ULTRARAPID SYNTHESIS OF MICROBEADS WITH TUNEABLE SURFACE PROBE DENSITY

Xiamo Chianty Zhou, Florian Suderleith, Sandra Stanke, Wouter van der Wijngaart*,
and Tommy Haraldsson

KTH Royal Institute of Technology, Stockholm, SWEDEN

ABSTRACT

We present, for the first time, a simplified and ultrarapid (~ 1 s) approach for synthesis of microbeads with thiol- functionalized surfaces as potential binding sites, whose density can be tuned according to the ratio of two components in the prepolymer. We verify the successful generation of microbeads and their tuneable surface density resulted from different prepolymer off-stoichiometric ratio. This novel approach can be used for bioassays that require rapid surface capture probe binding and customized probe density for suitable bioreactions.

INTRODUCTION

Microbeads are widely used as mobile solid capture surfaces in quantitative biosensing assays. Three bead parameters define the assay kinetics and require careful tuning: 1) the bead size; 2) the bead density; and 3) the surface probe density [1]. Today, the former two parameters are easy to tune during manufacturing and assay preparation, respectively; however, the surface probe density is indirectly defined either by:

- the feed rate and cross-reactivity (r_1 and r_2 values) of two monomers, in which a degree of homopolymerization occurs that is hard to control, resulting in variations in composition; or
- by post-manufacturing solution based functionalization protocols, typically in mixes of silanes (one inert, one active), that rely on a statistical distribution of surface groups, in a slow process, in which it is difficult to control that only a monolayer is formed.

Specifically, thiolated microbeads are of great interest since they offer stable covalent bonding sites for biomolecules [2], but their current synthesis lasts hours to several days [3], [4].

Droplet microfluidics is a commonly reported method for microparticle synthesis that allows precise control of the synthesis conditions [5].

The off-stoichiometry thiol-ene (OSTE) polymer system features excess of either thiol- or ene- functional groups in the prepolymer, which results in a native polymer surface with thiol- or ene- reactive groups, respectively, which can be used as anchors for covalent attachment of biomolecules [6]. Lafleur et al. demonstrated the relation between prepolymer composition and the surface reactive group density [7].

NOVEL CONCEPT

Here we demonstrate (Fig. 1):

- the microfluidic synthesis of thiolated and allyl-functional microbeads (instead of previously used suspension based methods);
- with native reactive surface probes, of which the density can be easily tuned (instead of depending on, previously used, difficult to control post-manufacturing solution based activation);
- in an ultrarapid manufacturing process (~ 1 s UV crosslinking instead of hours, as in previous methods).

This allows readily manufacturing of microbeads with capture kinetics optimised for their intended bioassay.

Figure 1: Overview of the novelties in this work.

EXPERIMENTAL

Novel Bead Manufacturing Process (Fig. 2)

We combine, for the first time, 1) the OSTE polymer system, which does not suffer from homopolymerization, in 2) the easily tuneable chemical environment of droplet microfluidic platforms, for the synthesis of microbeads with native reactive surfaces. Two syringes loaded with 1) different off-stoichiometric OSTE prepolymers (two components polymeric system containing PTMP thiol-monomer: pentaerythritol tetrakis (3-mercaptopropionate) and TATATO allyl-monomer: 1,3,5-triallyl-1,3,5-

triazine-2,4,6(1H,3H,5H)-trione from Mercene Labs AB, Sweden) with 30% w/w toluene (Sigma-Aldrich, Sweden) and 2) DI water with PVA (20% w/w) and Triton-X (2% w/w) as surfactant, were driven by syringe pumps (NE-100, New Era Pump Systems, Inc. NY, USA) as dispersed phase and continuous phase, respectively, into a readily fabricated microfluidics chip (injection molded using OSTEmer 322 from Mercene Labs AB, molding master was PDMS slabs casted from a in-house-milled PMMA piece using a variant of previously described protocol [8]).

Figure 2: OSTEmer droplets generation via flow-focusing mechanism and subsequent UV crosslinking. Different dispersed phase prepolymer mixture results in different surface functional group density (upper); visual colorimetric reagent adhesion and washing steps (lower).

OSTEmer prepolymer droplets in a DI water continuous phase were generated on chip by microfluidic flow-focusing. The droplets are on-chip polymerized by 1 s UV exposure (UV LED, 10 W, chemistry mechanism in Fig. 3) and thereafter collected in eppendorf tubes for further evaluation. Droplets were generated with five different mixtures of OSTEmer prepolymer, with 20%, 30%, 40%, 60% thiol excess and 60% allyl excess, resulting in microbeads with five different surface probe densities.

Figure 3: Thiol-ene “click” reaction mechanism

Probe density evaluation

The beads were submerged for 2 days at room temperature in visual light colorimetric reagent (0.2% w/w disperse red 1 acrylate, Sigma-Aldrich, Sweden) dissolved in toluene, with 1% w/w catalyst DMP-30 (2,4,6-Tris (dimethylaminomethyl) phenol; Sigma-Aldrich, Sweden). The colorimetric assay generates a colour intensity shift via the thiol-michael addition reaction (Fig. 4) at the thiol functional groups on the bead surfaces, which is proportional to the thiol surface probe density. The beads were extracted from the eppendorf tubes by centrifuge, dried at R.T., and

thereafter imaged (Fig. 3).

