MICRO- AND NANO-MATERIALS FOR DRUG DELIVERY AND BIOIMAGING APPLICATIONS

A dissertation submitted to Kent State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

by

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May, 2015

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# TABLE OF CONTENTS

**TABLE OF CONTENTS** .................................................................................................................. III

**LIST OF FIGURES** ...................................................................................................................... VII

**LIST OF TABLES** ....................................................................................................................... XIII

**ACKNOWLEDGEMENTS** ............................................................................................................ XIV

**CHAPTER 1 INTRODUCTION** .................................................................................................... 1

1.1 Motivation ................................................................................................................................. 1

1.2 Materials in smaller scales ....................................................................................................... 6

1.3 Foundation ............................................................................................................................... 8

1.3.1 Magnetism ......................................................................................................................... 8

1.3.3 Nanoparticle synthesis ....................................................................................................... 12

1.3.4 Nanoparticle suspension stability ..................................................................................... 14

1.3.5 Photoluminescence – Jablonski Diagram .......................................................................... 17

1.3.6 Hydrophilic-Lipophilic Balance: HLB ............................................................................... 19

1.4 Overview of my work ............................................................................................................. 22

1.5 References ............................................................................................................................. 23

**CHAPTER 2 ONE-STEP SOLVENT-FREE THERMAL TREATMENT TO GENERATE**

**CARBON NANODOTS** ............................................................................................................ 30

2.1 Introduction ............................................................................................................................. 30
2.2 Materials and Characterizations ................................................................. 31

2.2.1 Materials ............................................................................................... 31

2.2.2 Synthesis of PL-polymers and polymer-CNDs ....................................... 31

2.2.3 Characterizations .................................................................................. 32

2.3 Results and Discussion ............................................................................. 34

2.4 Conclusion .................................................................................................. 50

2.5 References .................................................................................................. 51

CHAPTER 3 PHOTOLUMINESCENT MAGNETIC IRON OXIDE NANOPARTICLES
.......................................................................................................................... 55

3.1 Introduction .................................................................................................. 55

3.2 Materials and Characterizations ............................................................... 57

3.2.1 Materials ............................................................................................... 57

3.2.2 Chacterizations .................................................................................... 57

3.3 Supermagnetic iron oxide nanoparticles (SPION) ..................................... 59

3.3.1 Synthesis of SPION .............................................................................. 59

3.3.2 Size distribution and morphologies ....................................................... 59

3.3.3 Heating efficiency ................................................................................ 60

3.4 Magnetic nanoparticles coated with PF127 ............................................ 64

3.4.1 ION@PF127 ......................................................................................... 64

3.4.2 Silica coated ION@PF127 .................................................................... 66
3.5 Nanoparticles coated with PVA ................................................................. 70
3.5.1 ION@PVA ......................................................................................... 70
3.5.2 Photoluminescent ION@PVA ............................................................. 70
3.5.3 Photoluminescent ION@PVA-MPEG ............................................... 72
3.5.4 Functionalization of Photoluminescent ION@PVA-MPEG ............... 75

3.6 Nanoparticles coated with PAA ................................................................. 81
3.6.1 ION@PAA ......................................................................................... 81
3.6.2 Photoluminescent ION@PAA ............................................................. 86
3.6.3 Functionalization of Photoluminescent ION@PAA ............................. 90

3.7 Conclusion ............................................................................................. 92

3.8 References ............................................................................................. 93

CHAPTER 4 MONODISPERSE MESOPOROUS SILICA MICROPARTICLES .......... 98

4.1 Introduction ............................................................................................. 98

4.2 Materials and Characterizations ............................................................ 101
4.2.1 Materials ............................................................................................ 101
4.2.2 Generation of O/W/O double emulsions ............................................ 101
4.2.3 Polymerization .................................................................................... 103
4.2.4 Characterization .................................................................................. 103

4.3 Results ..................................................................................................... 103

4.4 Discussion ............................................................................................... 113
CHAPTER 5 HIGH THROUGHPUT MICROFLUIDIC METHOD FOR MEASURING CELL STIFFNESS ........................................... 124

5.1 Introduction ................................................................................................................. 124

5.2 Microfluidic device fabrication .................................................................................. 125

5.2.1 Substrate Preparation ............................................................................................ 125

5.2.2 Photolithography - master mold ............................................................................ 126

5.2.3 Polydimethyl siloxane(PDMS) microchannels ..................................................... 127

5.2.4 PDMS - glass bonding ......................................................................................... 127

5.3 Evaluation of the homemade plasma treatment system .......................................... 129

5.4 Pressure sensing microfluidic manometers ............................................................. 133

5.4.1 Microfluidic manometer 1 .................................................................................... 133

5.4.2 Microfluidic manometer 2 ................................................................................... 137

5.5 Conclusions .............................................................................................................. 143

5.6 References ............................................................................................................... 144

CHAPTER 6 SUMMARY .............................................................................................. 147

6.1 Summary .................................................................................................................... 147

6.2 Future work .............................................................................................................. 148
LIST OF FIGURES

Figure 1.1 C-Dots (Carbon nanodots) with unique properties have great potential applications in bioimaging, optoelectronics, sensor, SERS, and photocatalysis [3]...........................................2

Figure 1.2 Recent diagnostic and therapeutic applications of SPIONs (Superparamagnetic iron oxide nanoparticles) [19]........................................................................................................3

Figure 1.3 Schematic illustration of the synthesis of uniform-size hollow silica microspheres through interfacial polymerization in monodisperse water-in-oil droplets [25]..........5

Figure 1.4 Photograph of gecko’s feet. Photograph by Mark Moffet/Minden Pictures; Courtesy Micheal Bartlett and Alfred J. Crosby/Umass Amherst [31]........................................7

Figure 1.5 M-H curves of (a) diamagnetic materials, (b) paramagnetic materials, (c) ferromagnetic materials and (d) superparamagnetic materials [40].......................... 11

Figure 1.6 “Top-down” and “Bottom-up” approaches to obtain nanostructured material [43]. .... 12

Figure 1.7 The overall scheme for producing monodisperse nanocrystals by using thermal decomposition method [44]............................................................... 13

Figure 1.8 Chelating bidentate interaction between the –COOH group of oleic acid and the iron atoms [47]. .............................................................. 15

Figure 1.9 Synthetic scheme for silica cross-linked pluronic F127 loaded with iron oxide nanoparticles [49]........................................................... 16

Figure 1.10 Schematic illustration of the ligand exchange of oleic acid on iron oxide nanocrystals with Poly (acrylic acid) (PAA) [50]. .............................................................. 17

Figure 1.11 Illustration of Jablonski Diagram. .............................................................. 18

Figure 1.12 HLB Scale showing classification of surfactant function, by MKD[53]........ 21

Figure 2.1 TEM images and size histograms of (a) PAA-CNDs; (b) MPEG-DW-CNDs; (c) MPEG-DB-CNDs and (d) PAA-24h-CNDs. The scale bars are 20 nm. .............35
Figure 2.2 Absorption (bottom dotted black lines) and PL excitation (top solid blue lines) spectra (λem = 450 nm) on the left, PL emission spectra with different excitation wavelengths in the middle, and normalized PL emission spectra on the right, of (a) MPEG-DB-CNDs, (b) MPEG-DW-CNDs and (c) PAA-CNDs, respectively. 

Figure 2.3 PL intensities vs. absorbances of acridine orange (AO), MPEG-DB-CNDs and PAA-CNDs. Lines are linear fits to the measured data. 

Figure 2.4 (a) Integrated PL intensity vs. processing time of PL-MPEG (filled blue circles) and PL-PAA (empty red circles) processed at 200 °C, and (b) integrated PL intensity vs. absorbances for various processing times that are shown next to some data points in hours. 

Figure 2.5 Photobleaching measurements for MPEG-CNDs under the TXRED filter set (red filled circles), PL-MPEG under the FITC filter set (blue empty circles), PL-PAA under the TXRED filter set (red filled squares), PL-PAA under the FITC filter set (blue empty squares), Texas-red aqueous solution (red filled triangles), fluorescein solution (blue empty triangles), respectively. Lines are drawn to guide eyes. 

Figure 2.6 Time-Correlated Single Photon Counting (TCSPC) measurement of the PL-MPEG (blue dots) at 500 nm by excitation at 360 nm, fitted with a double exponential function. The characteristic relaxation times (τ) are 0.9 and 3.2 ns. The background (x20) due to the solvent (water) and glass is shown in red dots with τ~ 0.9 ns. 

Figure 2.7 FT-IR spectra of (a) untreated MPEG (blue top line) and MPEG-CNDs (red bottom line); and (b) untreated PAA (blue top line) and PAA-CNDs (red bottom line). 

Figure 2.8 Photographs of a piece of paper with writing (KSU) by using PL-MPEG with UV-lamp off (a) and on (b). 

Figure 2.9 Images of eight types of PL-Polymers on a piece of paper under a UV-lamp (peak
wavelength = 365 nm). (a): the UV-lamp is off; and (b): the UV-lamp is on. The 1 and 3 rows are untreated polymers and 2 and 4 rows are PL-Polymers.

Figure 3.1 TEM image of SPION. .......................................................... 60
Figure 3.2 TG curve of SPION. .......................................................... 61
Figure 3.3 (a) Magnetization loop of SPION at 300 K; (b) Zero-field-cooled (ZFC) temperature dependence of the magnetization for SPION ........................................... 63
Figure 3.4 Chemical structure of Pluronic® F-127. ................................. 64
Figure 3.5 TEM images of ION@PF127 .............................................. 65
Figure 3.6 TEM images of silica coated ION@PF127 ................................ 67
Figure 3.7 Fluorescent Microscopy images of collected photoluminescent silica cross-linked ION@PF127 under (a) bright field, (b) TXRED filter set, and (c) FITC filter set. The scale bar is 50 μm .......................................................... 69
Figure 3.8 TEM images of photoluminescent ION@PVA .......................... 71
Figure 3.9 Fluorescent Microscopy images of collected photoluminescent ION@PVA-MPEG under (a) bright field, (b) TXRED filter set, and (c) FITC filter set. The scale bar is 25 μm .......................................................... 73
Figure 3.10 TEM images of photoluminescent ION@PVA-MPEG .............. 74
Figure 3.11 FT-IR spectra of untreated MPEG 550 and MPEG550-COOH ........................................... 76
Figure 3.12 Fluorescent Microscopy images of collected photoluminescent ION@PVA-MPEG-COOH under (a) bright field, (b) TXRED filter set, and (c) FITC filter set. The scale bar is 50 μm .......................................................... 77
Figure 3.13 Fluorescent Microscopy images of collected photoluminescent ION@PVA-MPEG-CO-EGF under (a) bright field, (b) TXRED filter set, and (c) FITC filter set. The scale bar is 50 μm .......................................................... 79
Figure 3.14 FT-IR spectra of photoluminescent MPEG carbon nanodots (blue line) and photoluminescent ION@PVA-MPEG-CO-NHS (red line).

Figure 3.15 TEM image of ION@PAA.

Figure 3.16 TG curve of ION@PAA.

Figure 3.17 (a) Magnetization loop of ION@PAA at 300 K; (b) Zero-field-cooled (ZFC) temperature dependence of the magnetization for ION@PAA.

Figure 3.18 TEM image of photoluminescent ION@PAA. The scale bars are 50 nm.

Figure 3.19 Fluorescent Microscopy images of dried photoluminescent ION@PAA under (a) bright field, (b) TXRED filter set, and (c) FITC filter set. The scale bar is 25 μm.

Figure 3.20 Photobleaching measurements for PLMNP under the TXRED filter set (red filled circles) and PLMNP under the FITC filter set (blue filled squares), Texas-red aqueous solution (red open circles), fluorescein solution (blue open squares), respectively. Lines are drawn to guide eyes.

Figure 3.21 FT-IR spectra of PLMNP (top blue line) and PLMNP-NHS (bottom red line).

Figure 4.1 Schematic illustration of a 3-capillary microfluidic device for the formation of double emulsions.

Figure 4.2 Optical microscope image of fluids passing through the exit orifice and breaking up into double emulsion droplets.

Figure 4.3 Optical microscope image of silica microshells obtained by polymerizing double emulsions of Composition A (Table 4.1) at room temperature on a glass slide.

Figure 4.4 Scanning electron image of silica microshells obtained from the double emulsions of Composition A polymerized on a glass slide at room temperature: (a) 45° view with a lower magnification and (b) 45° view with a higher magnification, the diameter is 220 ± 20 μm; and polymerized at 60 °C; (c) 45° view of radially-cracked microshells and
(d) top view, the diameter is $230 \pm 10 \ \mu m$. The scale bars are 200 \mu m.

Figure 4.5 SEM images of mesoporous silica microshells obtained from the double emulsions of Composition B polymerized on a glass slide: (a) Top and (b) 45° view of a microshell, the diameter is 233 \mu m and (c) 45° view of a cracked microshell.

Figure 4.6 SEM images of mesoporous silica shells obtained from the double emulsions of Composition B polymerized in a glass dish with extra outer fluid. The diameter of this particle is 200 \mu m.

Figure 4.7 Electron micrographs of mesoporous silica microdisks obtained from the double emulsions of Composition C polymerized on a glass slide: (a) a SEM 45° view image of a silica microdisk, (b) a SEM cross-section image of a microdisk, and (c) a transmission electron microscopy (TEM) image of the internal structure.

Figure 4.8 Electron micrographs of uniform silica microspheres obtained from the double emulsions of Composition D polymerized in a closed plastic bottle with outer fluid at room temperature: (a) a SEM image of silica microspheres and (b) a TEM image of internal nanostructure.

Figure 4.9 Schematically illustrations of the four different mechanisms for the structural variations: (a) microshell in Figure 4.3, 4.4 and 4.5, (b) crumbled microshell in Figure 4.6, (c) microdisk in Figure 4.7, and (d) microsphere in Figure 4.8.

Figure 4.10 Time lapse images of a double emulsion during the polymerization process. The double emulsions are made of Composition B (silicone oil as both inner and outer fluids) and polymerized on a glass dish with the extra outer fluid.

Figure 4.11 Graphic summaries about silica microparticles in different shapes.

Figure 5.1 Illustration of Homemade plasma system.

Figure 5.2 Relationship between the contact angle and time after a plasma treatment.
Figure 5.3 Photolithography pattern (a) and an optical microscopy image (b) of PDMS microfluidic manometer 1. Suspension of cells or particles pass through the channel in the middle from A to D. B and C are connected to a differential pressure sensor.  
134
Figure 5.4 Labview panels. ................................................................. 135
Figure 5.5 Data acquainted with pumping DI water. X axis is the time of the moment and Y axis is the amplitude of voltage (V). ................................................................. 136
Figure 5.6 Photolithography pattern (a) and an optical microscopy image (b) of the microfluidic manometer 2. The numbers are length scales of channel in micrometers. .......... 138
Figure 5.7(a) position of the interface when there’s no cell going through the upper channel. (b) The interface position changes when there’s a cell inside of the upper channel. ...... 139
Figure 5.8 Calibration data of the microfluidic mamometer................................................. 140
Figure 5.9 Experimental data of cells passing through the channel. X-axis is the height change [pixel] of the interface; y-axis is the number of cells has certain height change. ..... 141
Figure 5.10 A newly-designed device with smaller dimensions (4 µm channel width). ............. 142
## LIST OF TABLES

Table 2.1 List of eight polymers shown in Figure 2.9. ................................................................. 49

Table 3.1 SLP values of SPION in hexane. ......................................................................................... 62

Table 3.2 SLP values of ION@PAA in water.................................................................................... 83

Table 4.1 Combinations of inner and outer fluids......................................................................... 102

Table 5.1 Contact angle measurement for untreated PDMS (water is used as the liquid phase). 129

Table 5.2 Contact angle measurement for UV-Ozone cleanser treated PDMS (water is used as the liquid phase). ........................................................................................................ 130

Table 5.3 Contact angle measurement for homemade plasma generating system treated PDMS (water is used as the liquid phase). ................................................................. 131
ACKNOWLEDGEMENTS

There have been countless people who have inspired and helped me on my path to this degree. First and foremost, I would like to express my deepest gratitude to my dissertation advisor, Dr. Chanjoong Kim. His wisdom, guidance and patience have benefited me significantly during the dissertation work. His consideration and understanding have been provided to me not only in research but also many other aspects of my life.

