NANO FIBER BIOLOGICAL FILTER

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ABSTRACT

Electropositive nano alumina (NanoCeram™) filters retain >99.9999% of virus and bacteria at flowrates 500-1000 times greater than ultraporous membranes, even in salt water and sewage. The filters are rather resistant to clogging, particularly for small (e.g.-virus size) particles. The filter will sanitize BW-contaminated drinking water and could also be used for collection in BW detection devices for water and air. NanoCeram™ has been coated onto carbon and other substrates, and may provide a breathable biological barrier for collective protection. The filter will adsorb toxic metals such as chromium, arsenic, and radioisotopic metals that might be dissolved or in colloidal form.

INTRODUCTION

Alumina (AlOOH) nanofibers (NanoCeram™) only 2 nm in diameter were developed in Russia under a CRADA of Argonide with the Department of Energy. Argonide immobilized the fibers into a filter and subsequently was awarded an SBIR contract by NASA to develop a filter for space cabins. The filters, which received a "Best 100 New Product" award by R and D magazine, are being commercialized in January 2003 in the form of laboratory size filters for biotech applications. The filters are highly electropositive as a result of the high surface area, covered by hydroxide groups and attract and retain pathogens and virus that are principally electronegative.

PHYSICAL AND CHEMICAL CHARACTERISTICS

(NanoCeram™) fibers are shown in Figures 1 and 2. The fibers are produced via a proprietary sol-gel reaction, followed by filtering the sol and subsequent drying and heat-treating. X-ray diffraction shows that the fiber is principally composed of boehmite (AlOOH). The surface area ranges between 300 and 600 m^2/g. Computation of surface area assuming a 2 nm diameter suggests that most if not all of the

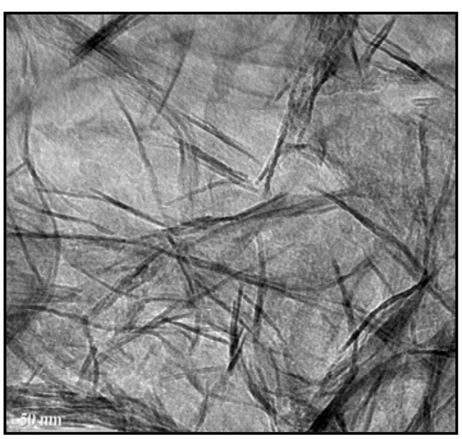


Figure 1. TEM of NanoCeram (50 nm scale).

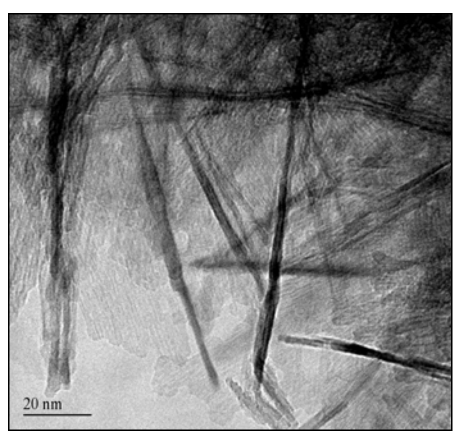


Figure 2. TEM of NanoCeram (20 nm scale).

area is external as compared to activated alumina where most of the available surface area can only be accessed through a capillary network. This would suggest that NanoCeram has rapid sorption kinetics.

The fiber was found to be an excellent adsorbent for trace dissolved metal. Table 1 shows the adsorption of several metals from challenge concentrations in the ppm level by relatively thin (~ 3 mm) layers of NanoCeram™ fibers. Other data (not shown) demonstrate efficient sorption of chromium, iron, nickel, tin, zinc, uranium (uranyl- UO_2) and vanadyl (VO) species. NanoCeram will adsorb dissolved halogen (7.5, 9.0, 17.0 and 39.0 mg/g respectively for F, Cl, Br and I) and fluoride ion (approximately 3 times that of activated alumina). The filter would appear to be capable of filtering radioactive metals, as well as lead, arsenic and other toxic metals. Argonide recently received an EPA SBIR for an arsenic filter.

