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Particulate Release From Nanoparticle-Loaded Shape Memory Polymer Foams

Highly porous, open-celled shape memory polymer (SMP) foams are being developed for a number of vascular occlusion devices. Applications include abdominal aortic and neurovascular aneurysm or peripheral vascular occlusion. A major concern with implanting these high surface area materials in the vasculature is the potential to generate unacceptable particulate burden, in terms of number, size, and composition. This study demonstrates that particulate numbers and sizes in SMP foams are in compliance with limits stated by the most relevant standard and guidance documents. Particulates were quantified in SMP foams as made, postreticulation, and after incorporating nanoparticles intended to increase material toughness and improve radiopacity. When concentrated particulate treatments were administered to fibroblasts, they exhibited high cell viability (100%). These results demonstrate that the SMP foams do not induce an unacceptable level of risk to potential vascular occlusion devices due to particulate generation.
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1 Introduction

There are a number of vascular abnormalities that introduce significant morbidity and mortality in which an effective occlusion device could improve patient outcomes. For example, an aneurysm is a localized abnormal dilation of a blood vessel due to a damaged or weakened vessel wall [1]. Aneurysms are typically asymptomatic until rupture, which causes high patient mortality rates and makes them difficult to treat [2]. However, due to an increase in the use of sophisticated imaging technology, prerupture aneurysm detection rates are rising [3]. Porous, low density shape memory polymer (SMP) foam is reported to be an effective aneurysm occlusion material with excellent biocompatibility as seen in a porcine model wherein rapid, stable thrombus formation and high volume occlusion was observed [4,5]. SMP foams are materials capable of maintaining a temporary shape and, upon application of a thermal, chemical, or optical stimulus, recovering their primary shape [6]. These materials possess tunable thermal

and mechanical properties [7–9] and are deliverable via catheter [10]. Other potential applications of SMP foams include peripheral vascular occlusion for disease treatment. While SMP foams could serve as highly valuable implantable biomaterials, there is a risk of microfracture of the foam matrix during SMP foam device placement that could generate emboli in downstream microvasculature and result in unintended ischemia [11,12]. Particulate matter generated by SMP foams has not been reported previously and must be characterized prior to their translation into biomedical devices.

When evaluating particulate generation of a material intended for implantation, each step in the manufacturing and preparation process must be considered and examined as a potential source for particulates. A technical information report (TIR) from the Association for the Advancement of Medical Instrumentation (AAMI) advises testing for particulates generated during manufacturing, packaging, acute application, sterilization, and degradation [13]. Other important considerations in device safety include particulate chemical composition and their potential chronic formation after in vivo deployment. If particulates form over time after implantation, it would present significant systemic risks. The FDA has yet

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to define specific tests detailing acceptable particulate limits for medical devices, but they strongly recommend measuring the size and quantity of particulates that a device may generate as an indication of the embolic risk that the device will pose [14]. The U.S. Pharmacopeial (USP) Convention's chapter 788, "Particulate Matter in Injections," is commonly referenced when evaluating particulates [15]. It provides a protocol for counting particulates in solution and quantitative limits for particles $\geq 10\ \mu\text{m}$ and $\geq 25\ \mu\text{m}$. However, the chapter does not outline a protocol for evaluating particulates generated by medical devices or provide acceptable thresholds for particle generation. In a guidance document concerning a Class II medical device, the FDA recommends reporting the total quantity and size of the particulates generated during simulated use of the device in three distinct size ranges ($\geq 10\ \mu\text{m}$, $\geq 25\ \mu\text{m}$, and $\geq 50\ \mu\text{m}$) to comprehensively report on any subvisible particulate matter present [14]. To this end, an appropriate protocol should be developed to ensure that the particles will be in suspension during evaluation. In this study, our group has developed a protocol, based on USP 788 and FDA guidance documents, for benchtop analysis of particulate matter in the SMP foam intended for use in vascular occlusion applications. While this work focuses on SMP foams, the methods described here could be utilized with a range of medical device types during preparation for submission to the FDA.