I would like to thank Dr. Lu Zou, who worked in the lab of Dr. Kim, was always willing to help and give her best suggestions. I would like to thank many friends and fellow students with whom I’ve shared parts of this journey, in the lab and in the classroom.

Many thanks go to my family for their love and unlimited support through my entire life.

And finally, I would like to thank all the members of my defense committee for their time, valuable comments and suggestions on the dissertation, and their participation in my most important event of my graduate career.

Huan Yan

February, 2015
CHAPTER 1

Introduction

1.1 Motivation

The objective of this work is to develop innovative, simple techniques for generating and utilizing several biomaterials in micro- and nano-scales for drug delivery and bioimaging applications.

Biomaterial is defined as “any material of natural or of synthetic origin that comes in contact with tissue, blood or biological fluids, and intended for use in prosthetic, diagnostic, therapeutic or storage applications without adversely affecting the living organism and its components.” [1]

A biomaterial [2] must be 1) biocompatible; 2) inert or specifically interactive; 3) mechanically and chemically stable or biodegradable; 4) processable; 5) nonthrombogenic and 6) sterilizable.

Types of biomaterials involved in this dissertation are carbon nanodots, fluorescent iron oxide nanoparticles and silica microcapsules. Carbon nanodots are generally accepted as biomaterial because of their low toxicity and good biocompatibility [3]. Most of the carbon nanodots are only formed by C, H and O elements. Their potential for use as replacements of toxic metal-based quantum dots is significant [4]. Possessing unique properties, carbon nanodots can be used in applications in bioimaging, optoelectronics, sensor and surface-enhanced Raman Scattering (SERS) (Figure 1.1) [3]. Commonly, carbon nanodots are generated by using techniques such as laser-ablation [5], arc discharge [6], microwave-assisted method [7] and combustion/thermal/hydrothermal/acidic oxidation [8, 9]. However, these techniques have extreme requirements, including complex synthesis procedures, expensive equipment and
precisely controlled experiment conditions. All these requirements add difficulties to the idea of industrial applications of carbon nanodots. Therefore, a simple, low-cost technique needs to be developed for commercialization of carbon nanodots.

![Figure 1.1 C-Dots (Carbon nanodots) with unique properties have great potential applications in bioimaging, optoelectronics, sensor, SERS, and photocatalysis [3].](image-url)
Superparamagnetic iron oxide nanoparticles (SPIONs) coated with polymers such as PEG [10, 11], PVA [12, 13] and PAA [14, 15] are biocompatible and they can be used for biomedical applications including magnetic resonance imaging (MRI) contrast enhancement [16, 17], targeted drug delivery [18, 19], hyperthermia [20, 21], etc. (Figure 1.2 [19]).

Figure 1.2 Recent diagnostic and therapeutic applications of SPIONs (Superparamagnetic iron oxide nanoparticles) [19].
Iron oxide nanoparticles with photoluminescence are dual-functional and possess both therapeutic and diagnostic capabilities [18-20]. Currently, conjugating some fluorescent chemicals to the surface of magnetic nanoparticles is the way to generate photoluminescent magnetic nanoparticles, which usually involves complex chemical reactions. In addition, depending on the fluorescent compound that is conjugated to the magnetic nanoparticles, these photoluminescent magnetic nanoparticles may be toxic or photo-bleachable, which limits the application of PLMNP. Therefore, a strategy to synthesize uniform, functionalizable, dual modality photoluminescent magnetic nanoparticles which show better photo-stability, high heating efficiency, and high water solubility would be promising and helpful for biomedical applications.

Biocompatible mesoporous silica microcapsules with controllable size and morphology have high surface areas and pore volumes, which make them great candidates for drug delivery [23]. Mesoporous silica microcapsules with spherical shape have already been generated and studied combining the microfluidic generation of uniform droplets with diffusion induced self-assembly in microchannels [24, 25]. For example, a schematic illustration of the synthesis of uniform-size hollow silica microspheres is shown in Figure 1.3 [25]. Firstly, water-in-oil droplets are generated by using a flow-focusing glass microfluidic chip with catalyst ammonia and cationic surfactant cetyltrimethylammonium bromide (CTAB) in the aqueous phase and silica precursor tetraethyl orthosilicate (TEOS) in the oil phase. The hydrolysis of TEOS occurs at the water-oil interface when TEOS in the oil phase diffuses to the interface and interacts with basic catalyst in the aqueous phase. After polymerization, silica hollow shell structure forms [25].
Figure 1.3 Schematic illustration of the synthesis of uniform-size hollow silica microspheres through interfacial polymerization in monodisperse water-in-oil droplets [25].

However, silica microparticles and microshells with different shapes and surface morphologies, which can be used in many applications such as drug delivery, sensing and energy-requiring high loading capacity, have not been studied much. It would be interesting if a simple method could be developed to generating size-controllable, monodisperse silica shells and particles with different shapes and morphologies.
1.2 Materials in smaller scales

My study mainly focuses on smaller scales, not only because it is useful to create smaller versions of larger systems that do the same work, but also by scaling down, especially down to nanoscales, the system may show properties which are not present in larger systems.

Firstly, optical properties may change at smaller scale. For instance, bulk gold appears yellow in color, while gold nanoparticles show red [26]. Large Zinc Oxide (ZnO) particles used as sun block scatter visible light and appear white, but Zinc Oxide nanoparticles used in sunscreen creams appears transparent on the skin because they are too small to scatter visible light [27]. Secondly, scale change affects the electrical properties. One great example is carbon nanotubes, which possess a unique combination of stiffness, strength and high electrical conductivity [28] because of their nanoscale structures. Thirdly, physical properties may also get influenced by the change of scale. Size dependent melting-point depression shows that small particles have a lower melting point than bulk material. The reason is that at macroscale most of the atoms are on the inside of the object, however, at nanoscale the atoms are split between the inside and the surface of the object. As the size of the structure decreases, the role of the surface, at which the atoms are more loosely bound than in bulk, is getting more and more important, which makes a lower melting point [29].

There are several factors contributing to the property differences between materials in macroscale and nanoscale. One of the most related factors is the surface area to volume ratio. Since the surface area changes by scale square while volume changes by scale cube, by scaling down, the surface area to volume ratio increases dramatically. For example, if the radius of a sphere is brought down by a factor of $10^6$, which is from 1 mm to 1nm, the surface area to volume ratio would increase by a factor of $10^6$.

It is important to increase the surface area to volume ratio for many reasons. One of the
examples is that because chemical reactions take place at the surface of a chemical or a material, the greater the surface area to volume ratio, the higher the chemical reactivity [30]. Another interesting example provided by nature is that the extraordinary adhesive ability of geckos is highly related to the millions of tiny hairs in the feet of the gecko (Figure 1.4) which creates much greater surface areas than smooth feet [31, 32].

Figure 1.4 Photograph of gecko’s feet. Photograph by Mark Moffett/Minden Pictures; Courtesy Micheal Bartlett and Alfred J. Crosby/Umass Amherst [31].
1.3 Foundation

1.3.1 Magnetism

Magnetism is a class of physical phenomena that are mediated by magnetic fields [33]. The most common ones are diamagnetism, paramagnetism and ferromagnetism. Diamagnetism is a type of magnetism, whereby certain materials are repelled by the applied magnetic field and generate internal, induced magnetic fields in the opposite direction of the applied magnetic field. In contrast of this behavior, paramagnetism happens when certain materials are attracted by the applied magnetic field and generate internal, induced magnetic field in the same direction of the applied magnetic field. Ferromagnetism happens when certain materials possess permanent magnets, or are attracted to magnets [33].

Diamagnetism is the consequence of Lenz’ Law applied to electric currents: “When a current loop is exposed to a magnetic field current flows so to oppose the change in flux through the loop.” [34] It occurs in materials consisting of atoms with no net magnetic moment [35]. Diamagnetism is a property of all the materials and it always has a weak contribution to the response to a magnetic field of the material.

Paramagnetic materials consist of unpaired electrons which give a magnetic moment with a constant magnitude. In the presence of an applied field, such a moment will experience a torque tending to align it with the magnetic field [35]. These materials do not retain the magnetic properties when the external magnetic field is removed.

Ferromagnetic materials exhibit parallel alignment of moments resulting in large net magnetization even in the absence of a magnetic field. One of the unique properties of ferromagnetic materials is that they show spontaneous magnetization. Spontaneous magnetization is the net magnetization that exists inside a uniformly magnetized microscopic volume in the
absence of a field [36]. If the magnetic moments of atoms or molecules are aligning in the opposite direction of the neighboring electron spins, the materials are called antiferromagnetic materials [37]. If the material has populations of atoms with unequal, opposing magnetic moments, and there’s a spontaneous magnetization remains, the material is ferrimagnetic [38].

For nanoparticles with sufficiently small sizes, magnetization can randomly flip directions in response to thermal energy, this type of nanoparticles is called superparamagnetic nanoparticles [39]. Superparamagnetic nanoparticles can be either ferromagnetic or ferrimagnetic. Superparamagnetic nanoparticles are single domain particles. All the individual magnetic moments of the atoms which form the nanoparticle can be approximately treated as one giant magnetic moment, which is the total magnetic moment of the nanoparticle [40].

If a magnetic field $H$ is applied, the individual atomic moments in the magnetic material contribute to its overall response, the magnetic induction:

$$B = \mu_0(H + M)$$

where $\mu_0$ is the permeability of free space.

Magnetization $M$ is the magnetic moment per unit volume:

$$M = \frac{m}{V}$$

where $m$ is the magnetic moment on a volume $V$ of the material [39].

The volumetric magnetic susceptibility, $\chi$, is dimensionless and defined by

$$M = \chi H$$

For diamagnetic materials, $\chi$ is in the range $-10^{-6}$ to $-10^{-3}$; for paramagnetic materials, $\chi$ is in the range of $10^{-6}$ to $10^{-1}$ [39]. However, for ferromagnetic materials, $\chi$ could be as high as $10^{6}$ [36].

Materials with different types of magnetisms behave differently in their magnetizations when varying the external magnetic field $H$. The schematic behavior of diamagnetic, paramagnetic, ferromagnetic or superparamagnetic materials in an external magnetic field is
shown in Figure 1.5 [40]. For diamagnetic materials (Figure 1.5a), the higher the magnetic field \( H \), the lower the magnetization \( M \). For paramagnetic materials (Figure 1.5b), the higher the magnetic field \( H \), the higher the magnetization \( M \). For ferromagnetic materials (Figure 1.5c), a hysteresis loop occurs. A hysteresis loop demonstrates that when a ferromagnetic material is magnetized in one direction, simply remove the external magnetic field is not adequate to drive the magnetization to zero. An external magnetic field in the opposite direction is required to drive the magnetization to zero. If an alternating magnetic field is applied to the material, its magnetization will trace out a loop [41]. This loop is called a hysteresis loop. A hysteresis loop shows the history dependent nature of magnetization of a ferromagnetic material. Larger particles have multi domains, they take relatively little field energy to make the domain walls move, which leads to a narrow hysteresis loop [39]. While for single domain, smaller particles, the loop is quite broad. For particles with even smaller sizes, superparamagnetism may occur. The hysteretic property disappears, because the magnetic moment of the particle as a whole is free to fluctuate in response to thermal energy, while the individual atomic moments maintain their ordered state relative to each other [39].
Figure 1.5 M-H curves of (a) diamagnetic materials, (b) paramagnetic materials, (c) ferromagnetic materials and (d) superparamagnetic materials [40].
1.3.3 Nanoparticle synthesis

To generate nanostructured materials, the “top-down” and the “bottom-up” approaches usually are used. The “top-down” approach begins with materials with larger initial structures and then reduced to nanostructures using physical methods, such as grinding and milling [42]. With the “top-down” approach, a larger quantity of nanostructures can be achieved easily. However, it’s quite difficult to control the size and uniformity of the nanomaterials. On the other hand, the “bottom-up” approach usually starts from very small components and then assembles to form nanostructures, which in most cases, chemical synthesis is involved (Figure 1.6) [43]. Compared to the “top-down” approach, it’s easier to form nanoparticles with controllable size for the “bottom-up” approach, but the amount of material that can be produced at one time is very limited.

![Nanostructured Material](image)

Figure 1.6 “Top-down” and “Bottom-up” approaches to obtain nanostructured material [43].
Numerous chemical methods have been developed to generate monodisperse magnetic nanoparticles from the “bottom-up” approach. One of the most popular ways to produce monodisperse and highly crystalline magnetic nanoparticle is thermal decomposition. Thermal decomposition is the method we use to synthesize magnetic iron oxide nanoparticles in this study. A scheme for thermal decomposition method is shown in Figure 1.7 [44].

![Figure 1.7 The overall scheme for producing monodisperse nanocrystals by using thermal decomposition method [44].](image)

Basically, the reaction between metal chloride and sodium oleate provides metal-oleate complex which is decomposed and acts as a growth source in synthesizing monodisperse nanocrystals. In here, the nucleation and growth processes of crystallization are separated. At first, the number of particles increases. Then it reaches a point where the particle concentration reaches to a maximum. After this point, the major growth stage occurs and the number of particles either remains constant or decreases. Nucleation occurs at a lower temperature (200 °C - 240 °C) at which one oleate ligand is dissociated from the metal-oleate complex precursor by CO₂
elimination. The major growth process happens at about 300 °C because of the dissociation of the remaining oleate ligands. Growth without additional nucleation is a necessary condition for assembling nanoparticles with a narrow size distribution. The size and morphology of the nanoparticles can be controlled by changing the reaction time, temperature, concentration of reactants, solvents and precursors [14, 42, 44]. For example, as the boiling temperature of the solvent increases, the size of the nanoparticles increases [44].

1.3.4 Nanoparticle suspension stability

Because of the nanoparticles’ large surface area to volume ratio, they tend to aggregate into larger clusters due to the attractive van der Waals force which tends to minimize the total surface energy [19]. The physical stability of nanoparticle suspensions is controlled by the attraction and repulsion of the particles, which depends on the particle size, surface properties and the environment of the nanoparticles [45]. Synthesizing nanoparticles with surfactant is a common way to obtain stable nanoparticle suspensions. In addition to preventing the agglomeration of the nanoparticles, the adsorption of the surfactant to the surface of the nanoparticles also controls the crystal growth, the crystal shape and the monomer concentration [46]. The surfactant controls the size of the nanoparticles by slowing down or stopping the growth of the crystal. It controls the shape of the nanoparticles by attaching to different crystal surfaces selectively during growth. And it controls the concentration of the monomer by capturing atoms and releasing them later during the growth [46].

