Dynamic Covalent Cross-linked Nanogel-stabilized Pickering Emulsion for Responsive Microstructures

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Designing new dynamic matrices in combination with a highly diverse material formation approach as Pickering emulsions provides us with the tools to engineer innovative dynamic porous microstructures in a highly controllable fashion. Here we make use of nanogels (nGels), which exhibits dynamic covalent cross-linking capabilities, as surface stabilizing agents in view of their highly controllable physiochemical properties. The method provides successful formation of dynamic covalent cross-linked hydrogel microstructures based on ketone and amine functionalized nGels using Pickering emulsions was shown. In this system we incorporated a pH-triggerable responsive behavior. The physiochemical properties of the resulting microstructure can be further tailored by modifying the intramolecular interactions at the interface, making this systems interesting for a wide range of applications.

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1. Introduction

Formation of functional materials is indispensable for a wide range of applications in the food industry, cosmetics, pharmaceutical, and biomedical engineering field. Hereby, high control over the physical and chemical properties are important to tailor the desired features. The main advantage of porous microstructures is the possibility to create excluded, isolated cavities in which storage, protection, and overall mechanical properties can be tuned. It possibly allows for the encapsulation of drugs, gases, or liquids. However, an important feature that displays great potential as a tool to broaden the scope of material design is the use of dynamic covalent properties that enables materials to be both responsive as well as dynamic where the response lies in the complete disassembly of a material. It provides the use of an external stimulus, such as temperature or pH in order to release the entrapped substances or change material properties in a controlled way upon changing the environmental conditions. So far, the dynamic covalent approach has been substantially applied to macroscopic hydrogels based on polymers and small molecules, but not to porous microstructures as is presented in current work.

Engineering stable porous microstructures can be done by making use of Pickering emulsions. These two-phase-systems are stabilized by solid particles at the interface of both solvents, leading to reduction of the interfacial energy. Pickering emulsion prevents phase separation, Ostwald ripening, and droplet coalescence, making them an important tool to form microstructures. This distinguishes Pickering emulsions from usual emulsions as those are generally unstable and phase separate overtime. The properties of the resulting emulsion can be tuned by modifying the solid particles used as surface stabilizers influencing the inter-droplet interactions. Furthermore, shear force, interfacial surface area, surface charge, and the energy used to form those emulsions, make it possible to precisely engineer the properties of the resulting microstructure. Therefore, Pickering emulsions are an important tool to form highly controllable microstructures.

Using nanogels (nGels) as emulsifiers has gained interest in the past decade, as they show good stability, can be easily functionalized, and are sensitive to external stimuli, such as pH or
temperature.\textsuperscript{[21–23]} Hereby, poly(N-isopropylacrylamide) (NIPAM) based nGels are widely studied, as they show high sensitivity to temperature.\textsuperscript{[24]} Previously, NIPAM nGels have been used as emulsifiers for Pickering Emulsions, investigating the influence of the physical properties of the nGel such as size and deformability on the stability of the resulting emulsion.\textsuperscript{[25,26]} Not only the physical properties of the emulsifiers play an important role, but also the chemical functionalization influences the characteristics of Pickering emulsions. Wu et al. demonstrated the possibility of forming a tuneable cross-linked network based on attractive forces of Pickering emulsions between carboxyl functionalized shellac nanoparticles and amino functionalized telechelic polymers, demonstrating the importance of inter-droplet interactions.\textsuperscript{[27]}

This work presents the formation of an external stimuli responsive microstructure via a dynamically covalent cross-linked nGel matrix formation using Pickering emulsions. For this purpose highly controllable NIPAM nGels in view of their physical and chemical properties were used as surface stabilizing agent. These nGels were functionalized with primary amines using N-(3-aminopropyl)-methacrylamide (NIPAM-co-APMA) and N-(1,1-dimethyl-3-oxobutyl)-acrylamide (NIPAM-co-DAA) for ketone modification respectively. Upon emulsification of oil containing NIPAM-co-DAA nGel and NIPAM-co-APMA aqueous suspension, successful formation of a pH responsive microstructure based on dynamically covalently cross-linked chemistry was formed. The dynamics were shown by pH dependent dynamic light scattering (DLS) analysis of mixtures of aqueous solutions of both nanogels as well as the response of the porous micro- and macrostructures engineered using Pickering emulsions.

