Effects of Nanobubbles on Cell HEK-293

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Abstract

Nanobubbles (NBs) have gained significant attention in biological applications due to their unique physicochemical properties. This study explores the effects of air, nitrogen, and oxygen nanobubbles on eukaryotic cell (HEK-293) viability and adhesion, alongside the characterization of nanobubble size and concentration. In Experiment 1, a cell scratching assay was performed on HEK-293 cells to assess their response to nanobubbles in phosphate-buffered saline (PBS). The experiment included control conditions using complete cell media. In Experiment 2, dynamic light scattering (DLS) analysis was employed to determine nanobubble size and concentration. Experiment 3 investigated ozone nanobubbles using a colorimetric reaction. HEK-293 cells detached from the wells within one minute of exposure to nanobubbles, likely due to the preparation of NBs in reverse osmosis (RO) water. Cells could not survive in PBS for more than two minutes, while those in complete media remained attached for at least five minutes. DLS analysis revealed that nanobubble solutions had particle sizes estimated at 40-65 nm in diameter depending on generation conditions. The presence of nanobubbles was corroborated by AFM imagery. Ozone nanobubble concentrations were estimated at 2.5E11 /mL. The presence of nanobubbles influenced cell adhesion and survival, with potential improvements achievable by preparing nanobubble solutions in cell media. Further studies should explore cell viability using fluorescent dyes and further quantification of nanobubbles. Additionally, optimizing nanobubble concentration for additional DLS and AFM analyses along with fluorescence studies of reactive oxygen species to unambiguously identify the respective species will enhance characterization and quantification accuracy.

Introduction

Nanobubbles (NBs) have gained significant attention in biological applications due to their unique physicochemical properties. This study explores the effects of oxygen nanobubbles on eukaryotic cell (HEK-293) viability and adhesion, alongside the characterization of nanobubble size and concentration.

Materials and Methods

Nutrient solutions prepared from standard chemicals for cell cultivation
Dynamic light scattering techniques
Fluorescence spectrometer for identification of reactive oxygen species
Atomic force microscopy for visualization of nanobubbles
N-type and p-type silicon substrates

Methodology

Nanobubble generation:

Air was concentrated by an oxygen concentrator to produce oxygen gas as a feed to a novel nanobubble aerator (NBA) that generated NB in the water sample.

Nanobubble Characterization:

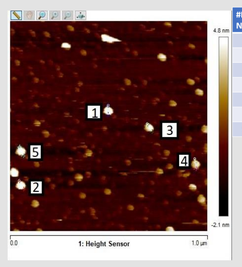
AFM: This cutting-edge technique was used to visualize and characterize the oxygen nanobubble samples.
Oxygen NB sample were imaged on the n-type and p-type silicon substrate and were imaged using different tips.

DLS: The dynamic light scattering technique was used to determine the particle size before and after oxygen aeration on day 0 and day 10.

ROS: Different reactive oxygen species (ROS) were determined in oxygen NB sample using different chemicals that react with various ROS giving fluorescence intensities being measured.

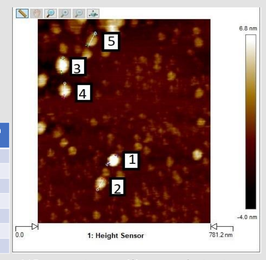
Results : A.) Characterization of O2 NB water.

AFM: Using the AFM, we successfully visualized the NBs. The size of NB ranges from 41 nm to 65 nm and the concentration of NB is 2.5 x 10^14 NB/L



#Bubble. No.	Diameter in nm
1	43.137
2	41.16
3	39.745
4	43.645
5	39.46
Mean	41.394
STDev	1.709

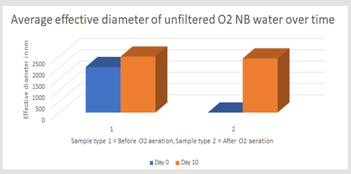
NB on n-type silicon substrate

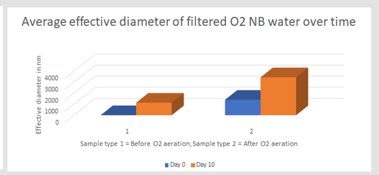


#Bubble. No.	Diameter in nm
1	58.555
2	58.735
3	74.887
4	66.671
5	65.784
Mean	64.652
STDev	6.743

NB on p-type silicon substrate

DLS: Average effective diameter is measured over period of time in filtered and unfiltered O2 NB samples, which clearly show that as time passes, the size of NB increases, suggesting growth of the particles over time, or aggregation.

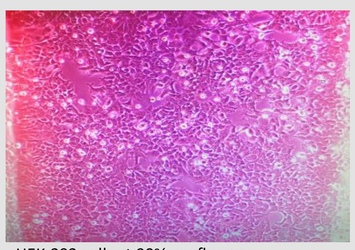




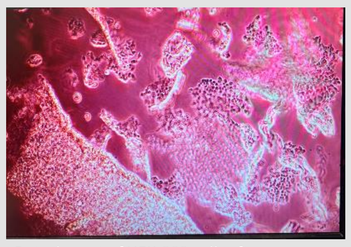
ROS: Oxygen NB water when reacted with APF and different chemicals show fluorescence. These chemicals only react with certain ROS, thus presence of ROS can be determined.

Sr. No	Oxygen NB water with (0.025 mm AFP)	Fluorescence	Conclusion
1	DMSO	935 to 740	Hydroxyl radicals were produced in low amounts.
2	ferrous ion	935 to 2196	H2O2 produced in significant quantity
3	SOD and ferrous ion	935 to 2830	O2- was produced in significant quantity as compared to ozone NB water.

B.) Cell line interaction with O2 NB water.



HEK-293 cells at 90% confluency



Detachment of HEK-293 cells after exposure to N_2, air and O_2 nanobubbles for 1 minute in phosphorus buffered saline solution

Solution	HEK-293 survival time
NaCl saline solution	~40 minutes
NaCl saline solution + 10% nanobubble	~40 minutes
EMEM	>24 hours
DMEM	>24 hours
EMEM + 10% nanobubbles	>40 minutes
DMEM + 10% nanobubbles	>40 minutes

Conclusion

HEK-293 cells detached from the wells within one minute of exposure to nanobubbles, likely due to the preparation of NBs in reverse osmosis (RO) water. Cells could not survive in PBS for more than two minutes, while those in complete media remained attached for at least five minutes. DLS analysis revealed that nanobubble solutions had particle sizes estimated at 40 nm to 65 nm in diameter.

Recommendations

Further studies should explore cell viability using fluorescent dyes and additional confirmation and quantification for various gas nanobubbles. Additionally, increasing nanobubble concentration for further instrumental analyses will improve characterization and quantification assurances.

Acknowledgements

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