The purpose of this research is to evaluate the particulate generation in SMP foams that serve as candidates for vascular occlusion devices. Particulates were evaluated in SMP foams with varied monomer and nanoparticle filler content and postreticulation. Reticulation allows for increased permeability into the foam matrix allowing more stable thrombus formation and cellular infiltration [16]. The foams were synthesized with varied monomer ratios and filler types, weight percentages (wt.%), and volume percentages (vol. %) to enable tailoring of the foam's physical and mechanical properties. Fillers are incorporated to increase toughness and wear-resistance to mitigate the risk of foam fracture and particulate generation while preserving the shape memory behavior of the materials [17,18]. The nanoparticle fillers, aluminum oxide (Al_2O_3), silicon dioxide (SiO_2), and tungsten (W) were chosen because they have been shown to improve mechanical properties in tissue scaffolds, implantable device coatings, and implantable devices [19–23]. Varying the monomer concentrations affects the hydrophobicity of the low density foams, and varying the weight and volume percentages of the fillers allows finer control of foam mechanical properties [18,24]. We aim to provide quantitative feedback on the particulates generated from the foams when subjected to cyclic inversion testing based on protocols developed in reference to those detailed in USP 788. Furthermore, 3T3 fibroblast viability was assessed upon administration of concentrated particulate solutions.

2 Materials and Methods

2.1 Materials. N,N,N',N'-tetrakis(2-hydroxypropyl)ethylene-diamine (HPED, 99%; Sigma-Aldrich, Inc., St. Louis, MO), triethanolamine (TEA, 98%; Sigma-Aldrich, Inc., St. Louis, MO), trimethyl-1,6-hexamethylene diisocyanate, 2,2,4- and 2,4,4-mixture (TMHDI; TCI America, Inc., Portland, OR), hexamethylene diisocyanate (HDI; TCI America, Inc., Portland, OR), DC 198 (Air Products and Chemicals, Inc., Allentown, PA), DC 5943 (Air Products and Chemicals, Inc., Allentown, PA), T-131 (Air Products and Chemicals, Inc., Allentown, PA), BL-22 (Air Products and Chemicals, Inc., Allentown, PA), Enovate 245fa Blowing Agent (Honeywell International, Inc., Houston, TX), 2-propanol 99% (IPA) (VWR, Radnor, PA), and de-ionized (DI) water (E-Pure water system, Barnstead International, Dubuque, IA) were used as received. Tungsten nanoparticles (W, 99.95%, 40–60 nm; U.S. Research Nanomaterials, Inc., Houston, TX), aluminum oxide nanoparticles (Al_2O_3 , alpha, 99+%, 80 nm, hydrophilic; U.S. Research Nanomaterials, Inc., Houston, TX), and

silicon dioxide nanoparticles (SiO_2 , 98+%, 60–70 nm, amorphous; U.S. Research Nanomaterials, Inc., Houston, TX) were dried under vacuum at 90 °C for 12 h prior to foam synthesis [18].

Penicillin/streptomycin (P/S, 1%; VWR, Radnor, PA), Dulbecco's modified eagle medium (DMEM; VWR, Radnor, PA), newborn calf serum (NBCS, 10%; Sigma-Aldrich, Inc., St. Louis, MO), fungizone (0.1%, VWR, Radnor, PA), calcium chloride (CaCl_2 , 0.1%; Sigma-Aldrich, Inc., St. Louis, MO), formaldehyde (0.5%; Sigma-Aldrich, Inc., St. Louis, MO), acetic acid (1%; Sigma-Aldrich, Inc., St. Louis, MO), ethanol (50%; Sigma-Aldrich, Inc., St. Louis, MO), 3T3 fibroblasts (ATCC, Manassas, VA), and a neutral red (NR) uptake assay (TOX4, Sigma-Aldrich, Inc., St. Louis, MO) were used as received.

2.2 Foam Synthesis. Twelve different variations of SMP foams, shown in Table 1, were synthesized using the three-step protocol previously described by Hasan et al. [18], Fig. 1. Isocyanate prepolymers were synthesized using appropriate molar ratios of HPED, TEA, TMHDI, and HDI, with a 35 wt.% hydroxyl (OH) composition. Nanoparticles were physically incorporated into the isocyanate prepolymers via high shear mixing prior to foam blowing at the appropriate concentrations. A OH mixture was prepared with the remaining molar equivalents of HPED and TEA, along with catalysts, surfactants, and DI water. During foam blowing, a physical blowing agent, Enovate, was mixed with the isocyanate prepolymer and the OH mixture using a speedmixer (SpeedMixer, FlakTek, Inc., Hauschild, Germany). The resulting foams were cured in a vacuum oven at 90 °C for 20 min. The SMP foam nanocomposites were cooled to room temperature ($21 \pm 1\ ^\circ\text{C}$) followed by a 24-h cold cure before further processing.