Regarding to the chemical synthesis procedure we use to obtain monodisperse magnetic nanoparticles, oleic acid serves as a surfactant [42]. A previous study [47] shows that the oleic acid is chemisorbed onto the iron oxide nanoparticles as a carboxylate via chelating bidentate interaction. The proposed chemical structure of the surface adsorption of oleic acid to iron oxide
nanoparticles is shown in Figure 1.8 [47].

![Image of chelating bidentate interaction](image)

**Figure 1.8 Chelating bidentate interaction between the –COOH group of oleic acid and the iron atoms [47].**

Oleic acid has a low HLB (hydrophilic–lipophilic balance) value of 1 [48]. HLB will be introduced later in this chapter. Basically, with this low HLB value, oleic acid is soluble in non-polar solvents which makes the iron oxide nanoparticles bonded with oleic acid on the surface able to disperse well in non-polar solvents. However, this hydrophobicity of the oleic acid coated nanoparticles highly limits the possibility of using iron oxide nanoparticles in biomedical applications, such as drug delivery, bioimaging and biodetection in aqueous systems. There are several methods have been used to transfer nanoparticles with hydrophobic surfaces into water. In this study, we will demonstrate transferring iron oxide nanoparticles with oleic acid coated surfaces into water with the following methods:

(a) Silica cross-linked Pluronic F127. Pluronic F127 is an ABA-type triblock copolymer which is consisted of a central hydrophobic block, polypropylene glycol (PPO), in between two hydrophilic blocks of polyethylene glycol (PEO). The hydrophobic block in the center provides an attraction to the surface of nanoparticles, while the two hydrophilic tails stretch out in the aqueous media. Tetraethyl orthosilicate (TEOS) is used as silica precursors, after crosslinking, it forms a double-layer coating of silica and PEO on the surfaces of the iron oxide nanoparticles, shown in Figure 1.9 [49].
Figure 1.9 Synthetic scheme for silica cross-linked pluronic F127 loaded with iron oxide nanoparticles [49].

Both of Pluronic F127 and silica are biocompatible. The layer of PEO makes nanoparticles disperse well in aqueous media and the silica cross-linking makes the nanoparticles possess high chemical and physical stabilities [49].

(b) Ligand exchange with PVA. Poly (vinyl alcohol) (PVA) is a common surfactant to use for transferring nanoparticles to water because it’s biocompatible and hydrophilic [39].

(c) A robust ligand exchange with PAA at an elevated temperature in a glycol solvent. Poly (acrylic acid) (PAA) is used because in addition to its biocompatibility, it is a polyelectrolyte whose repeating units disassociate in aqueous solutions making the polymer charged. Being a polyelectrolyte provides PAA a strong coordination of its functional groups to the nanoparticles surface [50]. In this study, we use diethylene glycol (DEG) as the polar solvent because of its high boiling point (~ 245 °C), high dissolving capability for PAA and high miscibility with both water and the organic solvent hexane [50]. A schematic illustration of the ligand exchange of the chemical structure is shown in Figure 1.10 [50]. The major ligand exchange happens at a
temperature close to the boiling temperature of the solvent. After the ligand exchange, the nanoparticles become water soluble.

Figure 1.10 Schematic illustration of the ligand exchange of oleic acid on iron oxide nanocrystals with Poly (acrylic acid) (PAA) [50].

1.3.5 Photoluminescence – Jablonski Diagram

In this study, we will discuss the photoluminescence properties of carbon nanodots as well as carbon nanodot coated iron oxide nanoparticles. Photoluminescence is light emission from any form of matter after the absorption of photons [51]. It can be divided into two categories: Fluorescence and Phosphorescence [52]. Fluorescence is emission of light from singlet excitation states, in which the electron in the excited orbital is paired (by opposite spin) to the second electron in the ground-state orbital. While phosphorescence is emission of light from triplet excitation states, in which the electron in the excited orbital has the same spin orientation as the ground-state electron [52].
Figure 1.11 Illustration of Jablonski Diagram.

Jablonski Diagram describes the processes that occur between absorption and emission of the light. A typical Jablonski Diagram is shown in Figure 1.11. $S_0$ is the singlet ground state, $S_1$, $S_2$ are first and second electronic states. Between these electronic states, there are rotation and vibration levels, depicted by 0, 1, 2, etc. When an electron absorbs energy (photons) and gets excited to some higher vibrational level of either $S_1$ or $S_2$, with a few rare exceptions, the electron rotates and vibrates to the lowest vibrational level of $S_1$. This relaxation process is called internal conversion. The electron has a tendency to go back to the most stable state - ground state $S_0$. If the electron emits photon when going back to $S_0$, this process is called radiative relaxation. The photon which the electron emits is within the emission spectrum of the specific fluorophore. The photon that the electron absorbs is within the absorption spectrum of the specific fluorophore. If the electron relaxes to the ground state by dissipating the excitation energy as heat, this process is
called non-radiative relaxation. The internal conversion is also non-radiative because it doesn’t emit photon during energy level change.

Another possible transition is after the electron gets excited to some higher vibrational level and goes through the internal conversion, instead of directly relaxing down to the ground state S₀, the electron undergoes a spin conversion to the first triplet state T₁, which has a slightly lower energy level than S₁. This spin conversion is called intersystem crossing. After this, if the electron may relax to the ground state S₀ through a non-radiative relaxation, which not emits photon. If the electron emits photon when going back from the triplet state T₁ to the ground state S₀, this emission is termed as phosphorescence. The intersystem crossing can happen in both directions, meaning that the electron may go from S₁ to T₁ as well as from T₁ back to S₁. If the electron travels back from T₁ to S₁, then going down to the ground state S₀ through radiative relaxation, the process is called delayed fluorescence.

The lifetime of a fluorophore is the average time between its excitation and return to the ground state. For fluorescence, because electron returning to a ground state is spin allowed, so it occurs rapidly by emission of a photon. The emission rates of fluorescence are typically $10^8$ s⁻¹, so that a typical lifetime of fluorescence is near 10 ns. However, transitions to the ground state are forbidden for phosphorescence, so the emission rates are slow ($10^3$ to $10^6$ s⁻¹) which make the lifetimes of phosphorescence milliseconds to seconds or even longer [52].

1.3.6 Hydrophile-Lipophile Balance: HLB

A sufficient knowledge of hydrophilicity of the surfactants is crucial to this study. For instance, in chapter 3, the hydrophilicity of the polymer coated on the surface of the nanoparticles highly influences the stability of the nanoparticle suspensions which influences the properties of the nanoparticle suspensions. In chapter 4, using the right surfactant for each phase is a must to
form monodisperse, stable double emulsions which provide scaffold for silica precursors to polymerize into silica with certain shapes.

HLB is a degree to measure the hydrophilicity of a surfactant. It provides a very useful guide to be familiar with the hydrophilicity of a surfactant. HLB values may be calculated or determined experimentally for non-ionic surfactants. For other types of surfactants, HLB values may be determined experimentally. Griffin’s method can be used for calculating HLB values for non-ionic surfactants:

$$HLB = 20 \times \frac{M_h}{M}$$

where $M_h$ is the molecular mass of the hydrophilic portion of the molecule, and $M$ is the molecular mass of the whole molecule. HLB values have a scale of 0 to 20. An HLB value of 0 corresponds to a completely hydrophobic molecule, and a value of 20 corresponds to a completely hydrophilic molecule [53]. An HLB scale is shown in Figure 12 [53].
Figure 1.12 HLB Scale showing classification of surfactant function, by MKD [53].

HLB system provides a very useful guidance for choosing emulsifiers. A “water-soluble” emulsifier is needed to make the final product exhibiting aqueous characteristics, such as to form an oil-in-water emulsion, or to obtain detergent action. On the other hand, a “oil-soluble” emulsifier is needed to make a water-in-oil emulsion, or couple water soluble materials into an oil [48].
1.4 Overview of my work

In this work, we will present four independent biomaterials and complex fluids related research projects. In Chapter 2, a one-step solvent-free thermal treatment method to generate photoluminescent carbon nanodots is developed. The morphologies, chemical structures and fluorescent properties of the carbon nanodots generated from two different types of polymers (MPEG and PAA) are presented. These carbon nanodots can be used for bio-detecting and labeling. In Chapter 3, a novel strategy that combines photoluminescent carbon nanodots with magnetic iron oxide nanoparticles is proposed. Monodisperse iron oxide nanoparticles are synthesized and the surface of the nanoparticles is modified with carbon nanodots originated from 3 different types of polymers (PF127, PVA and PAA). The functionalizability of the photoluminescent magnetic nanoparticles is demonstrated for bio-imaging and hyperthermia therapy applications. In Chapter 4, various types of monodisperse mesoporous silica microparticles are generated by glass-capillary microfluidic devices via double emulsion templates. These silica microcapsules can be used for drug delivery. In Chapter 5, a low-cost and high throughput method to detect the stiffness of the cells is presented. Two types of PDMS microfluidic manometers are constructed and studied. Finally, in Chapter 6, we will summarize our results.
1.5 References


[41] Hysteresis in magnetic materials http://hyperphysics.phy-astr.gsu.edu/hbase/solids/hyst.html.


CHAPTER 2

One-step solvent-free thermal treatment to generate carbon nanodots

2.1 Introduction

Recently, carbon nanodots (CNDs) have drawn recent research interest because of their strong and tunable photoluminescence, good water solubility, low toxicity and resistance to photobleaching [1, 2]. These attractive attributes make CNDs suitable for applications such as bio-imaging [3, 4] and chemical sensing [5, 6]. Several methods have been developed to generate CNDs, such as laser-ablation [7], arc discharge [8], microwave-assisted method [9] and combustion/thermal/hydrothermal/acidic oxidation [10, 11]. However, industrial applications have been limited due to the drawbacks of these approaches, including complex synthesis procedures, extreme production conditions, difficulty in further surface functionalization, and high cost. Therefore, a simple and efficient method to generate CNDs would expedite the scale-up and commercialization of CNDs. There have been a few studies on a one-step hydrothermal treatment to generate CNDs with nitrogen-doped carbon dots [12, 13] using water as solvent during heating [2, 14] or a mixture of sugars and acids [15, 16]. Among these methods, using water as solvent during heating may be the simplest method so far, though it still requires high-pressure processing in an autoclave [2, 14]. Moreover, this batch method may cause difficulties in the continuous flow production of CNDs.

Our group has recently reported that a simple polymer-coating process can generate photoluminescence in magnetic nanoparticles, and that the photoluminescence is originated from CNDs of the coating polymers [17, 18]. In this chapter, we present a simple thermal treatment
method to generate CNDs by directly heating hydrophilic polymers in open air without adding any other chemicals or solvents, and study their nanostructures and photoluminescent properties. This simple, fast method can be applied to a broad range of polymers, being suitable for the commercial mass production of CNDs.

2.2 Materials and Characterizations

2.2.1 Materials

All the chemicals are used as received without further purification. Poly(ethylene glycol mono-methyl ether)(MPEG, Mw ≈ 550) is purchased from Scientific Polymer Products, Inc. Poly(acrylic acid)(PAA, Mw ≈ 1800), Polyethylene glycol (PEG), Poly(ethylene glycol) bis(3-aminopropyl) terminated, Poly(ethylene glycol) diglycidyl ether, Poly(ethylene glycol) bis(carboxymethyl) ether, Polypropylene glycol (PPG), Polyvinylpyrrolidone (PVP), Poly(vinyl alcohol)(PVA), Pluronic® F-127, dimethyl sulfoxide-d6 and fluorescein sodium salt are purchased from Sigma-Aldrich Inc. Pluronic® P123 and Pluronic® L64 are purchased from BASF Corporation, Tween® 80 is purchased from Chemistry Connection, Texas Red BSA (TR-BSA) is purchased from Invitrogen.Spectra/Por®3 dialysis membrane, MWCO = 3.5 kD (pore size ≈ 1.2 nm) [19] and Spectra/Por®7 dialysis membrane, MWCO = 25 kD (pore size ≈ 4 nm) [19] are purchased from Spectrum Laboratories, Inc.

2.2.2 Synthesis of PL-polymers and polymer-CNDs

We note that throughout this chapter, the photoluminescent product of a polymer without dialysis is referred to as PL-polymer and the product after the dialysis is referred to as polymer-CNDs, where the polymer is either MPEG or PAA. PL-MPEG is produced as follows. 10 g of
liquid MPEG is added to a 25 mL round-bottom flask and heated in an oil bath at 200 °C for 24 h. After the heating process, the colorless original MPEG becomes a more viscous, brown liquid product: PL-MPEG. No photoluminescence is observed while nitrogen gas is bubbled in the MPEG during the heat process, confirming that oxygen in the air is essential for the generation of CNDs and photoluminescence [10, 11]. PL-MPEG is then dissolved in 10 mL deionized water and dialyzed with a 25 kDa cut-off dialysis bag against deionized water for 48 h. The deionized water outside of the dialysis bag is changed frequently and saved in another bottle. By evaporating all the water from the solution in the dialysis bag by rotary evaporation, a small amount of dried product is collected and labeled as MPEG-DB-CNDs, and the product collected from the solution outside of the dialysis bag is labeled as MPEG-DW-CNDs.

A similar process is applied to the production of PL-PAA and PAA-CNDs, as follows. 1 g of PAA is added into a 25 mL round-bottom flask and heated in an oil bath at 200 °C for 4 h. The white powder becomes waxy yellow amorphous solid PL-PAA. 10 mL of deionized water is added and the mixture is sonicated for 30 min for thorough mixing. Then, dialysis is conducted with a 3.5 kDa dialysis bag for 48 h. The deionized water is changed frequently and saved in a different bottle. The product, PAA-CNDs, is collected after the rotary evaporation of the solution obtained from the inside of the dialysis bag.

2.2.3 Characterizations

The nanoscale morphologies of the CNDs are obtained using a transmission electron microscope (TEM, FEI Tecnai F20). Each TEM sample is prepared by spreading a small amount of a dilute aqueous solution of CNDs on a copper grid coated with double layers of carbon and drying it in a fume hood.

Fluorescent spectra of the CNDs in clean quartz cuvettes are measured using a
SpectroMaxM5 Multi-Mode fluorometer (Molecular Devices, Sunnyvale, CA, USA). The PAA-CNDs, MPEG-DB-CNDs and MPEG-DW-CNDs are dissolved in water at concentrations of 18.25 mg/mL, 3.77 mg/mL and 1.61 mg/mL, respectively.

Photobleaching measurements are carried out using a fluorescent microscope (BX51, Olympus) by exposing a sample under a mercury lamp and measuring the emission intensity of the sample in grayscale under two different filter sets (FITC filter set: EX = 480/40, EM = 535/40, and TXRED filter set: EX = 559/34, EM = 630/69). In order to compare the photostability of PL-polymers to that of a common organic dye at each filter set, solutions of fluorescein sodium salt and TR-BSA are prepared at the concentrations of $9.82 \times 10^{-3}$ mg/mL and 0.442 mg/mL, respectively. These concentrations are selected in order not to saturate the imaging sensor (IPX-VGA210, Imperx) of the microscope. Each aqueous solution is drawn into a 1 mm square capillary and both ends of the capillary are sealed using epoxy resin. Also, the PL-MPEG is drawn into a square capillary and sealed. A small amount of the waxy PL-PAA is placed between two glass slides and all sides of the glass slides are sealed as well.

