and sulfuric acid. The overall chemical integrity of the CNTs is largely preserved, as demonstrated via physical and chemical characterizations, despite significant shortening and functionalization with oxygen-containing groups. This is attributed to the protected inner walls of double-walled CNTs (DWCNTs) in the samples. The resulting CNT dispersions show greatly enhanced dielectric properties compared to a CNT-free control. For example, at 3 GHz, the average relative permittivity and effective conductivity across several 20 mg/ml CNT samples were increased by ~70% and ~400%, respectively, compared to the control. These CNT dispersions exhibit the stability and extraordinary microwave properties desired in systemically administered theranostic agents for microwave diagnostic imaging and/or thermal therapy.

Index Terms—Microwave frequency, dielectric properties, carbon nanotubes, tumor detection, thermal therapy, theranostic agents.

I. INTRODUCTION

CARBON nanotubes (CNTs) are promising nanoparticle-based theranostic platforms for diagnostic imaging and thermal therapy due to their unique interactions with electromagnetic radiation over a broad range of frequencies [1]-[4]. In particular, recent microwave-frequency experiments show that aqueous CNT dispersions can significantly enhance the effective dielectric properties - relative permittivity $\epsilon_r$ and effective conductivity $\sigma_e$ - of tissue-mimicking materials [5] and breast tumor xenografts [6]. Such enhancement may be exploited for microwave-based detection [2] and thermal therapy of tumors mediated by CNT theranostic agents.

This work was supported by the Department of Defense through a Breast Cancer Research Program Idea Award (W81XWH-10-1-0521 and W81XWH-10-1-0522), the National Science Foundation under ECCS 1128049, and the Philip D. Reed Chair Professorship.

Shawn X. Xie, Sunny C. Patel, and Balaji Sitharaman are with the Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY, 11794 USA (email: shawn.xie@stonybrook.edu; sunnypatel19@gmail.com; balaji.sitharaman@stonybrook.edu).

F. Gao, J. H. Booske, and Susan C. Hagness are with the Department of Electrical and Computer Engineering, University of Wisconsin, Madison, WI, 53706 USA (e-mail: fgao4@wisc.edu; booske@engr.wisc.edu; hagness@engr.wisc.edu).

Copyright (c) 2013 IEEE. Personal use of this material is permitted. However, permission to use this material for any other purposes must be obtained from the IEEE by sending an email to pubs-permissions@ieee.org.

The appropriate in vivo dose of CNT theranostic agents has not yet been established for microwave-based tumor detection or therapy. For other in vivo theranostic technologies investigated using small-animal models, the potential CNT dose ranges from units to tens of mg/kg of body weight [1], [7]. For example, in a rat model with a blood volume of $\sim$10 ml and a weight of $\sim$0.2 kg, a dose of $\sim$10 mg/kg requires a CNT stock dispersion concentration of $\sim$2 mg/ml, assuming that the maximum injection volume is $\sim$1 ml (i.e. no more than 10% of the blood volume). Higher doses may prove to greatly enhance the efficacy of tumor detection or therapy at microwave frequencies. Furthermore, preclinical pharmacology guidelines established by the U.S. Food and Drug Administration (FDA) suggest assessing safety at doses that are at least 10 times higher than the projected therapeutic dose [8]. Thus future in vivo preclinical safety and efficacy studies of CNT theranostic agents for microwave technologies will require stable CNT stock dispersions that pass through injection needles without clogging at concentrations on the order of tens of mg/ml.

Natural or synthetic amphiphilic macromolecules that are biocompatible are widely employed as dispersing agents in the preparation of aqueous CNT dispersions. However, these functionalization strategies only allow stable dispersions of individual or debundled CNTs up to hundreds of $\mu$g/ml. Methods employing mixtures of strong acids that covalently functionalize CNT sidewalls with carboxyl or hydroxyl functional groups have been shown to yield stable dispersions of debundled CNTs at concentrations on the order of 10 mg/ml. However, this increased CNT dispersibility has generally been accompanied by changes in CNT chemical and physical properties [9], [10] that lead to changes in their intrinsic electromagnetic properties. In particular, both decreased length and increased oxidation of CNTs have been shown to reduce the microwave dielectric properties of CNT dispersions [10]. The degree of chemical and morphological change induced by acid-based oxidation methods is highly sensitive to the treatment conditions, such as temperature, time, and whether sonication was employed [11]. We have also recently shown that the dispersible concentration of non-isolated CNTs that can pass through small needles is limited to a few mg/ml [6].

