The FASEB Journal express article10.1096/fj.04-2029fje. Published online September 2, 2004.

Putting copper into action: copper-impregnated products with potent biocidal activities

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ABSTRACT

Copper ions, either alone or in copper complexes, have been used for centuries to disinfect liquids, solids, and human tissue. Today copper is used as a water purifier, algaecide, fungicide, nematocide, molluscicide, and antibacterial and antifouling agent. Copper also displays potent antiviral activity. We hypothesized that introducing copper into clothing, bedding, and other articles would provide them with biocidal properties. A durable platform technology has been developed that introduces copper into cotton fibers, latex, and other polymeric materials. This study demonstrates the broad-spectrum antimicrobial (antibacterial, antiviral, antifungal) and antimite activities of copper-impregnated fibers and polyester products. This technology enabled the production of antiviral gloves and filters (which deactivate HIV-1 and other viruses), antibacterial self-sterilizing fabrics (which kill antibiotic-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci*), antifungal socks (which alleviate symptoms of athlete's foot), and anti-dust mite mattress covers (which reduce mite-related allergies). These products did not have skin-sensitizing properties, as determined by guine pig maximization and rabbit skin irritation tests. Our study demonstrates the potential use of copper in new applications. These applications address medical issues of the greatest importance, such as viral transmissions; nosocomial, or healthcare-associated, infections; and the spread of antibiotic-resistant bacteria.

Key words: HIV-1 • filters • fabrics • dust mites • athlete's foot

The ancient Greeks were the first to discover the sanitizing power of copper thousands of years ago. Since then, copper has been used as a biocide by Celts, Hindus, American pioneers, and Japanese, as well as by inhabitants of Africa and Asia, for treating sores and skin diseases (1). Today, soluble copper compounds are used as bactericides in paints to render surfaces self-disinfecting (2), on ship hulls to decrease friction by reducing barnacle accumulation and microbial biofilm formation (3), in hospital water pumps to control *Legionella* in water distribution systems (4), and in agriculture as an algaecide, fungicide, or molluscicide (5, 6). Copper has also been shown to inactivate a variety of enveloped and nonenveloped viruses, such as bacteriophages (7), bronchitis virus (8), poliovirus (7, 9), herpes simplex virus (9, 10), and HIV-1 (11).

Several mechanisms for the biocidal activity of copper have been proposed. These include denaturation of nucleic acids by binding to and/or disordering helical structures and/or by cross-

linking between and within nucleic acid strands (12), alteration of proteins and inhibition of their biological assembly and activity (13, 14), plasma membrane permeabilization (15), and membrane lipid peroxidation (16).

Microorganisms, but not viruses, have developed several mechanisms to tolerate excess copper. These include exclusion by a permeability barrier, intra- and extracellular sequestration by cell envelopes, active transport membrane efflux pumps, reduction in the sensitivity of cellular targets to copper ions, and extracellular chelation or precipitation by secreted metabolites (15). However, prolonged exposure to high copper concentrations is toxic to microorganisms.

Based on the above, we hypothesized that introducing copper into many different types of products would provide them with biocidal qualities. Permanent or durable binding of inorganic compounds to organic substrates is extremely difficult, especially for mass production processes. Using the properties of copper, a durable platform technology was developed that introduces copper to textile fibers as well as latex and other polymer products (17). Our study demonstrates the broad-spectrum antibacterial, antiviral, antifungal, and antimite activities of copper-impregnated fibers and polyester products and discusses the possible impact of these products on various public health concerns.

MATERIALS AND METHODS

Production of cotton fibers, latex, or polyester impregnated with copper

Cationic copper is bound to Lyocel cotton fibers (Tencell Ltd., London, UK) by an electroless plating process (17), which includes the following steps: 1) cotton fibers having a diameter of ~11–13 μm are soaked for 5 s in 1% $SnCl_2$, pH 3.5, at room temperature; 2) the fibers are then soaked for 5 s in $PdCl_2$, pH 4, at room temperature, producing activated fibers; and 3) the activated cotton fibers are then exposed to formaldehyde, $CuSO_4$, and polyethylene glycol at pH 9. After 5 min, the cotton fibers are plated with cationic copper [Cu(II) and Cu(I)]. Finally, the fibers are dried and run through a textile carding machine, which separates and aligns them. Hereafter, these fibers will be referred to as copper fibers. Hereafter, fabrics containing 20% (weight/weight) copper fibers and 80% non-copper-treated cotton fibers will be referred to as copper fabrics. Impregnation of copper into latex is achieved by adding cuprous oxide powder mix (70% Cu_2O and 30% CuO) to 10 g/l of undiluted latex.

The characteristics of the copper coating on the cotton fibers and in the latex were examined by SEM (Jeol JMS 840 scanning electron microscope), TEM (Hitachi HU-11A), and XPS (Shimadzu XRD 6000, TN-5500 X-ray analysis system). The analysis was performed by Dr. Gilbert J. Sloan in the laboratories of Micron Analytical Services (Wilmington, DE).

Bactericidal activity of copper fabrics

Swatches of copper fabric and control non-copper-treated fabric were tested quantitatively for antibacterial activity according to the American Association of Textile Chemists and Colorists (AATCC) test method 100-1993. Briefly, sterile circular swatches of 4.8 ± 0.1 cm in diameter of both copper and control fabrics were placed in Petri dishes and exposed to 1 ± 0.1 ml of a 24 h broth culture of the test bacteria. Immediately afterwards ("0 h" contact time), or after different

exposure times of up to 120 min at room temperature, samples of the culture bacteria were transferred aseptically to 250 ml jars. One hundred milliliters of violet red bile agar (VRBA) was then immediately added. The jars were sealed tightly and shaken vigorously for 1 min. Serial 10-fold dilutions with water were made, and 1 ml aliquots were plated by standard bacteriological procedures, in duplicate, on tryptic soy agar plates. The plates were incubated at 35 ± 1°C for 24–48 h, and the number of bacteria colonies was determined by the standard pour plate count. The percent of bacterial reduction was determined according to the following formula: 100(B–A)/B = R, where R is % reduction, A is the number of bacteria recovered from the inoculated test specimen swatches after 60 or 120 min, and B is the number of bacteria recovered from the inoculated test specimen swatches immediately after inoculation ("0" min). The tests with *Staphylococcus aureus* and *Escherichia coli* were performed by AminoLab Laboratory Services (Nes Ziona, Israel), and the tests with methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) by Hy Laboratories Ltd. (Rehovot, Israel).