SMP foams synthesized with 100% TMHDI and no fillers were used as controls. Four foams were synthesized containing either Al_2O_3 or SiO_2 fillers with varied monomer ratios, as listed in Table 1. The two isocyanate monomers used were TMHDI and HDI. The remaining seven foams were synthesized with varying weight or volume percentages of W filler. Weight percentages ranged from 1 to 3 wt.%, as an unfavorable decrease in mechanical properties (ultimate tensile strength, Young's modulus, and strain at break) is exhibited by foams with higher wt.% fillers (>3 wt.%) [17]. Additionally, 3, 5, 6, and 8 vol. % W foams were assessed due to the increased X-ray visibility exhibited by these foams [18].

2.3 Mechanical Reticulation. The mechanical reticulation system includes a gravity-driven floating nitinol pin array and a vertically oscillating vibratory shaker (Fig. 2) [16]. Foam samples were cut to rectangular blocks 30 mm in height and 50 mm in length/width. The foam blocks were held in place by a custom vibratory mounting platform that is cyclically raised and lowered for 2 h. Then, the foam block was turned over, resecured, and subject to another 2 h reticulation cycle. Complete reticulation of the foam block took 4 h. One foam block of 100% TMHDI foam with no filler particles was mechanically reticulated and cut into five, 8 mm cylinders (30 mm in length) using a biopsy punch (Acupunch, Acuderm, Inc., Fort Lauderdale, FL) for use in cyclic testing.

2.4 Cyclic Testing of Particulate Generation. Foam cylinders ($n=5$) with 8 mm diameter and 30 mm length were cut from the 12 foam formulations (Table 1) using an 8 mm biopsy punch. Particulate data was collected for three distinct size ranges, $\geq 10\ \mu\text{m}$, $\geq 25\ \mu\text{m}$, and $\geq 50\ \mu\text{m}$, as recommended by the FDA. A procedure was developed for agitation of the foam samples in reference to USP 788. Briefly, foam sample agitation was achieved by successive inversion of a single foam cylinder 20 times in DI water. The resulting particulate levels in the DI water were measured using light obscuration (PC5000, Chemtrac, Inc., Norcross, GA). Prior to testing, the particle counter was validated by introducing microspheres of a known size and quantity into the system

Table 1 Filler type, monomer ratio, and weight or volume percentage used in foam syntheses

Foam name	Monomer ratio	Filler particle	Filler wt. %	Filler vol. %
Control	100% TMHDI	—	—	—
50:50 1 wt.% Al ₂ O ₃	50% HDI 50% TMHDI	Al ₂ O ₃	1	—
20:80 1 wt.% Al ₂ O ₃	20% HDI 80% TMHDI	Al ₂ O ₃	1	—
0:100 1 wt.% Al ₂ O ₃	100% TMHDI	Al ₂ O ₃	1	—
1 wt.% SiO ₂	100% TMHDI	SiO ₂	1	—
1 wt.% W	100% TMHDI	W	1	—
2 wt.% W	100% TMHDI	W	2	—
3 wt.% W	100% TMHDI	W	3	—
3 vol. % W	100% TMHDI	W	—	3
5 vol. % W	100% TMHDI	W	—	5
6 vol. % W	100% TMHDI	W	—	6
8 vol. % W	100% TMHDI	W	—	8

Fig. 1 Three-step foaming process. (a) Prepolymer solution is synthesized and cured for 36 h. **(b)** The resulting prepolymer is mixed with surfactants and nanoparticle fillers to make the side A mixture. The hydroxyl components, water, and catalysts are added to the side B mixture. **(c)** Side A and side B are mixed and a blowing agent is added to initiate the foam blowing process. Foams cured in the oven at 90 °C for 20 min.

Fig. 2 Mechanical reticulation process. (a) Scanning electron microscopy (SEM) image of SMP foam prior to reticulation, **(b)** SMP foam block is repetitively raised and lowered on a vibrating platform allowing the floating nitinol pin array to mechanically reticulate the foam, and **(c)** SEM image of SMP foam postreticulation.

Table 2 Size and quantity of microspheres inputted, acceptable value for $\geq 75\%$ recovery, and average $\pm$ standard deviation of particle levels outputted by the particle counter

Input microsphere size (μm)	Input microsphere number	Output size range (μm)	Output (75% = 11,250)	% recovery
15	15,000	10–25	12,870 $\pm$ 75	86
70	15,000	65–80	12,326 $\pm$ 1074	82

Table 3 USP 788 acceptable limits for injection or parenteral infusion [15]

Volume	Particle size range	Acceptable number of particles
≤100 ml	≥10 μm	6000
≤100 ml	≥25 μm	600

and ensuring that >75% were recovered (Table 2) [14]. Particle counts from the SMP foams were compared to the limits for small volume infusions stated in USP 788 (Table 3) to characterize the foams on the basis of particulate generation.

