POLYETHYLENEIMINE CAPPED POROUS SILICA NANOPARTICLES FOR NEOVASCULAR AGE-RELATED MACULAR DEGENERATION: RELEASE AND TOXICITY EVALUATION

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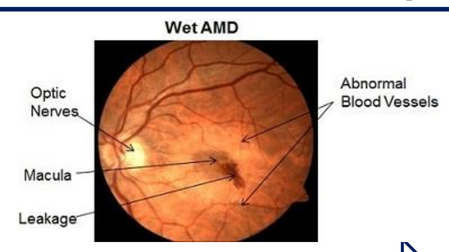
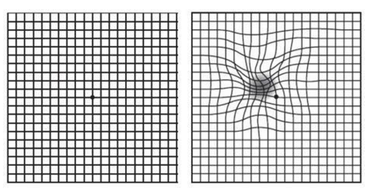
Age-related Macular Degeneration (AMD) - Background

- Age-related Macular Degeneration (AMD), a condition with progressive loss of central visual acuity creates blind spots on the retina with increasing age.
- Irregular angiogenesis characterised by weak and leaky blood vessels become prominent due to the over-expression of the vascular endothelial growth factor A (VEGFA) in the wet form of AMD.
- The currently available clinical regimens are commonly invasive, patient incompliant, along with numerous side effects and pharmacokinetic variations in individuals.
- As many as 11 million people in the United States have some form of age-related macular degeneration. This number is expected to double to nearly 22 million by 2050[1].
- 7% of the Irish population aged 50 years and over are current AMD patients.





Amsler grid viewed with normal vision (left) and with wet AMD (right)

Objective

- To develop large-pore mesoporous silica nanoparticle (MSN) carrier system coated with different concentrations of a cationic polymer, polyethyleneimine (PEI), which will be further used to load VEGFA siRNA to form MSN@VEGFAsiRNA@PEI (Fig 1.).
- To synthesize and characterize the extracted MSNs and functionalized MSNs viz, FMSN 1 (0.5 mg PEI/mg MSN) and FMSN 2 (0.75 mg PEI/mg MSN)
- To understand the *in-vitro* release of rhodamine B from FMSN 1 and FMSN 2 in the acidic lysosomal extract obtained from rabbit liver tissue.
- To assess the cytotoxicity of FMSN 1 and FMSN 2 in the ARPE-19 retinal cells

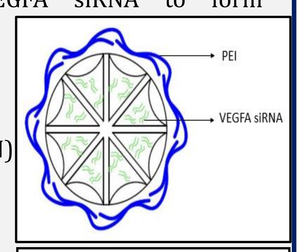
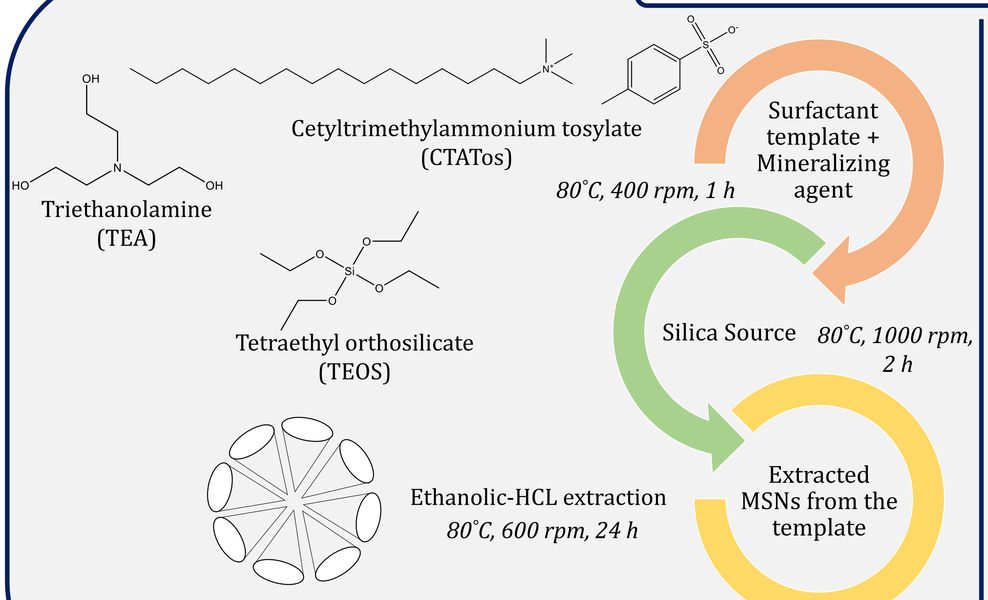


