KU LEUVEN

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Biomaterials and Tissue Engineering research group
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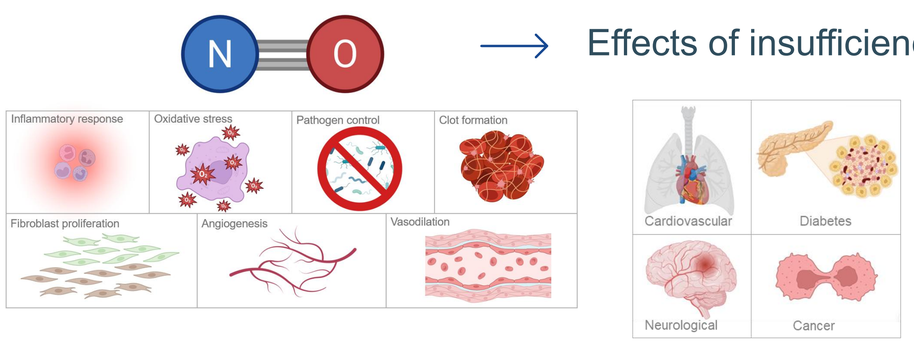
Evaluating nitric oxide release kinetics from electrospun hydrogels via the haemoglobin assay

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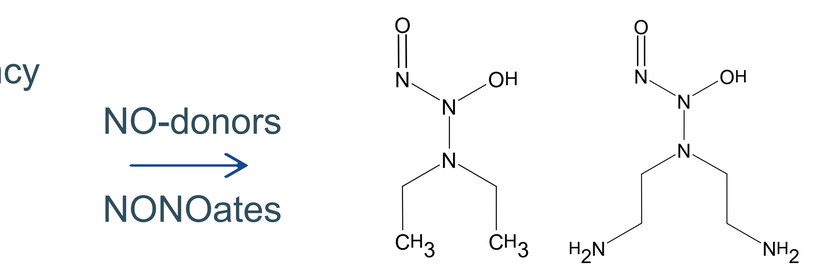
Nitric oxide (NO) delivery from diazeniumdiolate donors is a promising strategy in biomaterial design for wound healing and regenerative medicine, yet quantifying release remains challenging due to NO's gaseous nature, short half-life, and high reactivity. The haemoglobin assay offers simplicity, scalability, and direct correlation with NO concentration. Here, we adapted the assay to a 96-well plate format for time-resolved monitoring of NO release from electrospun methacrylated alginate hydrogels, with and without mesoporous silica nanoparticles as donor carriers. This miniaturized approach enables continuous measurement, reduced reagent waste, and increased throughput [1].

NITRIC OXIDE

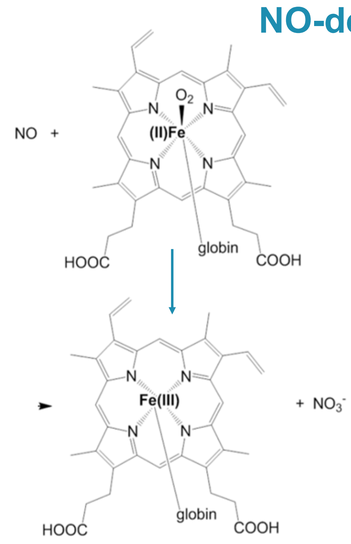
Therapeutic relevance [2,3]



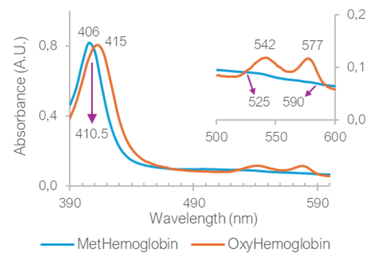
NO-donors and delivery



NO-detection



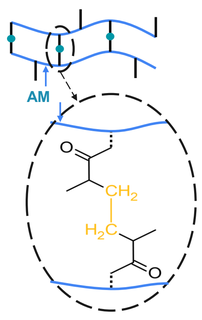
Hemoglobin Assay



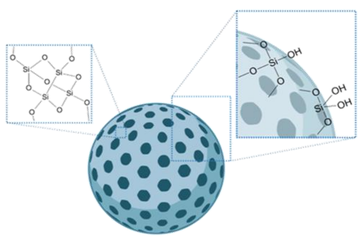
Absorbance spectra HbO_2 & metHb
Overlapping regions and isosbestic points at 410.5, 525 and 590 nm.

