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Controlled network structures of chitosan-poly(ethylene glycol) hydrogel microspheres and their impact on protein conjugation

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ABSTRACT

We demonstrate a facile approach to manufacture hydrogel microspheres with controlled and macroporous network structures via a simple yet versatile micromolding-based technique. Specifically, highly uniform poly(ethylene glycol) (PEG) hydrogel microspheres containing chemically functional chitosan are readily fabricated using the micromolding-based technique that utilizes surface tension-induced droplet formation of aqueous prepolymer solution followed by photo-induced interfacial polymerization. Network structures of the hydrogel microspheres are readily controlled by simple addition of inert porogen, which induces phase separation during the polymerization. Fluorescent labeling studies reveal tunable distribution of the chitosan within the PEG networks (i.e., uniform and core-shell like distributions) depending on the content of the porogen in the prepolymer solution. In addition, protein conjugation studies show tunable pore sizes and 3D network structures by adjusting content of the porogen, and formation of macroporous networks leading to significantly improved protein conjugation kinetics. The macroporous network structures are further supported with scanning electron microscopy (SEM) results that correspond well with confocal microscopy results. We believe that our fabrication strategy offers a simple and robust route to construct diverse 3D hydrogel network structures with chemical functionality, enabling production of a variety of biofunctionalized hydrogel microspheres that can be utilized in various biomedical applications.

1. Introduction

Hydrogel microparticle-based suspension arrays hold significant potential as powerful alternatives to planar and bead-based arrays for medical diagnostic and biosensing applications [1–4]. Specifically, hydrogel microparticles offer several advantages over solid bead-based ones such as solution-like environment for ideal target-probe binding with minimal non-specific adsorption, rapid assay time, and reduced signal to noise ratio by enhanced loading and capture capacity of probes and targets, respectively [4,5]. Recent advances in the fabrication techniques of the hydrogel microparticles have enabled convenient shape-based encoding and rapid production of complex and/or multicompartmental microparticles, opening doors for multiplexed sensing or diagnostic assays. Such techniques include stop-flow lithography [6–8], photolithography [9–11], capillary microfluidics [12–15] and electrohydrodynamic co-jetting [16,17].

Despite such advances, there still exist several critical challenges in simple and programmable fabrication of the hydrogel microparticles with desired properties. First, most current techniques including microfluidic methods require complex equipment and delicate control of microflows and solution properties (e.g., viscosity and surface tension). Second, the most commonly used polymerizable unit poly(ethylene glycol) diacrylate (PEGDA) with low molecular weight is often not suitable for the creation of large pores that permit rapid diffusion and binding of large biomolecular targets [18–21]. Lastly, the common co-polymerization technique to incorporate biomolecular probes (e.g., antibodies) with the hydrogel network faces challenges in preserving their biospecific affinities due to the harsh radical polymerization environment [22–24]. There thus exist critical needs for simple and reliable techniques to produce hydrogel microparticles with controlled and tunable macroporous network structures as well as chemical func-

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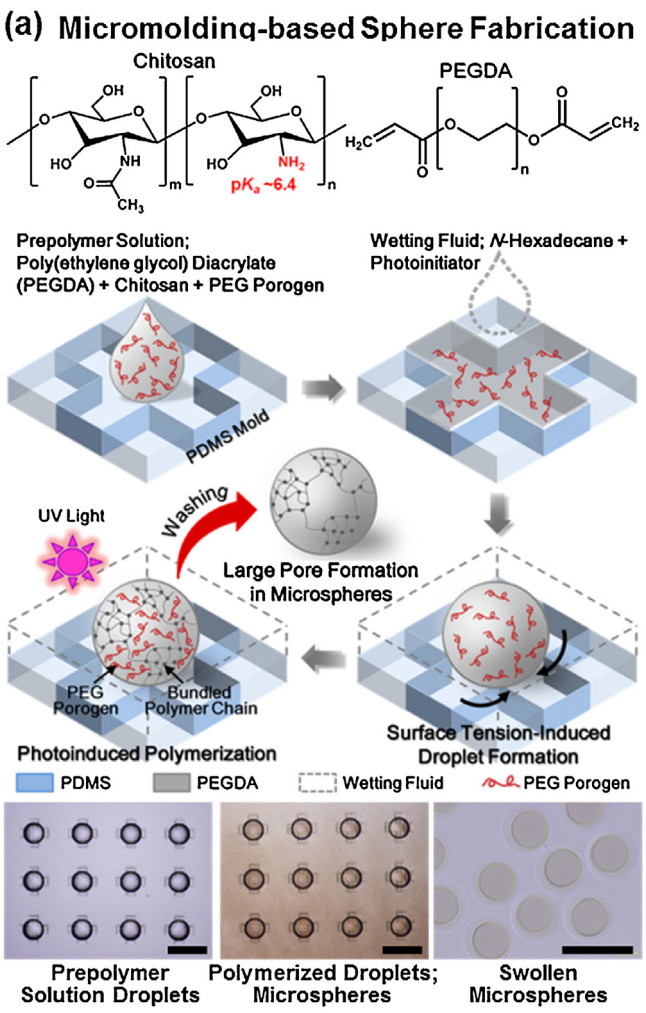
tionalities that allow for facile and efficient conjugation of the biomolecules.