Antifungal activity of copper fabrics

Swatches of copper fabric and control fabric were tested quantitatively for antifungal activity basically as described above for antibacterial activity (AATCC test method 100-1993). The tests were carried out by AminoLab Laboratory Services.

Acaricidal efficacy of copper fabrics on house dust mites

Dermatophagoides farinae were cultured by using a medium of human dander/medical yeast mixture (2:1) kept at a temperature of 25 ± 1°C and 75 ± 5% relative humidity. Copper fabric and control fabric swatches of 3.5 ± 0.1 cm in diameter were introduced into vial microtiter plates and glued with regular plastic glue (Omega Cat) to the bottom of the vial. After 24 h, ~200 mites at various stages of development and 5 mg of culture medium were transferred to each vial. The opening of each vial was sealed with nonhardening glue, which prevented the mites from escaping. During the entire experiment, the mites were incubated under the above-described conditions. Mortality was recorded every 2–4 days by counting the living mites under a stereomicroscope. At the end of each experiment, each vial and the fabric in it were rinsed thoroughly with 40% alcohol. The liquid was then filtered through filter paper (7 cm diameter), and all mites (dead and alive) were counted under the microscope. Each experiment was repeated three times. These experiments were conducted under a subcontract agreement by Dr. Kosta Y. Muncuoglu (Department of Parasitology, Hebrew University-Hadassah Medical School, Jerusalem, Israel).

Determination of antiHIV-1 activity

Latex impregnated with copper

Aliquots of 50 µl of Roswell Park Memorial Institute (RPMI) 1640 medium containing HIV-1_{IIIB} (0.36 pg of p24 viral core antigen) were placed on top of UV-sterilized latex gloves, containing 0.2–3% copper (weight/weight). As a positive control, similar 50 µl aliquots containing HIV-1 were placed on a glove without copper. As a negative control for viral activity, 50 µl aliquots of medium, without any HIV-1, were placed on both regular and copper-impregnated gloves. After 20 min of incubation at room temperature, the 50 µl drops were mixed with 450 µl fresh RPMI

medium containing 10% fetal calf serum (FCS), and the mixtures were added to 2×10^5 MT-2 cells (T cells) in 1 ml medium containing 10% FCS. The virus-cell mixtures were then incubated in 24 well plates in a CO_2 humidified incubator at 37°C. After 4 days of incubation, the amount of virus present per well was quantified by HIV-1 RT assay, as described by Borkow et al. (18).

Copper filters

Ten milliliters of RPMI medium, to which 2×10^6 $TCID_{50}$ (tissue culture infectious dose that causes 50% infectivity) units of HIV-1 (see below for details) were added, were passed through syringes containing 4 ml of packed polyester or copper-polyester fibers. Filtering was done at a flow rate of 5 ml/min. Immediately after filtration, MT-2 cells (when T-tropic HIV-1 isolates were tested) or cMAGI cells (when M-tropic HIV-1 isolates were tested) in medium containing 10% FCS were exposed to 10-fold sequential dilutions of the filtered virus. As a negative control for HIV-1 infectivity, the cells were exposed to medium only. Each dilution of the virus was exposed to target cells prepositioned in six separate wells in a 96-well plate. After 3–6 days of culture at 37°C, viral infectivity was determined in MT-2 cells or in cMAGI indicator cells. MT-2 infection by T-tropic HIV-1 isolates results in syncytia formation. Each well in which even one syncytia was seen was considered as a positive well, that is, infected with HIV-1.

The cMAGI cell line is a lymphocyte cell line stably transfected with a plasmid containing the HIV-1 LTR fused to β-galactosidase (19). These cells stain blue in the presence of X-gal only when infected with HIV-1. As some spontaneous β-galactosidase may occur, the number of cells stained blue in wells exposed to medium alone, without HIV-1, served as the background levels of β-galactosidase expression. Each well in which the number of blue-stained cells was two times that of the background was considered as positive, that is, infected with HIV-1. MT2 and cMAGI cell lines were cultured in RPMI 1640 medium containing 10% FCS and antibiotics. Aliquots of cell-free culture supernatants were used as viral inocula. The following HIV-1 isolates were tested with the filters in separate experiments: $HIV\text{-}1_{IIIB}$, HIV-1 M461/L63P/V82T/184, HIV-1 L10R/M461/L63P/V82T/184, $HIV\text{-}1_{RTMC}$/MT-2, HIV-1 96USNG20, $HIV\text{-}1_{IIIB}$ A17 variant, saquinavir-resistant HIV-1, $HIV\text{-}1_{Ba\text{-}L}$, $HIV\text{-}1_{JR\text{-}FL}$, and macrophage tropic (M-Tropic) and T cell tropic (T-tropic) HIV-1 clade C clinical isolates. The first nine HIV-1 isolates listed were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, and the clade C clinical isolates were isolated at Kaplan Medical Centre (Rehovot, Israel). These isolates represent a wide variety of strains and include viruses that are protease resistant, AZT resistant, and non-nucleoside resistant; syncytia inducing (SI) and non-syncytia inducing (NSI); clade A, clade B, and clade C; T-tropic and M-tropic.

The studies related to HIV-1 were performed under a subcontract agreement by Dr. Gadi Borkow, from the Ruth Ben-Ari Institute of Clinical Immunology, Kaplan Medical Center (Rehovot, Israel).