MATERIALS AND METHODS

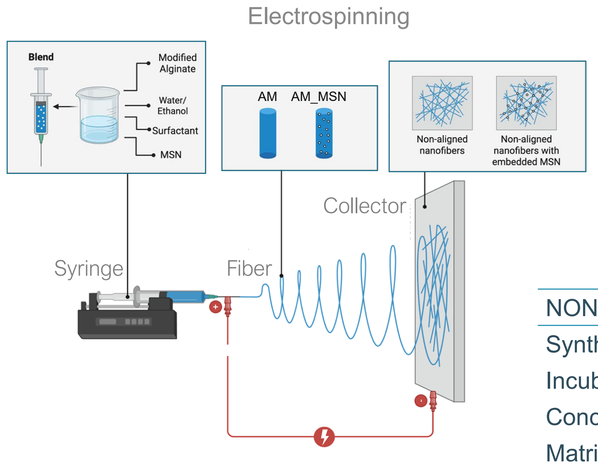
Alginate methacrylate (ALMA) UV-crosslinking



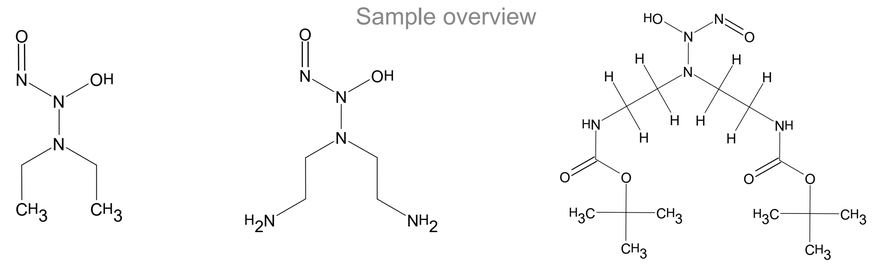
Mesoporous silica nanoparticles (MSN)



Electrospinning



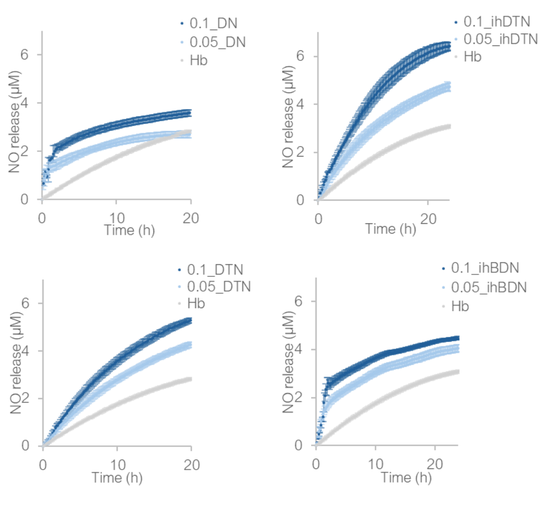
Sample overview



NONOate amine	Diethylamine DN	Diethylenetriamine DTN	Di-Boc-diethylenetriamine BDN
Synthesis	commercial	commercial + in-house (ihDTN)	In-house (ihBDN)
Incubation time	30, 60 min	60 min	60 min
Concentration	0.05, 0.1 µM	0.05, 0.1 µM	0.05, 0.1 µM
Matrix	AM, AM_MSN	AM, AM_MSN	AM, AM_MSN