Fig 1. Illustration of MSN@VEGFAsiRNA@PEI

Synthesis and Characterization of Extracted MSNs and Functionalized MSNs



Co-condensation Process

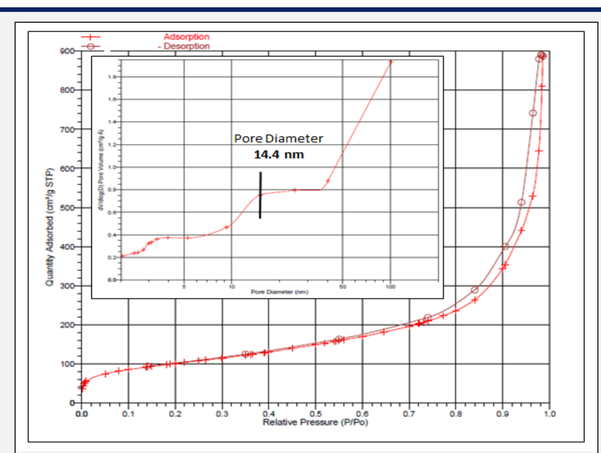


Fig 2. N_2 adsorption-desorption isotherm and pore size distribution curve (inset) for extracted MSNs.

A type-IV isotherm confirms the center-radial nature of the extracted MSNs with

- Pore diameter- 14.4 nm
- BET surface are – 358 m^2/g
- Pore volume - 1.4 cm^3/g

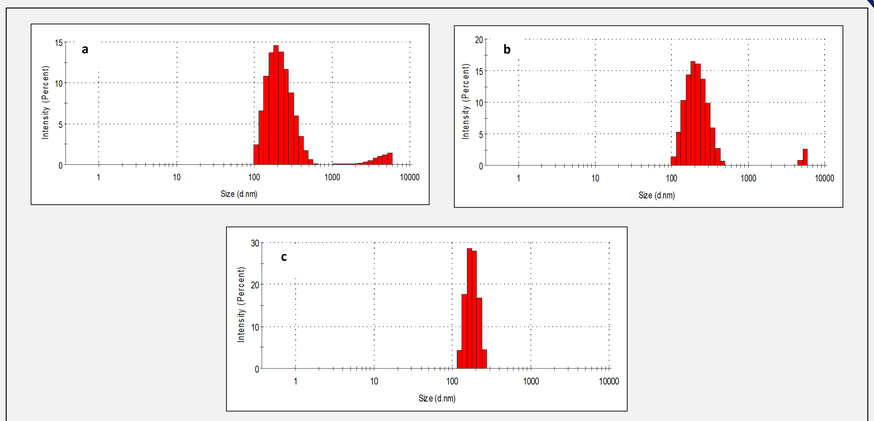
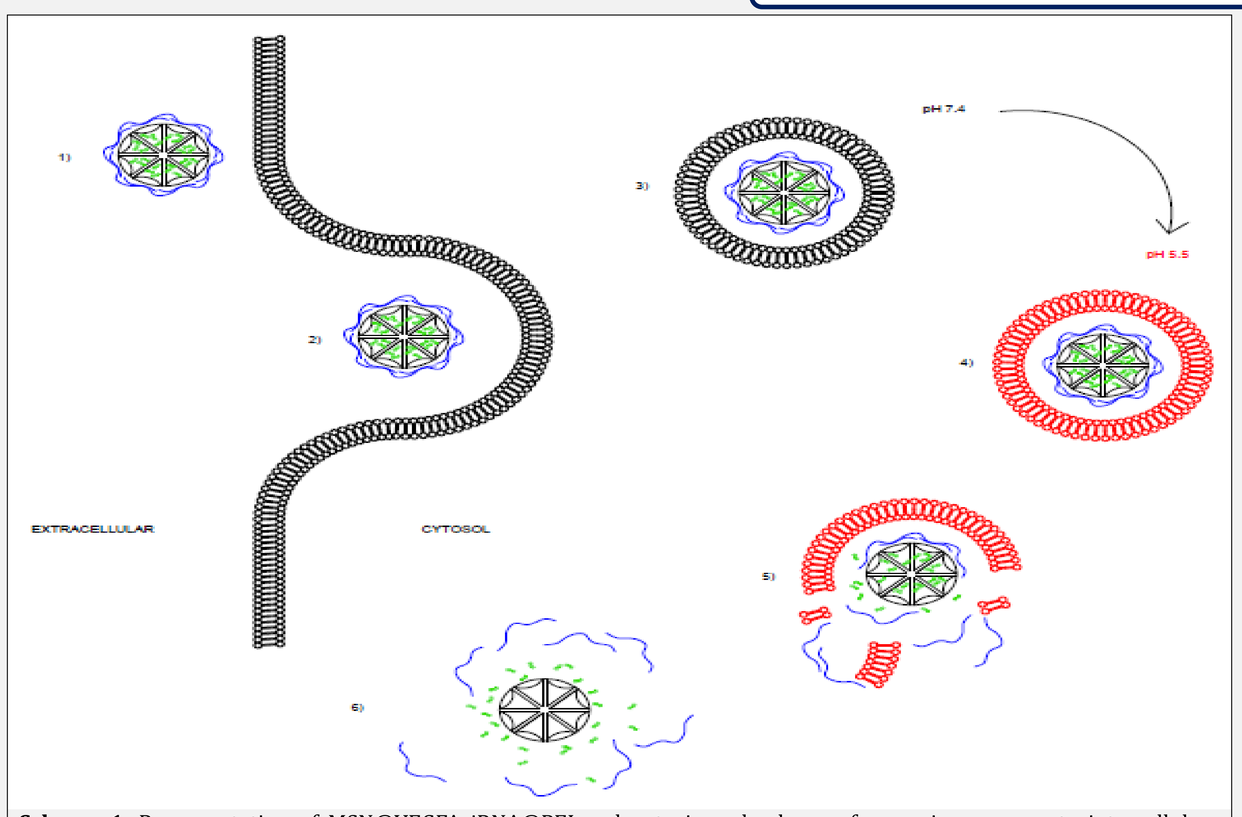