Determination of anti-West Nile virus (WNV) activity by copper filters

WNV (10^6 $TCID_{50}$ units) were filtered at a flow rate of ~5 ml/min through syringes containing 4 ml of polyester or copper-polyester fibers. Immediately after filtration, Vero cells in culture medium were exposed to 10-fold sequential dilutions of the filtered virus. Each dilution of the

virus was exposed to the Vero target cells prepositioned in six separate wells in a 96-well plate. After 7 days of culture, the cytopathic effect was determined by microscopic assessment. The studies related to WNV were performed by Harlan Biotech Israel (Kiryat Weizmann, Rehovot, Israel).

Guinea pig maximization test

This sensitization test, conducted according to the ISO 10993-10 (1994) guideline, consists of an induction phase during which test animals were intradermally injected and topically treated with a polar extract of the copper fabric (see below). After a latency of 14 days, the animals were challenged with the extracts of the test item on their skin. The degree of skin reaction was compared with control animals, which were treated with the extraction medium during the induction phase and with the extract of the copper fabric during the challenge phase.

Extracts of copper fabrics (containing 20% w/w copper fibers) were prepared with extraction medium (isotonic saline, 0.9% NaCl) for 72 h at 37 ± 1°C, according to guideline ISO 10993-12. The amount of fabric sample to extraction medium used was 120 cm^2/ml.

Fifteen female, nonpregnant guinea pigs, weighing 301–310 g at the commencement of the study were used for this test. Ten animals were used for the copper-fabric group and five animals for the control group. The animals were barrier maintained in an air-conditioned room at 22 ± 3°C. During the first induction step (day 0), the animals were intradermally injected in the shoulder region ("test area"), which was cleared of hair before injection. Each animal received three pairs of injections; each injection was 0.1 ml. The injections constituted the following: pair 1, complete Freund's adjuvant; pair 2, 100% test extract; pair 3, 50% (v/v) test extract in complete Freund's adjuvant. The control group was injected with the extraction media instead of the test extract. During the second induction step, at day 6, 24 h before the topical induction application, the test area was painted with 0.5 ml of 10% sodium lauryl sulfate in Vaseline, in order to create a local irritation. At day 7, a patch was fully loaded with the extract of the test item and applied to the test area and held in contact by an occlusive dressing for 48 h. After 20 days, the flanks of the test and control animals were cleared of hair. A patch loaded with the extract of the respective test item was applied to the left flank of each animal. A patch loaded with the extraction medium was applied to the right flank of each animal as an intraspecific control. The patches were held in place by an occlusive dressing for 24 h. Approximately 21 h later, the challenged area was cleaned and cleared of hair. Approximately 24 h after removing the patches, the skin reaction (edema and erythema) was observed and recorded. Two additional observations were recorded 48 and 72 h after patch removal. This study was performed by BSL Bioservice Scientific Laboratories GmbH (Munich, Germany).

Rabbit skin irritation test

Three New Zealand white female rabbits were used for this study. On the day before the test, the dorsal area of the animal's trunk and flanks was carefully clipped free of hair, as close to the skin as possible. Copper fabric specimens (25 × 25 mm) were moistened with water to ensure contact with the skin. The test materials were applied directly to the skin, one fabric specimen on each side of the test rabbit. Subsequently, the application sites were covered with nonocclusive gauze patches measuring 50 × 50 mm that were kept in place by adhesive surgical tape and then with a

semiocclusive bandage wrapped around the rabbit's entire trunk. After 4 h, the dressings were removed and the sites of application marked. Any residual test material was removed by washing with lukewarm water and careful drying. Dermal reactions for edema and erythema were monitored at 1, 24, 48, and 72 h after removal of the patches. This study was performed by Harlan Biotech Israel (Kiryat Weizmann, Rehovot , Israel).

RESULTS

Impregnating cotton fibers with copper

As determined by scanning electron microscopy (SEM), cotton fibers are smooth and nearly featureless (Fig. 1*A*). X-ray photoelectron spectra of untreated fibers show only carbon and oxygen to be present at high levels (~99%), with chlorine detectable at a very low level (Fig. 1*B*). In contrast, cotton fibers treated with cationic [Cu(II) and Cu(I)] copper showed irregular surfaces (Fig. 1*C*). X-ray spectra of the copper fibers showed large amounts of copper, with low levels of carbon (Fig. 1*D*), implying that the fibers were nearly completely coated by copper.

To test for adhesion of the copper coating to the cotton substrate, we subjected a small sample of fiber to ultrasonication for 10 min in a cleaning bath containing a mildly alkaline detergent solution (pH 8). The sample was then cleaned by ultrasonication in distilled water for 10 min and rinsed with methanol. Although this sequence removed a considerable part of the copper-containing layer, a significant presence of copper was still noted in the SEM picture and X-ray photoelectron spectrum (Fig. 1*E*, 1*F*, respectively). As determined by transmission electron microscopy (TEM) of copper fibers, a thick layer of copper appears at the fiber's periphery (Fig. 1*G*, solid arrows). The thickness of this layer was ~0.1–0.2 μm and extended through the substructure to a depth of up to 0.5 μm (Fig. 1*G*, broken arrows). Copper deposits extending into the fiber structure were also noted when exposed circular copper fiber sections were plasma etched, coated with gold to ensure electrical conductivity, and then characterized by backscattered-electron imaging (BEI) (Fig. 1*H*).

Bactericidal, antifungal, and acaricidal activity of fabrics containing copper-treated fibers

In the present study, unless otherwise specified, the results described were obtained by independent testing laboratories (see Materials and Methods) using two kind of fabrics: 1) fabrics containing only nontreated cotton fibers (referred to as control fabrics) and 2) fabrics containing 20% (weight/weight) copper fibers and 80% non-copper-treated cotton fibers (referred to as copper fabrics) (Fig. 1*I*).

The antibacterial effectiveness of control and copper fabrics on *S. aureus (*American Type Culture Collection [ATCC] 6538), a gram-positive organism, and on *E. coli* (ATCC 8739), a gram-negative organism, are shown in Figure 2*A*. The copper fabrics reduced by more than 2 logs the number of recoverable *E. coli* and *S. aureus* bacteria within 2 h of their being exposed to copper fabrics. Similar results were obtained in three separate experiments.