TABLE 1. Adsorption of heavy metals

Metal	Zn	Cd	Pb	Cu	Au	Ag
Challenge concentration, mg/L	50	35	35	35	1	1
Concentration in filtrate, mg/L	0.001	0.001	0.001	0.001	0.001	0.001
Filtration Efficiency, %	99.998	99.997	99.997	99.997	99.9	99.9
Dynamic Sorption Capacity, g(metal)/kg(NanoCeram)	3.3	1.0	5.0	5.6	5.0	5.0

Bioactivity and Virus Filtration

The fibers were found [1] to attract and retain osteoblast (bone) cells better than hydroxyapatite (natural bone mineral). NanoCeram powders were also found to adsorb virus from water. Controls of Millipore HA, 0.4 μ microporous membranes showed <1% retention when challenged with MS-2 or ϕ-x-41 virus (bacteriophage) at pH=7.5, but when a thin layer (0.7 g, or about 3 mm thick over 25 mm diameter) was spread over the membranes, retention soared to 99.6 + %. These data led to our being awarded a Phase I SBIR by NASA to develop a filter for removing pathogens from recycled water in space cabins. Our first filters removed 99.5% of virus from neutral water. Subsequent improvements were to 99.99% (4 logs) then 5 logs and the newest filters can clear 6-7 logs (Table 1).

TABLE 2. Retention of virus by NanoCeram/Microglass Filters

Thickness, mm	Weight % NanoCeram™	MS-2 % removal	PRD-1 % removal
1.1	0	8	
1.2	2	14	
1.0	5	29	
1.1	10	94	
1.1	20	>99.9999	99.99999
1.3	30	>99.9999	>99.99999
1.3	40	>99.9999	99.99991
1.2	50	>99.9999	>99.99999
1.3	60	>99.9999	99.99999
1.2	70	>99.9999	99.99999

These data were obtained at water flow velocities of approximately 0.3 cm/sec (for the heavier loadings of NanoCeram™) and 0.5 cm/sec for lighter (<30 wt %) loadings. This flow capability is about 2 orders of magnitude greater than ultraporous membranes could maintain. For instance, water flow velocities through Millipore VM (50 nm rating) and VS (25 nm rating) are respectively 0.01 and 0.0025 cm/sec at a ΔP of 0.7 bar. The very high retention at high flow rate is indicative of very rapid adsorption kinetics.

Attraction and Adhesion of Pathogens

"Partition coefficients of virus and bacteria were measured between the sorbent and neutral water. Bacteriophages MS-2 and PRD-1 were diluted in 150 mL of 0.02M imidazole/0.02M glycine buffer (pH 7.2) to give an effective concentration of approx. 8×10^5 PFU/ml. NanoCeram™ fibers (0.5 grams) were placed into a 15 mL conical centrifuge tube containing 10 mL of the bacteriophage solution. The powder was dispersed by vortex mixing and was shaken on a rocking table for 15 minutes. The mixture was centrifuged at low speed (2500 rpm) to collect the powder at the bottom of the tube and the supernatant was assayed for the presence of viruses. An initial and final aliquot was taken from the buffer solution containing bacteriophage, to verify that virus concentration remained constant throughout the experiment. Analysis showed that 99.99% of the MS-2 and 99.5+% of PRD-1 virus were removed from the solution.

Similar testing was done with bacteria and bacillus. The microbes were of the genus Micrococcus (spherical, non-motile), bacillus (minor motility, spore-forming, rod-shaped) and Pseudemonas (rod-shaped and motile). A suspension of the cells was prepared in a sterile 0.5% saline solution. Initial concentration of the microorganisms was determined by the method of limiting dilutions followed by seeding on meat-peptone agar medium (MPA). The mixture was passed through a column 0.8 cm diam, 15 cm height, filled with the sorbent. The non-sorbed biomass was then washed out of the column using buffered solution with a volume that was 3-4 times the volume of the column. The sorbent was then transferred to a test tube containing 9 ml of sterile 0.5% NaCl containing 1-2 drops of TWIN-80 surfactant to desorb the microorganisms. The sample was then agitated for 1 min and after 10 min at rest, the supernatant was sampled and inoculated onto MPA to determine the number of microorganisms that had been on the sorbent. Table 3 shows a high capacity for the different microbes.