In this paper, we present a method for producing CNT dispersions that satisfy previously unmet criteria for systemically administered CNT-based microwave theranostic agents. Namely, the dispersions of highly debundled CNTs are...
achieved at high concentrations (i.e. tens of mg/ml) and yet do not clog injection needles; furthermore, they exhibit extremely high dielectric properties at microwave frequencies. Both the stability and microwave properties of these high-concentration dispersions represent dramatic improvements over what has been achieved previously. Additionally, we report physical and chemical characterizations that reveal why this particular processing method results in CNT dispersions with such desirable properties.

II. METHODS

The CNTs chosen for this study were synthesized by catalytic chemical vapor deposition over cobalt catalysts (Cheap-tubes, Inc.). The pristine CNT samples comprised an approximately 50:50 mixture of single- and double-walled CNTs (SWCNTs and DWCNTs), and were treated as follows. For each treatment batch, 60 mg of CNTs were thoroughly mixed in 60 ml of 3:1 H₂SO₄:HNO₃ with a glass rod, and bath-sonicated at 100 W for 4 h. The temperature of the bath water was initially 25°C. Sonication raised the water temperature to 50°C after two hours, at which point the temperature was maintained at 50±5°C until completion. The mixture was then cooled to room temperature, diluted with deionized (DI) water at a 1:4 ratio, and pelleted by centrifugation at 4000 rpm for 15 min at 10°C. The supernatant mixture was discarded and the CNT pellets were collected and resuspended in 50 ml of DI water. The centrifugation, discarding, and resuspension steps were repeated until the supernatant color remained dark, typically between pH 1.0 - 2.0. The mixture was then neutralized with NaOH and filtered through a 0.1 µm pore-sized filter. The product on the filter was washed thoroughly with excess DI water (≥ 2 ml per mg CNT) and collected in 15 ml of DI water. The suspensions were then aliquoted, and after flash-freezing the aliquots in liquid N₂, the dry product was collected by lyophilization for 24 h. The osmolality was confirmed by a single-sample osmometer (Advanced Instruments 3D3). No surfactant or sonication was required in the preparation of the samples. Control samples (CNT-free mannitol) were prepared by dispersing treated CNTs in DI water at these high concentrations to establish [12]. The focus of these experiments is on the impact of CNTs on heating efficiency.

For measurement comparisons. The concentration of mannitol in the AFM samples and analysis of the length distributions [10]. 70 objects per group from multiple spots on the substrates were measured from the micrographs. Lastly, thermogravimetric analyses (TGA) were performed using a thermogravimetric/differential thermal analyzer (Perkin Elmer) in the range of 20-800°C in air at a scan rate of 5°C/min.

III. RESULTS

Figure 1 shows the measured dielectric properties of several samples for each of the two test sample types (10 and 20 mg/ml concentrations), before and after passing through the 25G needle, in comparison to the control (CNT-free mannitol solution). Each 20 mg/ml curve (blue) represents the average across 12 measurements (four samples, three measurements each). Each 10 mg/ml curve (red) represents the average across 15 measurements (five samples, three measurements each). The vertical bars span the maximum and minimum values, we refer to this spread in the data as the variability in the measured properties. The bars are staggered around 3 GHz to avoid overlap; the bars at 2.9 GHz show the variability before passing through the needle, while the bars at 3.1 GHz show the variability after passing through the needle.

The dielectric properties of the CNT dispersions shown in Fig. 1 are significantly higher than those of the control samples. For example, at 3 GHz, the average ε_r across all dispersions of a fixed CNT concentration, before passing the needle (i.e. the solid colored curves of Fig. 1(a)), is increased

IEEE TRANSACTIONS ON BIOMEDICAL ENGINEERING
The greatly enhanced effective conductivity of the samples with CNTs, compared to the CNT-free control, leads to more efficient microwave heating of those samples, as illustrated in Fig. 2. Each curve represents the average temperature of multiple samples at a fixed concentration (four samples for 20 mg/ml, and five samples for 10 mg/ml). The bars span the minimum and maximum temperatures across samples after heating for one minute. The heating rate and the steady state temperature are greatly increased for samples with CNTs. For example, the average heating rate during the first 20 seconds is 0.14°C/s for the control, 0.26°C/s for the 10 mg/ml samples, and 0.63°C/s for the 20 mg/ml samples.

