Development of a 3D-printed single-use separation chamber for mRNA vaccine purification via magnetic microparticles

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Introduction

mRNA-based vaccines are produced via cell culture methods. There are laboratory protocols for mRNA purification via magnetic beads after cell lysis. Therein, magnetic particles funcionalized with thymidine (oligo(dT)-beads) have the complementary base to adenine of the mRNA. In lysis/binding buffer only the selective hybridization between the mRNA molecules and the oligo(dT)-beads takes place. Magnetic separation can be utilized to remove the impurities, wash the magnetic particles and change the buffer. Thereafter, the temperature is increased to elute the mRNA and the magnetic beads can be regenerated. For producing mRNA amounts needed for clinical studies, time saving aspects play an important role. So, it seems to be beneficial to scale these laboratory protocols up. Therefore, high-gradient magnetic separation (HGMS) is suitable.

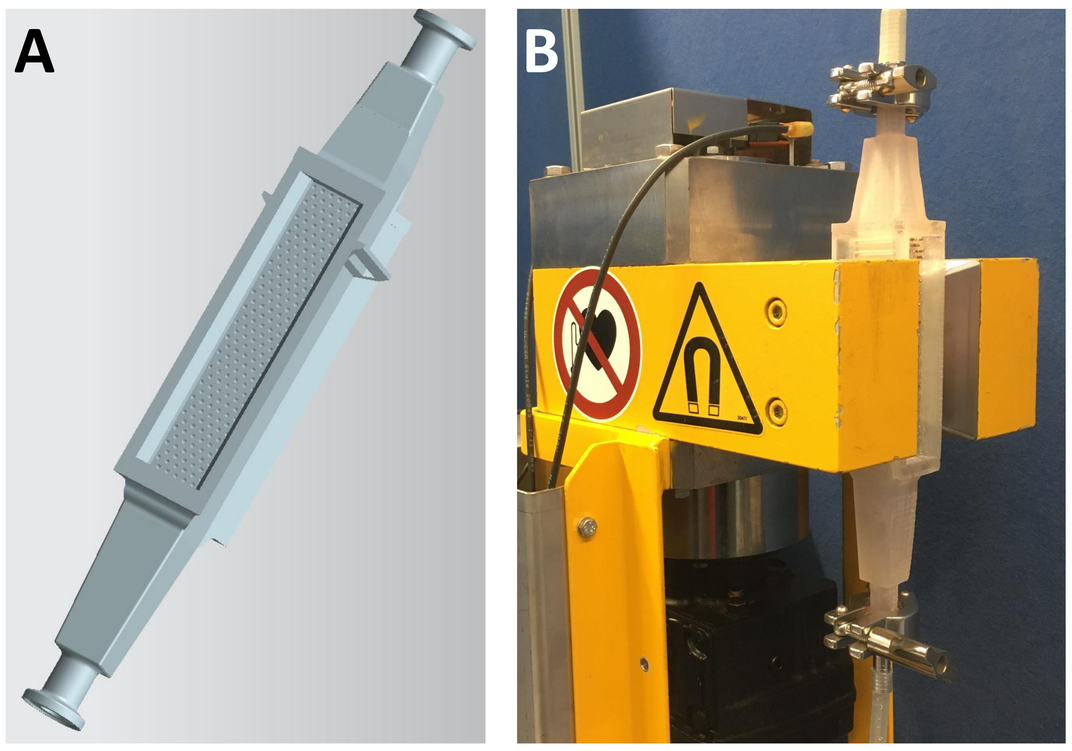

Figure 1: CAD model of the single-use-HGMS separation chamber (A), magnetic separator equipped with the 3D-printed SU-HGMS chamber (B)

Results

To evaluate the separation efficiency of the developed separation chamber, the turbity in the magnetic filtrate (OD_{600}) was measured and normalized to the turbity in the feed (figure 2). At volumetric flow rates up to 100 mL/min in lysis/binding buffer as well as PBS buffer, the cumulative particle loss was < 0.3 %. Flow rates of 150 mL/min resulted in particle losses of < 0.7 %. Even in the foaming lysis/binding buffer separation was sufficient. Average particle recoveries were 92 – 102 %. Buffers improved the wettability of the 3D-printed resin (figure 3). The contact angles were 47 ° with water, 32 ° with PBS buffer and 30 ° with lysis/binding buffer.

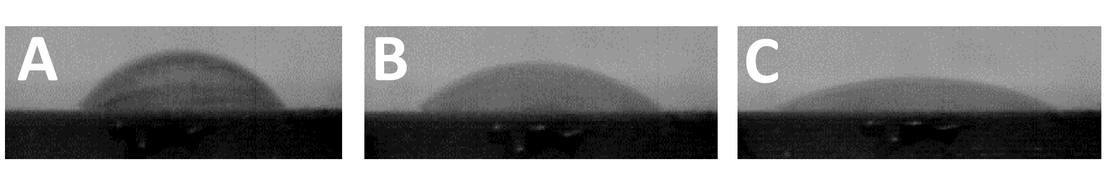

Figure 3: Contact angles of water (A), PBS buffer (B) and lysis/binding buffer (C) on the resin

mRNA purification via HGMS with a single-use separation chamber

Single-use (SU) equipment could be used to reduce cross-contaminations. Therefore, a SU-HGMS chamber (figure 1) was developed and 3D-printed via digital light processing. The 3D printer Vida (EnvisionTEC GmbH) was used with an USP Class VI certified resin fullfilling pharmaceutical regulations. Due to the special design, the product-containing liquid only comes into contact with the 3D-printed material and not with the magnetizable steel of the filter matrix.

