ABSTRACT

The aim of this work was to generate monodispersed protein microbubbles and understand the in-vitro dissolution stability in an air saturated aqueous environment. Compared to existing microbubble production methods, the microfluidic device is an efficient technique for generating monodispersed microbubbles. Microbubbles synthesized by microfluidics have a narrow size distribution but are large in diameter and have less stability. Hence, in this work, we first optimize the parametric space to elucidate the effects of flow parameters and fluid properties on microbubble formation in a crossflow type T-junction microfluidic device. In addition to the above, we also investigated the effect of mixing zone on the size of microbubbles produced. Microbubble generation was facilitated by an aqueous phase consisting of Bovine serum albumin (BSA) as the model protein, and Sodium Dodecyl Sulphate (SDS) and Glycerol as additives. The gas stream for the microbubble formation was nitrogen (N_2). Optimization of mixing zone size from 200 to 50 µm, the size of the microbubbles reduced from 280 ± 2.3 µm to 160 ± 1.7 µm. Further, we investigated the effect of individual as well as combined chemical and thermal crosslinking on the in-vitro dissolution of microbubbles. The dissolution behavior of microbubbles was captured in an closed air-saturated aqueous environment until complete dissolution. The recorded microbubbles dissolution behavior was processed to obtain diameter v/s time curves. The dissolution time of non-crosslinked microbubbles was observed to be 63 ± 2.6 min, whereas the dissolution time of 179 ± 4.9, 210 ± 7.5, and 334 ± 13.1 min was observed for chemically crosslinked (CC), thermally crosslinked (TC), and combined chemical and thermal crosslinking (CC & TC) respectively. Circular Dichroism (CD) spectroscopy analysis was carried out to study the extent of crosslinking on the structure of BSA. It was found that the % alpha helices for non-crosslinked microbubbles was 40.6 ± 0.6 and that reduced to 33.6 ± 0.16, 17.6 ± 0.42 and 15.6 ± 0.32 for chemically crosslinked (CC), thermally crosslinked (TC), and combined (CC & TC), respectively. The results imply that higher crosslinking of BSA shells was achieved in a combination with chemical and thermal crosslinking that improved the in-vitro stability of the microbubbles. Moreover, in contrast to non crosslinked microbubbles, shell detachment was observed during the final phase of the dissolution for crosslinked microbubbles. The phenomenon of shell release was observed at higher magnifications and dependent upon the extent of crosslinking of the shell.

Keywords: Microbubbles, Microfluidics, Crosslinking, Dissolution.