As shown in Fig. 3, each SMP foam cylinder was placed in a bottle containing 100 ml DI water, agitated, removed from the water, and placed into a subsequent bottle of fresh DI water. The suspension of particles was then analyzed using the particle counter. This process constituted one wash cycle. Each sample was subjected to six wash cycles or the number of cycles required to reach a plateau in the level of particulate generation ($92 \pm 3\%$ decrease). Decreases in particulates from wash to wash were quantified using the following formula:

$$\text{Percent Decrease} = \frac{|N_{n-1} - N_n|}{N_{n-1}} \times 100\%$$

where N is the number of particulates and n is the wash number. Due to the high sensitivity of the particle counter, baseline water counts were taken prior to each experiment to ensure that the DI water used met the criteria outlined in USP 788 (i.e., for water to be deemed particle-free, it must contain less than 1 particle $\geq 10 \mu\text{m}$ in size per ml) [15].

2.5 Cytocompatibility Assay. Control foams (100% TMHDI, no filler), 1 wt.% Al_2O_3 filler foams, 1 wt.% SiO_2 filler foams, 1 wt.% W filler foams, and 3 vol. % W filler foams were washed with DI water according to the protocols outlined in Sec. 2.4. Forty ml of particulate solution was collected after the first and sixth washes for each sample, and 40 ml of fresh DI water was collected as a control. P/S was added to each solution at 1% between collection and cell treatment to reduce contamination. The solutions were centrifuged for 10 min at 4400 rpm to pellet particulates; then, the supernatant was aspirated and exchanged for 2 ml of cell culture media to make $20\times$ concentrated particulate solutions. Cell culture media was composed of DMEM, 10% NBCS, 1% P/S, and 0.1% fungizone.

3T3 fibroblasts were seeded in 96-well tissue culture plates at a concentration of 5000 cells/well and incubated in a humidified

incubator at 37°C with 5% carbon dioxide (CO_2) for 24 h. Cell morphology was observed in all wells using a Nikon Eclipse TE 2000-S inverted microscope (Nikon, Melville, NY) and even cell distribution was confirmed before addition of the treatments. Cell culture media was aspirated from the wells, and $200 \mu\text{L}$ of the designated treatment solutions were added to each well. Cells were incubated with particulate treatments for 48 h at 37°C with 5% CO_2 .

Following incubation, cell morphology was observed in all wells using the Nikon inverted microscope to qualitatively evaluate changes induced by addition of the treatments. The extract treatments were then removed and a NR uptake assay was utilized to quantify cell viability. NR is a red dye that is actively transported across the cell membrane where it accumulates within lysosomes [25]. After incubation with NR for 3 h, the cells were fixed with a solution of 0.1% CaCl_2 in 0.5% formaldehyde. The dye was solubilized in a solution of 1% acetic acid in 50% ethanol for measurement. A plate reader (Tecan Infinite M200 Pro, Morrisville, NC) was used to measure the optical density at 540 nm to quantify the amount of remaining dye in each well. Cell viability is expressed as a percentage using the following equation:

$$\text{Cell Viability } (X) = \frac{OD_{540}(X)}{OD_{540}(\text{Negative Control})}$$

where X is any treatment group and the negative control for cytocompatibility (DI water) is used as a standard that equals 100% viability.

2.6 Statistical Calculations. Data was compiled and expressed as mean $\pm$ standard deviation (SD) (Table 4), and statistical analysis was performed using student's t-tests (Microsoft Excel, Microsoft, Redmond, WA). The 12 foam compositions were divided into subgroups and compared with other foams in their subgroup to determine statistical differences in particulate generation with a 95% confidence level ($p < 0.05$). The particulates $\geq 10 \mu\text{m}$ generated after the first wash were found to be most characteristic of the foam compositions. For this reason, first wash particulate levels were used when performing the t-tests.