TABLE 3. Dynamic Sorption Capacity of Microbes by NanoCeram ™

Mass of sorbent, g	Microbe	Volume of suspension, ml	Initial concentration of microorganisms, cl/ml	Specific sorption, cl/g
4.25	Bacillus	7	$40.4 \cdot 10^6$	$67.3 \cdot 10^6$
5.07	Micrococcus	7	$400 \cdot 10^6$	$558.9 \cdot 10^6$
10.33	Pseudomonas	8	$0.85 \cdot 10^6$	$0.66 \cdot 10^6$

Another series of tests were done to determine the affect of pH and temperature on the static adsorption of Micrococcus and bacillus. These data (Fig 3) show that three different strains are adsorbed to > 99% over the temperature range 20-50 C. (The lower capacity for bacteria as compared to virus might be because the agglomerated fibers excluded much of the larger bacteria from available surfaces. Because there was some concern about the pressure drop with very fine powder, the powders were granulated and the series in Table 4 show excellent bio adsorption. In addition there was some concern about loss of adsorption in the presence of oil contamination. Yet, the data in Table 4 showed in fact an increase in adsorption in the presence of oil.

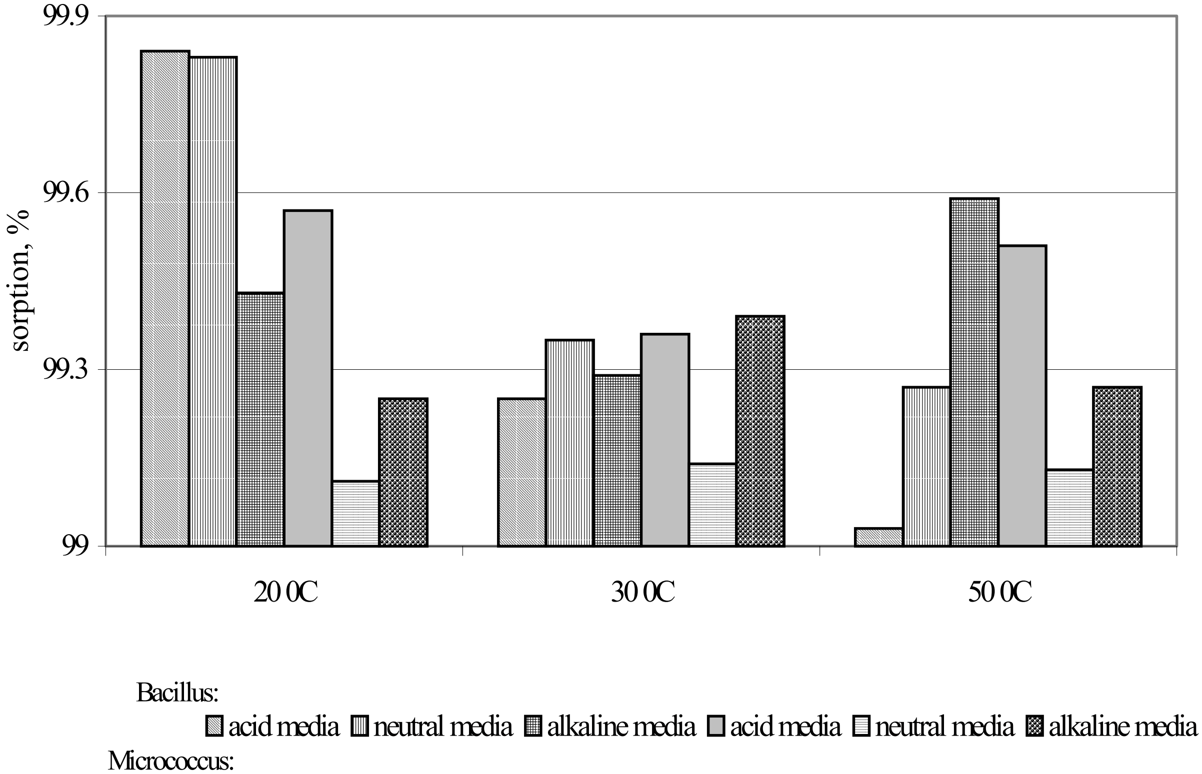


Figure 3. Adsorption of Bacillus and Micrococcus by NanoCeram.

