Antimicrobial efficacy and durability of copper formulations over one year of hospital use

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ABSTRACT

Objective: To evaluate three formulations of copper (Cu) based self-sanitizing surfaces for antimicrobial efficacy and durability over one year in inpatient clinical areas and laboratories.

Design: Randomized control trial.

Setting: Three Cu formulations were assessed a) solid alloy 80% Cu 20% Ni (integral copper), b) spray–on 80% Cu 20% Ni (spray-on) and c) 16% composite Cu-impregnated surface. Coupons ($1cm^2$) of the three products and control surgical grade (AISI 316) stainless steel (SS) were inserted into gaskets and adhered onto clinical carts used in patient care areas (including Emergency and Maternity units) (n=480) and on microbiology laboratory bench workspaces (n=240). The microbial burden and assessment of resistance to wear, corrosion, and material compatibility were determined every three months. Three tertiary care Canadian adult and one paediatric/maternity hospital participated.

Results: Cu formulations used on inpatient units statistically significantly reduced bacterial bioburden compared to SS at months 3 and 6. Only the integral product had significantly less bacteria compared to SS at month 12. There were no statistically significant differences in microbial burden between Cu formulations and SS coupons on microbiology laboratory benches where bacterial counts were low overall. All mass changes and corrosion rates of the formulations were acceptable by engineering standards.

Conclusions: Cu surfaces vary in their antimicrobial efficacy after one year in-hospital use. Frequency of cleaning and disinfection influences the impact of copper with the greatest reduction in microbial bioburden seen in clinical areas compared to the microbiology laboratory where cleaning/disinfection occurred multiple times daily.

INTRODUCTION

There is increasing interest in copper (Cu) formulations on high-touch patient room surfaces and hospital equipment in an effort to reduce healthcare associated infections, however questions remain regarding their impact and durability. Three clinical trials – a cross-over study on an acute care medical ward, a randomized control trial in three American intensive care units (ICUs), and a nonrandomized, unmasked, controlled clinical trial in a pediatric ICU– have suggested that Cu surfaces reduce the rate of healthcare associated infections/colonization.[1-7] The potential utility of this metal as a self-sanitizing surface has resulted in the development of different Cu formulations for healthcare use. These can be classified into three categories according to how the Cu has been applied: a) *integral*, where Cu is the primary material (metallic copper or Cu alloys); b) *spray applications* that cover a device or furnishing surface (Cu-containing coatings that may include metallic Cu or oxides of Cu); and c) *composite*, where metallic Cu or Cu oxides are part of a multiphase solid, normally involving a polymeric matrix (polymer-Cu alloy composites).

The decision to invest in self-sanitizing surfaces requires considerations as to the impact on HAI's, feasibility for design/engineering, long-term impact, and durability. The latter features include resistance to wear, corrosion, and compatibility with hospital-grade disinfectants. Answering these fundamental questions will assist decision makers not only in selecting the appropriate materials for high-touch surfaces but informing future studies assessing the role of Cu surfaces in the reduction in healthcare acquired infections.[8]

This evaluation assessed the durability and antimicrobial efficacy of different Cu surfaces over a one-year period of use in two settings where frequency and compliance with cleaning varies: patient care areas, where daily cleaning/disinfection should occur, and medical microbiology work

benches, where meticulous cleaning/disinfection occurs at least three times daily. Three formulations of Cu and stainless steel controls were embedded as coupons in removable gaskets adhered onto clinical carts on inpatient units (including emergency and maternity units) and onto microbiology laboratory bench workspaces at four hospitals. This article details the assessment of antimicrobial efficacy, development of Cu resistance, surface wear, corrosion, and material compatibility with advanced hydrogen peroxide (AHP) cleaning/disinfection over one year of use.