The quantum yield ($\Phi$) of each CND sample is calculated using acridine orange ($\Phi = 0.46$) [20] as a reference. In order to find the slope of the PL intensity to the absorbance, six concentrations of each CND sample are made. Acridine orange and MPEG-DB-CNDs are dissolved in ethanol (refractive index, $\eta_{\text{ethanol}} = 1.361$) while PAA-CNDs is dissolved in water ($\eta_{\text{water}} = 1.333$). PL emission spectra are measured with excitation at 366 nm for all samples, and the PL intensity is obtained by integrating each PL emission spectrum between 400 and 700 nm. Absorbance is obtained at 366 nm using a UV-Vis spectrometer (LAMBDA 25, PerkinElmer).

A PicoHarp 300 system is used to measure the time-correlated single photon counting (TCSPC) of PL-MPEG sealed in a vitrotube. The incident wavelength is 360 nm, and the emission is set to 500 nm. A Bruker Avance 400 spectrometer is used to obtain 1-H and 13-C
NMR spectra. FT-IR spectra are recorded with a Bruker Hyperion FT-IR microscope.

2.3 Results and Discussion

The TEM images of CNDs are shown in Figure 2.1. PAA-CNDs are well dispersed and have an average diameter around 4 nm with a narrow size distribution (Figure 2.1a). MPEG-CNDs with two different sizes are separated by dialysis with a 25 kDa membrane, and collected by rotary evaporation. MPEG-DW-CNDs are smaller (average diameter ≈ 2.7 nm) with good monodispersity (Figure 2.1b), while larger MPEG-DB-CNDs (average diameter ≈ 20 nm) and have crystalline structures (Figure 2.1c). In contrast to PL-MPEG, PL-PAA does not contain any large CNDs, probably because the thermal treatment time for PL-PAA (4 h) is not long enough to form large CNDs. During the heat treatment, small CNDs may form first, and then CNDs grow larger by coalescence. To test this hypothesis, we generate PAA-24h-CNDs by treating PAA for 24 h using the same heating condition. Figure 2.1d shows a few large PAA-24h-CNDs (average diameter ≈ 30 nm). We note that the water solubility of PAA-24h-CNDs is quite poor compared to PAA-CNDs and MPEG-CNDs, so no further analysis is carried out for PAA-24h-CNDs.
Figure 2.1 TEM images and size histograms of (a) PAA-CNDs; (b) MPEG-DW-CNDs; (c) MPEG-DB-CNDs and (d) PAA-24h-CNDs. The scale bars are 20 nm.
In order to study the optical properties of CNDs, the UV-Visible absorption, emission and excitation spectra of MPEG-DB-CNDs, MPEG-DW-CNDs and PAA-CNDs are measured as shown in Figure 2.2. All three CNDs show strong absorption in the UV region with an extended tail in the visible range, which is typical for CNDs[6]. The PL excitation spectra of both MPEG-CNDs with an emission wavelength of 450 nm exhibit two excitation peaks at 275 nm and 360 nm, while that of PAA-CNDs show one excitation peak at 358 nm and a shoulder around 290 nm. All the PL emission spectra exhibit asymmetric peaks, which may indicate there are two different emission mechanisms in the CNDs [21, 22]. With shorter excitation wavelengths (250 – 380 nm), the first emission peak remains at 450 - 460 nm and the second emission peak remains at 490 – 500 nm for MPEG-DB-CNDs and MPEG-DW-CNDs. With longer excitation wavelengths (400 – 550 nm), only the second peak is observed because the range of excitation wavelength is above the first emission peak position. This second peak shifts with the excitation wavelength, which is a typical PL behavior of CNDs [23]. PAA-CNDs shows similar trends: the second peak shift with the excitation wavelength while the first peak remains at 420 - 450 nm. The reason for the excitation-dependence of the PL of CNDs is still not clear. Plausibly, both the size distribution and the surface structure of CNDs may affect this excitation-dependence behavior. The relatively broader size distribution of CNDs provides different energy gaps, which causes smaller particles to get excited at shorter wavelengths while larger ones get excited at longer wavelengths. The variation of surface functional groups generates different levels of energy traps, which may also lead to the variation of excitation wavelengths [15, 16].
Figure 2.2 Absorption (bottom dotted black lines) and PL excitation (top solid blue lines) spectra ($\lambda_{em} = 450$ nm) on the left, PL emission spectra with different excitation wavelengths in the middle, and normalized PL emission spectra on the right, of (a) MPEG-DB-CNDs, (b) MPEG-DW-CNDs and (c) PAA-CNDs, respectively.
To characterize the quantum yields of the CNDs, the integrated PL emission intensities ($\lambda_{ex} =$ 366 nm) of a standard (acridine orange), MPEG-DB-CNDs and PAA-CNDs are plotted with respect to their absorbances at 366 nm, as shown in Figure 2.3. The data show a good linearity and the intercept is close to zero for each case. The quantum yield is calculated by using the following equation [24, 25]:

$$\Phi_X = \Phi_{ST} \left( \frac{a_X}{a_{ST}} \right) \left( \frac{\eta_X^2}{\eta_{ST}^2} \right)$$

where $\Phi$ is quantum yield, $a$ is the slope, $\eta$ is the refractive index of the solvent, and the subscripts, ST and X, stand for the standard and a sample, respectively. The quantum yields of MPEG-DB-CNDs and PAA-CNDs are 14% and 15%, respectively. The quantum yields of the CNDs by our heat treatment are significantly higher than those of CNDs derived from PEG ($\Phi =$ 3.5%) [14] and cornflour ($\Phi =$7.7%) [2] by the autoclave hydro-heating method.
In order to find out if the CND are formed at a specific time of reaction or continuously converted from the original polymers, we measure the integrated PL intensities ($\lambda_{ex} = 360$ nm, integration between 400 nm and 700 nm) and absorbances at 360 nm of the *in situ* products of MPEG and PAA processed at 200 °C for different times. The values of the integrated PL intensities increase almost linearly with the heating time (Figure 2.4a), except for one PL-PAA outlier at ~ 75 h. This implies the overall concentrations of the CNDs increase in a constant rate, since the *in situ* products are mixtures of the original polymer and the CNDs. Figure 2.4b shows that the plots of the integrated PL intensities vs. the absorbances maintain almost the same slopes for ~ 20 h of heating for both MPEG and PAA, and then start to bend down with the heating time.

![Figure 2.3](image.png)

*Figure 2.3 PL intensities vs. absorbances of acridine orange (AO), MPEG-DB-CNDs and PAA-CNDs. Lines are linear fits to the measured data.*
Because the quantum yield is proportional to the ratio between the integrated PL intensity and the absorbance, these results suggest that the quantum yields first keep at a constant then decrease with the heating time. It may be related to the formation of larger CNDs at longer processing times (Figure 2.1), which may have lower quantum yields than smaller ones.

Figure 2.4 (a) Integrated PL intensity vs. processing time of PL-MPEG (filled blue circles) and PL-PAA (empty red circles) processed at 200 °C, and (b) integrated PL intensity vs. absorbances for various processing times that are shown next to some data points in hours.
The photobleaching behaviors of the PL-MPEG and PL-PAA are studied by exposing them to mercury lamp light without any filter. Their PL intensities are measured occasionally under both TXRED and FITC filter sets, and compared to those of two commonly used organic fluorescent dyes, Texas red and fluorescein. Figure 2.5 clearly demonstrates that the PL-polymers are more photostable than Texas red and fluorescein. Within the first 60 minutes of the light exposure, the intensity of Texas-red dye decreases down to 35%, and that of fluorescein down to almost zero. In contrast, PL-MPEG maintains its intensity at ~ 95%, and PL-PAA at ~ 80%. Although CNDs have been demonstrated to have better photostability than conventional fluorescent dyes [6, 26], our CNDs, especially PL-MPEG, show excellent photostability against photobleaching.

In order to find out the photoluminescence mechanism of the CNDs generated by our heat treatment method, we carry out a time-correlated single photon counting (TCSPC) measurement on pure PL-MPEG that is processed at 200 °C for 70 min. The characteristic decay time of the correlation function, \( \tau \), is closely related to the basic photoluminescence mechanism [27]. Typical lifetimes of fluorescence are in the range of 0.5 to 20 ns, while those of phosphorescence are much longer in the range of 1 ms to 10 s [27]. As shown in Figure 2.6, by fitting a double-exponential decay function, we find that \( \tau \)'s are about 1 and 3 ns, where the short 1 ns decay is due to the solvent (water) and the longer 3 ns decay corresponds to fluorescence.
Figure 2.5 Photobleaching measurements for MPEG-CNDs under the TXRED filter set (red filled circles), PL-MPEG under the FITC filter set (blue empty circles), PL-PAA under the TXREFD filter set (red filled squares), PL-PAA under the FITC filter set (blue empty squares), Texas-red aqueous solution (red filled triangles), fluorescein solution (blue empty triangles), respectively.

Lines are drawn to guide eyes.
Figure 2.6 Time-Correlated Single Photon Counting (TCSPC) measurement of the PL-MPEG (blue dots) at 500 nm by excitation at 360 nm, fitted with a double exponential function. The characteristic relaxation times \( \tau \) are 0.9 and 3.2 ns. The background (x20) due to the solvent (water) and glass is shown in red dots with \( \tau \sim 0.9 \) ns.
The changes in the chemical structures during the thermal treatment are studied using FT-IR and NMR. The FT-IR spectra of the untreated MPEG and PL-MPEG are shown in Figure 2.7a. PL-MPEG develops a new strong peak centred at 1710 cm\(^{-1}\) (stretching C=O of ester) that indicates the formation of esters during the heating treatment. In Figure 2.7b, the untreated PAA has a very sharp peak at 1705 cm\(^{-1}\) (stretching C=O of carboxylic acid), which weakens and becomes broader in the spectrum of PL-PAA. This indicates that a significant portion of carboxylic acid groups in the untreated PAA are converted to ester groups in PL-PAA during the heat treatment. 1-H and 13-C NMR spectra are obtained to further confirm the change in the chemical structures. PL-MPEG shows peaks at 8.22 ppm (H-COO-) in the 1-H NMR spectrum and 162.19 ppm (-C=O) in the 13-C NMR spectrum, but not the untreated MPEG, indicating the formation of formate ester groups during the heat treatment. PL-PAA shows peaks at 4.85 ppm (R-COO-CH) in the 1-H NMR spectrum and 173.74 ppm (-COO) in the 13-C NMR spectrum, but not the untreated PAA, also indicating the formation of ester groups. CNDs have been commonly accepted as nanoparticles consisting of an amorphous or nanocrystalline core with predominately sp\(^2\) C=C carbon atoms [6]. However, both FT-IR and NMR spectra of our PL-MPEG and PL-PAA that contains CNDs show no evidence of the existence of C=C. Thus, we propose that CNDs synthesized with our thermal method do not contain C=C bonds, but rather just sp\(^3\) carbon and sp\(^2\) hybridized carbon in the core with –OH and C=O groups on the surface. Although there is no current theory supporting that this type of chemical structures can exhibit fluorescence, we believe that further investigation such as quantum mechanics calculations may reveal a new fluorescence mechanism.
Figure 2.7 FT-IR spectra of (a) untreated MPEG (blue top line) and MPEG-CNDs (red bottom line); and (b) untreated PAA (blue top line) and PAA-CNDs (red bottom line).
In addition to the conventional idea that CNDs are good candidates for bio-imaging, we explore a new use of CNDs as invisible ink. For example, after the heat treatment, PL-MPEG forms brownish viscous liquid at room temperature. When written on a paper with brownish background, the liquid gets absorbed into the paper and becomes invisible under room light (Figure 2.8a). Under UV light, however, the writing (KSU) becomes visible, as shown in Figure 2.8b.

Finally, in order to examine if the original end group and the molecular weight affect the emergence of the fluorescence, we test our one-step, solvent-free heat treatment on PEGs with various end groups(-OH, -COOH, epoxide), polypropylene glycol (PPG), and PEG-PPG-PEG triblock copolymers, as listed in Table 2.1, which also contains a variation in molecular weight (300 – 12,500 g/mol). Figure 2.9 shows that none of original polymers are fluorescent, but after 1-2 h of heat treatment at 200 °C all of them exhibit fluorescence under the UV lamp.
Figure 2.8 Photographs of a piece of paper with writing (KSU) by using PL-MPEG with UV-lamp off (a) and on (b).
Figure 2.9 Images of eight types of PL-Polymers on a piece of paper under a UV-lamp (peak wavelength = 365 nm). (a): the UV-lamp is off; and (b): the UV-lamp is on. The 1 and 3 rows are untreated polymers and 2 and 4 rows are PL-Polymers.
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<th>Molecular weight</th>
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<td><img src="image" alt="L64" /></td>
<td>2900</td>
<td>200</td>
<td>1 h</td>
</tr>
</tbody>
</table>

Table 2.1 List of eight polymers shown in Figure 2.9.
2.4 Conclusion

We present an open-air thermal treatment method to generate carbon nanodots (CNDs) from commonly used hydrophilic polymers, and study the photoluminescence (PL) and chemical structures of CNDs. The PL intensities of PL-Polymers that contain the CNDs increase almost linearly with the heating time. These CNDs show broad excitation and emission spectra, excellent photostability and relatively high quantum yields. These unique properties may open up applications beyond bio-imaging. Surprisingly, the chemical structure analyses of our CNDs show no evidence of C=C bonds that are commonly associated with fluorescence. This method may be used for the in-line mass production of carbon nanodots for industrial applications.
2.5 References


[19] Anon Relative Pore Sizes for Dialysis Membranes
http://www.spectrumlabs.com/dialysis/PoreSize.html


CHAPTER 3

Photoluminescent magnetic iron oxide nanoparticles

3.1 Introduction

For decades, nanoparticles have drawn much attention because of their unique optical [1], structural [2] and magnetic properties [3, 4] due to their small size. Nanoparticles have been proposed for various applications including biomedical [5] and sensor devices [6]. In particular, their magnetic properties make them ideal candidates in biomedical applications such as magnetic resonance imaging contrast agents [7, 8], magnetic field controllable drug delivery agents [9, 10] and agents for hyperthermia generated by electromagnetic fields [11, 12]. Superparamagnetic iron oxide nanoparticles (SPION), which exhibit superparamagnetism, high field irreversibility [3] and biocompatibility [13], lead to a range of new biological [13, 14], therapeutic [15, 16] and diagnostic applications [14, 17]. Fluorescent nanoparticles are great candidates for detection and labelling [1, 9, 18] due to unique advantages over conventional organic fluorescent molecules, such as their higher photostability and stronger fluorescence [19].

Photoluminescent magnetic nanoparticles (PLMNP) are promising candidates as dual modality (MRI and optical) imaging contrast agents, and as theranostic agents that have both therapeutic and diagnostic capabilities [18 - 20]. In order to function properly in these applications, PLMNP have to satisfy several requirements. First, they should have high susceptibility without permanent magnetization. SPION are broadly accepted as great candidates for MR probes and hyperthermia treatment agents because of their superparamagnetic properties. Second, PLMNPs should disperse well and be stable in aqueous media [21 - 27]. Third, high
photostability against photobleaching is desirable, so that they can provide sufficient signal intensity and high imaging quality for an extended period of time during an operation [18].

Commonly, the way to generate PLMNP is to conjugate some fluorescent chemicals to the surface of magnetic nanoparticles, but it usually requires complex chemical reactions. Depending on the fluorescent compound that conjugated to the magnetic nanoparticles, PLMNP may be toxic or photo-bleachable, which limits the application of PLMNP.

In Chapter 2, we demonstrate that photoluminescent carbon nanodots possess advantages such as strong and tuneable photoluminescence, good water solubility, low toxicity and resistance to photobleaching. Inspired by the idea that simply heating certain types of polymers can generate photoluminescent carbon nanodots, we present a unique strategy for the synthesis of uniform, functionalizable, dual modality SPION which show better photo-stability, high heating efficiency, and high water solubility. To the best of our knowledge, this is the first work that combines photoluminescent carbon nanodots with magnetic nanoparticles.