3 Results

All foam types generated the highest average number of particulates after the first wash, and the average number of particulates declined exponentially with each subsequent wash. The magnitude of generated first-wash particulates varied with foam composition, with the control foam generating the lowest number of particulates of the tested foam compositions. All of the foams

Fig. 3 Schematic representation of the cyclic wash protocol used to evaluate foam samples for particulate generation

Table 4 Mean $\pm$ standard deviation of particulate numbers from the first wash, and percent decrease after first and second washes for particles $\geq 10 \mu\text{m}$ and $\geq 25 \mu\text{m}$

Foam ($N = 5$)	Mean particulates $\geq 10 \mu\text{m}$	Mean particulates $\geq 25 \mu\text{m}$	Mean % decrease after first wash	Mean % decrease after second wash
Control	760 $\pm$ 140	70 $\pm$ 20	80 $\pm$ 10	50 $\pm$ 30
Reticulated control	1990 $\pm$ 490	187 $\pm$ 58	55 $\pm$ 80	50 $\pm$ 30
50:50 1 wt.% Al_2O_3	1470 $\pm$ 550	83 $\pm$ 40	70 $\pm$ 20	30 $\pm$ 25
20:80 1 wt.% Al_2O_3	2300 $\pm$ 520	260 $\pm$ 330	90 $\pm$ 5	50 $\pm$ 40
0:100 1 wt.% Al_2O_3	1470 $\pm$ 860	50 $\pm$ 20	70 $\pm$ 20	35 $\pm$ 30
1 wt.% SiO_2	1100 $\pm$ 240	40 $\pm$ 20	80 $\pm$ 15	45 $\pm$ 30
1 wt.% W	800 $\pm$ 220	40 $\pm$ 10	90 $\pm$ 10	30 $\pm$ 20
2 wt.% W	1070 $\pm$ 350	60 $\pm$ 20	90 $\pm$ 5	50 $\pm$ 25
3 wt.% W	1140 $\pm$ 550	30 $\pm$ 10	75 $\pm$ 10	45 $\pm$ 30
3 vol. % W	1430 $\pm$ 460	130 $\pm$ 60	80 $\pm$ 10	50 $\pm$ 25
5 vol. % W	1380 $\pm$ 320	90 $\pm$ 40	90 $\pm$ 5	60 $\pm$ 10
6 vol. % W	1480 $\pm$ 440	130 $\pm$ 110	90 $\pm$ 5	55 $\pm$ 30
8 vol. % W	2080 $\pm$ 460	120 $\pm$ 100	85 $\pm$ 10	65 $\pm$ 30

tested exhibited a decrease in all three particulate size ranges after their initial wash. On average, there was an $80 \pm 11\%$ decrease in particulates $\geq 10 \mu\text{m}$ and $\geq 25 \mu\text{m}$ after the first wash (Table 4). Subsequently, a $47 \pm 11\%$ decrease was observed between the second and third washes. This trend was observed with all foam chemistries. Quantification of decreases in particulates observed after the first and second washes and average particulate counts for particles $\geq 10 \mu\text{m}$ and $\geq 25 \mu\text{m}$ are shown in Table 4. Particulate counts for particles $\geq 50 \mu\text{m}$ were very low (average of three particles across all compositions tested) and exhibited no discernable trends (Fig. 4). The results from NR uptake assay supported the cytocompatibility of particulates generated from all tested foam compositions.

3.1 Effects of Mechanical Reticulation. Reticulation of the control foam resulted in significantly higher particles levels than the nonreticulated control foam. This difference is likely due to the fact that the process involves physical perforation of the foam-cell membranes using nitinol wires. This physical perforation resulted in statistically significant differences between the nonreticulated and reticulated control foams as shown in Fig. 5. Comparing the reticulated versus the nonreticulated control foam, it is evident that the reticulated foams require a higher number of

washes to reduce the particle levels. This is supported by the percent decreases between the first and second cycles shown in Table 4. The reticulated foam recorded a 55% decrease in particulates, while the other foam chemistries averaged an 85% decrease. This difference in particulate release pattern can be attributed to the reticulation of the foams. Nonreticulated foams have trace amounts of chemical and particulates trapped within the body of the foam; the majority of those particulates are able to be washed out during one inversion cycle because the majority of the foam-cells are still closed. Reticulation opens up the majority of the foam-cells, enabling release of more particulates that are trapped deep within the body of the foam.