Fabrics that were subjected to very drastic washing conditions (35 industrial washings at 85°C in a tumble cycle using abrasive salts and soaps) also reduced by more than 2 logs the viability of *S. aureus* within 2 h of the bacteria being exposed to the copper fabric (Fig. 2*A*). More than 2 logs

reduction in *E. coli* viability occurred within 20 min of their exposure to copper fabric. Similar results (>2 log reduction) were obtained when MRSA and VRE bacteria were tested (Fig. 2*A*).

As depicted in Figure 2*B*, copper fabric reduced the number of viable fungi (*Candida albicans*) in a time-dependent manner. Complete inhibition was noted within 60 min after the fungi were exposed to the fabric. In contrast, the control fabric did not affect the fungus's viability.

On the basis of this result, we gave socks containing 10% (weight/weight) copper-coated fibers to noncoated fibers to 50 individuals suffering from the fungal infection tinea pedis, or athlete's foot. All 50 individuals reported the disappearance of the burning and itching that accompanies athlete's foot within 1–2 days of wearing the socks. Moreover, within 2–6 days of using the socks, the blistering and fissures characteristic of athlete's foot disappeared, and the skin returned to normal. None of the 50 individuals reported any adverse effects after using the copper-impregnated socks.

Figure 2*C* shows the result of an experiment in which the effect of two fabrics, one containing 20% copper fibers and one containing 100% copper fibers, were tested for acaricidal activity. The house dust mite tested was *Dermatophagoides farinae*. Whereas during the first 12 days of the experiment all mites exposed to control fabrics were alive, >60% and 100% of the mites exposed to the 100% copper fabric were dead after 1 and 5 days, respectively. Approximately 50% of the mites exposed to the 20% copper fabrics died within 12 days of exposure to the fabrics. After 47 days of culture, 86% and 67% of the mites in the absence of any fabric and in the control fabric containers were alive, while all mites exposed to the 20% copper fabric were dead. Similar results were obtained when the mites were put on top of the fabrics and when the fabrics were put on top of the mites. This latter configuration imitates what occurs in mattresses.

Antiviral activity of latex and polyester impregnated with copper

Copper ions have been reported to inactivate HIV-1 (11). We therefore investigated whether our copper-impregnated latex would reduce HIV-1 infectivity. Indeed, HIV-1 infectivity was reduced in a dose-dependent manner when HIV-1_{IIIB} was exposed to latex gloves containing increasing concentrations of copper (Fig. 3*A*). As determined by SEM and X-ray photoelectron spectroscopy, none of the copper was found on the surface of the latex gloves. We therefore assume that due to the hydrophilic nature of latex, the copper ions permeated out from the latex. Significantly, introduction of Cu(II) and Cu(I) into the latex gloves during the manufacturing process did not damage the glove's mechanical characteristics.

HIV-1 is transmitted through body fluids (e.g., blood and mother's milk). Accordingly, syringes, filled with polyester fibers impregnated with copper (Fig. 1*J–L*), were prepared to serve as "filters." As depicted for one representative experiment in Figure 3*B*, filtration of 10 ml of medium containing saquinavir-resistant HIV-1 through syringes containing 4 ml of copper polyester fibers, at a flow rate of 5 ml/min, resulted in at least a 5 log reduction of the viral infectious titers. The filters deactivated all other 11 HIV-1 viral isolates tested. These included T-tropic and M-tropic isolates, laboratory and clinical isolates, and nucleoside, nonnucleoside and protease resistant-viral isolates (see Materials and Methods for more details). Each virus isolate was tested at least twice. Similar results were obtained when WNV was filtered (Fig. 3*C*).

Test for skin sensitization

Two animal studies were conducted to determine whether extracts of the copper-impregnated fabrics have skin-sensitizing properties. In the first, the guinea pig maximization test used to determine the allergenicity of new chemicals and products (20, 21) was conducted. Ten guinea pigs were sensitized by intradermal injections to extracts of copper fabrics (prepared as described in Materials and Methods), and 14 days later (to allow for a potential reaction of the immune system), the animals were challenged with the extracts of the copper fabric on their skin. The degree of skin reactions was compared with five guinea pigs (control animals), which were treated with only the extraction medium (saline) during the induction phase and with the extract of the copper fabric during the challenge phase. None of the 10 animals exposed to extracts of copper fabrics showed allergic skin reactions as compared with the intraspecific application of the extraction vehicle and when compared with the animals of the control group. Animals of both groups showed normal food intake and weight gain throughout the test period.

The second test, the rabbit skin irritation test, is recommended by the Biological Evaluation of Medical Devices, ISO 10993-10: "Tests for irritation sensitization." Three rabbits were exposed to both control and copper fabrics. Each fabric was applied to both sides of the test rabbits for 4 h, as described in Materials and Methods. The animals were then monitored for 72 h. None of the animals exhibited any skin irritation, other toxic effects, or clinical signs resulting from the treatment throughout the entire observation period.

DISCUSSION

Using the properties of copper, an inexpensive platform technology was developed that binds copper to textile fibers from which woven and nonwoven fabrics can be produced. Similarly, copper may be integrated into latex and other polymeric products during manufacture. As demonstrated here, these copper-impregnated products possess broad-spectrum antimicrobial properties. This technology, for example, enables the production of antiviral gloves and filters (which inter alia deactivate HIV-1 and other viruses), antibacterial self-sterilizing fabrics (which inter alia kill antibiotic-resistant bacteria, including MRSA and VRE), antifungal socks (which inter alia alleviate symptoms of athlete's foot), and anti-dust mite mattress covers (which reduce mite-related allergies).

Copper is considered safe to humans, as demonstrated by the widespread and prolonged use by women of copper intrauterine devices (IUDs) (22–24). Copper ions released by the IUD act as a prefertilization spermicide and as a postfertilization inhibitor of uterine implantation. Animal testing has demonstrated that copper fabrics do not possess skin-sensitizing properties. In addition, none of the 50 individuals who used socks containing copper-impregnated fibers to alleviate their athlete's foot conditions reported any negative effects caused by the socks. These findings are in accordance with the very low risk of adverse skin reactions associated with copper (25).

