Temperature-induced assembly and direct drug loading of covalently cross-linked, charged, smart poly(N-isopropylacrylamide) microgels based on oligomeric precursors

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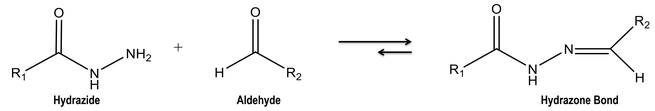
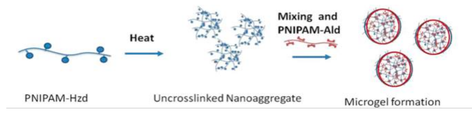
Introduction

Microgels, solvent-swollen cross-linked gel particles with sub-micron diameters, have been widely investigated as potential materials of interest for many biomedical applications. However, the conventional precipitation based free-radical polymerization method used to make microgels offers challenges in terms of degradability (and thus ultimate clearance of the microgels from the body). To counter this problem, microgels can instead be formed from well-defined polymeric precursors of controlled molecular weight and structure, with the microgel pre-polymers directly representing the degradation products.

Hydrazone chemistry offers the ideal solution, as the cross links formed are hydrolytically degradable (via hydrazone bond hydrolysis) over the course of weeks to months, and cleavable with a strong acid within hours.



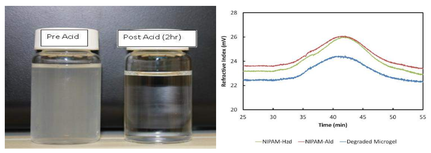
Here, a simple, rapid, solvent-free, and scalable thermally-driven self-assembly approach is used to produce highly monodisperse, covalently cross-linked, and degradable poly(N-isopropylacrylamide) (PNIPAM) microgels based on mixing hydrazide (PNIPAM-Hzd) and aldehyde (PNIPAM-Ald) functionalized PNIPAM precursors. Pre-heating of a seed PNIPAM-Hzd solution above its phase transition temperature produces nanoaggregates that are subsequently stabilized and cross-linked by the addition of PNIPAM-Ald.



Incorporation of pH-ionizable monomers into the precursor oligomers results in a dual pH/temperature-responsive microgels. Such microgels have particular potential utility in drug delivery applications, as drug payloads can be released selectively in areas of lower local pH (e.g. inflammation sites, cancerous sites, or inside cells). Dual sensitivity with both pH and temperature responses offers further potential for drug localization at highly metabolic sites within the body (e.g. cancers).

Results

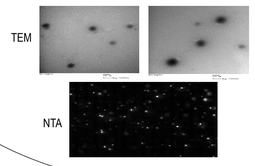
Degradable



1M HCl: Microgels degrade fully within 2 hours into precursor polymers

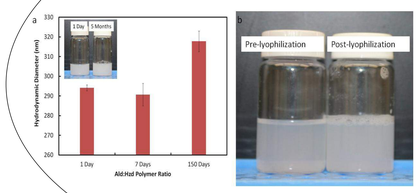
After two hours of exposure of 1M HCl, the visual appearance of the microgels changed from slightly opaque (indicating particles) to transparent. Gel permeation chromatography on the degraded microgel product indicates that the microgels are fully cleaved back into the starting PNIPAM-Ald and PNIPAM-Hzd oligomeric building blocks. The capacity for such well-defined degradation lies in contrast to microgels prepared with conventional precipitation techniques.

Monodisperse



Transmission electron microscopy (TEM) indicated that the microgels were consistently spherical, and nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS) confirmed the monodispersity of the resulting microgel suspension (polydispersity < 0.1 on DLS, D_w/D_n < 1.13 on NTA)).

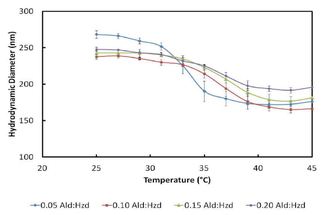
Colloidally stable



The self-assembled microgels are stable over at least 5 months with no aggregate present. The increased size after 5 months is due to the degradation of hydrazone cross-links. Furthermore, microgels can be re-suspended following lyophilisation with no change in particle size.