3.2 Effects of Monomer Ratio. Particulate analysis of foams with varied monomer ratios of HDI to TMHDI (0:100, 20:80, 50:50) revealed that foams containing a 20:80 HDI:TMHDI ratio (Fig. 6) generated the highest number of particulates after their first wash cycle. T-tests were performed to quantify differences in particulate generation in relation to monomer ratios. There was a statistically significant difference between the 20:80 HDI:TMHDI and the 50:50 HDI:TMHDI foams ($p = 0.02$) for $\geq 10 \mu\text{m}$ particle generation (Fig. 6(a)). By increasing the ratio of TMHDI, the more hydrophobic of the two monomers, the foams become stiffer due to the reduced rate of water permeation into the foam matrix [24]. This increased stiffness could be the cause of higher particulate levels in the foams with higher TMHDI ratios ($\geq 80\%$). There were no significant differences exhibited in the $\geq 25 \mu\text{m}$ size range (Fig. 6(b)).

3.3 Effects of Filler Type. Variation in filler type resulted in differences in first wash particulate generation (Fig. 7). Of the three fillers, foams with W generated the lowest number of first wash particulates and foams with Al_2O_3 produced the highest number of particulates. T-tests were performed to assess differences in particulate generation among the filler particles. Statistical analysis returned that the only significant difference ($p < 0.05$) was between the SiO_2 foams and the W foams ($p = 0.04$) (Fig. 7(a)). In general, adding fillers at low concentrations increased the small particulates ($> 10 \mu\text{m}$) but reduced the larger ($> 25 \mu\text{m}$) particulates. Filler incorporation reinforces the polymer matrix and, at low concentrations (0.5–1%), provides physical net points that allow for improved resistance to mechanical deformation [18]. The SiO_2 foam most likely differed from the control foam because of the lower mass of the SiO_2 nanoparticles relative to the W and Al_2O_3 nanoparticles; due to this reduced mass, a higher number of SiO_2 nanoparticles were required to achieve the same weight percent loading as the W and Al_2O_3 loaded foams [18]. The increased numbers of $\geq 10 \mu\text{m}$ particulates (Fig. 7(a)) seen in the filler foams relative to the control foam could be due to less stable areas of the foam with aggregates of filler

Fig. 4 Particulates $\geq 50 \mu\text{m}$ generated over three inversion cycles for all foam chemistries tested. $n = 5$; Mean $\pm$ standard deviation displayed. No USP or FDA limit for particulates $\geq 50 \mu\text{m}$.

Fig. 5 Number of particulates (a) $\geq 10 \mu\text{m}$ and (b) $\geq 25 \mu\text{m}$ from control foams that were reticulated and nonreticulated over three inversion cycles. $n = 5$; Mean $\pm$ standard error displayed; * $p < 0.05$ relative to control. USP limit for particulates $\geq 10 \mu\text{m} = 6000$; limit $\geq 25 \mu\text{m} = 600$.

Fig. 6 Numbers of particulates (a) $\geq 10 \mu\text{m}$ and (b) $\geq 25 \mu\text{m}$ from 1 wt.% Al_2O_3 foams with varying monomer ratios of 50:50 HDI:TMHDI, 20:80 HDI:TMHDI, and 100% TMHDI. $n = 5$; Mean $\pm$ standard deviation displayed; * $p < 0.05$ relative to control; ** $p < 0.05$ between the bracketed groups. USP 788 limit for particulates $\geq 10 \mu\text{m} = 6000$; limit $\geq 25 \mu\text{m} = 600$.

Fig. 7 Numbers of particulates (a) $\geq 10 \mu\text{m}$ and (b) $\geq 25 \mu\text{m}$ from foams with W, SiO₂, and Al₂O₃ filler types. $n = 5$; Mean $\pm$ standard deviation displayed; * $p < 0.05$ relative to control; ** $p < 0.05$ between bracketed groups. USP 788 limit for particulates $\geq 10 \mu\text{m} = 6000$; limit $\geq 25 \mu\text{m} = 600$.

nanoparticles, and the reduced numbers of $\geq 25 \mu\text{m}$ particulates (Fig. 7(b)) could be related to the increased net points that the fillers provide.

3.4 Effects of Filler Weight Percent. Variation in the weight percentage of W incorporated into the foam resulted in differences

in particulate generation (Fig. 8). Statistical analysis returned that the only significant difference ($p < 0.05$) was between the 2 wt.% W foams and the 3 wt.% W foams ($p = 0.03$) in the $\geq 25 \mu\text{m}$ size range (Fig. 8(b)). The average magnitude of generated particulates $\geq 10 \mu\text{m}$ recorded after the first wash cycle generally increased as weight percentage W was increased (Fig. 8(a)). However, T-tests returned that the number of generated particulates $\geq 10 \mu\text{m}$ did not

Fig. 8 Numbers of particulates (a) $\geq 10 \mu\text{m}$ and (b) $\geq 25 \mu\text{m}$ from foams with varying weight percentages of W. $n = 5$; Mean $\pm$ standard deviation displayed; * $p < 0.05$ relative to control; ** $p < 0.05$ between bracketed groups. USP limit for particulates $\geq 10 \mu\text{m} = 6000$; limit $\geq 25 \mu\text{m} = 600$.

