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Cationic, anionic, and amphoteric dual pH/temperature-responsive degradable microgels via self-assembly of functionalized oligomeric precursor polymers

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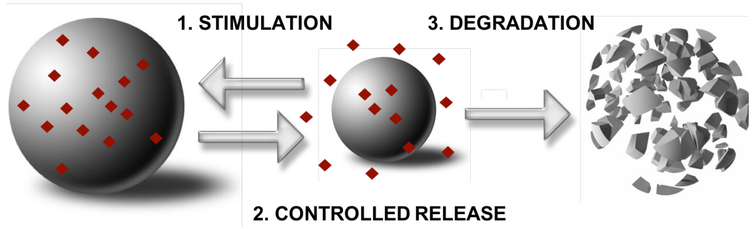
Introduction

Microgels are colloidal particles that consist of cross-linked water-soluble polymers and have potential as materials with unique functionality in a wide range of biomedical and environmental applications including emulsion stabilizers, separation supports, and triggered drug delivery vehicles. With their micro- or nanoscale dimensions, microgels provide the ability to control and design drug delivery mechanisms and/or specific binding affinities for a host of therapeutic or environmental targets. Microgels can be loaded with a therapeutic agent and transported via circulation to deliver drug at a rate and/or location tunable based on the structure of the microgel. In particular, microgels made from thermoresponsive polymers like poly(N-isopropylacrylamide) (PNIPAM) or poly(oligoethylene glycol methacrylate) (POEGMA) are of interest in a variety of these applications due to their switchable thermoresponsive properties. Further functionalization allows the fabrication of multi-responsive "smart" microgels that can switch properties under certain environmental conditions (e.g. temperature or pH) appropriate for site-specific drug delivery *in vivo* (e.g. cancer, infection sites, etc.)



Problem Statement

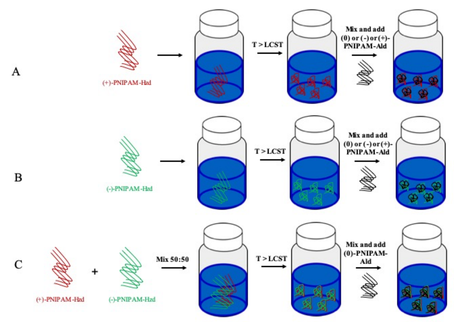
The conventional precipitation polymerization method used to prepare "smart" PNIPAM microgels has two main challenges:

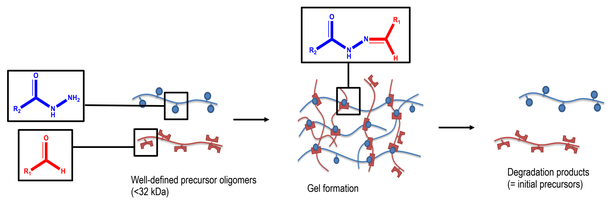
1. the chemical nature of the degradation products is not controlled, leading to potential for bioaccumulation and/or chronic inflammation as a result of microgel exposure and
2. the typically low encapsulation efficiency of moderately hydrophobic drugs into the generally hydrophilic microgel matrix

There is a substantial need to develop delivery vehicles that can both load hydrophobic drugs but also are themselves highly hydrophilic to minimize protein adsorption and thus inflammatory/immune responses. Microgels have been proven to enable long circulation times and low protein deposition, but their general lack of controllable degradation and poor capacity for loading moderate to highly hydrophobic drugs have limited their utility. Our technology provides a solution to this problem that can enable the safe and effective delivery of more hydrophobic therapeutics using microgels.

Proposed Solution

Self-assembly is a simple microgel fabrication method that is an alternative to the conventional precipitation-based method. Unlike conventional microgels that lack the ability to be degraded into compounds of known molecular weights, self-assembled microgels can be degraded at low pH in a short period of time (within hours to days) or at neutral conditions over months into well-defined products. Hydrazide-functionalized oligomers are pre-heated above their lower critical solution temperature (i.e. the temperature at which the chains collapse), after which the aldehyde-functionalized oligomer is added to induce crosslinking. This fabrication method is useful in synthesizing microgels with charge that allows the microgels to respond not only to changes in temperature but also in pH, expanding the range of biomedical applications, and/or enhance affinity loading of the microgel with charged drugs to enhance drug loading efficiency and prolong drug release kinetics.





Significance

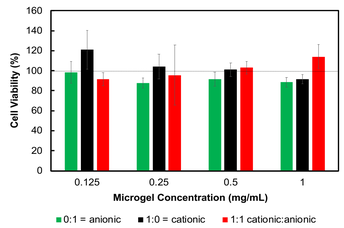
There are three advantages of the self-assembly microgel fabrication method:

(1) **Faster/more efficient** – can fabricate microgels within a couple of minutes and encapsulate moderately hydrophobic drugs directly during the self-assembly process to save time and eliminate additional purification steps;
(2) **Flexible** – can easily incorporate modified precursor polymers, i.e. cationic or anionic charged polymers, and can engineer amphoteric microgels by simply mixing the cationic and anionic precursor polymers
(3) **Scale up** – given the insensitivity of the process to mixing, we have demonstrated the potential to scale up this process to pharmaceutically-relevant bulk scales

Current work remains underway to assess the release kinetics of drug from these microgels as well as confirm the degradation rates of the microgel in more physiologically-relevant applications, with animal tests planned using these microgels as carriers for anti-psychotic drugs (typically hydrophobic) and/or cancer targeting.