Fig 3. Hydrodynamic particle size distribution of a) MSNs, b) FMSN 1 and c) FMSN 2.

Sample name	Average particle size (nm)	PdI	Zeta potential (mV)
MSNs (a)	213 ± 77	0.208	-13.6
FMSN 1 (b)	249 ± 61	0.331	+13.8
FMSN 2 (c)	226 ± 34	0.381	+36.3

In-vitro Release Kinetics for Rhodamine B loaded FMSN



Scheme 1. Representation of MSN@VEGFAsiRNA@PEI endocytosis and release of cargo in response to intracellular microenvironment. 1) Nanocarrier in the extracellular region, 2) Cellular uptake by endocytosis, 3) MSN within the endosome, 4) Acidification of transiting endosome, 5) Triggered escape of the MSN from endosome, 6) VEGFA siRNA release in the cell.

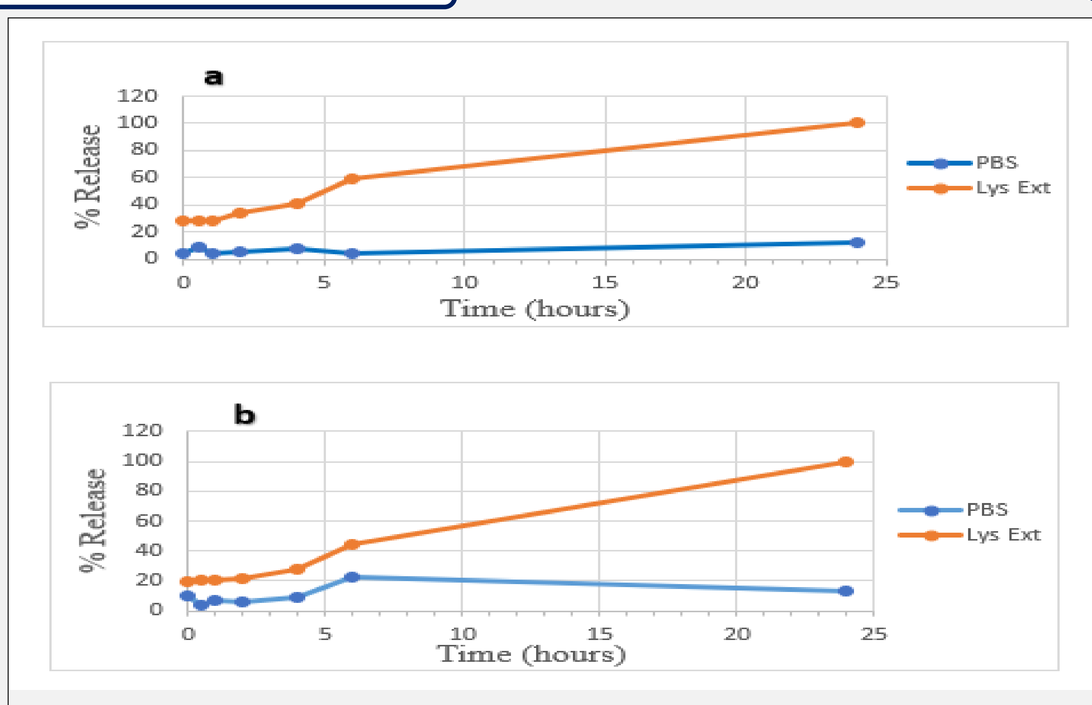


Fig 4. Release of rhodamine B (%) from a) FMSN 1 and b) FMSN 2 in lysosomal extract and PBS (n =2).