In contrast to the low sensitivity of human tissue (skin or other) to copper (25), microorganisms are extremely susceptible to copper. Copper toxicity to microorganisms, including viruses, may occur through the displacement of essential metals from their native binding sites; from interference with oxidative phosphorylation and osmotic balance; and from alterations in the

conformational structure of nucleic acids, membranes, and proteins. For example, Cu^{2+} has been shown to have a specific affinity for DNA and can bind and disorder helical structures by cross-linking within and between nucleic acid strands. The protective effects of metal chelators and catalase, the lack of effect of superoxide dismutase, and the partial protection conferred by free-radical scavengers suggest that the mechanism of copper-mediated deactivation of herpes simplex virus and of bacteriophage phy X174 DNA are due to copper-mediated DNA damage (9, 10). In addition, exposure of intact *S. cerevisiae* to Cu^{2+} causes a loss of the permeability barrier of its plasma membranes within 2 min at 25°C (26), and exposure of viral HIV-1 protease to stoichiometric concentrations of Cu^{2+} results in immediate inactivation of the enzyme, with inactivation being dependent on the presence of cysteine residue(s) in the enzyme (13). Furthermore, the redox properties of copper (the redox cycling of copper between Cu^{2+} and Cu^{1+}) catalyze the production of highly reactive hydroxyl radicals, which subsequently damage lipids, proteins, DNA, and other biomolecules, as demonstrated for both microbes and viruses (12, 27). Extensive metal-induced disruption of membrane integrity inevitably leads to loss of cell viability. However, even relatively small alterations in the physical properties of biological membranes can result in marked changes in the activities of many essential membrane-dependent functions, including transport protein activity (28), phagocytosis (29), and ion impermeability (28).

In most microorganisms, but not in viruses, there is an integrated set of proteins that delivers copper to specific subcellular compartments and copper-containing proteins without releasing free copper ions (30). Although some organisms have mechanisms of resistance to excess copper (15, 31), generally, exposure of most microorganisms to high concentrations of this trace element results in damage to cellular components. Viruses lack DNA repair mechanisms, permeability barriers, intra- and extracellular sequestration of metals by cell envelopes, active metal transport membrane efflux pumps, and enzymatic metal detoxification mechanisms, such as those found in bacteria and cells. These reduced capabilities of viruses may explain their high vulnerability and susceptibility to copper.

The possibility of introducing copper into fabrics, paper, latex, or other polymers may have significant ramifications. One example is the reduction of nosocomial infections in hospitals. Nosocomial infection ranks fourth among causes of death in the United States, behind heart disease, cancer, and stroke. Nearly 2 million patients annually contract an infection while hospitalized. More than 90,000 deaths in the United States are attributed to these infections each year, and one out of four deaths in intensive care units is caused by an infection unrelated to the initial cause of hospitalization. Nosocomial infections are estimated to add $5 billion to U.S. hospital and insurance costs each year (32).

Nosocomial infections can be bacterial, viral, fungal, or even parasitic. These infections are largely device-associated or surgically related (33). The main sources for contamination are the patient's skin flora, the flora on the hands of medical and nursing staff, and contaminated infusion fluids. However, recently it has been demonstrated that sheets that are in direct contact with a patient's skin and his bacterial flora are an important source of infection (34, 35). Moreover, sheets were significantly more contaminated by patients carrying infection than by noninfected patients ($P<0.01$) (34). Therefore, use of self-sterilizing fabrics in pajamas, sheets, pillow covers, and robes in a hospital setting may reduce the spread of microorganisms in hospital wards, resulting in a reduction of nosocomial infections. Similarly, the use of gloves

with antibacterial and antiviral properties by hospital personnel may also aid in reducing transmission of infectious microbes and viruses while providing increased protection to hospital personnel.

Another possible use of copper fabrics is related to allergies and asthma. It is estimated that 15% of the general population suffer from one or more allergic disorders of which allergic rhinitis is the most common (36). Allergic rhinitis affects an estimated 20–40 million people in the United States alone. Similarly, nearly 15 million Americans have asthma, including almost 5 million children. Approximately 5500 persons die each year from asthma (37). Dust mites are considered to be an important source of allergen for perennial rhinitis and asthmatic attacks (38). Thus, elimination of house dust mites in mattresses, quilts, carpets, and pillows would be an important step in improving the quality of life of those suffering from dust mite-related allergies.

An additional potential use of copper-impregnated fabrics is related to foot ulcerations, a common complication of type 2 diabetes, which afflicts ~130 million individuals around the world (39). In many cases, these ulcerations can become severe due to cuts/bruises that do not heal, or heal only slowly, and become infected. An infection that does not heal can cause the tissue to die (gangrene). In severe cases, the toes may have to be amputated in order to save the rest of the foot or even leg. Use of socks containing copper-impregnated fibers by diabetics may significantly reduce the risk of foot infection.

Use of copper-impregnated socks by the wider population may also be beneficial in more benign conditions. About 15–20% of the population suffers from tinea pedis (40, 41). Although there are many clinical presentations of tinea pedis, the most common are found between the toes and on the soles, heels, and sides of the foot. Although this fungal infection is not usually dangerous, it can cause discomfort, may be resistant to treatment, and may spread to other parts of the body or other people. Affected feet can also become secondarily infected by bacteria. It has been found that copper-impregnated socks may be useful in preventing and treating tinea pedis.

An important potential application of copper-impregnated materials is related to the reduction of bacterial and viral transmission during transfusion of blood or blood-related products. The safety of whole blood and its components is a continuing global problem (42). A growing number of viral, bacterial, and protozoa pathogens have been identified in blood products, and new pathogens are regularly identified as being present. While the current estimate of HIV transmission through blood transfusions in the United States is ~1 in 2,000,000 units (43), new viruses are constantly being discovered in the blood supply. Inexpensive rapid screening tests for emerging viruses generally take time to develop. One need only remember the recent panic relating to WNV in the blood supply where it has been estimated that 1 in 5000 (44) units were infected before the problem was even detected**.** Recent findings reveal that while quarantine-stored fresh frozen plasma contains physiologically active, therapeutically relevant, plasma proteins, it also carries a risk of transmitting HIV and other viruses (45). The capacity of HIV-1 to be transported in whole blood by platelets and red blood cells has also been demonstrated (46, 47).