Table 4. Affect of mineral oil on adsorption of Micrococcus

Sorbent type	Initial microbe suspension, 10^6 cells	Sorption efficiency, %	Specific sorption efficiency, 10^6 cell/g
Powder	60	98	1.9
Powder + 30% petroleum	105	99.9	3.5
Granulated powder	25.2	99.9	5.51
Granulated powder + 10% petroleum	25.2	99.9	5.03

Adsorption in the Presence of Salt and Sewage-Contaminated Water

Filters containing 40 wt% NanoCeram with micro glass fibers were challenged with virus titers spiked into simulated salt water and also into a sewage mixture at pH 7.8. (The sewage mixture was prefiltered through a 100 μ filter to remove large sediment). The filters were found to be very effective in removing virus from salty or sewage laden water. The adsorption of E. coli O157H7[2] was also tested in similar backgrounds and no loss of retention was observed.

TABLE 5. Filter performance in salt water and sewage backgrounds

Thickness, mm	Weight % fibers	Background Solution	MS-2	Polio 1
			% removal	% removal
1.6	40	Instant Ocean*	99.95	99.9
1.4	40	Instant Ocean	99.995	99.98
1.4	40	Instant Ocean	99.994	99.99
1.4	40	Instant Ocean	>99.999	99.95
1.5	40	Sewage	99.99	99.95
1.6	40	Sewage	99.993	99.992
1.5	40	Sewage	99.998	99.993
1.4	40	Sewage	99.98	99.98
2.1	40	Sewage	>99.999	Not Done
0.8	40	Sewage	99.2	"
1.3	40	Sewage	99.95	"

Filter Capacity Measurements

The dynamic capacity of NanoCeram/microglass filters for nano particles was determined using known concentrations of monodisperse latex spheres in water. The technique, practiced by others [2], allowed us to attain high contaminant loadings (billions of particles per ml) reducing testing time to breakthrough of the filter, which was determined by an optical turbidimeter. The 30 nm diameter latex simulated virus particles. Figure 4 shows that the capacity of the filters is directly proportional to the ratio of NanoCeram in the filter. We also presume that the filter capacity for a fixed ratio of NanoCeram in the filters would be directly proportional to the filter thickness (number of layers). Computations showed that a 25 mm. diameter filter approximately 1 mm thick was able to absorb $4 \cdot 10^{14}$ particles before spheres started to show in the effluent. We were then able to calculate that the filter absorbed $8 \cdot 10^{13}$ particles/cm^2 at breakpoint.

Filter Clogging

The clogging caused by particles was measured (Figure 5) by loading with 30 nm latex spheres and measuring flow velocity on the NanoCeram filter intermittently during the loading process. These data were compared to a similar set with 25 nm ultraporous membranes (Millipore VC grade), capable of filtering MS-2 and polio. The initial flow velocity was found to be several hundred times as great as that of the membrane. The low flow evidenced in the membranes declined even more (about 70% of initial) during the interval until filter breakthrough while there was no measurable flow decay noted with the NanoCeram until well past breakthrough (~8 X10^{12} beads), indicating its relative tolerance of heavy loads of ultra fine particles.

The filters were then challenged with 3 micron size latex spheres, (Figure 6) simulating bacteria. Retention was again very high although in the case of these larger size spheres, we noticed they were being deposited as a film on the surface of the filter, causing a decrease in flow velocity, much like the membrane. We believe that the surface-deposited particles create a filter cake, controlling pressure drop.