- The acidic lysosomal pH (≈ 5.5) triggers the uncapping of the PEI as compared to the neutral PBS environment.
- %release in Lys Ext >> %release in PBS
- %release from FMSN 1 >> %release from FMSN 2

Cytotoxicity Study

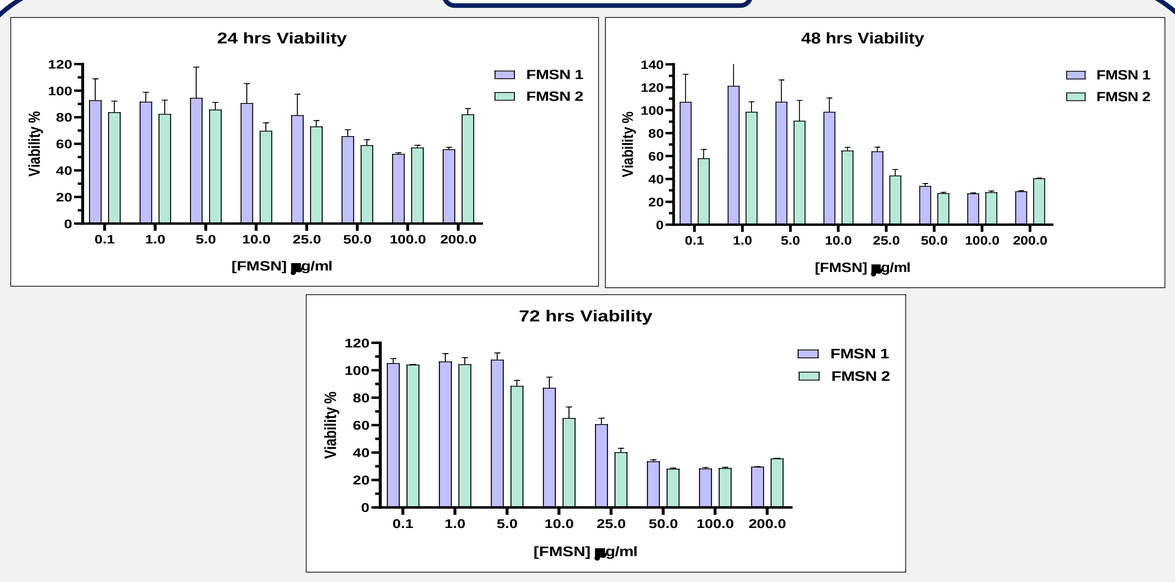


Fig 5. Effect of FMSN 1 and FMSN 2 on ARPE-19 cells viability at 24, 48 and 72 h after treatment. The results are representation of mean ± SD.

- FMSN 2 is more toxic than FMSN 1.
- For FMSN 1, more than 80% cell viability was seen at a cut-off concentration of 25 µg/mL at 24 h (~82%) incubation which fell down to 10 µg/mL at 48 h (~98%) and 72 h (~87%) incubation.

Conclusion

- The MSNs were synthesized using the co-condensation process, functionalized with 2 different concentrations of PEI and were characterized for various parameters successfully.
- The microenvironment triggered release hypothesis was confirmed from the *in-vitro* release of rhodamine B from the functionalized MSNs.
- The cytotoxicity study revealed a further scope of MSN surface modification to ensure more safe, targeted and efficient siRNA delivery in wet AMD.

Future work

- MSN surface modification to develop more biocompatible and targeted cargo system.
- Cellular uptake and *in-vitro* particle tracking studies and silencing assay [2].
- Haemato-compatibility studies.
- siRNA leakage studies from the nanocarrier system.
- Preclinical studies.

Acknowledgement

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1. https://www.brightfocus.org/macular/article/age-related-macular-facts-figures (accessed on October 6th, 2020).
2. Q. He, Z. Zhang, Y. Gao, J. Shi, Y. Li, *Small* **2009**, *5(23)*, 2722.