Magnetic microparticles Dynabeads™ MyOne™ Carboxylic Acid (Life Technologies AS) were functionalized with amino-modified oligo$(dT)_{25}$ (Invitrogen Life Technologies AS) and loaded with synthetic mRNA. For separation with the SU-HGMS chamber, these 1 µm magnetic beads and the magnetic separator HGF 10 (Steinert Elektromagnetbau GmbH) were used. The HGMS was performed with lysis/binding buffer of commercial mRNA purification kits and a PBS storage buffer at different volumetric flow rates. Thereby, the concentration of magnetic beads was 1.6 – 2.1 g/L.

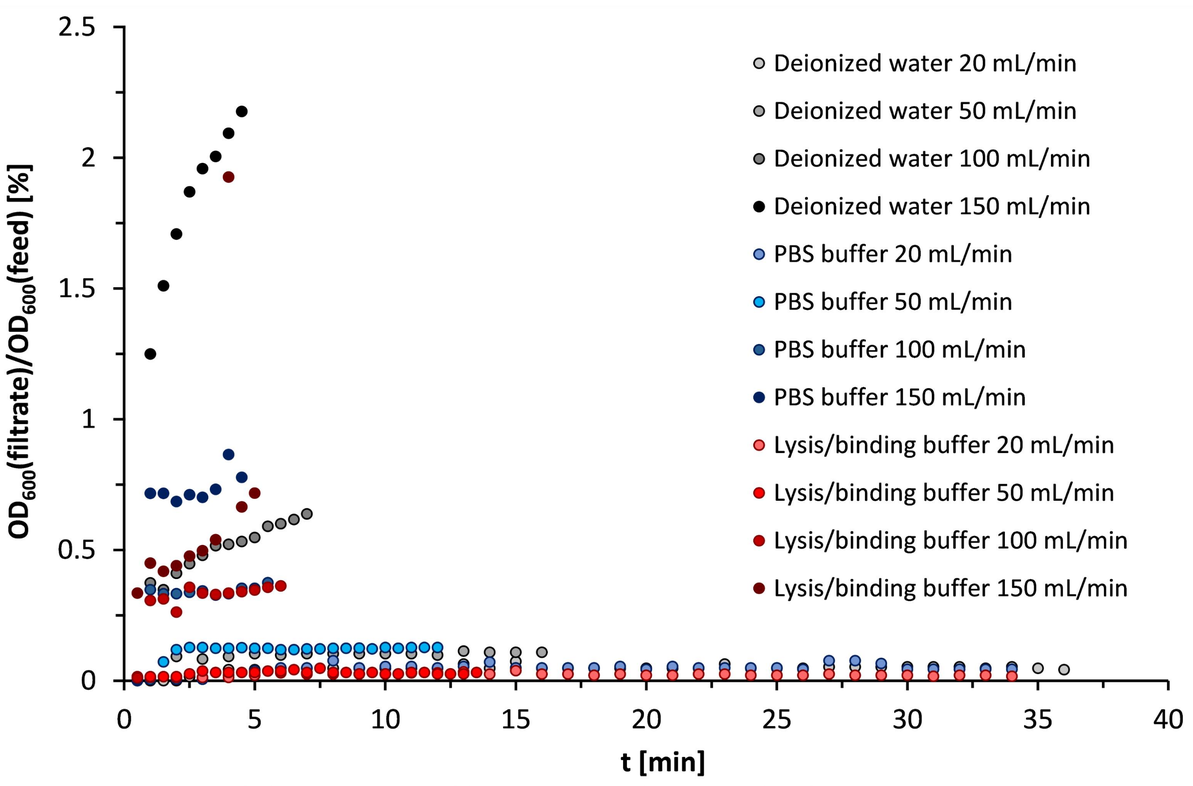

Figure 2: OD_{600} of the magnetic filtrate normalized to the feed during HGMS

Conclusion and Outlook

The developed SU-HGMS separation chamber is suitable for separation of mRNA-loaded Dynabeads™ MyOne™ out of relevant buffers used in magnetic microparticle-based mRNA purification. Excellent particle separation efficiencies of > 99.7 % or > 99.3 % were achieved in buffers at flow rates up to 100 mL/min or of 150 mL/min, respectively. Also, high particle recoveries were possible. The influence of increased viscosity of cell lysates and its components on the separation have to be investigated in the future. Even better separation results can be expected or higher volumetric flow rates are possible by using commercially available 2.8 µm oligo$(dT)_{25}$-Dynabeads™.

Acknowledgement: This work is funded by the European Union from the European Regional Development Fund and the state of Rhineland-Palatinate