We recently reported facile fabrication of chemically functional hydrogel microparticles via simple micromolding techniques and incorporation of chitosan [18,25,26]. As shown in the chemical structure in Fig. 1(a), chitosan is a naturally derived aminopolysaccharide that provides abundant and highly reactive primary amines as an efficient conjugation handle due to their low pK_a value [27–29], making it an enabling biofabrication component that imparts potent chemical functionality [30]. With facile post-fabrication biofunctionalization approaches using the primary amines and bioorthogonal conjugation reactions, we demonstrated utilization of the chitosan-incorporated hydrogel microparticles as protein conjugation and biosensing platforms [18,25]. However, there still exist limitations in mass transfer of large biomolecules into the microparticles leading to slow protein conjugation kinetics owing to tightly crosslinked hydrogel networks. Such slow mass transfer leads to longer incubation and assay time, which in turn can cause compromised results on labile biomolecular probes and targets. In addition, imparting a diverse range of 3D network structures including core-shell geometries with tunable shell thicknesses and mesh sizes could open doors for the development of more versatile sensing platforms. For example, such sensing platforms may be suitable for accurate size discrimination and/or detection of larger biomolecular assemblies such as viruses and protein aggregates (e.g., beta amyloid oligomers implicated in Alzheimer's disease or alpha-synuclein in Parkinson's disease) [31,32]. Therefore, it is imperative that more thorough examination of fabrication parameters and routes to impart wider ranges of 3D network structures should be conducted toward potent hydrogel microparticle design and manufacturing principles.

In this report, we demonstrate a simple approach to drastically increase mesh sizes for improved protein conjugation kinetics and to further fine-tune the network structures of hydrogel microspheres, by exploiting inert porogens from crosslinking PEGDA (scheme in Fig. 1(a)) [33,34]. Briefly, aqueous prepolymer solution containing PEGDA, chitosan and inert short chain PEG porogen is added into PDMS molds, and then the prepolymer solution droplets are spontaneously formed by surface tension upon placing hydrophobic wetting fluid on the mold [26,35–37]. The droplets are crosslinked by photo-induced polymerization reaction of PEGDA, while the crosslinked PEG chains create excluded volume by PIPS during the polymerization reaction [26]. The PEG porogens are then simply washed out during rinsing, leaving large pores within the microspheres. Notably, this approach enables facile tuning of 3D network structures (i.e., core-shell like microspheres with different shell thicknesses and mesh sizes) using simple fabrication parameters such as the PEGDA and PEG porogen content, as well as addressing the mass transfer limitation that allows for improvement in protein conjugation. Specifically, addition of the short PEG porogen (MW 600 Da) at varying concentrations (10–30 v/v%) in the prepolymer solution leads to diverse 3D network structures of the resulting hydrogel microspheres and varying distribution of chemical conjugation handles, allowing for programmable biomolecule conjugation profiles within the microspheres. The fluorescent labeling and protein conjugation results show not only tunable network structures but also enhancement in mass transfer and conjugation of large biomolecules into the microspheres, highly desirable for bioassays in practical clinical settings. In-depth characterization via confocal microscopy and SEM further supports the observed tunable 3D network structures that lead to controllable protein conjugation profiles and kinetics, providing deep insights into the detailed structural features of the hydrogel microspheres. These insights should thus offer promising routes to construct hydrogel microscale materials with varying 3D network structures, and lead to a significant advancement toward facile manufacturing



(b) Swelling Capacity of Microspheres

PEGDA (vol%)	PEG600 (vol%)	D_{wet} (μm)	D_{dry} (μm)	Swelling Ratio	Water Content (vol%)
10	0	140±6	81±5	5.2	81
	10	146±5	83±5	5.4	81
	20	148±5	87±6	4.9	80
	30	150±4	89±6	4.9	80
15	0	143±5	89±2	4.2	76
	10	148±5	90±4	4.5	78
	20	151±4	91±4	4.6	78
	30	157±3	92±3	4.9	80

Fig. 1. Fabrication of chitosan–PEG hydrogel microspheres via a simple micromolding-based technique. (a) Chemical structures of main components in prepolymer solution, and schematic diagram describing procedure of the fabrication along with corresponding bright-field micrographs to each step. Scale bars represent 200 μm. (b) Summary of swelling capacity of the as-prepared microspheres ($n \geq 20$).