Mortality and morbidity associated with blood transfusions that are contaminated by pathogens have been well established. In response, regulatory agencies and blood bank standards groups have called for efficient new technologies to improve the safety of blood products (48). In areas

of the world where screening tests are too expensive to be performed regularly, a cheap, rapid virus inactivation filter would be extremely helpful. Even in the United States, hospitals can no longer afford to pay for expensive tests for each "pathogen du jour" (49). Accordingly, a filter that can inactivate a broad spectrum of viruses in blood products would be very valuable.

Preliminary results showing the neutralization of HIV-1 and WNV infectivity when viruses were passed through our copper-containing syringes indicate the possibility of producing a generic antiviral filter. These filters may be constructed of copper-impregnated polyester fibers. However, it must first be established that these filters do not damage filtered plasma and other blood components and that they do not harm individuals infused with these blood products. We are currently doing these studies.

A further possible use of copper-impregnated filters is related to the reduction of HIV-1 transmission through breast feeding. Transmission of HIV during lactation accounts for one-third to one-half of all HIV mother-infant transmissions (50). Breast milk may be passed through a copper fiber-containing filter, reducing HIV infectivity. If there is no degradation to the milk's essential nutrients as a result of filtration, the filtered milk may be fed to infants, thereby reducing the risk of HIV transmission. Admittedly, implementing such measures may be very difficult because of sociological factors existing in developing countries. However, HIV-1, as well as other viruses, will be with us for many years, and methods to reduce their impact must be developed.

In conclusion, our study demonstrates the potential uses of copper in new applications that address medical concerns of the greatest importance. Implementation of even a few of the possible applications of this technology may have a major effect on our lives.

ACKNOWLEDGMENTS

We are grateful to Dr. Irving Treitel for his help, advice, and careful review of the manuscript.

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Received April 23, 2004; accepted July 13, 2004.