2. Results

In order to form a pH responsive dynamic covalent microstructure, using dynamically covalently cross-linked nanoparticles, nGels were used as emulsifiers. These nGels were functionalized with primary amines using p(NIPAM-co-APMA) and p(NIPAM-co-DAA) for
ketone modification respectively (Figure S1, S2). They were formed via precipitation polymerization.\textsuperscript{[28,29]} The synthetic approach is demonstrated in Scheme 1a. Free radical polymerization induced by thermosensitive initiators, led to the formation of small oligomers, which then precipitate and form larger colloids with ongoing reaction time.\textsuperscript{[30]} Using NIPAM as a monomer promotes the precipitation of the resulting colloids and introduces a thermoresponsive behaviour of the formed nanogels. Incorporation of functional groups within nGels can be achieved by using different comonomers. The volume phase transition temperature (VPTT) of NIPAM-co-APMA nGel is 32°C and 28°C for NIPAM-co-DAA respectively, and is in a good agreement with previous literature (Figure S3).\textsuperscript{[31,32]} The VPTT, and therefore the swelling/de-swelling behaviour, is dependent on the ratio of hydrophobic attraction of isopropyl groups introduced by the monomer NIPAM and the charge and hydrophilic properties introduced via the functional groups of comonomers APMA and DAA respectively. The difference in the VPTT for both nGels can therefore be explained by the different hydrophilic property of each co-monomer.\textsuperscript{[31,33]} Figure S4 shows the pH dependent hydrodynamic diameter and zeta potential of p(NIPAM-co-APMA) with an isoelectric point at pH > 7. At low pH an increased quantity of protonated primary amine groups is observed, leading to an increase in the zeta potential, high pH leads to a decreased zeta potential, respectively.
Scheme 1. Synthetic approach of NIPAM-co-APMA core shell and NIPAM-co-DAA nGel through controlled precipitation polymerization with a schematic description of the dynamically covalently cross-linked hydrogel matrix formation using Pickering emulsion.

To form dynamically cross-linked porous microstructures, an oil-in-water emulsion was used. Hereby, a NIPAM-co-DAA oil suspension and an aqueous NIPAM-co-APMA suspension are mixed together. Emulsification of NIPAM-co-DAA and NIPAM-co-APMA facilitates the reaction of both nGels at the oil/water interface (Figure S6). This reaction is promoted by two driving forces: (a) the reduction of the interfacial tension between oil and water by surface stabilizing nGel particles and (b) the attractive forces between amine groups of NIPAM-co-APMA nGel and ketone groups of NIPAM-co-DAA nGel. By nucleophilic attack of primary amines on the carbonyl group of NIPAM-co-DAA, a hemiaminal is formed. Subsequently, this intermediate is dehydrated leading to an imine bond between both nGels. The resulting microstructure based on dynamically covalently cross-linked nGels, allows the introduction of a pH responsive system that results in complete loss of material integrity rather than just size change due to dehydration. Matrix formation via Pickering emulsions is in general
advantageous since the physical properties of the resulting microstructure can be easily tuned by e.g. changing the interfacial area, oil composition, or concentration of nGels but additionally combined with dynamic covalent chemistry one can tune stability by switching between covalent and physical stabilization and controlling the destabilization kinetics by playing on the reaction conditions. 

Figure 1 shows the dynamically covalent cross-linked microstructures after emulsification using fluorescence microscopy. Hereby, Rhodamine B labelled NIPAM-co-APMA nGel is depicted in Figure 1a, whereas Figure 1b shows Nile Blue labelled NIPAM-co-DAA nGel. Figure 1c shows the overlay of fluorescent signals of both nGels. The latter demonstrates that interactions between amine- and ketone-functionalized particles are strongly facilitated at the oil/water interface. Hereby, the porous, physically unfavourable non-spherical structures within the matrix supports the successful formation of a stable covalently cross-linked system. When exposing the matrix to air, comparable structures have been observed (Figure S5).

Figure 1. Fluorescence images of nGel emulsion: (a) NIPAM-co-DAA nGel (b) NIPAM-co-APMA core shell nGel (c) overlay of (a) and (b); Scale bar 100 µm.

The influence of pH on the attractive forces between amine and ketone functionalized nGels was investigated in an aqueous suspension without the presence of the emulsion. Hereby, we measured the hydrodynamic diameter of a NIPAM-co-DAA and NIPAM-co-APMA suspension at different pH with DLS at a constant temperature of 25°C (Figure 2). An
increase in the hydrodynamic diameter can be explained by the formation of imine bonds between ketone and amine functionalized nGels. The highest hydrodynamic diameter was found for pH 5 where an increase of over 200nm of the hydrodynamic radius was found when comparing the radius measured at high and low pH. This value is in agreement to previously found behaviours. Slightly acidic conditions are favourable to promote nucleophilic attack of primary amines on the carbonyl group of ketones, ultimately leading to imine formation. At low pH, high amount of primary amines from NIPAM-co-APMA nGel are protonated leading to non-nucleophiles and thus, preventing nucleophilic attack on the carbonyl group of NIPAM-co-DAA nGel. Therefore, imine formation is not facilitated, which results in lower hydrodynamic diameters. Increasing the pH towards basic conditions, primary amines initiate a nucleophilic attack on the carbonyl groups.