The main goal of the work in this chapter is to synthesize functionalizable PLMNP and characterize their properties. A general routine is first synthesizing magnetic iron oxide nanoparticles which disperse well in hexane. Then by coating a hydrophilic polymer layer, nanoparticles are transferred into water. Then, photoluminescent carbon nanodots are generated by heating the polymer coating layer on the nanoparticles. The nanoparticles are then functionalized by conjugating to other chemicals. In this chapter, 3 different types of polymers (PF127, PVA and PAA) have been used to coat magnetic nanoparticles and the morphologies have been studied. We find that the type of polymer affect the morphology of resulting products greatly. For some case, large aggregations form. In addition, the functionalizability of PLMNP is demonstrated by conjugating N-hydroxysuccinimide (NHS) to the surface of the PLMNP.
3.2 Materials and Characterizations

3.2.1 Materials

All chemicals are used as received without further purification. Iron(III) chloride hexahydrate, sodium oleate, dioctyl ether (99%), ethanol, hexane, oleic acid, poly(acrylic acid) (PAA) with $M_w \approx 1800$ g/mol, diethylene glycol (DEG), poly (vinyl alcohol) (PVA), Poly(ethylene glycol mono-methyl ether) (MPEG) with $M_w \approx 550$ g/mol, Pluronic®F-127, sodium hydroxide, fluorescein sodium salt, N-hydroxysuccinimide (NHS), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), Tetraethyl orthosilicate (TEOS), diethoxydimethylysilane (DEDMS) are purchased from Sigma-Aldrich. Texas-red/BSA is purchased from Invitrogen.

3.2.2 Characterizations

The morphologies of the nanoparticles are obtained using a transmission electron microscope (TEM, FEI Tecnai F20). An appropriate amount of nanoparticles is spread on a copper grid coated with double layers of carbon and dried in a hood. The hydrodynamic diameters of the nanoparticles are measured using the dynamic light scattering (DLS) setup of a Zeta potential and particle size analyzer (ZetaPALS, Brookhaven). The fluorescent microscope images are taken using a camera (IMPERX) mounted on a fluorescent microscope (BX51, OLYMPUS). Thermogravimetric analysis is performed using a Q500 thermogravimetric analyzer (TA Instruments). $1H$ NMR spectrum is recorded with a Bruker Avance 400 spectrometer. FT-IR spectra are recorded with a Bruker Hyperion FT-IR microscope.

In order to evaluate the magnetic heating efficiency of nanoparticles, specific loss power
(SLP) is measured in a following system. A 200 µL suspension of nanoparticles is inserted in a water-cooled magnetic coil. A high-frequency, high-amplitude alternating magnetic field (AMF) is applied at a frequency of 2.1 MHz and at amplitude of 20 kA/m. Using a fiber optic temperature probe (Neoptix), the time-dependent temperature change of the suspension \( (dT/dt) \) is simultaneously recorded. SLP is calculated as \( \text{SLP} = (C \cdot V_s/m) \cdot (dT/dt) \) [11], where \( C \) is the volumetric specific heat capacity of the sample solution (4.186 J/(g·°C) for water, and 2.26 J/(g·°C) for hexane), \( V_s \) is the sample volume, and \( m \) is the mass of the magnetic material (iron oxide cores) in the sample. In order to obtain the mass of iron oxide cores, TG analysis are performed for both SPION and ION@PAA.

In addition, a SQUID magnetometer (Quantum Design, MPMS XL) is used to measure the magnetization hysteresis data at 300 K and zero-field cooled (ZFC) temperature dependence of the magnetization of the samples.

Photobleaching measurements are carried out by exposing samples under a mercury lamp and measuring the emission intensity of the sample under two different filter sets (FITC filter set: \( \text{EX} = 480/40, \text{EM} = 535/40 \), and TXRED filter set: \( \text{EX} = 559/34, \text{EM} = 630/69 \)) using the fluorescent microscope. In order to compare the photostability of PLMNP to that of common organic dyes at each filter set, solutions of fluorescein sodium salt and Texas-red are prepared at the concentrations of \( 9.82 \times 10^{-3} \text{ mg/ml} \) and \( 0.442 \text{ mg/ml} \), respectively. These concentrations are chosen in order not to saturate the imaging sensor of the microscope. Each solution is drawn into a square capillary and both ends of the capillary are sealed by epoxy. PLMNP are first dispersed in isopropanol. The suspension is drawn into a square capillary and the solvent is dried by heating. Then both ends of the capillary are sealed by epoxy.
3.3 Supermagnetic iron oxide nanoparticles (SPION)

3.3.1 Synthesis of SPION

Superparamagnetic iron oxide nanoparticles are synthesized according to literature [22, 23]. Briefly, 0.903 g FeCl$_3$·H$_2$O and 3.046 g sodium oleate are dissolved in a mixture of 25 mL ethanol, 20 mL deionized water and 45 mL hexane. After refluxing for 4 h at 62 °C, the suspension of iron oleate complex is washed with deionized water 3 times by extraction. 3.13 g dried iron oleate complex and 0.6 g oleic acid are dissolved in 20 mL dioctyl ether at 70 °C. Then the mixture is gradually heated to 290 °C for 1.5 h. Iron oxide nanoparticles are then collected by centrifugation at 6000 rpm in ethanol. Particles are redispersed in hexane and extra ethanol is added, and the mixture is centrifuged at 6000 rpm for 30 min. This step is repeated 3 times to remove all impurities. SPION are then redispersed in 40 mL hexane with 100 μL oleic acid and stored in a refrigerator for future use.

3.3.2 Size distribution and morphologies

Because of the coating of hydrophobic hydrocarbon chains from ligands of oleic acid, SPION can be dispersed very well in organic solvent, such as hexane and toluene. TEM images of SPION are shown in Figure 3.1. The average diameter of the SPION in Figure 3.1 is 13.5 ± 1 nm. The hydrodynamic diameter of SPION is 14.7 nm with a polydispersity of 0.062, determined by the DLS measurement of SPION in hexane.
3.3.3 Heating efficiency

The Specific loss power (SLP) is defined as the thermal power dissipated by a material per unit mass during exposure to an AMF [11]. The samples are prepared into different concentrations to show the SLP values are irrelevant to the concentrations. In order to know the mass of the magnetic core, TG analysis is used. TG curve of SPION is shown in Figure 3.2. The major weight loss is ~ 33%, which is similar to the report in the literature [24]. SLP values of SPION in hexane are shown in Table 3.1. Using the mass of iron oxide cores, the SLP value for SPION is found to be around 110 W/g. The SLP value depends on the size of the nanoparticles and the magnetic field of the measurements. According to a previous work [11], the SLP value of
commercial 100 nm SPION measured using the same setup is ~ 320 W/g. Our SPION seem to have reasonably high heating efficiency, considering their size is about 1/7 of the commercial 100 nm SPION, while the SLP value of our nanoparticles is more than 1/3 of that of the 100 nm SPION. The magnetization loop at 300 K of SPION is shown in Figure 3.3(a). It shows typical superparamagnetic behaviors [25]. The saturation magnetic moment (Ms) of SPION is 28.36 emu/g. Figure 3.3(b) shows zero-field cooled (ZFC) temperature dependence of magnetization for SPION measured under a magnetic field of 100 Oe in a temperature range from 5 K to 350 K.

![Figure 3.2 TG curve of SPION.](image)
<table>
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<th>Sample</th>
<th>Solvent</th>
<th>dT/dt (°C/s)</th>
<th>C/(g·°C))</th>
<th>Vs(ml)</th>
<th>m(mg)</th>
<th>SLP (W/g)</th>
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<tr>
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</tbody>
</table>

Table 3.1 SLP values of SPION in hexane.
Figure 3.3 (a) Magnetization loop of SPION at 300 K; (b) Zero-field-cooled (ZFC) temperature dependence of the magnetization for SPION.
3.4 Magnetic nanoparticles coated with PF127

3.4.1 ION@PF127

In order to transfer SPION into water, we coat SPION with a triblock copolymer, Pluronic® F-127 (PF127). PF127 is consisted of a central hydrophobic block, polypropylene glycol, in between two hydrophilic blocks of polyethylene glycol, as the chemical structure is shown in Figure 3.4.

\[
\text{HO-(CH}_2\text{CH}_2\text{O)}_{100}(\text{CH}_2\text{CHO})_{65}(\text{CH}_2\text{CH}_2\text{O)}_{100}\text{H}
\]

\[\text{CH}_3\]

Figure 3.4 Chemical structure of Pluronic® F-127.

The procedure to coat PF127 on SPION is modified according to literature [26]. 1 mL hexane solution of SPION is mixed with 8 mL 4% PF127 aqueous solution in a 40 mL glass vial. The mixture is then vigorously stirred at room temperature overnight in an open glass vial.
Figure 3.5 TEM images of ION@PF127.
After the coating, there is neither sedimentation nor phase separation in the nanoparticle aqueous suspension, this means the nanoparticles are coated with PF127. TEM images of ION@PF127 (Figure 3.5) further confirm that ION@PF127 disperse well in water, and all the nanoparticles are coated with PF127 separately.

3.4.2 Silica coated ION@PF127

Silica coated ION@PF127 is prepared as following [27]: 0.075 g TEOS and 0.15 g HCl(0.3M) are added into 3 mL ION@PF127 water solution and stirred for 48 h. Then 0.0375 g DEDMS is added and the mixture is stirred for 3 more hours. The silica coated ION@PF127 is dialysis with a 25,000 Da membrane against water for 48 h to remove silica precursors.

TEM images of silica coated ION@PF127 are shown in Figure 3.6. Some of the nanoparticles are dispersed well with an average diameter of 9 nm. But there are some nanoparticles are grouped together. That is because of the silica precursor crosslinked in between nanoparticles.
Figure 3.6 TEM images of silica coated ION@PF127.
To generate photoluminescence to the nanoparticles, silica coated ION@PF127 water suspension is heated to 215 °C for 4 h.

After heating treatment, the mixture becomes less water-soluble. But most of the mixture can be dispersed in ethanol. Centrifugation at 1500 rpm for 20 min is used to collect photoluminescent silica coated ION@PF127 nanoparticles.

The fluorescent microscopy images are shown in Figure 3.7. After heating treatment, these nanoparticles show photoluminescence under both FITC and TXRED filter sets. The main reason that all these large aggregates is because the images are taken with centrifugation collected dried nanoparticles. But the existence of a few small glassy pieces that don’t show photoluminescence indicates there are still some free silica precursors remain after dialysis and crosslink into small silica pieces during heating.
Figure 3.7 Fluorescent Microscopy images of collected photoluminescent silica cross-linked ION@PF127 under (a) bright field, (b) TXRED filter set, and (c) FITC filter set. The scale bar is 50 μm.
3.5 Nanoparticles coated with PVA

In order to make separated coated PLMNP, we use a different polymer PVA to transfer SPION into water.

3.5.1 ION@PVA

As mentioned earlier, there are lots of ligands of oleic acid to make SPION disperse well in organic solvent. But in order to coat SPION with PVA, the ligands have to be washed away first. The following procedure is developed to wash the nanoparticles: 1 mL ethanol is added into 1 mL of SPION hexane solution followed by centrifugation at 10,000 rpm for 5 min and the supernatant is discarded. The precipitate is then dispersed in 1 mL toluene and precipitated with 1 mL of acetone. The mixture is centrifuged at 12,500 rpm for 5 min, and the supernatant is discarded. The precipitate is then redispersed in 1 mL of toluene and further precipitated by adding 1 mL of ethanol. The precipitate is redispersed in toluene and precipitated again by adding ethanol and the mixture is heated at 65 °C for 1 min. The mixture is centrifuged at 12,500 rpm for 5 min to collect the nanoparticles. This precipitation-redispersion procedure is repeated 2 more times, and the nanoparticles obtained afterward are dispersed in 1 mL of toluene. To coat PVA to SPION, 1 mL SPION toluene solution is added into 10 g PVA water solution (0.15 wt%) dropwise with stirring. The mixture is heated at 90 °C with stirring for 40 min [28]. The nanoparticles are collected by centrifugation at 8000 rpm for 5 min. The sedimentation is washed with DI water 3 times to get rid of free PVA polymer. Then ION@PVA is redispersed in 1 mL DI water for future use.

3.5.2 Photoluminescent ION@PVA

The ION@PVA water suspension is directly heated to 200 °C overnight to generate
photoluminescence.

Figure 3.8 TEM images of photoluminescent ION@PVA.
Photoluminescent ION@PVA shows a broad size distribution from DLS measurement. TEM images (Figure 3.8) also show the nanoparticles aggregate because of the heating treatment.

3.5.3 Photoluminescent ION@PVA-MPEG

In order to generate well-dispersed PLMNP, it is important to prevent aggregation during the heating treatment. So we try a different protocol to generate photoluminescence by coating MPEG polymer onto the surface of ION@PVA with heating, which concomitantly generates photoluminescence [28]. The procedure is: 1 mL ION@PVA water suspension is added into 1 g of MPEG-550 dropwise while stirring. The mixture is heated to 60 °C followed by keep adding NaOH aqueous solution (5 wt%) until phase separation occurs. Then, the mixture is heated at 60 °C with stirring for 15 min. The pH is adjusted to 6 by adding acetic acid. The mixture is heated at 170 °C overnight, followed by 3 h of heating at 195 °C. 5 mL of DI H2O is added to disperse the nanoparticles. The nanoparticles are then collected by centrifugation at 8000 rpm for 5 min and washed with DI water 3 times to remove free polymers.

The fluorescent microscopy images of photoluminescent ION@PVA-MPEG are shown in Figure 3.9. Same as all other cases, the heat treatment generates photoluminescence onto the nanoparticles.
Figure 3.9 Fluorescent Microscopy images of collected photoluminescent ION@PVA-MPEG under (a) bright field, (b) TXRED filter set, and (c) FITC filter set. The scale bar is 25 μm.
Figure 3.10 TEM images of photoluminescent ION@PVA-MPEG.
TEM images of photoluminescent ION@PVA-MPEG nanoparticles (Figure 3.10) show that there are some separated coated nanoparticles with a very thick polymer coating layer and also aggregated nanoparticles.

3.5.4 Functionalization of Photoluminescent ION@PVA-MPEG

To make the nanoparticles useful in real applications, they have to be further functionalizable. To study the further functionalizability of these photoluminescent ION@PVA-MPEG, we ignore the aggregation of the nanoparticles for now and study the possibility of functionalization.

3.5.4.1 Primary alcohol oxidation to carboxylic acids for polymers

In Chapter 2, we studied the structure of photoluminescent carbon nanodots generated from MPEG. Despite the formation of ester groups, there still are a sufficient amount of hydroxyl group remain. So the strategy here to further functionalize this type of PLMNP is to first transform –OH groups to –COOH groups. Because hydroxyl groups are not so reactive other functional groups except for carboxyl groups through esterification which is a reversible reaction usually leading to a low yield. In contrast, carboxyl groups can easily react with functional groups like amine and alcohol groups which are common in many chemicals and biomaterials, such as drugs, bacteria, proteins, sugars, etc.