Figure 4: Mechanism of visual colorimetric reagent “thiol-michael addition” reaction to bond thiol- groups

RESULTS

OSTEmer polymer droplets with five different mixtures were generated successfully at a rate of 350 droplets / min with a homogenous size distribution centered at 300 μm . The droplets collected from the outlet tubing after on-chip UV polymerization showed a well-polymerized structure under microscope.

Different off-stoichiometric beads were bound with different amount of visual colorimetric reagent to the

thiol-groups on their surfaces, thus showing different colour intensity (Fig.5). The measured colour intensity of OSTE beads, after background subtraction, was evaluated versus the off-stoichiometry of the prepolymers. Assuming a linear relation between colour intensity and surface probe density allows comparing our results with previous measurements of surface probe density on flat OSTE surfaces as described by Lafleur et al. [7]. The microbeads generated with allyl excess prepolymer showed no colour shift, indicating no functional thiol groups present on the surface (Fig.6). The relation between microbeads surface probe density and thiol excess in the prepolymer illustrates the ease of probe density tuning for specific requirements.

Figure 5: OSTE beads with different off-stoichiometric ratio (A: OSTE-Allyl-60%; B: OSTE-Thiol-20%; C: OSTE-Thiol-30%; D: OSTE-Thiol-40%; E: OSTE-Thiol-60%)

Figure 6: Plot of measured color intensity of OSTE beads (red dots: A-E; evaluated after background subtraction), and thiol-surface density from previous work [7] (blue cross) versus the thiol polymer excess (negative values ~ allyl excess) in the prepolymer. Assuming a linear relation between color intensity and probe density allows comparing our work with that of Lafleur et al. [7]

CONCLUSIONS

We have demonstrated a rapid, repeatable and well-controlled method of microfluidic microbeads synthesis. We manufactured beads with 300 μm in diameter, at a rate of 350 beads/min and with synthesis time ≈ 1 s, with tuneable surface probe density. We also visualized the microbeads surface probe density by attaching colorimetric reagent to thiol- functional groups on the surfaces. The proportional relation between the beads surface color intensity and the thiol excess in the prepolymer agreed with the surface thiol density studies conducted previously. This novel microbeads manufacturing approach has potential impacts on bioapplications where on-demand chemical reactive surfaces in microscale are required.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support by Barncancerfonden, Sweden and the Stockholm Läns Landsting (Stockholm County Council). We also express our gratitude to Mercene Labs AB for providing the OSTE prepolymers and our technician Mikael Bergqvist for setting up the UV station.

REFERENCES

- [1] R. J. Heaton, R. M. Georgiadis, and A. W. Peterson, "The effect of surface probe density on DNA hybridization.," *Nucl. Acids Res.*, vol. 29, no. 24, pp. 5163–5168, Dec. 2001.
- [2] T. B. Mehta, A. R. Kopf-Sill, J. W. Parce, A. W. Chow, L. J. Bousse, M. R. Knapp, T. T. Nikiforov, S. Gallagher, and C. T. Corp, "Manipulation of microparticles in microfluidic systems," Oct. 2003.
- [3] R. A. Prasath, M. T. Gokmen, P. Espeel, and F. E. Du Prez, "Thiol - ene and thiol - yne chemistry in microfluidics: a straightforward method towards macroporous and nonporous functional polymer beads," *Polymer Chemistry*, vol. 1, no. 5, pp. 685–692, 2010.
- [4] A. Storha, E. A. Mun, and V. Khutoryanskiy, "Synthesis of thiolated and acrylated nanoparticles using thiol-ene click chemistry: towards novel mucoadhesive materials for drug delivery," *RSC Adv.*, vol. 3, no. 30, pp. 12275–12279, 2013.
- [5] J. Il Park, A. Saffari, S. Kumar, and A. Günther, "Microfluidic Synthesis of Polymer and Inorganic Particulate Materials," *Annual Review of Materials Research*, vol. 40, pp. 415–443, Jul. 2010.
- [6] C. F. Carlborg, T. Haraldsson, K. Öberg, M. Malkoch, and W. van der Wijngaart, "Beyond PDMS: off-stoichiometry thiol-ene (OSTE) based soft lithography for rapid prototyping of microfluidic devices," *Lab Chip*, vol. 11, no. 18, p. 3136, 2011.
- [7] J. P. Lafleur, R. Kwapiszewski, T. G. Jensen, and J. P. Kutter, "Rapid photochemical surface patterning of proteins in thiol-ene based

microfluidic devices,” *Analyst*, vol. 138, no. 3, p. 845, 2013.

- [8] X. Zhou, T. Haraldsson, and W. van der Wijngaart, “One-Step Injection Molding of Oste+ Microfluidic Devices with Screw Threaded Ports.” San Antonio, pp. 1671–1673, 16-Sep-2014.

CONTACT

*Wouter van der Wijngaart,

Tel: +4687906613

Email: wouter@kth.se