Fig. 9 Numbers of particulates (a) $\geq 10 \mu\text{m}$ and (b) $\geq 25 \mu\text{m}$ from foams with varying volume percentages of W. $n = 5$; Mean $\pm$ standard deviation displayed; * $p < 0.05$ relative to control; ** $p < 0.05$ between bracketed groups. USP 788 limit for particulates $\geq 10 \mu\text{m} = 6000$; limit $\geq 25 \mu\text{m} = 600$.

differ significantly for the three weight percentages tested despite the observed positive correlation. The relatively low filler concentrations tested here were previously shown to have improved foam toughness and lowered particulate levels [18]. Thus, particulate levels that were generally less than or equal to the control foam was expected.

3.5 Effects of Filler Volume Percent. Variation in volume percentage of W incorporated into the foam also resulted in differences in first wash particulate generation (Fig. 9). T-tests showed that the 8 vol. % W generated a statistically higher number of particulates than the 3, 5, and 6 vol. % W foams ($p < 0.05$) in the smaller size range (Fig. 9(a)). Despite the weak positive correlation demonstrated between increasing vol. % and particulate levels (Fig. 9(a)), none of the 3, 5, or 6 vol. % W foams differed significantly from one another. Increased filler concentrations (>4 vol. %) were previously seen to lower material toughness [18] and, as observed in this study, generated higher particulate levels in both the $\geq 10 \mu\text{m}$ and $\geq 25 \mu\text{m}$ size ranges (Figs. 9(a) and 9(b)). High filler content decreases foam stiffness due to disruption of the foam matrix when filler aggregates replace the polymer within the foam struts and, ultimately, makes the foams more susceptible to brittle fracture [18].

3.6 Cytocompatibility Assay. The results of the NR uptake assay are shown in Fig. 10. No morphologic changes were observed after 48 h of incubation with particulate solutions. Cell viability greater than 100% was observed for all treatment groups

and ranged from 107% to 117%. This evidence supports the cytocompatibility of all particulate samples. Means were compared using student's t-tests and no statistically significant differences were observed in viability between washes 1 and 6 for any group.

Fig. 10 Cell viability of 3T3 fibroblasts determined by neutral red uptake assay. $n = 5$; Mean $\pm$ standard error displayed. No significant differences were observed between the treatment groups.

4 Discussion

The overarching goal of this study was to develop a protocol for quantification of particulate levels in SMP foams intended for use in vascular occlusion devices. These SMP foams were not cleaned postsynthesis so that they could be analyzed independent of the cleaning method. The results from this benchtop study indicate that particulates in the two ranges addressed by USP 788 ($\geq 10\ \mu\text{m}$ and $\geq 25\ \mu\text{m}$) are below the stated limits. While these limits do not directly address medical devices, they allow comparison to the most relevant standard. The third size range ($\geq 50\ \mu\text{m}$) tested here does not have a limit defined by either the USP or FDA, but the level of particulates was very low for this size range. The results from the NR assay support the cytocompatibility of particulates generated from the SMP foams.

Reticulation of the foams was shown to increase the number of particulates in both size ranges, as expected. The reticulation process opens up the pores throughout the foams, allowing inner particulates to be washed out. Additionally, the process mechanically breaks up the foams and produces additional particulates that were washed out during the inversion cycles. Although the particulate count was increased after reticulation, it is recommended to reticulate foams prior to cleaning, as it will allow for a more thorough removal of particulates before sterilization and implantation.

As the concentration of HDI relative to TMHDI was increased, the number of particulates increased in both size ranges. These results are attributed to increased water uptake and reduced modulus of HDI-containing foams [7]. As foams increase their water uptake (due to increased hydrophilicity) during the wash cycles, it is likely that the washing will be more effective at removing foam debris. Additionally, reduced foam modulus results in reduced force required to deform the material and break off particulates during wash cycles.

