Rapid Nucleic Acid Concentration from Wastewater with Minimal Equipment: New Strategy for COVID-19 Surveillance

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Background

Surveillance of wastewater for SARS-COV-2, the virus that causes COVID-19, is a promising tool for monitoring disease trends and catching outbreaks early.

However, one challenge is concentrating the virus from large volumes of wastewater into the tiny volume needed for COVID-19 testing.

A rapid, cheap, and easy-to-use technique for concentrating SARS-COV-2 RNA would facilitate widespread use of wastewater surveillance of COVID-19.

Hollow silica microspheres, or microscopic glass bubbles, have previously been functionalized and used to capture *Cryptosporidium* from stool (Weigum, Xiang et al. 2016), which sets the stage for their potential use in RNA concentration.

Purpose

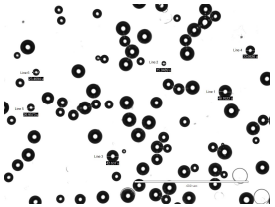
To optimize the use of functionalized hollow silica microspheres for RNA capture and subsequent nucleic acid testing

Research Questions and Goals
What amount of microspheres and volume of liquid should be used?
How long does it take for nucleic acids to bind to the functionalized microspheres?
How can the captured nucleic acids be released from the microspheres after concentration?
Is this method compatible with PCR, the nucleic acid test that is used to detect SARS-CoV-2?

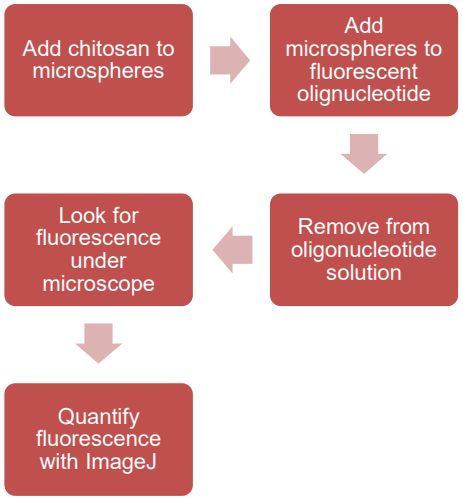
Goal: Use hollow silica microspheres to rapidly concentrate nucleic acids from aqueous solution so they can undergo PCR.

Methods

- For functionalization, the microspheres are mixed with chitosan at a high pH
- To test nucleic acid binding, the functionalized microspheres are mixed with a short, fluorescent oligonucleotide
- Next, the microspheres are viewed with epifluorescent microscopy to observe binding of the fluorescent oligonucleotide
- Binding of the fluorescent oligonucleotide can be quantified using ImageJ, which measures the fluorescence around each microsphere



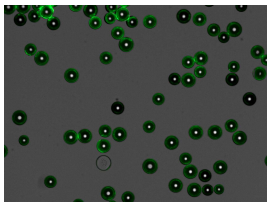
Microscopic picture of hollow silica microspheres before chitosan functionalization or nucleotide capture. The microspheres have been sieved to isolate those that are 25-50 micrometers in diameter.



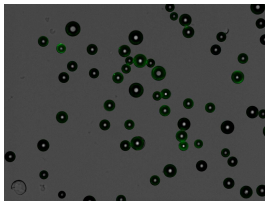
Findings

Speed of Nucleic Acid Capture
Preliminary experiments show that nucleic acids bind to the microspheres in as little as ten minutes. Current experiments are aiming to examine nucleic acid capture in sub-ten-minute amounts of time.

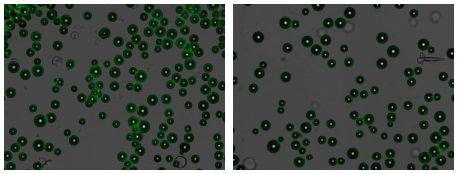
Conclusion
Chitosan-functionalized hollow silica microspheres merit further exploration as a potential tool for rapid, cheap, and easy-to-use tool for concentrating RNA



Chitosan-functionalized microspheres after ten minutes of incubation with a fluorescent oligonucleotide. The green rings around the microspheres show that the oligonucleotide has already bound to the microspheres.



Chitosan-functionalized microspheres after twenty minutes of incubation with a fluorescent oligonucleotide. As in the ten-minute picture, green rings show that the oligonucleotide has been captured.



Chitosan-functionalized microspheres after six (left) and 24 (right) hours of incubation with a fluorescent oligonucleotide. The oligonucleotide has been captured, as shown by the green rings around the microspheres.

Implications and Future Directions

It is encouraging that nucleic acid capture appeared to be so rapid, as this would reduce turnaround time for testing. However, releasing the nucleic acids for PCR is another challenge that must be tackled.

So far, experiments have been performed with hollow silica microspheres in 0.5mL of liquid, so efforts should be made to demonstrate efficacy with higher volumes.

If this method is successful, not only could it aid in surveillance of COVID-19 outbreaks, but it could also potentially be harnessed for other nucleic acid detection applications, such as wastewater surveillance of other pathogens.

Value
Chitosan-functionalized hollow silica microspheres could potentially be a cheap, rapid, and easy tool with which to concentrate RNA from wastewater for COVID-19 testing and other applications.

Acknowledgements

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References

Weigum, S. E., et al. (2016). "Hollow silica microspheres for buoyancy-assisted separation of infectious pathogens from stool." Journal of Chromatography A **1466**: 29-36.

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