To start, we use untreated MPEG550 to change –OH groups to –COOH groups by oxidation of alcohol [29]. A 5 mL K$_2$CrO$_4$ water solution (6 wt %) is added into a mixture of 0.55 g MPEG-550, 8 mL concentrated H$_2$SO$_4$ and 17 mL DI H$_2$O. The mixture is stirred at room temperature for 4 h. After the reaction, 25 mL DI H$_2$O is added followed by adding CH$_2$Cl$_2$. The organic layer is washed with DI H$_2$O 3 times by extraction. Then a saturated NaCl solution (36 wt% at 25 °C) is
used to wash the organic layer and MgSO$_4$ is added to dry remaining water after removing most of the water out. Filtration is performed and the organic layer is dried with rotary evaporation.

Then FT-IR spectra (Figure 3.11) are measured to confirm the formation of –COOH groups. After oxidation, a peak centered at 1710 cm$^{-1}$ shows up which corresponding to C=O stretching of carboxylic acid groups. This result confirms that the oxidation method can be used to generate –COOH groups from –OH groups.

Figure 3.11 FT-IR spectra of untreated MPEG 550 and MPEG550-COOH.
3.5.4.2 Surface functionalization of photoluminescent ION@PVA-MPEG

So we use the same alcohol oxidation method to functionalize photoluminescent ION@PVA-MPEG. A 2.5 mL K₂CrO₄ water Solution (2.4 wt%) is added into a mixture of 0.03 g photoluminescent ION@PVA-MPEG, 4 mL concentrated H₂SO₄ and 12.5 mL DI H₂O. The mixture is stirred at room temperature for 4 h. After washing with water 3 times by centrifugation, sedimentation of photoluminescent ION@PVA-MPEG-COOH is obtained. The fluorescent microscopy images show the nanoparticles still show strong photoluminescence after oxidation. (Figure 3.12)

Figure 3.12 Fluorescent Microscopy images of collected photoluminescent ION@PVA-MPEG-COOH under (a) bright field, (b) TXRED filter set, and (c) FITC filter set. The scale bar is 50 μm.
As an example to show the functionalizability, we functionalize nanoparticles by attaching NHS to the surface, which is a commonly used coupling agent with primary amines for protein labeling. The procedure is as following [30]: 38.34 mg EDC and 28.40 mg NHS are added into 1 mL acetate buffer solution (pH = 6) containing 10 mg photoluminescent ION@PVA-MPEG-COOH with stirring. Then the mixture is stirred at room temperature for 15 min. Centrifugation at 4400 rpm is used to wash the nanoparticles with DI water 3 times to remove all the impurity. Then the nanoparticles are dried in an oven for characterizations.

Fluorescent microscopy images of collected photoluminescent ION@PVA-MPEG-CO-NHS (Figure 3.13) which show photoluminescence after the whole procedure.

FT-IR spectra of carboxyl groups-ended photoluminescent MPEG carbon nanodots and photoluminescent ION@PVA-MPEG-CO-NHS are shown in Figure 3.14. The major differences are around 1600 - 1800 cm\(^{-1}\). The peak centered at 1750 cm\(^{-1}\) (C=O stretching of carboxyl acid) in MPEG-COOH shifts to 1600 cm\(^{-1}\) (C=O stretching of amide) in ION@PVA_MPEG-CO-NHS proves the conjugation of NHS to the nanoparticles.
Figure 3.13 Fluorescent Microscopy images of collected photoluminescent ION@PVA-MPEG-CO-EGF under (a) bright field, (b) TXRED filter set, and (c) FITC filter set. The scale bar is 50 μm.
Figure 3.14 FT-IR spectra of photoluminescent MPEG carbon nanodots (blue line) and photoluminescent ION@PVA-MPEG-CO-NHS (red line).
3.6 Nanoparticles coated with PAA

Although we demonstrate further functionalization of PVA-coated PLMNP, the remaining problem is that the nanoparticles are aggregated into big clusters. The large size limits the application of these nanoparticles. So we try to coat SPION with PAA polymer to get individual PLMNP.

3.6.1 ION@PAA

In order to render originally hydrophobic SPION water-solubility, we prepare ION@PAA that are SPION coated with hydrophilic poly (acrylic acid) following a previous work[27]. Briefly, a mixture of 0.5 g PAA with 8 mL DEG is heated to 110 °C with vigorous stirring under nitrogen gas. Then, 1 mL of ION hexane solution is injected into the mixture. The system is heated to 200 °C for 2 h. After the mixture cools down, a sufficient amount of hydrochloric acid water solution (pH = 2) is added in order to precipitate the nanoparticles. ION@PAA are then collected by centrifugation at 14,000 rpm. Then the sedimentation is washed with deionized water 3 times to remove all impurities. ION@PAA are then dispersed in sodium hydroxide water solution (pH = 12). The high pH ION@PAA water solution is centrifuged at 14,000 rpm and the ION@PAA are then redispersed into deionized water and stored at room temperature for future use.
Because the PAA layer is hydrophilic, ION@PAA can be dispersed well in water, as evident in Figure 3.15 that shows well dispersed ION@PAA that have an average diameter of ~ 18 nm and are individually coated with a ~ 2 nm thick PAA layer. The DLS result shows the hydrodynamic diameter of the particles in water is ~ 28.7 nm with a polydispersity of 0.171, which is larger than the average diameter from the TEM. This may be because the PAA coating uptakes water and swells in water.
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<th>Solvent</th>
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<th>$C$ (J/(g·°C))</th>
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<th>$m$ (mg)</th>
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</table>

Table 3.2 SLP values of ION@PAA in water.

Figure 3.16 TG curve of ION@PAA
To prove the heating efficiency does not change with or without polymer coating, we measure SLP values of ION@PAA in water (Table 3.2). The samples are prepared into different concentrations to show the SLP values are irrelevant to the concentrations. In order to know the mass of the magnetic core, TG analysis is used. TG curve of ION@PAA is shown in Figure 3.16. The major weight loss is ~ 30%, which is similar to the report in the literature [31]. Using the mass of iron oxide cores, the SLP value of ION@PAA is found to be around ~ 110 W/g which is consistent with SLP value we got from SPION samples.

The magnetization loop at 300 K of ION@PAA is shown in Figure 3.17 (a). It shows typical superparamagnetic behaviors [25]. The saturation magnetic moment (Ms) of ION@PAA is 23.39 emu/g. Figure 3.17 (b) shows zero-field cooled (ZFC) temperature dependence of magnetization for ION@PAA measured under a magnetic field of 100 Oe in a temperature range from 5 K to 350 K. These nanoparticles show similar magnetic behavior as PVP-stabilized iron oxide nanoparticles [32, 33].
Figure 3.17 (a) Magnetization loop of ION@PAA at 300 K; (b) Zero-field-cooled (ZFC) temperature dependence of the magnetization for ION@PAA.
3.6.2 Photoluminescent ION@PAA

By direct heating, we generate photoluminescent ION@PAA. TEM images are shown in Figure 3.18. TEM images show that photoluminescent ION@PAA are dispersed either individually or in small groups (1-5 particles), which is far less severe than the aggregates of all the previously discussed photoluminescent nanoparticles.

Figure 3.18 TEM image of photoluminescent ION@PAA. The scale bars are 50 nm.
Figure 3.19 Fluorescent Microscopy images of dried photoluminescent ION@PAA under (a) bright field, (b) TXRED filter set, and (c) FITC filter set. The scale bar is 25 μm.
Figure 3.19 shows the fluorescent microscopy images of the dried photoluminescent ION@PAA. The fluorescent intensity of these nanoparticles is high enough be imaged under FITC (EX: 495 nm; EM: 517 nm) and TXRED (EX: 596 nm; EM: 613 nm) filter sets without any special arrangement. As the emission spectrum is as broad as all the previous cases, it is also possible to image photoluminescent ION@PAA with both filter sets.

In Figure 3.20, we compare the photostability of PLMNP to that of two commonly used organic fluorescent dyes, Texas red and fluorescein, under the TXRED filter set and the FITC filter set, respectively. Within the first 60 minutes of mercury lamp exposure, the intensity loss is 65% for Texas-red dye, almost 100% for fluorescein, while only about 16% for PLMNP under TXRED filter set and 25% under FITC filter set. This indicates that the PLMNP are much more photostable than those conventional fluorescent dyes.
Figure 3.20 Photobleaching measurements for PLMNP under the TXRED filter set (red filled circles) and PLMNP under the FITC filter set (blue filled squares), Texas-red aqueous solution (red open circles), fluorescein solution (blue open squares), respectively. Lines are drawn to guide eyes.
3.6.3 Functionalization of Photoluminescent ION@PAA

Photoluminescent ION@PAA meet most aims we set for photoluminescent magnetic nanoparticles in this project which combines photoluminescent carbon nanodots with magnetic nanoparticles and well dispersed in aqueous solutions. So the next thing we need to achieve is the further functionalization ability of photoluminescent ION@PAA. In Chapter 2, the chemical structure of carbon nanodots generated from PAA polymer shows remaining –COOH groups. Using this functional group, we will show that photoluminescent ION@PAA can be functionalized through reactions with amine and hydroxyl groups.

3.6.3.1 Photoluminescent ION@PAA-NHS

As an example to show photoluminescent ION@PAA can react with amine groups, we functionalize photoluminescent ION@PAA by attaching NHS to the surface. The procedure is as following [30]: 3 mg of PLMNP are dispersed in 3 mL acetate buffer solution (pH = 6), to which 12 mg EDC and 8.52 mg NHS are added. The mixture is then stirred at room temperature for 15 min, followed by washing with deionized water and centrifuging at 14,000 rpm for 1 h. The washing step is repeated two more times. Then the nanoparticles are dried in an oven for characterizations.
Figure 3.21 FT-IR spectra of PLMNP (top blue line) and PLMNP-NHS (bottom red line).

The FT-IR spectra of photoluminescent ION@PAA (PLMNP) and photoluminescent ION@PAA-NHS (PLMNP-NHS) are shown in Figure 3.21. The relatively broad band centered at 1715 cm\(^{-1}\) of PLMNP indicates the presence of remaining carboxyl groups and ester groups (stretching C=O of carboxylic acid and ester). The FT-IR spectrum of PLMNP-NHS confirms the presence of amide groups, as evidenced by the peak centered at 1670 cm\(^{-1}\) (C=O stretching of tertiary amide bond). The decrease of the peak at 1005 cm\(^{-1}\) (O-H bending of carboxylic acid) from PLMNP to PLMNP-NHS also indicates that a significant portion of \(\text{--COOH}\) groups are converted to the NHS-ester.
3.7 Conclusion

In summary, we have synthesized photoluminescent magnetic nanoparticles with high heating efficiency and water-solubility by coating 3 different polymers (PF127, PVA and PAA) on hydrophobic SPION cores and heat treating to convert the polymer coating layer to a photoluminescent carbon nanodot layer. There is no other published study that combines photoluminescence carbon nanodots with magnetic nanoparticles. For some of the cases, the heating process may cause the nanoparticles stick together and form large clusters. But with PAA coated nanoparticles, the heating treatment can generate single photoluminescent nanoparticle as well as small clusters of 1-5 nanoparticles. After generating photoluminescence, we demonstrate these nanoparticles can be further functionalized. That is significant because photoluminescent nanoparticles are appealing candidates for various therapeutic and diagnostic biomedical applications, such as bioimaging, hyperthermia, etc. This novel strategy of generating photoluminescence may not be exclusive to the presented materials, but has the potential to be used with a wide selection of polymer-coated nanoparticles.
3.8 References


CHAPTER 4

Monodisperse mesoporous silica microparticles

4.1 Introduction

Surfactant-templated ordered mesoporous silica materials have many applications such as delivery of DNA [1] and drugs [2, 3] into animal cells and plant cells [4], catalysts for selective organic transformations [5], and electronics with integral organic functionality [6]. Uniform monodisperse mesoporous silica spheres have attracted particular interest due to potential applications in sensors [7], encapsulation and biomolecule delivery [8]. Synthesis of mesoporous silica materials via lyotropic liquid crystal templates was discovered several years ago, by utilizing the self-assembly structure of amphiphiles as a template [9, 10].

Since Stöber’s first synthesize of monodisperse non-porous silica nanoparticles using hydrolysis and condensation of tetraalkoxysilane in alcoholic solution in 1968 [11], many groups have tried to modify Stöber’s method to synthesize monodisperse mesoporous silica particles that have very high surface area. Andersson et al. [12] designed an emulsion and solvent evaporation (ESE) method by combining emulsification with the evaporation-induced self-assembly (EISA) method [13, 14]. Using this method, they synthesized well-ordered 2D hexagonal spherical mesoporous silica particles. Because they used inhomogeneous vigorous stirring to prepare emulsions, the resulting droplets and final particles have a broad size distribution. A modified synthesis method was reported that can generate sub-micrometer scale monodisperse supermicroporous silica spheres whose sizes can be controlled by varying the synthesis temperature, the solvent mixture ratio and the source chemicals of silica [15]. Lee et al. have reported the
formation of monodisperse mesoporous microspheres combining the microfluidic generation of uniform droplets with diffusion induced self-assembly in microchannels [16]. Recently, a one-step microfluidic method was reported to synthesize hollow silica microshells [17]. This work used interfacial polymerization of silica precursors on water-in-oil droplets generated by a microfluidic device. Specifically, they use cationic surfactant cetyltrimethylammonium bromide (CTAB) dissolved in a 1:1 water/ethanol phase. Silica precursors in the oil phase then diffuse onto the water/oil interface and polymerize there. The hollow silica microspheres are more desirable for the use of encapsulation because of their high capacity.

Another convenient common method to generate a shell structure is using double emulsion templates [18]. Double emulsions are emulsions with smaller droplets of a third fluid within the larger drops. Although emulsions are not in an equilibrium state, if an emulsion’s interface is stabilized by a suitable surfactant the droplets can maintain their integrity for a long time [19]. Because of the intermediate fluid, the innermost fluid can be separated from the continuous phase. This feature makes double emulsions highly desirable in many areas, such as controlled release [20, 21], separation, and encapsulation [21, 22].

Traditionally, emulsions are produced by vigorously shaking two immiscible liquids with a small amount of surfactant. The shaking generates enormous shear force that breaks up a fluid in another fluid, resulting emulsion droplets. However, this method results in droplets with a wide size distribution. In addition, the resulting emulsions cannot be finely controlled so it is hard to study the properties of individual droplets.

Similarly, double emulsions can be produced in two steps: first, the inner droplets are emulsified in the middle phase, and then the first emulsion dispersed in another phase [23, 24]. Each step results in an emulsion with a high polydisperse distribution. Therefore, these double emulsions are poorly controlled in both size and structure, which limits their application. By
using flow focusing, coaxial T-shape microchannels, monodisperse double emulsions can be achieved [25, 26]. But it requires a two-step droplet breakup and re-emulsified into continuous phase.

3-capillary microfluidic devices can generate uniform double emulsions in a single step [27-29]. This microfluidic device can control not only the outer and inner drop sizes precisely, but also the number of droplets encapsulated in each large drop. This device is constructed with three glass capillaries, two cylindrical ones and a square one, as shown in Figure 4.1. The innermost fluid flows through the injection tube, the middle fluid is pumped through the middle part between the outside of the cylindrical tube and the inside of the square tube, and the outer fluid is pumped from the opposite direction of the other side of the square capillary coaxial region. When the drag force overcomes the interfacial tension due to the coflow of liquids, this technique can produce droplets with a consistent size. The three flows break into either oil-water-oil (O/W/O) or water-oil-water (W/O/W) double emulsions. However, this 3-capillary technique has never been applied for the production of monodisperse mesoporous silica microshells.