Self-assembled microgels have the same key properties of conventional precipitation-based microgels

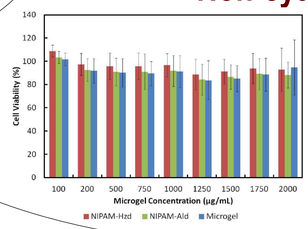
Thermosensitive



VPTT = 35-38 °C

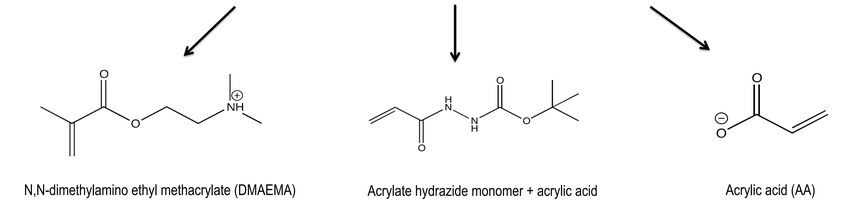
Self-assembled microgels exhibit similar thermal phase transition behavior to precipitation-based microgels, with decreased transition magnitudes observed at higher cross-link densities.

Non-cytotoxic



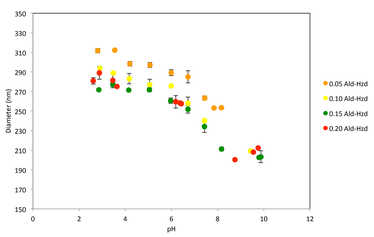
No significant cytotoxicity was observed using the MTT metabolic assay and 3T3 mouse myoblast cells when the cells were exposed to the polymer precursors or base case microgels at concentrations from 0.1 mg/mL to 2 mg/mL. These results suggest that the self-assembled microgels have high potential to be used *in vivo*.

Functionalization of Microgels with pH-ionizable Groups



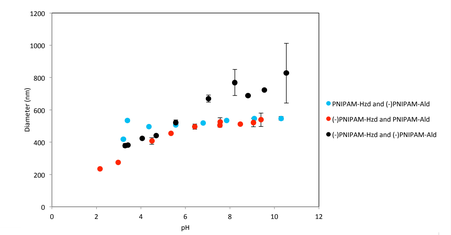
Functionalized/pH responsive microgels can be synthesized via simple functionalization of precursor polymers

Cationic-Functionalized



- As the pH increases, the diameter decreases for microgels with any cross-link ratio
- Size decreases with increasing cross-link ratio
  - *Both consistent with conventional non-degradable microgel behaviour*

Anionic-Functionalized



- As the pH increases, the diameter increases
- More anionic charge → increased swelling as a function of pH
  - *Both consistent with conventional non-degradable microgels*
- Significantly higher swelling observed when the core is anionic

Applications

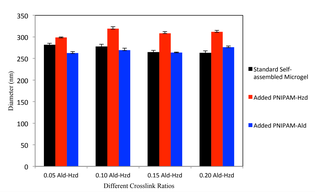
Direct Drug Loading of Microgels

Moderately hydrophobic drugs, e.g. dexamethasone, can be co-self-assembled with the polymers directly during the particle fabrication process if the drug is soluble in water (dexamethasone: 0.09 mg/mL)

Large drug encapsulation efficiencies **(>90%)** consistently observed
→ *Significantly higher than that achieved with conventional partition/diffusion-based drug loading processes using a much faster, single-step process (if drug is stable at assembly temperature)*

Layer-by-Layer Self-Assembly

Self-assembled, uncharged can be used as a core (seed) and covalently layer-by-layer cross-linked with a complementary precursors. The core is re-heated above its LCST and cross-linked with either PNIPAM-Hzd or PNIPAM-Ald. This process is analogous to polyelectrolyte multilayer construction, but with covalent bonds.



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Acknowledgements and References

For more information: Sivakumaran, Daryl; Mueller, Eva; Hoare, Todd. **2015**. "Temperature-Induced Assembly of Monodisperse, Covalently Cross-Linked, and Degradable Poly(N-isopropylacrylamide) Microgels Based on Oligomeric Precursors". *Langmuir,* 31, 5767-5778.

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