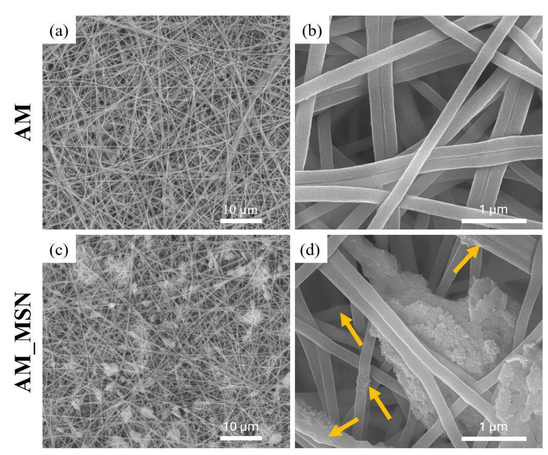
PROCESSING THROUGH ELECTROSPINNING AND NO-DONOR RELEASE

Release from free NO-donors



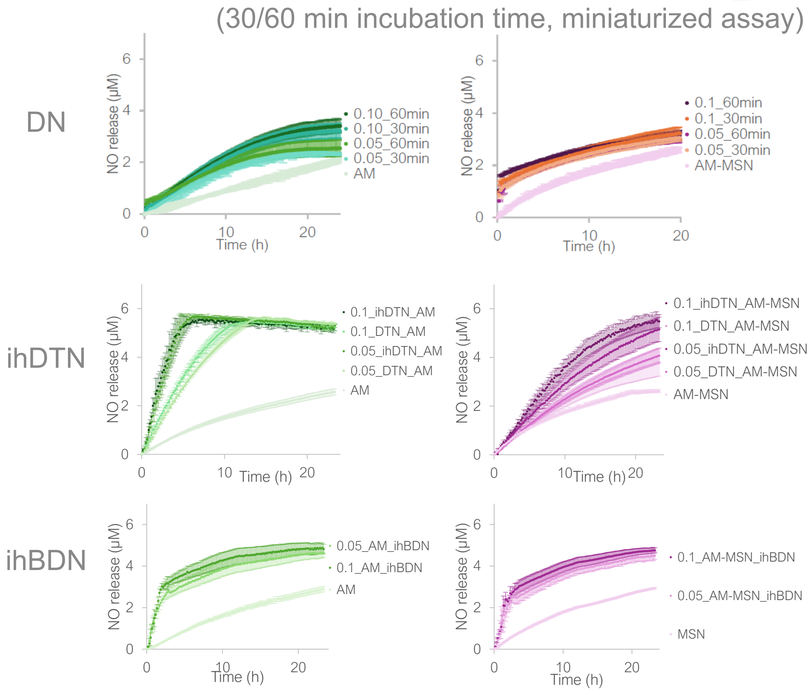
- Base amine structure dictates release behavior
- Concentration mainly affects total output, not rate

Electrospinning ALMA with(out) MSN



- Mat thickness AM 0.227 ± 0.025 mm & AM_MSN 0.297 ± 0.068 mm
- Fiber Ø 212 ± 18 nm (AM) & 183 ± 17 nm (AM_MSN)
- Particles homogeneously distributed

Continuous release of NO from AM and AM_MSN (30/60 min incubation time, miniaturized assay)



DN: - Negligible effect of loading time and concentration
- Limited effect of MSN

DTN: - Increased release rate at higher concentrations
- Release modulation through MSN

BDN: - Negligible effect of MSN and concentration

CONCLUSION

- Electrospun fibers of alginate methacrylate and MSN successfully developed
- Successful monitoring of NO release from free NO-donors
- Successful monitoring of NO release from AM and AM_MSN
- Spectrophotometric miniaturized assay (96 well)
- Limit of detection within physiological levels
- Real-time monitoring of NO: 24h continuous measurements

References
1. C. Cisneros et al, Microscale hemoglobin assay for time-resolved kinetic analysis of nitric oxide release from electrospun hydrogels, Under revision
2. S.M. Andrabi et al, Nitric Oxide: Physiological Functions, Delivery, and Biomedical Applications, Advanced Science 10 (2023) 2303259.
3. W. Xu et al, The role of nitric oxide in cancer, Cell Res 12 (2002) 311–320.
4. M.J. Thomson et al, Measuring Nitric Oxide Metabolism in the Pathogen Neisseria meningitidis, Methods Enzymol 437 (2008) 539–560

Acknowledgements
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