In this chapter, we present a simple method to generate various types of monodisperse mesoporous silica particles, including smooth and crumbled microshells, microdisks, and microspheres. This method utilizes 3-capillary microfluidic devices to produce double emulsion templates and take advantage of the diffusion induced self-assembly of silica precursors. The shape and morphology of silica particles can be tuned by varying just a few key parameters, such as temperature and the surrounding oil, because these parameters control the polymerization rate of silica precursors and the evaporation rate of ethanol.
4.2 Materials and Characterizations

4.2.1 Materials

Hexadecane (99%), ethanol (denatured, reagent grade), tetraethyl orthosilicate (TEOS, 98%), hydrochloric acid (37%, A.C.S reagent), light mineral oil, heavy silicone oil (viscosity 350 cSt at 25 °C, Dow 200® fluid), and light silicone oil (viscosity 20 cSt, Dow 200® fluid) are purchased from Sigma-Aldrich Inc. A silicone-based surfactant, Dow 749® fluid, is purchased from Dow Corning, and Span® 80 is from Uniqema. Surfactant ABIL®-EM90 is kindly provided by Evonik Goldschmidt Corporation, and Pluronic® P123 triblock copolymer by BASF Corp. (Parispany, NJ).

4.2.2 Generation of O/W/O double emulsions

A microfluidic device is constructed with three glass capillaries, two cylindrical ones and a square one [27]. The two cylindrical glass capillary tubes are nested within the square glass tube, forming a coaxial geometry (Figure 4.1). Both of the cylindrical glass capillaries are tapered by first pulling 1 mm circular capillaries with a microcapillary puller (P-97, Sutter Instrument), and then breaking the tapered capillaries at points with a desired diameter using a scoring tile (CTS, Sutter Instrument). The sharp cuts are smoothened by heat-trimming with a microforge (MF-830, Narishige International). The combinations of the inner and outer fluidics are listed in Table 4.1. The middle phase consists of a silica precursor solution prepared by mixing 1 g of P123, 2 g of TEOS, 4.1 g of ethanol, and 1 g of HCl (37 wt%) at 400 rpm at room temperature for 1 h. O/W/O double emulsions are formed by pumping the inner oil phase, the middle silica precursor solution and the outer continuous oil phase into the 3-capillary microfluidic device using 3 digitally controlled syringe pumps (NE-1010, New Era Pump Systems Inc.). The typical pumping rates for
the inner, middle, and outer phases are 0.1, 1.0 and 4.9 mL/h, respectively.

**Figure 4.1** Schematic illustration of a 3-capillary microfluidic device for the formation of double emulsions.

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<th>Surfactant for outer fluid</th>
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<td>90% light silicone oil and 10% heavy silicone oil</td>
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<tr>
<td>B</td>
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<td>Light silicone oil</td>
<td>3% Dow 749</td>
<td>90% light silicone oil and 10% heavy silicone oil</td>
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<td>Light silicone oil</td>
<td>4% ABIL-EM90</td>
<td>hexadecane</td>
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</table>

**Table 4.1** Combinations of inner and outer fluids.
4.2.3 Polymerization

The O/W/O double emulsions are transferred to either a clean glass slide or a glass dish with outer fluid (10 mm in height) and polymerized typically at room temperature for 1 day or occasionally at 60 °C for 1 day. Typically, the particles are then washed, followed by calcination in air at 440 °C for 4 h to remove organic templates and any remaining surfactants.

4.2.4 Characterization

The double emulsion formation and the polymerization of silica are monitored using a high-speed camera (EXILIM EX-F1, Casio) mounted on an inverted optical microscope (Olympus IX51). Polymerized silica particles are imaged using the optical microscope, a scanning electron microscope (SEM, Hitachi S-2600N) and a transmission electron microscope (TEM, FEI Tecnai F20).

4.3 Results

The coaxial, hydrodynamic flow focusing geometry enables the fluids to pass through the exit orifice and to break up into double emulsion droplets, as shown in Figure 4.2. Using the microfluidic device, we obtain double emulsion droplets. After polymerizing silica precursors in the double emulsion droplets, monodisperse silica particles are obtained. Figure 4.3 shows exemplar silica particles obtained by polymerizing the double emulsions of the Composition A in Table 4.1 at room temperature on a glass slide. The average diameter of the microparticles is 210 ± 10 µm.
Figure 4.2 Optical microscope image of fluids passing through the exit orifice and breaking up into double emulsion droplets.
Figure 4.3 Optical microscope image of silica microshells obtained by polymerizing double emulsions of Composition A (Table 4.1) at room temperature on a glass slide.

Using a SEM, we obtain the three-dimensional shape of the microparticles, as shown in Figure 4.4a and b, which reveals that the microparticles have a dimpled pancake shape, and are surrounded by some residual oil on the substrate. We also polymerize the same double emulsions (Composition A in Table 4.1) at higher polymerization temperature (60 °C) to accelerate both the evaporation of ethanol and the polymerization of silica precursors, obtaining radially-cracked microshells (Figure 4.4c and d).
Figure 4.4 Scanning electron image of silica microshells obtained from the double emulsions of Composition A polymerized on a glass slide at room temperature: (a) 45° view with a lower magnification and (b) 45° view with a higher magnification, the diameter is 220 ± 20 µm; and polymerized at 60 °C: (c) 45° view of radially-cracked microshells and (d) top view, the diameter is 230 ± 10 µm. The scale bars are 200 µm.
In order to investigate the effect of inner fluid of the double emulsion, we use light silicone oil as the inner fluid (Composition B) instead of mineral oil. Quite similar silica microparticles are obtained when the double emulsions are polymerized at the same condition: at room temperature on a glass slide. Figure 4.5a and b show the SEM images of calcinated silica microparticles from the top and 45°, respectively. In order to observe the internal structure of the silica microparticles, we break some and find a shell structure as shown in Figure 4.5c.
Figure 4.6 SEM images of mesoporous silica shells obtained from the double emulsions of Composition B polymerized in a glass dish with extra outer fluid. The diameter of this particle is 200 µm.
Also, we try to reduce the evaporation rate of ethanol from the middle phase by polymerizing the double emulsions in a glass dish with extra outer fluid (heavy silicone oil). As shown in Figure 4.6, the shape and pore structure of resulting microparticles are crumbled and highly porous presumably due to the bubbles of evaporated ethanol. They are significantly different from those smooth microdisks without any large pores in Figure 4.5.

Then, we increase the evaporation rate of ethanol by using hexadecane as outer fluid, in which ethanol is more soluble than in silicone oil. Figure 4.7 shows the images of silica particles obtained by polymerizing double emulsions with a hexadecane-rich outer fluid (Composition C) at room temperature on a glass slide. We broke some particles during SEM characterization, one of them happened to be cut from the middle. The cross-section image (Figure 4.7b) shows that solid microdisks are obtained in this case. These particles do not form a shell structure, but a pancake shape with closed pores all over the particles. Figure 4.7c is a TEM image which shows mesoporous structure inside of a microparticle.
Figure 4.7 Electron micrographs of mesoporous silica microdisks obtained from the double emulsions of Composition C polymerized on a glass slide: (a) a SEM 45° view image of a silica microdisk, (b) a SEM cross-section image of a microdisk, and (c) a transmission electron microscopy (TEM) image of the internal structure.
We also find that solid spherical particles can be obtained (Figure 4.8) by polymerizing double emulsions (Composition D) at room temperature in a closed plastic bottle with extra outer fluid (20 mm in height). The TEM image (Figure 4.8b) shows these microspheres have mesoporous structure internally.

![Figure 4.8 Electron micrographs of uniform silica microspheres obtained from the double emulsions of Composition D polymerized in a closed plastic bottle with outer fluid at room temperature: (a) a SEM image of silica microspheres and (b) a TEM image of internal nanostructure.](image_url)
4.4 Discussion

We produce remarkably different types of silica microparticles by changing the composition and the polymerization environment such as temperature and the thickness of the outer oil layer. The whole polymerization process is affected critically by the competition between the polymerization rate of silica and the evaporation rate of ethanol from the middle phase. Ethanol is a byproduct of silica polymerization and therefore evaporation of ethanol would increase the polymerization rate. The evaporation rate of ethanol depends on the solubility of ethanol in the surrounding fluids. When ethanol evaporates fast due to a thin outer layer, we find that smooth surfaces are formed. It is a distinctive contrast to the silica particles polymerized in a polydimethylsiloxane (PDMS) microfluidic channel [16], in which smooth surfaces were formed in mineral oil as outer fluid while rough surfaces were formed in hexadecane. Hexadecane has higher ethanol solubility than mineral oil [30]. For those silica particles polymerized in a PDMS microfluidic channel, first, an interfacial subphase was formed because of the diffusion of the precursor solution from droplets [16]. Then, ethanol diffused fast due to a sharp concentration gradient of ethanol to the outer fluid that was continuously replenished through the channel. Due to the continuous flow of outer fluid, an interfacial subphase can be formed in hexadecane, but not in mineral oil, which has lower ethanol solubility. Such a polymerization condition is quite different from those of our silica particles. In our case, droplets are first formed in the microfluidic device, and then exported out of the device to a rather static environment for polymerization. Therefore, when particles are polymerized in a thin layer of outer fluid, either silicone oil (Figure 4.5) or hexadecane (Figure 4.7) on a glass slide, ethanol evaporates through the thin oil layer to air without the formation of interfacial subphases that contain a high concentration of ethanol [16]. Due to the lack of interfacial subphases, rather smooth surfaces are formed in these cases. In contrast, a thick layer of silicone oil in a glass dish helps the formation
of interfacial subphases, resulting rough surfaces (Figure 4.6).

Figure 4.9 Schematically illustrations of the four different mechanisms for the structural variations: (a) microshell in Figure 4.3, 4.4 and 4.5, (b) crumbled microshell in Figure 4.6, (c) microdisk in Figure 4.7, and (d) microsphere in Figure 4.8.
Schematic illustrations of the four different cases of the microparticle formation are presented in Figure 4.9. In three cases (Figure 4.9b, 4.9c and 4.9d), we hypothesize that the inner fluid droplet is expelled out through the middle-phase shell. The depletion force that pushes the inner droplet out is generated by the micelle formation of the triblock copolymer in the middle phase. As ethanol evaporates in the middle phase, the concentration of P123 starts to increase and P123 forms micelles. These micelles push the inner fluid droplet out through the middle phase due to the depletion force [18, 19, 31]. For the case, in which silica particles are made from the double emulsions with silicone oil as the outer fluid (Compositions, A and B) and polymerized on a glass slide, ethanol evaporates fast and thus the polymerization of silica advances fast as well. The fast polymerization makes the middle phase hard, and depletion force is not large enough to push out the inner fluid through the middle phase; therefore, the inner fluid is staying inside during the polymerization. After silica is completely polymerized and the inner oil is removed by calcination, an empty space is left inside and the shell structure is formed (Figure 4.9a, Figure 4.4a and b, and Figure 4.5). Varying the inner fluid does not seem to affect the structure of silica microparticle much, because ethanol evaporates mainly outward. Silica particles in Figure 4.4a and b, and Figure 4.5 are produced in the same condition except for the inner fluid: mineral oil for Figure 4.4a and b, and silicone oil for Figure 4.5. Both cases result in compressed silica microshells. When the temperature is elevated to 60 °C, the polymerization rate increases and partially polymerized silica shells start to form. Meanwhile, the concentration of P123 in the middle phase keeps increasing due to the ethanol evaporation, and the depletion force due to the P123 micelles becomes high enough to push the inner oil through partially polymerized shells[18, 19, 31]. Finally, radially-cracked microshells are formed due to the collapse of the partially polymerized shells (Figure 4.4c and d). When the double emulsions of Composition B are polymerized on a glass dish with extra outer fluid, the silica particles form a crumbled structure
(Figure 4.6). Double emulsions are immersed in the outer fluid during polymerization, and thus the rates of ethanol evaporation and polymerization get slower. At certain point, the inner fluid droplet can be expelled out through a still soft shell due to the depletion interaction discussed above. The partially-polymerized middle fluid forms the crumbled shape and the microporous shell structure after further silica polymerization (Figure 4.9b). When the outer fluid is changed from silicone oil to hexadecane-rich oil (Composition C) and the double emulsions polymerize on a glass slide, disk-shaped particles are formed. That may be related to the fact that ethanol is highly soluble in hexadecane [30], while almost insoluble in silicone oil. Due to the relatively faster ethanol evaporation through hexadecane than silicone oil, the concentration of P123 in the middle phase increases even faster. Thus, a sufficiently-high depletion force that can push the inner droplet out is generated much earlier than the polymerization. Probably right after these droplets are produced, the inner droplet is pushed out (Figure 4.9c and d). When these droplets without the inner fluid are polymerized on a glass slide, they form solid microdisks (Figure 4.9c). Moreover, when they are polymerized in a closed plastic bottle with extra hexadecane outer fluid, they maintain their spherical shape during polymerization, and form solid microspheres after polymerization (Figure 4.9d). The smooth surface generated in this case is in agreement with the silica particles generated with hexadecane as outer fluid in ESE method [12].

Finally, we observe the polymerization process in time in order to test the hypothesis that the ejection of the inner phase happens only in the case of Figure 4.9b, 9c, and 9d, but not in 4.9a. Our observations cooperate with this hypothesis. Figure 4.10 shows exemplar time-lapse optical images of a double emulsion droplet during the polymerization on a glass slide. After about 1 min, the inner droplet is pushed out and the middle layer, which is not completely polymerized yet, is crumbled down due to higher density than surrounding fluid.
Figure 4.10 Time lapse images of a double emulsion during the polymerization process. The double emulsions are made of Composition B (silicone oil as both inner and outer fluids) and polymerized on a glass dish with the extra outer fluid.
4.5 Conclusion

![Figure 4.11](image)  

**Figure 4.11** Graphic summaries about silica microparticles in different shapes.

We demonstrated that size-controllable, monodisperse silica shells and particles with different shapes and morphologies can be fabricated by using microfluidics. The shapes and morphologies of silica particles can be controlled by adjusting the polymerization rate of silica precursors and the evaporation rate of ethanol. The size of the silica particles can be easily controlled by changing the flow rates and viscosities of the three phases. This method provides new opportunities to generate silica particles and shells with different shapes and surface morphologies, for example, spherical shells with mesoporous surface morphology,
ellipsoid shells with microporous surface morphology, mushroom shells, all of which can be used in many applications such as drug delivery, sensing and energy requiring high loading capacity.
4.6 References


CHAPTER 5

High throughput microfluidic method for measuring cell stiffness

5.1 Introduction

The occurrence of abnormal stiffness of certain cells indicates certain diseases, such as osteoporosis [1], atherosclerosis [2], etc. [3] Palpation is clinically used to examine unusual stiffness of tissues based on the relationship between abnormal stiffness of the tissue and diseases [3]. Previous studies also show that for different cell types, the mechanical properties such as the stiffness of the cells and cell viscosity have distinct differences which can be related to their specific roles in living bodies [4, 5]. This relation can be used to distinguish cancer cells from healthy cells [6]. Cancer is characterized as a group of diseases involving uncontrolled cell division, cell invasion and disrupting healthy body tissues [7, 8]. The enhanced softness of cancer cells compared to non-malignant cells is one of the important pathological characteristics of cancer cells [8, 9]. Therefore, quantification methods to probe the difference of cell stiffness between cancer cells and healthy cells can be reliable and promising for cancer diagnosis.