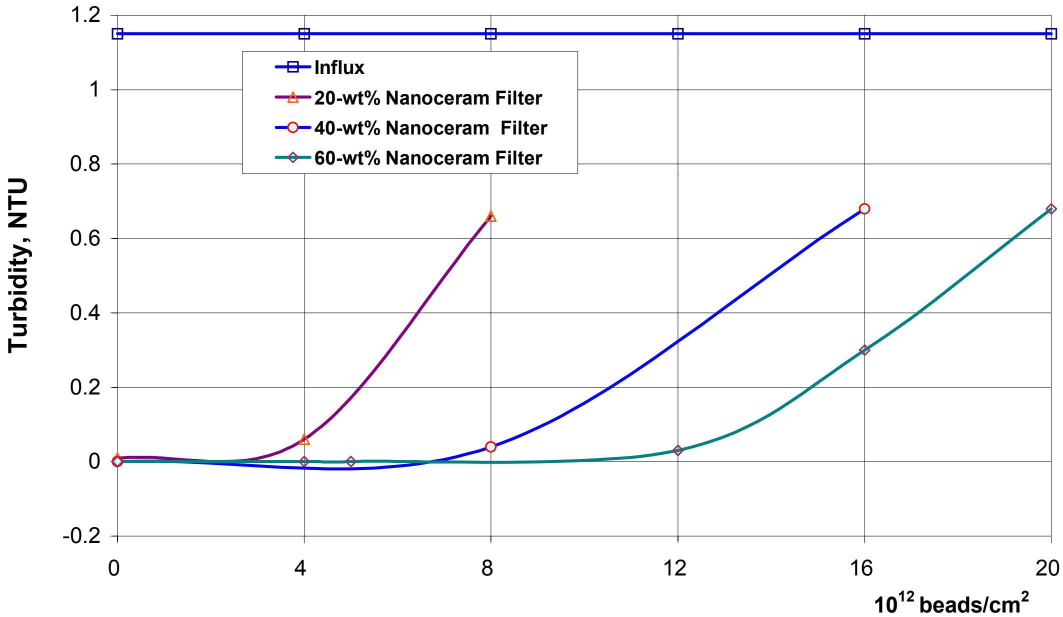


Figure 4. Filter capacity as a function of NanoCeram content.

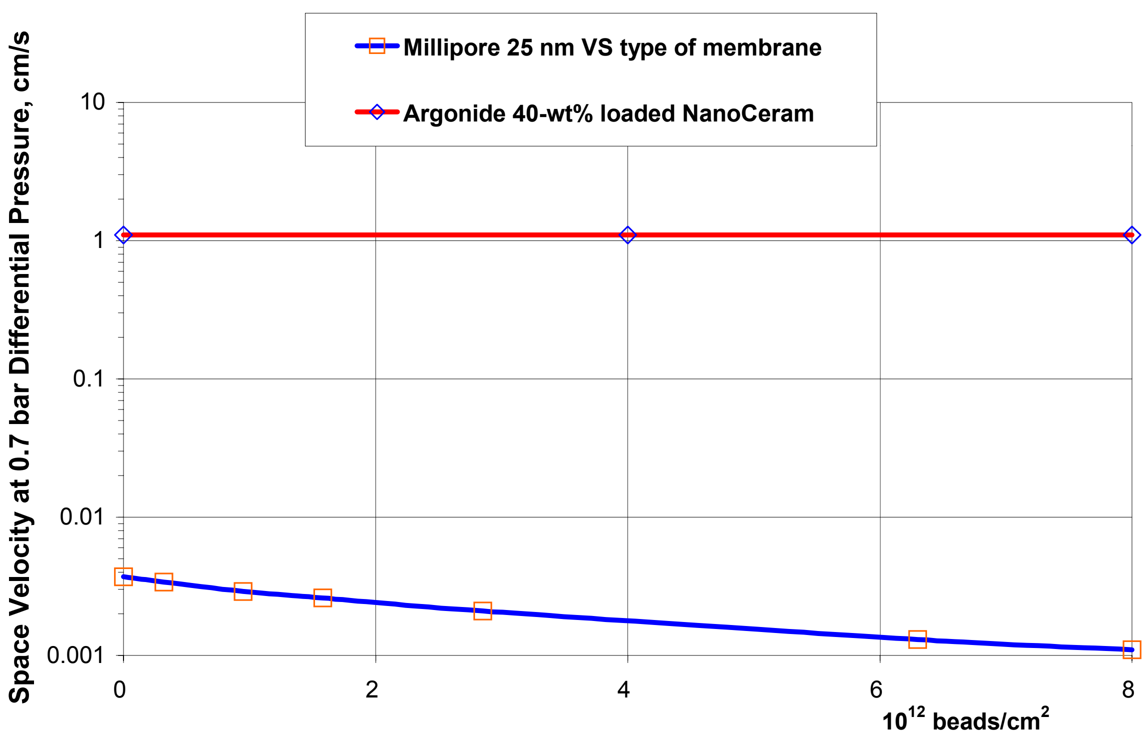


Figure 5. Flow velocity as a function of loading with 30 nm latex spheres.

