Application of metal-doped graphene quantum dots in biomedical imaging

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Abstract

With the development of personalized cancer medicine and moving away from a conventional biopsy, there is a need in creating a multifunctional platform for cancer diagnosis and treatment monitoring. Sonography offers many advantages over standard methods of therapeutic imaging due to its non-invasiveness, deep penetration, high spatial and temporal resolution, low cost, and portability. The benefits of the ultrasound method make contrast agents an ideal platform for the efficient strategy of cancer diagnostic and therapy. In this work, we developed metal-doped graphene quantum dots (Me-NGQDs) that demonstrate high-contrast properties in ultrasound brightness mode. The successful imaging enhancement was observed in tissue phantom and chicken breasts tissue. The relatively small size of the metal-doped graphene quantum dots makes them easily be internalized into the cells, while functional groups on their surface allow binding a cancer-targeted marker and therefore be used as a cancer-targeted delivery. By a combination of imaging and targeting capabilities, ultrasound contrast agents based on metal-doped graphene quantum dots enable desired cancer-focused nanotherapeutic and imaging approaches.

Introduction

Biomedical imaging: ultrasound (sonography)

- non-invasive
- portable
- cheap and affordable
- deep penetration and high resolution

Contrast enhanced ultrasound (CEUS) applications:

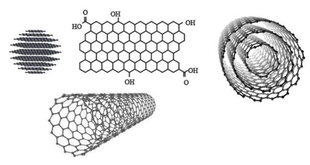
- cardiac imaging [1]
- accessing liver lesions [2]
- renal carcinoma [3]

CEUS requires using ultrasound contrast agents (UCAs) that have:

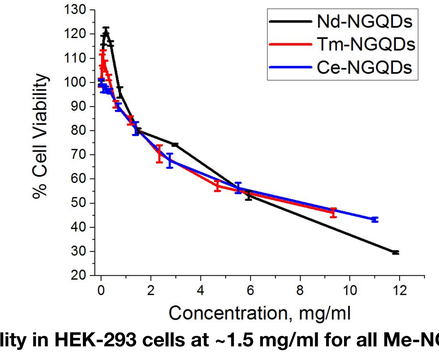
- good echogenicity
- high stability in the blood pool
- high biocompatibility and biodegradability

Carbon based nanomaterials – promising platform for developing UCAs:

- biocompatible and biodegradable
- nanoscale size
- good echogenic properties
- drug delivery vehicles
- optoelectronic properties



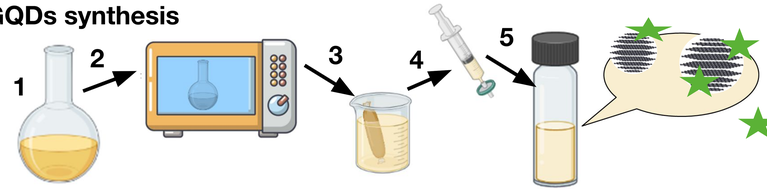
Results: Biocompatibility of Me-NGQDs



- 80% viability in HEK-293 cells at ~1.5 mg/ml for all Me-NGQDs.

Materials and Methods

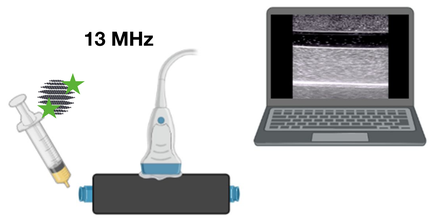
GQDs synthesis



Metal-doped NGQDs (Me-NGQDs), where Me is Ce, Nd or Tm; NGQDs – no metal dopant

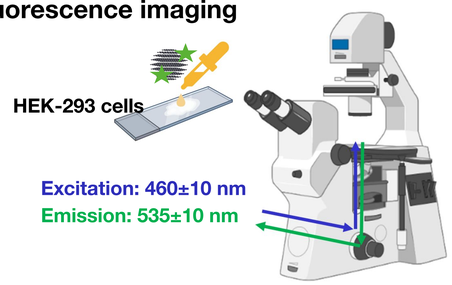
- $CeCl_3$ (0.005 M), $Tm(O_2C_2H_3)_3 \cdot 4H_2O$, $Nd(NO_3)_3 \cdot 6H_2O$ (0.008 M) and Glucosamine HCl (0.04 M) were mixed (1), microwaved (2), purified (3) and filtered (4) to get Ce-NGQDs, Nd-NGQDs and Tm-NGQDs (5).

Ultrasound imaging

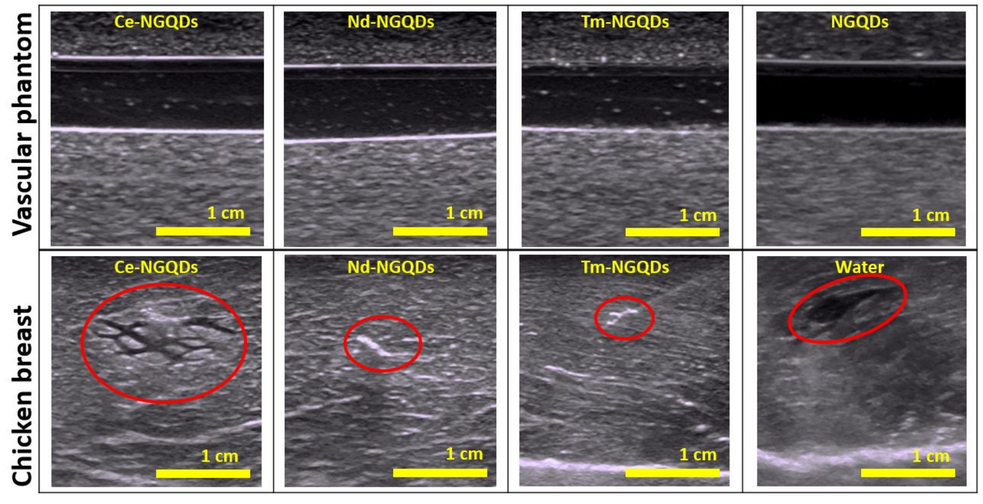


Vascular phantom (or animal tissue)