We performed AFM, Raman, FTIR, and TGA analyses to characterize the physical and chemical properties of pristine and treated CNTs. Figure 3 displays histograms of the CNT length distributions obtained by AFM measurements. Figure 3 indicates that, after the acid sonication treatment, the average length (AL) of the CNTs decreased significantly by 72% from 2.43 µm to 0.68 µm, indicating a greater length-shortening effect than previous methods employing either HCl+H2O2 or HNO3 reflux [10], as summarized in Table I.

Figure 4 displays the Raman spectra of the CNTs. There are two distinct peaks below and above 210 cm⁻¹ in the radial breathing mode (RBM) frequencies [Fig. 4(a)], which indicate the presence of DWCNTs [13]. The decrease in the intensity of the lower frequency RBM post-treatment signifies a chemical alteration to the outer diameter of the DWCNTs [14]. Figure 4(b) shows the relative G (∼1590 cm⁻¹)- and D (∼1340 cm⁻¹)-band intensities. As a measure of disordered sites and indicator of oxidation level in CNTs [15], [16], the ratio of the D-to G-band intensity (I_D/I_G) increases only mildly from 0.12 to 0.15 (a 25% increase) post-treatment. This relatively small increase in I_D/I_G may be attributed to the fact that the inner tubes within the DWCNTs are largely protected from chemical alteration. Indeed, larger diameter CNTs are generally less prone to destruction by acids and sonication [17].

Evidence of CNT functionalization is present in the FTIR transmittance spectra of the CNTs, as shown in Fig. 5. The pristine CNT band around 1593 cm⁻¹, which is assigned to
As summarized in Table I. We note likely facilitate the CNTs’ use in also

Finally, in the pristine CNTs, the C=C stretching mode [18], is shifted to a lower frequency around 1570 cm\(^{-1}\) after treatment, which may suggest a chemical change in the CNT structure. Indeed, the appearance of new bands around 1348 cm\(^{-1}\) and 1715 cm\(^{-1}\) in treated CNTs is assigned to the asymmetric SO\(_2\)-stretching mode of sulfonic acid (-SO\(_2\)-OH) groups and C=O stretching in carboxylic acid (-CO-OH) groups, respectively [19]. Furthermore, bands between 1000-1300 cm\(^{-1}\) are typically associated with C-O stretching and O-H bending modes of alcoholic, phenolic, and carboxylic groups; specifically, the higher frequency band (∼1219 cm\(^{-1}\)) present in the pristine CNTs is assigned to phenolic O-H groups, whereas the lower frequency band (∼1094 cm\(^{-1}\)) present in the treated CNTs is assigned to carboxylic O-H groups [20]. Finally, in the pristine CNTs, there exists a weak band around 1410 cm\(^{-1}\), which is most likely due to sorbed water (O-H in-plane deformation) [21].

Typically for CNTs, thermal decomposition between 150-350°C is attributed to loss of functional groups such as -CO-OH groups present on the CNT walls, and decomposition at ∼425°C is attributed to the thermolysis of SWCNTs; the leftover mass at 800°C is attributed to residual metal catalyst [10]. It is apparent that the thermal stability for the pristine CNTs is actually centered at ∼570°C, which indicates the presence of both SWCNTs and DWCNTs in the sample, as the thermal stability of pristine DWCNTs is centered at ∼630°C [22]. The TGA traces show that the acid sonication led to increased mass fraction of functional groups from ∼1% to ∼30% that are present on SWCNT sidewalls and the outer layer of DWCNTs; the thermal decomposition between 350-480°C can be attributed to leftover and inner layer SWCNTs in the sample.