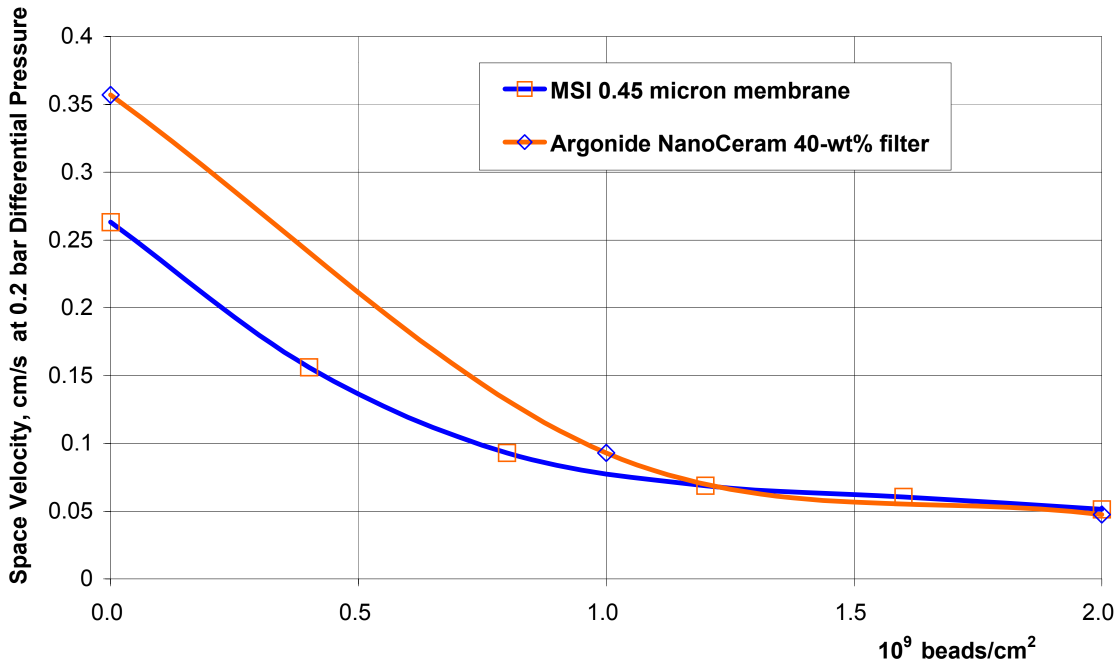


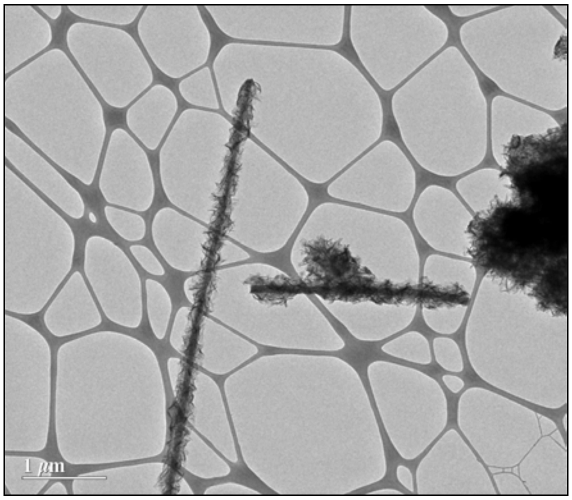
Figure 6. Flow decay (clogging) of Filters by 3 μ latex beads.

Filtration Mechanisms

Filters are generally classified as fibrous depth, surface or membrane filters. Since NanoCeram filters are based on fibers, they would be classified as depth filters, although such filters are generally incapable of sanitizing (> 4 logs) virus from water. Microporous membranes (0.22 μ pore size) will sanitize water of bacteria by size exclusion and ultraporous membranes (25 nm pore size) would exclude virus. However, such membranes are prone to clogging by limited amounts of particles on their surface.