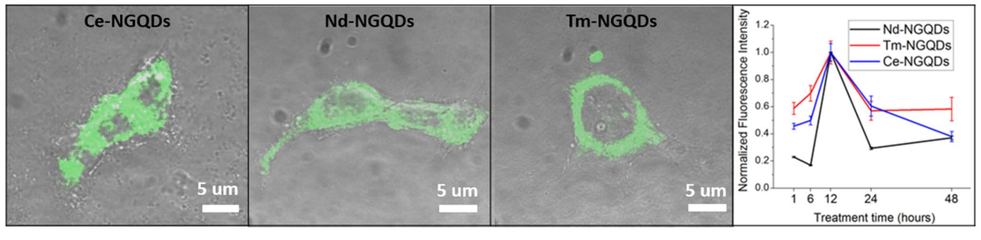
Fluorescence imaging



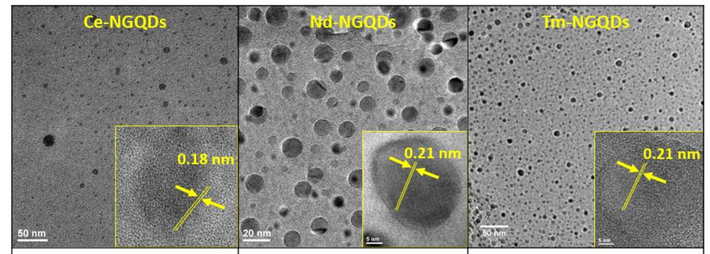
Results: Ultrasound images of contrast enhanced Me-NGQDs



Results: Fluorescence images and internalization study of Me-NGQDs in HEK-293 cells

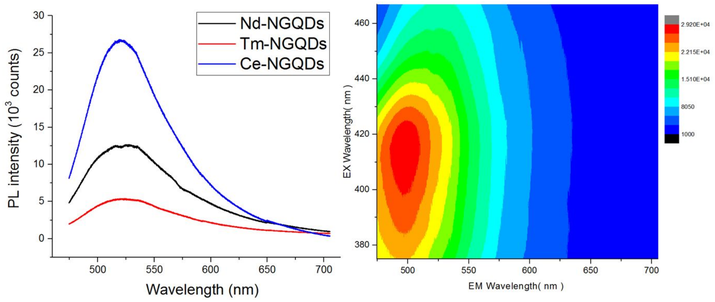


Results: TEM, HRTEM and EDX of Me-NGQDs



Ce-NGQDs	Nd-NGQDs	Tm-NGQDs
Size: 5.5±3.0 nm	Size: 7.2±2.0 and 13.3±2.1 nm	Size: 6.3±2.4 nm
Atomic percentage of elements:	Atomic percentage of elements:	Atomic percentage of elements:
C 63.80 % N 6.43 %	C 89.99 % N 4.67 %	C 93.51 % N 1.12 %
O 24.45 % Ce 5.31 %	O 4.73 % Nd 0.61%	O 4.88 % Tm 0.48 %

Results: Optical properties of Me-NGQDs



- Left: Fluorescence spectra of Me-NGQDs with excitation 460 nm.
- Right: Photoluminescence excitation-emission map of Ce-NGQDs.

Conclusion

- Nd-NGQDs, Tm-NGQDs, Ce-NGQDs show high ultrasound contrast properties in vascular phantom: good for vascular imaging. Nd-NGQDs and Tm-NGQDs are detected in chicken breast: soft tissue imaging.
- All Me-GQDs demonstrate high biocompatibility up to 1.5 mg/ml and efficient cell internalization showing potential for drug delivery/imaging.
- Nd-NGQDs, Tm-NGQDs and Ce-NGQDs exhibit intrinsic fluorescence offering high precision tracking of therapeutic, while ultrasound imaging allows for deeper tissue observation. These techniques can complement each other to provide deterministic imaging.

References

1. Porter, T.R., et al., *Guidelines for the cardiac sonographer in the performance of contrast echocardiography: a focused update from the American Society of Echocardiography.* Journal of the American Society of Echocardiography, 2014. **27**(8): p. 797-810.
2. Dietrich, C.F., et al., *Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) in the liver–update 2020–WFUMB in cooperation with EFSUMB, AFSUMB, AIUM, and FLAUS.* Ultraschall in der Medizin-European Journal of Ultrasound, 2020. **41**(05): p. 562-585.
3. Dong, X.-q., et al., *Contrast-enhanced ultrasound for detection and diagnosis of renal clear cell carcinoma.* Chinese medical journal, 2009. **122**(10): p. 1179-1183.