Fig. 1

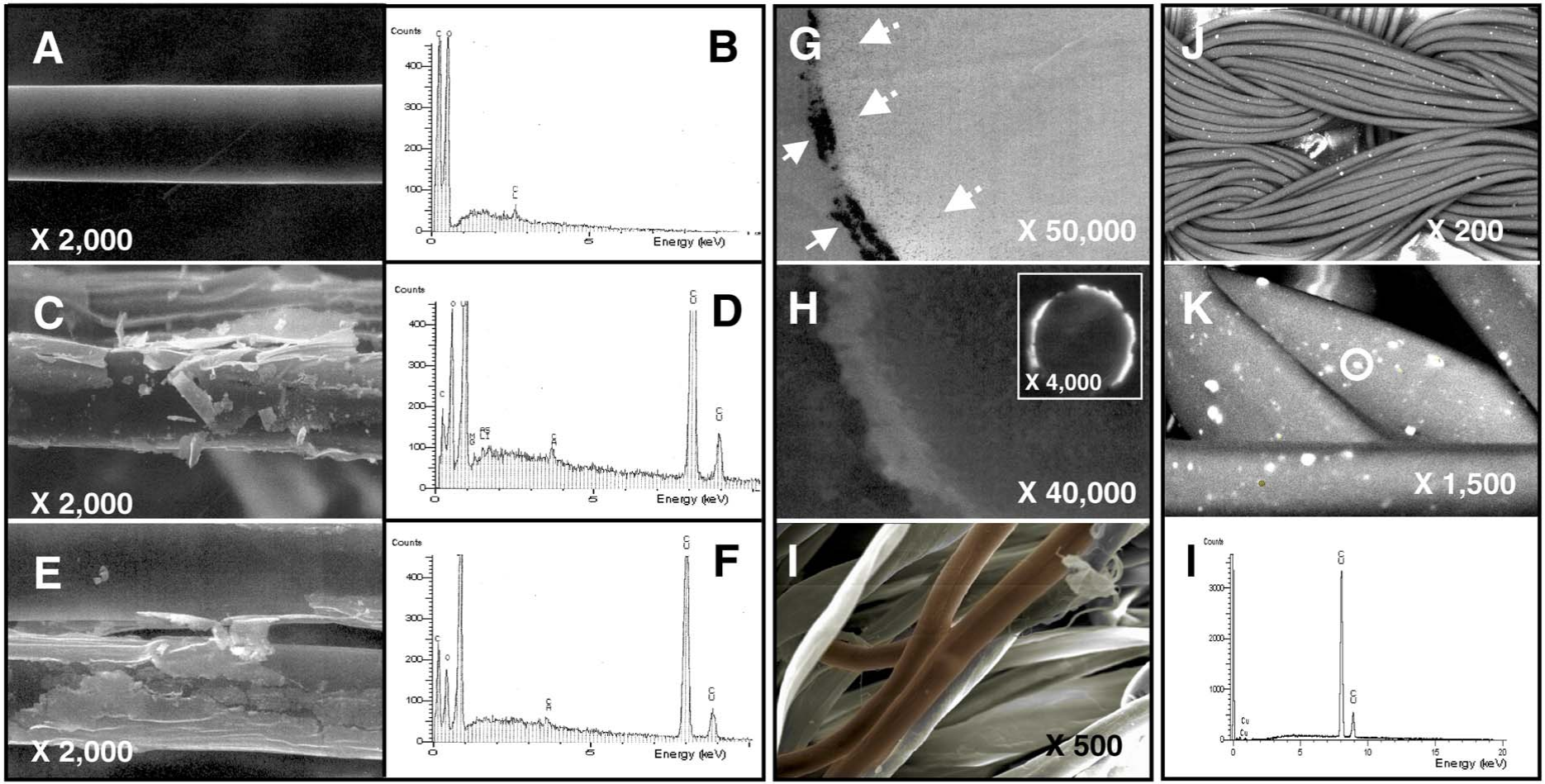

Figure 1. Copper plating. ***A)*** Scanning electron microscopy (SEM) of an untreated cotton fiber that serves as the substrate for copper plating. ***B)*** X-ray photoelectron spectrum of the untreated fiber shown in ***A***. ***C)*** SEM of a Cu(II)- and Cu(I)-treated fiber. ***D)*** X-ray photoelectron spectrum of the copper-treated fiber shown in ***C***. ***E)*** SEM of a Cu(II)- and Cu(I)-treated fiber that was subjected to ultrasonication in alkaline detergent solution. ***F)*** X-ray photoelectron spectrum of the copper-treated "washed" fiber shown in ***E***. ***G)*** Transmission electron microscopy (TEM) of a copper fiber. Dense layers of copper appear on the fiber periphery (closed arrows). Copper particles are visible (e.g., open arrows) below the surface of the fiber to a depth of ~0.5 μm. ***H)*** Backscattered-electron image (BEI) of copper-treated fibers coated with gold. ***I)*** Mixture of Cu(II)- and Cu(I)-treated fibers and untreated fibers. Dark fibers are the copper-treated fibers. ***J, K)*** SEM of copper-treated polyester used in the copper filters. The Cu_2O and CuO mixture was added during polyester production, and the polyester was then stretched to enable the protrusion of the copper. ***L)*** X-ray photoelectron spectrum of the visible white dots, one of which is shown in the white circle. The spectrum reveals that the dots are copper.

Fig. 2

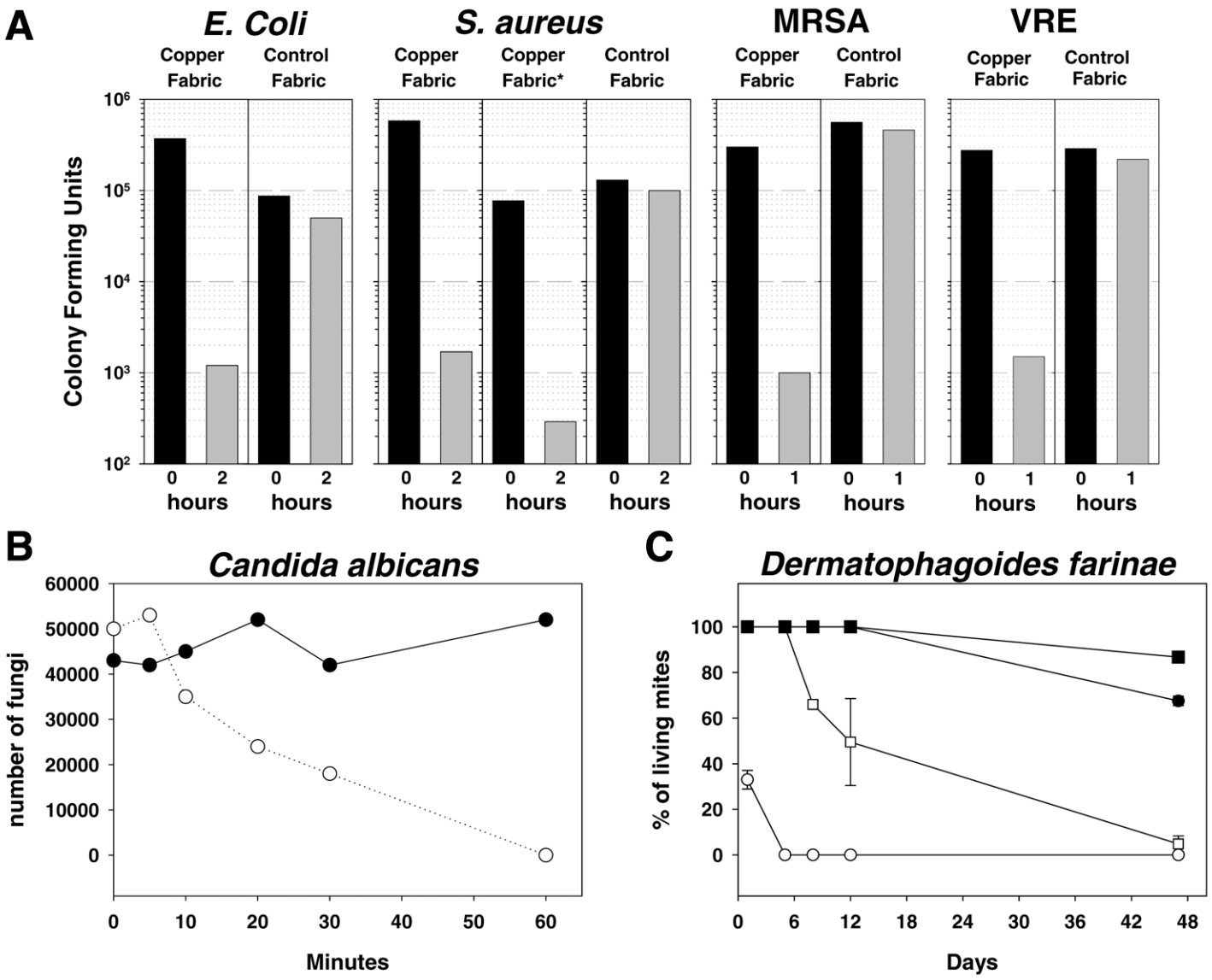

Figure 2. Antibacterial, antifungal, and acaricidal activity of copper fabrics. ***A)*** 1 ± 0.1 ml of a 24 h broth/bacteria culture were exposed to swatches of 20% copper fabrics or control fabrics for ~1 min (0 h) and 2 h (*Escherichia coli* and *Staphyloccus aureus*). (Methicillin-resistant *S. aureaus* (MRSA) and vancomycin-resistant S. *enterococci* (VRE) were exposed for ~1 min and 1 h). The bacterial titer was then determined by standard pour plate count. *Copper fabrics were subjected to 35 subsequent washes at 85ºC before testing. Control fabrics subjected to the same washing treatment did not affect bacteria growth (not shown). ***B)*** 1 ± 0.1 ml of a 24 h broth containing *Candida albicans* were exposed between 0 and 60 min to swatches of control fabric (●) or 20% copper fabric (○). The total number of fungal colonies is shown. ***C)*** Approximately 200 dust mites (*Dermatophagoides farinae*) were cultured for 48 days in the presence of swatches of control fabric (●), 20% copper fabric (□), 100% copper fibers (○), or any swatches (■). Mortality was recorded by counting the living mites under a stereomicroscope.