None of the three fillers resulted in particulate levels that would eliminate them as candidates for endovascular applications. In comparing filler types (all at 1 wt.%), SiO_2 and Al_2O_3 fillers had increased particulates $\geq 10\ \mu\text{m}$ relative to the control, while all filler types reduced the number of particulates $\geq 25\ \mu\text{m}$. In previous work, we showed that these fillers aggregate within the foams. The lowest aggregate size that we could measure was $50\ \mu\text{m}$, but it is hypothesized that smaller nanoparticle aggregates form and/or that aggregates are broken up during the wash cycles. Thus, the increase in smaller particulates from nanoparticle-loaded foams is attributed to nanoparticle aggregates that are released during the wash cycles. The reduction in larger particulates is likely due to the increased ultimate tensile strength and toughness that is observed with the addition of low concentrations of nanoparticles [18,26]. The improved mechanical properties are due to the formation of physical crosslinks within the foam matrix by the particulates, and likely prevent foam tearing and breakdown during the wash cycles.

For lower concentrations of W nanoparticles (1–3 wt.%), the trends in the number of particulates $\geq 25\ \mu\text{m}$ match those of previously published data on foam mechanical properties. Namely, 1 and 3 wt.% W nanofiller foams had increased toughness and strain at break than 2 wt.% W foams [26]. The relationship between nanofiller concentration and mechanical properties is due to nanoparticles forming physical crosslinks within the foam matrix. There are some concentrations of nanofillers (i.e., $>3\ \text{vol. \%}$) for which dispersion throughout the foam is reduced due to nanoparticle–nanoparticle interactions, which results in reduced toughness and an increased likelihood of particulate formation.

When volume percentage of W is altered in the foams, significant increases in particulate levels were observed. These large increases are due to nanoparticle aggregate formation at higher concentrations, which disrupts the foam matrix to reduce strength and elasticity. Previous studies have shown that as W vol. % is increased in this range, pore size, modulus, and ultimate tensile strength decrease [18]. For particulates $\geq 25\ \mu\text{m}$, the 3 vol. % W foams had greater particulate levels than the 5 vol. % W foams

and similar levels to the highest W concentration foams. This result is attributed to the balance between pore size and mechanical properties. Larger pore sizes in the lower concentration foams allow for more effective washing to more thoroughly remove particulates. As previously reported for tungsten-loaded foams, pore sizes decrease from, approximately, $2100\ \mu\text{m}$ for unloaded to $1000\ \mu\text{m}$ for the highest concentrations (10 vol. %) foams [18]. Increased modulus and tensile strength in the lower concentration foams reduces the chance of foam strut breakage and subsequent particulate formation. Thus, the pore size of the 3 vol. % W foams likely allowed for increased particulate removal, while the reduced mechanical properties of the 6 and 8 vol. % W foams increased particulate generation during washing. However, when comparing pore size effects for different fillers in this study, the pore sizes for different fillers in this study for the same weight-loaded foams were statistically similar. For example, the 1 wt.% transverse pore sizes for Al_2O_3 , SiO_2 , and W foams were $1500 \pm 125\ \mu\text{m}$, $1551 \pm 206\ \mu\text{m}$, and $1686 \pm 206\ \mu\text{m}$, respectively (mean $\pm$ std. dev., $n = 10$, method of Ref. [18] using light microscope).

The foams tested here were uncleaned as an example of the worst case scenario for particulate formation. However, all foams that are used in devices are reticulated and subjected to extensive cleaning processes [5] that serve to remove the particulates along with unreacted monomers and catalysts prior to implantation. Future work will focus on characterizing prolonged particulate release from foams under physiological flow conditions and under device delivery conditions. Particulate chemical composition will also be assessed, which is an important consideration in device safety evaluation.

In comparison to alloy-based embolization devices, which are the most comparable existing devices, SMP foams promote significantly improved clotting and healing [27]. Additionally, their unique shape memory properties enable their catheter delivery through tortuous vasculature to the desired treatment site, where they expand to fill the site and promote clotting. Overall, these results combined with the positive prior in vivo study results indicate that the SMP foams present minimal risks from particulate release that may result in unintended downstream emboli formation and have high potential for use as effective and safe embolization devices [4,5,16,28].

5 Conclusions

In summary, the SMP foams evaluated were in compliance with the most relevant standard and guidance documents. The protocol for quantifying particulate matter can be used for evaluating particulate risk in vascular occlusion devices during future benchtop testing. Future work will focus on characterizing prolonged particulate release from foams under physiological flow conditions and under device delivery conditions. Particulate chemical composition will also be assessed, which is an important consideration in device safety evaluation.

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