Cell Cytocompatibility

All the self-assembled microgels tested (regardless of charge) maintained high cytocompatibility, with cell viabilities of >80% observed at all microgel concentrations tested using C2C12 mouse myoblast cells. Ongoing studies are investigating the drug loading and release profiles of charged drugs, i.e. doxorubicin hydrochloride and naproxen sodium.

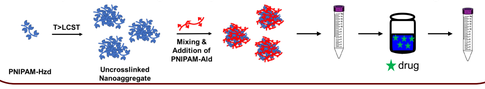


Passive Diffusion vs. Direct Loading of Drugs

Hydrophobic drug delivery, in particular, remains a challenge for conventional precipitation-based microgel systems due to the hydrophilicity of the crosslinked network and required use of diffusion/partitioning-based processes for loading drugs with conventional microgels. With the novel self-assembly method, it is possible to either (1) directly load the moderately hydrophobic drug in the self-assembled microgels or (2) use the conventional passive diffusion process:

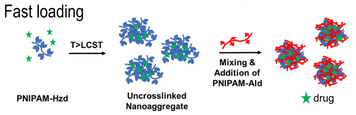
Passive Diffusion

1. Low encapsulation efficiency of moderately hydrophobic drugs
2. Longer purification and more optimization (two centrifugation steps required)
3. Only available drug loading technique for conventional microgels

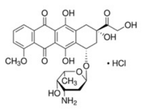


Direct Loading

1. Ease of encapsulating moderately hydrophobic drugs
2. Simple purification (only one centrifugation step required)
3. Fast loading

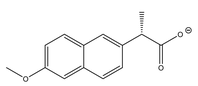


Moderately hydrophobic drugs, like dexamethasone (uncharged), have been successfully loaded into neutral self-assembled PNIPAM microgels at a 5-fold higher encapsulation efficiency compared to passive diffusion. Charged drugs, i.e. doxorubicin hydrochloride (pKa = 8.2) and naproxen sodium (pKa = 4.1), have also been successfully encapsulated in anionic and cationic self-assembled PNIPAM microgels, respectively:



Doxorubicin Hydrochloride
chemotherapy, cationic drug

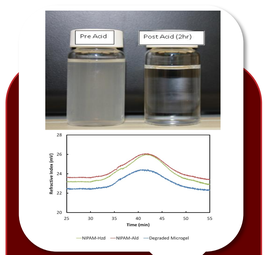
Passive Diffusion (EE%)	Direct Loading (EE%)
37 ± 4	84 ± 8



Naproxen Sodium
pain reliever, *anionic drug*

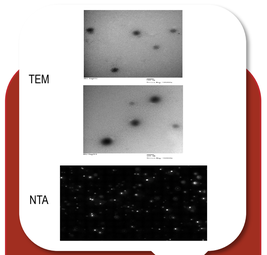
Passive Diffusion (EE%)	Direct Loading (EE%)
8 ± 7	35 ± 5

Self-assembled PNIPAM Microgel Properties



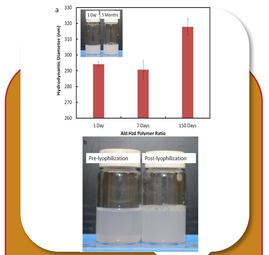
Degradable

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.



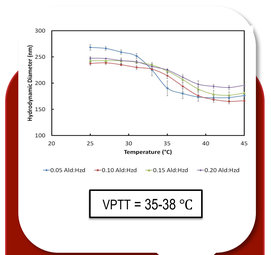
Monodisperse

Transmission electron microscopy (TEM) indicated that the microgels were consistently spherical, and nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS) confirmed their monodispersity.



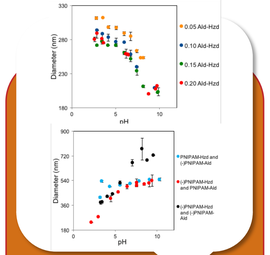
Colloidally Stable

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.



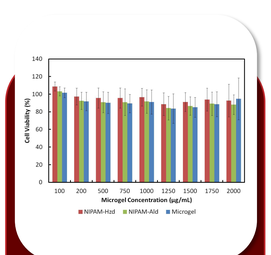
Thermoresponsive

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



pH-responsive

Functionalized/pH responsive microgels can be synthesized via simple functionalization of precursor polymers to produce cationic, anionic and amphoteric PNIPAM microgels.



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.

Acknowledgements and References

NSERC CRSNG

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