Fig. 3

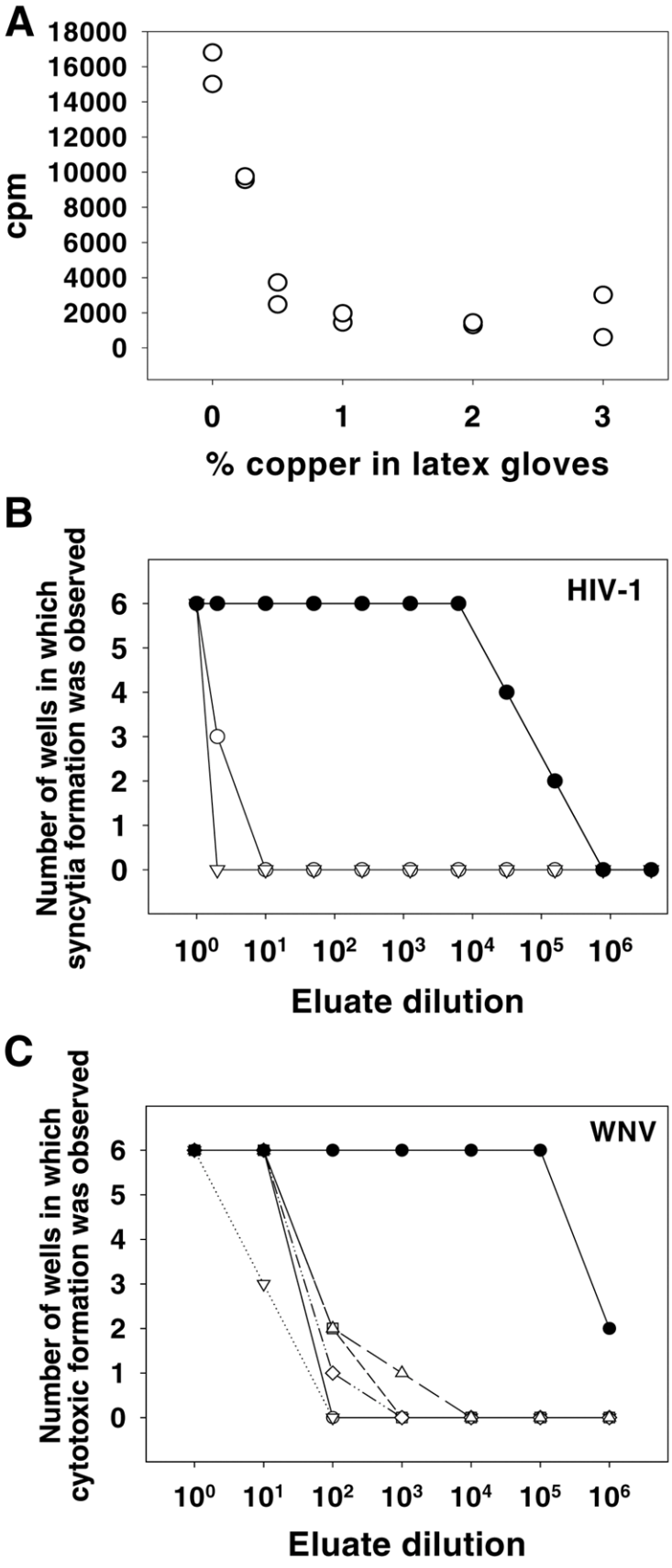
A
cpm
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
1
2
3
% copper in latex gloves
B
Number of wells in which syncytia formation was observed
HIV-1
6
5
4
3
2
1
0
10^0 10^1 10^2 10^3 10^4 10^5 10^6
Eluate dilution
C
Number of wells in which cytotoxic formation was observed
WNV
6
5
4
3
2
1
0
10^0 10^1 10^2 10^3 10^4 10^5 10^6
Eluate dilution

Figure 3. Antiviral activity of copper-impregnated latex and copper-impregnated polyester. ***A)*** 50 µl drops containing HIV-1_{IIIB} were placed on top of latex gloves impregnated with different amounts of copper. After 20 min of incubation at room temperature, the drops were mixed with MT-2 cells. The HIV-1_{IIIB}-exposed cells were incubated for 4 days at 37°C, and then the reverse transcriptase activity in the supernatant was determined. ***B)*** Saquinavir-resistant HIV-1 in medium (10 ml) was passed through syringes containing 4 ml of polyester (dark symbols) or copper-polyester fibers (light symbols: each line/symbol represents a different examined filter). The reduction of infectivity of the filtered virus was determined basically as previously described (51, 52) as follows: Immediately after filtration, sequential 10-fold dilutions of the filtrate were done in RPMI medium in six separate rows of wells in a 96-well plate. Subsequently, MT-2 target cells in medium containing 10% FCS prepositioned in six separate wells in a 96-well plate were exposed to each dilution of the virus. Thus, for each viral dilution, six replicate wells were used. After 6 days of culture at 37°C, viral infectivity was determined by microscopic assessment. MT-2 infection by T-tropic HIV-1 isolates results in syncytia formation. Each well in which even one syncytia was observed was considered as a positive well, that is, infected with HIV-1. The results depicted represent the number of positive wells infected with HIV-1 for each viral dilution. ***C)*** WNV was filtered and then diluted as detailed above in ***B***. All viral dilutions were then added to precultured Vero cells, so for each viral dilution, six replicate wells were used. After 6 days of culture at 37°C, the cytopathic effects caused by the virus were monitored. Each well in which cytopathic effects were observed was considered as a positive well (i.e., an infected well). The results depicted represent the number of positive wells infected with WNV for each viral dilution.