Figure 2. Hydrodynamic diameter measurement of aqueous mixture of NIPAM-co-DAA and NIPAM-co-APMA nGel suspension at different pH using DLS

However, protonation of the intermediate hemiaminal is prevented, and thus, the imine formation is limited, leading to a decrease in the hydrodynamic diameter. While the individual nanogels have a temperature dependent behaviour as expected (Figure S3), when
the temperature dependency was combined with the optimum pH for coupling, as shown in Figure 2, the size drastically increases (Figure S7). During the collapse, the cross-linking is facilitated, most likely due to partial aggregation, which force nanogels together and enhance the imine formation that then prevents particles from diffusing away from one another.

Following the investigation of pH responsive imine formation on the nanometer scale, the responsive behaviour of a nGel matrix in the macroscopic scale was examined. For this, the microstructures after emulsification was collected and same amount of matrix were placed in a vial filled with 10 ml MilliQ water at different pH. Hereby, we tested the stability of the microstructure at pH 2, 5, and 8. To adjust the required pH conditions, NaOH or HCl was added accordingly.

Figure 3a depicts the microstructures in aqueous environment at pH 2, 5, and 8 immediately after placing the matrix inside the vial. All three matrices show similar coherent structures. After 48h the aqueous suspensions at pH 2 and pH 8 turned turbid (Figure 3b). It can be said that, imine bonds between the dynamically cross-linked nGels were destabilized, leading to diffusion of the previously coherent structure. The matrix stored at pH 5 shows no significant changes, demonstrating high stability in slightly acidic conditions. These results are in correspondence to the results obtained by DLS when measuring the hydrodynamic diameter an aqueous mixture of NIPAM-co-DAA and NIPAM-co-APMA nGel at different pH. Hereby, pH 2 and pH 8 show low hydrodynamic diameters, impeding imine formation, whereas pH 5 promotes imine formation, leading to high hydrodynamic diameters.

As the individual nanogels are also likely to stabilize emulsions via the Pickering effect, the stabilization of the individual components were also compared to the dynamic covalent system (mixed nGels) at different pH (Figure S8). It was found that also the NIPAM-co-APMA nGel and NIPAM-co-DAA were able to initially stabilize the emulsion in a similar fashion as the mixed system. However, the NIPAM-co-APMA displayed a relative fast disappearance of the emulsion at all pH conditions while the NIPAM-co-DAA showed fast destabilization at pH 2 and 8 while being more stable at pH 5. Although NIPAM-co-DAA remains more stable at pH 5, it still diminishes in volume with respect to the mixed system and while in both single nGel systems pH 2 and pH 8 showed
complete destabilization, for the mixed nGel system is remains stable for longer times. It illustrates that the dynamic covalent approach, as presented here, allows for tuning the time of stabilization and degradation providing the system with a temporal control. This exhibits that the stability of the dynamically covalently cross-linked matrix can be triggered by changing the environmental properties and that the dynamic covalent nature can be harnessed at both the nano-, micro-, and macroscale in a time-dependent manner.

Figure 3. Stability of nGel matrix in aqueous solution at pH 2, 5 and 8 after (a) 0h and (b) 48h.

3. Conclusion

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In this work the formation of a dynamically cross-linked nGel matrix based on imine formation between NIPAM-co-DAA and NIPAM-co-APMA functionalized nGels was shown. Hereby, functionalization of NIPAM nGels allow the incorporation of a triggered response upon matrix formation to the external pH environment in view of the physiochemical properties of the resulting microstructure. Stable matrices were obtained in slightly acidic condition, whereas high and low pH lead to diffusion of the previously coherent matrix due to de-stabilization of the dynamically cross-linkage. In particular, high control over the size, temperature responsive behaviour and functionality of the surface stabilizing nGels enable tuning the resulting properties of the microstructure, making this system promising for a wide range of applications.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Dynamic covalent chemistry has made an impact on various chemical systems but to a less extent on porous soft materials. Here dynamic covalent imine formation was combined with nanogel-based interface stabilized emulsions to provide a Pickering emulsion templated materials design creating responsive and dynamic structure controlled by pH and as shown to be controllable on the nano- to macroscale.

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