Porphyrin free organic contrast agents for photoacoustic imaging

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Background

While the use of endogenous contrast agents in photoacoustic imaging (PA) provides morphological and functional information, exogenous contrast agents not only allow to further enhance the quality of the obtained images, i.e., contrast and penetration depth, but also, when combined with biomarkers, provides the tools to visualize biological processes in the body. Gold nanoparticles are the most common contrast agent. Although possessing very good optical and physicochemical properties, their biocompatibility is limited as their accumulation causes oxidative stress and may lead to permanent cellular damage. In this work, study porphyrin-free covalent organic framework nanoparticles (nCOFs) as exogenous contrast agents for photoacoustic imaging. These nanostructures are 100% organic, biodegradable, present strong stability in physiological fluids, and are non-cytotoxic in a wide range of concentrations [1, 2].

Methods

The feasibility of the 2D-π conjugated materials LZU-1 and TAPB-PDA (1,3,5-tris(4-aminophenyl)benzene-terephthaldehyde and 1,3,5-Benzenetricarboxaldehyde polymer with 1,4-benzenediamine, respectively) as exogenous contrast for photoacoustic imaging has been analysed. The PA response of nCOFs was compared with the following reference nanoparticles diluted in phosphate buffered saline (PBS): gold nanoparticles (AuNP) and mesoporous silica nanoparticles labelled with the ATTO532 chromophore (MSN-ATTO532).

Two experimental studies have been carried out. Since the optimum laser excitation wavelength for the two references (AuNP, ATTO532) is 532 nm, we first selected this excitation wavelength and compared the measured photoacoustic amplitude of the four samples diluted at a fixed concentration of 0.1 mg/mL. Secondly, since the NIR excitation window is widely used in photoacoustic imaging, we also characterised the nCOFs response in this range of the spectrum, even though it is far away from their absorption peak. For this test, all the samples with a concentration of 0.1 mg/mL were excited from 660 to 940 nm laser wavelengths at 20 nm steps.

The experimental setup consisted of a tuneable optical parametric oscillator (OPO) laser (EKSPLA NT352A, Vilnius, Lithuania), emitting the excitation pulses at a repetition rate of 10 Hz and 6 ns duration, synchronised with the Verasonics Vantage 256™ acquisition system coupled to a linear ultrasound probe (256-channel, 7.6-MHz central frequency, model L11-5v, Verasonics) for measuring the PA signals.

Results

The PA response of the four samples, which differ from the PBS reference level, is shown in Fig1(a). As expected, the highest response is provided by AuNP. The performance of the MSN-ATTO532 is also not surprising, as the grafted fluorochrome shows its maximum absorption at the selected laser wavelength. Attention is drawn, however, to the high level of photoacoustic amplitude generated by the two nCOFs. This could be explained by their strong π-π interlayer interaction, which absorbs over a wide range of wavelengths and releases vibrational energy producing a sufficiently large PA signal.

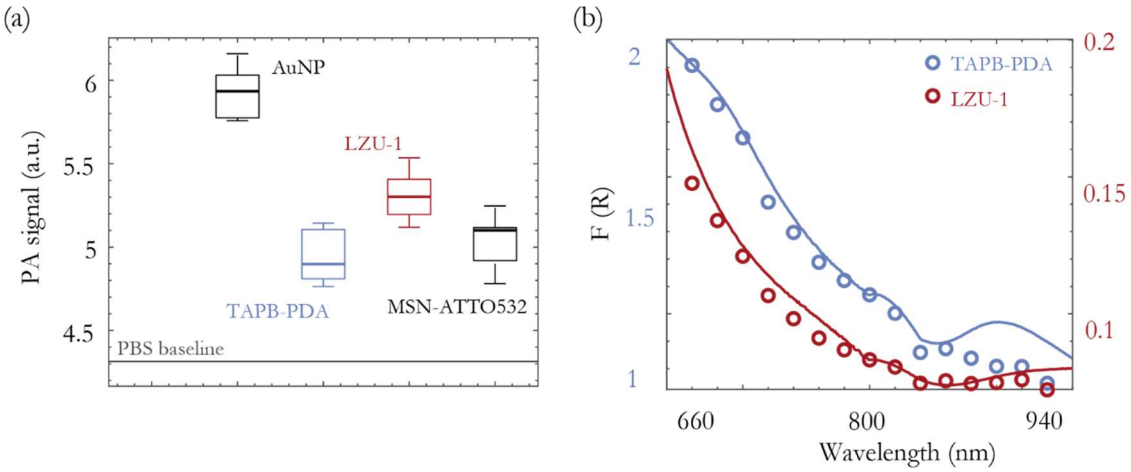


Fig. 1. (a) Comparative PA amplitude of the three samples at 0.1 mg/ml and excited by 532 nm laser wavelength. (b) Overlap of the PA spectrum between 660 and 940 nm of TAPB-PDA and LZU-1 (markers) and the NIR optical absorption (lines).

The optical absorption spectrum in the NIR window of the studied nCOFs, which were obtained using a commercial spectrophotometer, is shown in Fig. 1(b) solid lines. Although these nCOFs have a residual absorbance at NIR wavelengths that is less than an order of magnitude compared to their strong absorbance in the UV and visible bands, they still provide enough absorption to obtain detectable PA signals. The spectra have been overlapped and the amplitude normalised to allow a straightforward comparison of their profiles. Data points for the PA spectrum were taken as the average of six measurement replicates from laser excitation wavelengths from 680 to 940 nm at 20 nm steps.

Conclusions

The obtained results show that both nCOFs show promising behaviour as contrast agents for PAI, which is dependent on the interlayer stacking of their 2D-structure. It was also demonstrated that these nCOFs could be used to acquire deeper PA images using the NIR optical window, despite being outside their optimal absorbance band.

References

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