### IV. DISCUSSION

In this paper, we demonstrate that high-concentration dispersions of CNTs functionalized via a H\(_2\)SO\(_4\):HNO\(_3\) treatment with sonication exhibit dramatically enhanced dielectric properties. Chemical and physical characterizations of these functionalized CNTs highlight the properties that yield these high-concentration formulations, as summarized in Table I. We note that alternate reflux methods (HCl+H\(_2\)O\(_2\) and HNO\(_3\) reflux) [10] yielded CNTs that could not be dispersed in water at high concentrations; the highest concentration achievable with no surfactants was ∼1 mg/ml. In stark contrast, the H\(_2\)SO\(_4\):HNO\(_3\) sonication method yields CNTs that are readily dispersed in water at concentrations as high as 20 mg/ml. Based on the data, we attribute the increased CNT dispersibility to a stronger length-shortening effect (indicated by the larger percent decrease in AL) by the sonication method than the alternate reflux methods, as it has been shown that CNT dispersibility only correlates qualitatively but not quantitatively with \(\frac{I_D}{I_G}\) [23]. Indeed, nanotube length has been shown to be important to dispersibility [24], [25]. The shortened CNT lengths (<1 \(\mu\)m on average) will also likely facilitate the CNTs’ use in biomedical applications due to both increased biocompatibility [26] and dispersion stability. Successful functionalization by the addition of carboxylic and sulfonic acid groups on the outer CNT sidewalls is largely responsible for the stable and high CNT concentrations (≥10 mg/ml) reported in this study. Empirical evidence thus far and theoretical principles of strong microwave field enhancement by large-aspect-ratio conductors suggest that the use of DWCNTs for microwave medical applications may be preferred over SWCNTs. This
is because DWCNTs offer both an exterior layer that can be functionalized for water solubility and an interior layer that can mitigate changes to the CNT’s intrinsic electromagnetic properties by chemical treatments. In summary, the CNT formulations reported in this paper show high potential as microwave-frequency theranostic agents.

REFERENCES


Shawn X. Xie received the B.S. degree in Biomedical Engineering from the Johns Hopkins University, Baltimore, MD, in 2011. He is currently a Ph.D. candidate in the Department of Biomedical Engineering at Stony Brook University, Stony Brook, NY. His research is focused on using materials chemistry to develop carbon-based nanomaterials for tissue engineering with microwave-based imaging and thermal therapy applications.

Fuqiang Gao (S’09) received the B.S. degree in electrical engineering from Zhejiang University, China, in 2008; and the M.S. degree in electrical engineering from the University of Wisconsin-Madison, in 2010. He is currently working toward the Ph.D. degree in electrical engineering at the University of Wisconsin-Madison. His current research interests are in bio-electromagnetics, microwave medical imaging algorithms, and nanomaterials as theranostic agents for cancer imaging and treatment.

Mr. Gao received an honorable mention in the student paper competition at the 2012 IEEE International Symposium on Antennas and Propagation, and was a finalist in the student paper competition at the 2013 IEEE International Symposium on Antennas and Propagation.

Sunny C. Patel received the B.E. degree in Biomedical Engineering from Stony Brook University, Stony Brook, NY, in 2011. He is currently a Ph.D. candidate in the Department of Biomedical Engineering at Stony Brook University, Stony Brook, NY. His research entails creating novel fabrication techniques for developing all carbon nanomaterial thin films and coatings for coating-tissue integration, tissue engineering, and sensor development applications.
John H. Booske (S’82-M’85-SM’93-F’07) received the Ph.D. degree in nuclear engineering from the University of Michigan, Ann Arbor, MI, USA, in 1985. From 1985 to 1989, he was a Research Scientist with the University of Maryland, College Park, MD, researching magnetically confined hot ion plasmas and sheet-electron-beam free electron lasers. Since 1990, he has been with the faculty of the Department of Electrical and Computer Engineering, University of Wisconsin-Madison (UW), Madison, WI, USA, where he is currently the Chair of the department, Director of the Wisconsin Collaboratory for Enhanced Learning (a learning space that supports IT-assisted, peer-collaborative learning) and the Duane H. and Dorothy M. Blumenke Professor of Engineering. From 2001 to 2005, he served as Director of the UW Interdisciplinary Materials Science Program. His research interests include experimental and theoretical study of coherent electromagnetic radiation, its sources and its applications, spanning the RF, microwave, millimeter-wave, and THz regimes. His recent research activities include vacuum electronics, microfabrication of millimeter-wave and THz regime sources and components, high-power microwaves, advanced cathodes, physics of the interaction of THz radiation and materials, microwave-generated plasma discharges and biological applications of electric and electromagnetic fields.