The mechanical properties of a single cell have been studied using various techniques [10], such as micropipette aspiration [11], cell poker [12, 13] silicon micromachines [14], biointerface probe [15], microplates [16] and optical tweezers [17]. In addition, atomic force microscopy (AFM) is one of the most advanced techniques to study mechanical properties of cells in present [18, 3, 19]. However, most of the techniques can examine only local cell properties. These measurements may collect statistically meaningful data by probing several local points on a
single cell or testing a large number of cells, both of which demand lots of work. Additionally, though the mechanical behaviors of living cells are highly related to the environment of the cells, most techniques require specific substrates or microenvironment in which the cells might not behave properly. For example, standard AFM cell elasticity measurements require firm attachment of the cells on a substrate in order to get reliable results. The attachment changes the environment of the cells, which may change cell structure, viability and elasticity as well [20].

In this chapter, we present a low-cost and high throughput method to detect the mechanical properties of individual cells using a microfluidic device. This microfluidic manometer utilizes the hydrodynamic correlation between deformation of soft materials caused by strong structural confinement and pressure variation with the flow motions. Ultimately, this method will be able to isolate the most aggressive, highly metastatic tumor cell from the more latent cells, and to rapidly quantify the percentage of most aggressive/metastatic cells in the circulation, leading to the development of diagnostic tools. The method can be expanded to the investigation of the stiffness of various human-derived epithelial cell lines, and to the development of a drug-screening method.

5.2 Microfluidic device fabrication

5.2.1 Substrate Preparation

1.5 x 1.5 square inch glass substrates are cleaned by using soap solution with ultrasonic cleaning (Zenith Ultrasonic Tank) for 15 min. Then the glass substrates are rinsed with DI water and isopropanol a few times. The glass substrates are dried at 80 °C in an oven (Precision™ Mechanical Convection Oven) and covered with aluminum foil to prevent any contamination.

Positive photoresist (AZ 9260, 520 CPS, AZ Electronic Materials USA Corp.) is used to coat one side of the glass substrates. The pre-spin speed is 600 rpm for 6 s and spin speed is 2,000 rpm
for 30 s using a spin coater (ASS-301, ABLE Co., LTD.). Then photoresist-coated glass substrate is rested at room temperature for 3 min followed by 20 s of pre-baking at 65 °C and 6 min of baking at 110 °C on a hot plate (HT-1800, JAPAN PULSE LABO. INC.). The thickness of the photoresist coating layer depends on the spin coating speed, the viscosity of photoresist and the number of coating layers. The viscosity of photoresist changes with temperature. Therefore, during the experiments, the temperature of the photoresist is tuned to room temperature before spin coating.

The thickness of the photoresist is measured with a Tencor Profilometer. For one layer of coating using the standard parameters, the photoresist thickness is about 10 μm.

### 5.2.2 Photolithography - master mold

For this project, two photolithography machines have been used for different cases: Maskless UV Pattern Generator (belonging to Dr. Hiroshi Yokoyama’s lab) and Karl Suss Mask Aligner (Cleanroom). For the Maskless UV Pattern Generator, the pattern is drawn using Inkscape software and directly loaded to the digital micromirror device (DMD) and projected through an infinity corrected UV objective [21]. The resolution of the upper stage is about 10 μm/pixel and the resolution of the lower stage is about 2.2 μm/pixel. The Karl Suss Mask Aligner has a higher resolution down to less than 1 μm and is used to make microfluidic devices with smaller dimensions.

A typical photolithography process (exposure - developing – post baking) is used to generate the master mold containing microfluidic channels. For each of these two different exposing systems, various exposure and developing times are tested, and the best condition is selected after a set of trials. For the Maskless exposing system, 90 s of exposure time and 8 min of developing time are used for one layer of photoresist coating. For Karl Suss exposing system, 5 s of exposure
time and 6 min of developing time are used for one layer of photoresist coating.

5.2.3 Polydimethyl siloxane (PDMS) microchannels

Sylgard 184 Silicone Elastomer Kit (Dow Corning Co.), which contains two bottles: Sylgard 184 silicone elastomer base and Sylgard 184 silicone elastomer curing agent, is used to form PDMS microchannels replicating the pattern of a master mold. The two parts are mixed thoroughly at a ratio of 10:1, and poured on a master mold containing microfluidic channels. After waiting for a few hours, the bubbles trapped in the PDMS mixture disappear and PDMS is cured at 70 °C for 1 h in an oven (VWR International). Then the cured PDMS is peeled off from the substrate and cut into desired sizes. Harris Uni-Core™ is used to cut holes on the PDMS part to form inlets and outlets.

5.2.4 PDMS - glass bonding

In order to bond PDMS channels on a glass substrate and seal irreversibly to form a leak-free microfluidic device, surface oxygen plasma treatment is performed. Plasma treatment renders surface hydrophilicity to PDMS and glass substrate by plasma oxidation.

We build a homemade plasma cleaner (Figure 5.1) according to literature [22]. The system is made of a microwave, a vacuum pump, a vacuum gauge, an oxygen gas tank and a chamber. The chamber is a glass funnel (KIMAX®). A piece of PDMS replica mold (with the channels facing up) and a piece of clean glass are placed in the middle of the chamber, and vacuum is applied to remove the air and water inside of the chamber. Then, the pump is turned off and a small amount of oxygen is added into the chamber followed by giving a pressure level approximately 900 milliTorr. Then microwave is applied at 10 % of total power (900 W) for 5 s. Then the vacuum of the chamber is released to atmosphere and the PDMS part is placed onto the glass substrate.
with a gentle pressure by a finger. After this, the bonded PDMS microfluidic device is put in an oven at 70 °C for 10 min, it is covered with a piece of Scotch tape and stored at room temperature for future use.

Figure 5.1 Illustration of Homemade plasma system.
5.3 Evaluation of the homemade plasma treatment system

The homemade plasma generating system is evaluated by comparing with a UV-Ozone cleaner. The contact angle measurement results are shown in Table 5.1, 5.2 and 5.3.

### Drop Shape Image Analysis

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Mean: 106.7  106.6  106.7  0.1  1.962  3.724
Stand.dev.: 0.03  0.04  0.04  0.01  0.000  0.000

Table 5.1 Contact angle measurement for untreated PDMS (water is used as the liquid phase).
## Drop Shape Image Analysis

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| Mean:  | 78.0 | 79.1 | 78.6 | 0.5  | 1.072  | 2.767 |
| Stand.dev.: | 0.02 | 0.02 | 0.02 | 0.01 | 0.000  | 0.000 |

Table 5.2 Contact angle measurement for UV-Ozone cleanser treated PDMS (water is used as the liquid phase).
Table 5.3 Contact angle measurement for homemade plasma generating system treated PDMS (water is used as the liquid phase).
The contact angle between untreated PDMS surface and water is about 106.7°, which shows the PDMS is hydrophobic. After 10 min of UV-Ozone treatment the contact angle decreases to 78.6°. With our homemade plasma generating system, the contact angle dramatically decreases to 17.6°, which is the most hydrophilic. This validates the efficiency of the homemade plasma generating system.

Figure 5.2 Relationship between the contact angle and time after a plasma treatment.
The stability of hydrophilicity of the homemade plasma generating system treated PDMS is shown in Figure 5.2. The contact angle increases with time after a plasma treatment. Therefore, experiments are performed right after a plasma treatment.

5.4 Pressure sensing microfluidic manometers

In order to characterize the elasticity of cells and soft particles, we have designed and constructed a couple of different designs of microfluidic pressure sensing manometers using the soft photolithography technique.

5.4.1 Microfluidic manometer 1

The design of the PDMS microfluidic manometer 1 (Figure 5.3) detects the pressure difference between the inlet and outlet of a small channel in the middle, using a differential pressure sensor (ASDX030D44R, sensitivity is 0.133V/psi) connected to the ports B and C of the microfluidic device, while a suspension of particles or cells are pushed through the channel from the port A to the port D.
Figure 5.3 Photolithography pattern (a) and an optical microscopy image (b) of PDMS microfluidic manometer 1. Suspension of cells or particles pass through the channel in the middle from A to D. B and C are connected to a differential pressure sensor.
A Labview program for data acquisition and analysis has been developed (Figure 5.4). Then the sensitivity of this system has been tested by injecting DI water with a digitally controlled syringe pump (NE-1010, New Era Pump Systems Inc.). With 2.0 mL/h pumping rate, an acquired voltage data is shown in Figure 5.5.

Figure 5.4 Labview panels.
The voltage fluctuations may be caused by the instability of the mechanical syringe pump we use. In order to minimize the fluctuation of the flow and measure the voltage change as well as the pressure change when a particle or a cell passing through the channel in the middle, a more stable way to pump the flow into the device has to be developed. Considering the sensitivity of the pressure sensor and the technical difficulties of connecting and sealing the pressure sensor to the system, we come up with a different design [23] without the usage of a differential pressure sensor.

**Figure 5.5** Data acquainted with pumping DI water. X axis is the time of the moment and Y axis is the amplitude of voltage (V).
5.4.2 Microfluidic manometer 2

As shown in Figure 5.6, by injecting two liquids into the left two channels (40 µm x 200 µm), an interface is formed between the two liquids in the large 300 µm x 2000 µm channel [23]. The position of the interface changes when the pressure difference between the two channels (40 µm x 200 µm) is varied. When a particle passes through the top channel, there is a pressure drop in the top channel and the position of the interface moves up (Figure 5.7). The red lines indicate the positions of the interfaces and the blue circles indicate the cells.

We calibrate the device by injecting water into both inlets without any cells. When the pressures of the both channels are the same, the interface is in the middle of the device, and the interface height change ΔH is 0. When a pressure difference higher than 1 psi between the two liquid channels is introduced, the height change ΔH can be observed corresponding to the pressure change. The calibration data is shown in Figure 5.8.
Figure 5.6 Photolithography pattern (a) and an optical microscopy image (b) of the microfluidic manometer 2. The numbers are length scales of channel in micrometers.
Figure 5.7(a) position of the interface when there’s no cell going through the upper channel.

(b) The interface position changes when there’s a cell inside of the upper channel.
After that, we inject a cell/RPMI solution (provided by Professor Gail Fraizer) and a green food coloring/water solution through the two inlets to the channels and measure the height change ΔH when the cell passing through the channel (Figure 5.9). The passage of cells and the change of interface are monitored using a high speed camera (Phantom v211, Vision Research). Instead of using mechanical syringe pumps, we use pressurized air with regulators to push liquids into the channels to avoid the pressure instability generated by the syringe pumps. The cells are polydisperse and have an average diameter about 20 ± 5 µm. A recent study clearly indicates that the process of cell death significantly changes the stiffness of the cells [24]. To keep the elasticity of the cells, the cells have to be alive. So the experiments are conducted right after the cell culture.
and the temperature is kept at 37 °C by wrapping heating wires around the syringes to keep the cells alive. Because the cells have different sizes, when the cell size is smaller than the channel width, the passing-through of the cell doesn’t generate any pressure change, so the height change is 0. Those large height changes are probably because of the aggregation of the cells, several cells pass through together generate larger pressure change.

Figure 5.9 Experimental data of cells passing through the channel. X-axis is the height change [pixel] of the interface; y-axis is the number of cells has certain height change.
In order to detect cells with smaller sizes, a new design that has a zigzag channel with smaller dimensions is developed, as shown in Figure 5.10. The width of the thin channels is only 4\( \mu \text{m} \). Presumably, the device with this dimension will provide higher sensitivity and accuracy.

Figure 5.10 A newly-designed device with smaller dimensions (4 \( \mu \text{m} \) channel width).
5.5 Conclusions

In this chapter, we have demonstrated two out of many microfluidic manometer designs that can be great candidates for cell stiffness measurements. Further works need to be done in order to apply these designs into real cell stiffness measurements. For the first design, a way to provide a more stable flow is necessary to be usable. Better flow stability may be provided by a gas pressure pump instead of a mechanical pump. PDMS microparticles with various elasticities can be obtained by mixing the elastomer base and curing agent at various ratios. The elasticity of the PDMS microparticles can be easily characterized with a rheometer. After injecting the PDMS microparticles with the same size as the cells to the microfluidic manometer under the same condition, the height change (pressure drop) can be related to the elasticity of the PDMS microparticles. For example, if the height change is 5 pixel for PDMS microparticles with a 50 kPa linear shear moduli $G'$ value, presumably, $G'$ of the cells with 5 pixel’s height change should be about 50 kPa as well. By using PDMS microparticles with various elasticities, the microfluidic manometer system can be calibrated and the elasticity of cells can be measured. The second design is more promising because it provides meaningful statistical data in a short amount of time. A smaller dimension design which we have fabricated at the end of this chapter may provide higher sensitivity and provide more accurate data.
5.6 References


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CHAPTER 6

Summary

6.1 Summary

In Chapter 2, we present a simple, one-step thermal treatment that generates carbon nanodots (CNDs) from various hydrophilic polymers without involving complicated equipment or toxic chemicals. Two widely used polymers, polyethylene glycol and polyacrylic acid are selected as examples to present the properties of carbon nanodots generated by this method. Unique features of CNDs include broad excitation and emission spectra, relatively high quantum yields, and excellent stability against photobleaching. The emission spectra of CNDs show a dependence on the excitation wavelength. The size distribution of CNDs depends on the type of polymer and the duration of the thermal treatment. This one-step thermal treatment method may be applied for the mass-production of CNDs from a broad selection of polymers.

In Chapter 3, we present functionalizable photoluminescent magnetic iron oxide nanoparticles (PLMNP) produced by heating magnetic nanoparticles coated with non-photoluminescent hydrophilic poly (acrylic acid) (PAA), but without any add-on photoluminescent chemicals. Photoluminescence of PLMNP is originated from a carbon nanodot layer that is converted from the PAA polymer coating layer during the heat process. Interestingly, PLMNP are more photo-stable than conventional organic dyes. Further functionalization of PLMNP is easily achieved through the coupling reaction with carboxyl groups of the coating layer on the surface. PLMNP can be remotely heated by applying an alternating magnetic field due to the superparamagnetic property, and are found to have good heating efficiency. All these
advantages make these nanoparticles appealing to various biomedical applications such as dual modality imaging and hyperthermia treatment.

In Chapter 4, a simple method to generate monodisperse mesoporous silica particles from double emulsion templates generated by glass capillary microfluidic devices is present. Silica precursors in the middle phase of double emulsion templates are polymerized and self assembled to mesoporous structures of triblock copolymer templates. We find that various types of silica microparticles, including smooth and crumbled microshells, microdisks, and microspheres, can be generated by varying polymerization temperature and the outer fluid. This method provides a convenient strategy to generate silica mesoporous microparticles with a variety of shapes and surface morphologies by tuning a few key parameters.

In Chapter 5, a low-cost and high throughput method to detect the mechanical properties of individual cells using a microfluidic device is presented. Microfluidic PDMS channels are designed and built with photolithography technique. The manometer can characterize cell elasticity of cancer cells based on the pressure change.

6.2 Future work

For carbon nanodots project, future work would focus on determining the chemical structures of the carbon nanodots and the photoluminescence mechanism. X-ray Photoelectron Spectroscopy is a more sensitive instrument for detecting C = C structure, it may be used to further prove the absence of C=C structure in our carbon nanodots or to detect the existence of C = C structure. E-beam photolithography technique may be utilized to form a single layer of polymer which generates a single layer of carbon nanodots. The results may be helpful for studying the mechanism and chemical structure of the photoluminescent carbon nanodots. For photoluminescent nanoparticles project, future work would focus on the biocompatibility of the
nanoparticles. For example, it would be helpful to test the cell death rate after introducing nanoparticles into the cell culture. For cell elasticity project, microfluidic devices with smaller dimensions would benefit the results.