Streaming potential studies show that NanoCeram fibers are highly positively charged, and can therefore retain negatively charged particles. We believe that the hydroxide groups on the surface (with the proton projecting outward) create a high density of surface positive charges. In water, bacteria are attracted to the positive charges because they and their fragments are negatively charged due to their cell wall chemistry. Gram negative bacteria, abundant in water, contain negatively charged $-COO^-$ groups associated with proteins and lipopolysaccharides in their cell walls. Gram-negative bacterial endotoxins (pyrogens) are also negatively charged as are virus and most colloids. We also found that NanoCeram has a high affinity for DNA, RNA and proteins, suggesting that proteinous toxins such as botulin and ricin would also be retained.

The strong electropositive charges on the surface may be seen in Figures 7 and 8. Figure 7 is a TEM view of a composite NanoCeram/microglass composite fiber typical of as-produced NanoCeram filters. Note the fine nano alumina fibers (appearing as a fuzz) adhering to the microglass. Figure 8 shows a fiber that was extracted from a spent filter typical of that tested with 30 nm latex spheres as in Figure 4. Latex spheres completely cover the composite fiber and appears to be tightly adherent to it.



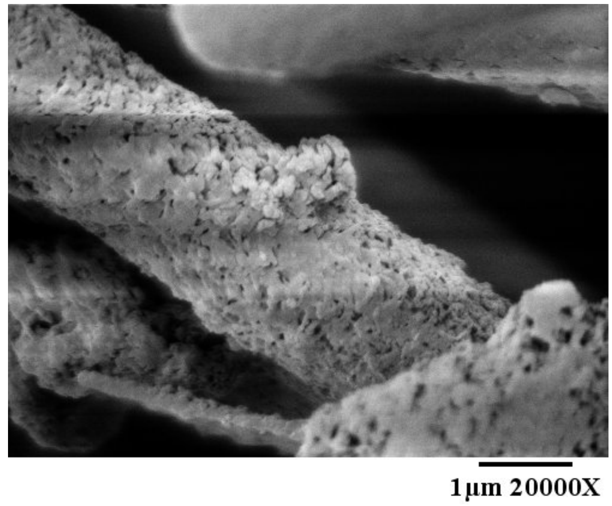


Figure 7. NanoCeram on Microglass.

Figure 8. Fiber Composite Covered with 30 nm Latex.

CONCLUSIONS

A fibrous filter has been developed that has the following characteristics:

- High (>99.9999%) virus and bacteria removal efficiency
- Flow capability is several orders of magnitude greater than equivalent membrane filters
- Higher capacity for particulates and less clogging particularly with nano size or colloidal particles. Capacity can be increased by thickening filter
- Robust - Point defects in a depth filter are compensated by fibers randomly spaced behind such defects, while there is no such redundancy in a membrane filter
- Chemisorbs dissolved heavy metals by ion exchange
- Filtration of sub-micron and nano particulates including radioisotopes

NanoCeram™ fibers and filters have the following NBC protection applications:

- Filter-sterilization of biologically contaminated water
- Decontamination of surfaces – The sorbent, as the active component of a slurry can be applied to surfaces and subsequently collected after the BW agent is attached.
- As a component of fabrics and non-wovens to impart improved biological and radiological protection
- Concentration/collection of biological agents for detection and assay in water
- Concentrating biological agents in air for detection and assay – Aerosols would be filtered from airstreams and the pathogens (including virus) would be attached to the filter. Excessive water could be blown out.

R & D TOPICS

Our current program is focused on the following topics that are relevant to collective protection:

- Develop processes to regenerate (decontaminate) filters
- Develop cartridge filters (larger area) for sterilizing drinking water in point of use, principally for residential, industrial and military
- Endotoxin and protein filtration – their efficient filtration would indicate the capability to adsorb protein toxins such as ricin and botulin
- Develop biocidal capability
- Reduce manufacturing cost

REFERENCES

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2. Hou, K. *et al*. (Nov. 1980), Capture of Latex Beads, Bacteria, Endotoxin and Viruses by Charge-Modified Filters, Appl. And Environmental Microbiology, 892-96.

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