Prof. Booske is a Fellow of the IEEE (2007) and the American Physical Society (2011). He was a coeditor of Microwave and Radio Frequency Applications (American Ceramic Society, 2003) and Microwave and Millimeter-Wave Power Electronics (IEEE/Wiley, 2005). He has been a guest editor of the IEEE TRANSACTIONS ON PLASMA SCIENCE. He received the University of Wisconsin Vilas Associate Award for research and the U.S. National Science Foundation Presidential Young Investigator Award. He has received many teaching awards, including the UW Chancellor’s Distinguished Teaching Award.

Susan C. Hagness (S’91-M’98-SM’04-F’09) received the B.S. degree with highest honors and the Ph.D. degree in electrical engineering from Northwestern University, Evanston, IL, USA, in 1993 and 1998, respectively. Since 1998, she has been with the Department of Electrical and Computer Engineering at the University of Wisconsin-Madison, where she currently holds the title of Philip D. Reed Professor and serves as Associate Dean for Research and Graduate Affairs in the College of Engineering. She is also a faculty affiliate of the Department of Biomedical Engineering and a member of the UW Carbone Cancer Center. Her current bioelectromagnetics research interests include microwave breast imaging, microwave thermotherapy, nanoparticles as electromagnetic theranostic agents, and computational electromagnetics theory and applications in biology and medicine.

Dr. Hagness served as an elected member of the IEEE Antennas and Propagation Society (AP-S) Administrative Committee from 2003 to 2005 and as an Associate Editor for the IEEE Antennas and Wireless Propagation Letters from 2002 to 2007. She also served as Chair of Commission K of the United States National Committee (USNC) of the International Union of Radio Science (URSI) from 2009 to 2011, and Technical Program Chair of the 2012 IEEE International Symposium on Antennas and Propagation and USNC/URSI National Radio Science Meeting. She currently serves as Chair of the IEEE AP-S Fellows Committee. She was the recipient of the Presidential Early Career Award for Scientists and Engineers presented by the White House in 2000. In 2002, she was named one of the 100 top young innovators in science and engineering in the world by the MIT Technology Review magazine. She is also the recipient of the UW-Madison Emil Steiger Distinguished Teaching Award (2003); the IEEE Engineering in Medicine and Biology Society Early Career Achievement Award (2004); the URSI Isaac Koga Gold Medal (2005); the IEEE Transactions on Biomedical Engineering Outstanding Paper Award (2007); the IEEE Education Society Mac E. Van Valkenburg Early Career Teaching Award (2007); the UWSysAlliant Energy Underkofler Excellence in Teaching Award (2009); the Physics in Medicine and Biology Citations Prize (2011); the UW-Madison Kellett MidCareer Award (2011); and the UW-Madison College of Engineering Benjamin Smith Reynolds Award for Excellence in Teaching Engineers (2014). She was elected Fellow of the IEEE in 2009.

Balaji Sitharaman is an Associate Professor of Biomedical Engineering at Stony Brook University. He received his B.S. (2000) from the Indian Institute of Technology at Kharagpur. He received his M.A and Ph.D. (2005) from Rice University, where he also completed his postdoctoral research (2005-2007) as the J. Evan Attwell-Welch Postdoctoral Fellow at the Richard E. Smalley Institute for Nanoscale Science and Technology. Sitharaman lab’s research program aims to develop materials, tools, and methods that can be employed for basic and applied research at the interface of nanotechnology and other emerging fields such as molecular imaging, targeted therapeutics and regenerative medicine. He is the author of over 50 peer-reviewed publications. He has received several awards for his research including NIH Director’s New Innovator Award from the National Institute of Health, Translational Research Award from Wallace H. Coulter Foundation, Idea Award from the Department of Defense, the Carol M. Baldwin Breast Cancer Research Award from the Carol Baldwin Foundation and the George Kozmetsky Award from the Nanotechnology Foundation of Texas.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TBME.2014.2326398, IEEE Transactions on Biomedical Engineering