METHODS

Sampling: Four hospitals participated; BC Children's and BC Women's Hospitals (BCCH), North York General General Hospital (NYGH), Toronto Mount Sinai (MSH), and Vancouver General Hospital (VGH). Three Cu formulations previously characterized[9] were assessed; a) a *spray-on* 80% Cu 20% Ni product (Aereus Technologies, Rosemont, Ontario, Canada) b*) integral* 80% Cu-20% Ni alloy (Trimco, Oceanside, California USA) and, c) a Cu-impregnated surface (CIS) containing 16% Cu oxide product embedded in polymer (EOS^{CU}, Norfolk, Virginia USA). Surgical grade 316 stainless steel (SS) was the control. The formulations of Cu and the SS controls were cut as coupons (10 mm by 10 mm by 3.12 mm in thickness); each coupon was engraved with a unique identifier on the back for purposes of tracking and randomization. Coupons from each Cu formulation and SS control were randomly embedded in triplicate in cleanable strip gaskets (20 mm x 190 mm, 1/8" Santoprene 90D with 12 square holes, Custom Gaskets, Vancouver, British Columbia, Canada). Ten strip gaskets containing 12 coupons each were adhered to phlebotomy cart handles, laundry carts, computer stations on wheels, and mobile weighing scale handles. Coupons were also embedded in triplicate in cleanable gaskets (40.64 cm square 3.17mm Teflon pads with 12 holes 10 mm by 10 mm); five gaskets containing 12 coupons each were adhered on to the specimen work-up benches in the medical microbiology laboratory at each hospital (Figure 1). Gaskets went through several iterations to ensure user comfort and were placed centrally on handles to encourage contact with hands. Their placement on laboratory benches was dictated by the users who decided where the highest contact areas were. The biosafety committee reviewed the gasket for ability to be adequately cleaned.

All clinical units used AHP with microfiber cloths to daily clean and disinfect surfaces. Cleaning and disinfection with AHP wipes or liquid was done at least three times daily in the microbiology

laboratories as per biosafety procedures.[9] No attempt was made to monitor or alter cleaning/disinfection practices. Every three months, the gaskets were removed, and the Cu-containing and SS coupons assessed for microbial bioburden. They were also assessed for resistance to wear, corrosion, and materials compatibility with AHP by the UBC Department of Materials Engineering. Coupons were replaced in their same position in the gaskets at each hospital within five days of assessment.

Assessment of microbial bioburden: At each hospital site, coupons were placed in a sterile 15 mL tube, covered with two mL of Dey-Engley Neutralizing broth (HiMedia, India) and either sonicated for five minutes or vortexed for 30 seconds, after which one mL was plated onto 5% sheep's blood agar plates (Oxoid, Nepean, Ontario). Plates were incubated for 48 hours at 37°C and colony counts performed. Individual bacterial isolates were collected onto Eswab transport media (Copan Diagnostics, USA) for overnight shipping to the Vancouver General Hospital Medical Microbiology laboratory where identification was performed using Matrix assisted laser desorption/ionization – time of flight assay (MALDI-TOF) (Bruker Ltd, Milton, Ontario). Bacteria were then frozen at -70°C for further analysis. Coupons were sterilized in 95% ethanol for 10 min and sent to UBC Department of Materials Engineering for durability testing before being reinstalled.

Screening for Copper resistant strains: The large number of gram positive bacteria collected from the Cu coupons necessitated stratified screening for Cu resistance to obtain proportional representation of bacteria from all hospitals. All the *Staphylococcus aureus* isolates, gram negative organisms and yeast and 20% of other gram positive bacteria from each hospital, (selected randomly from the freezer list for each site) were screened. Frozen samples were retrieved from storage and subcultured twice onto 5% sheep blood agar plates. Three colonies were then

suspended in 0.85 % NaCl, adjusted to a McFarland of 0.5 and 20 uL (~10^6 cfu/mL) subcultured to Muller Hinton Agar (Sigma Aldrich, Oakville Ontario) containing 0, 2, 4, 6, 8, 10, 12 or 14 mM of $CuCl_2$ (Fisher Scientific, United States). For some isolates, embedded six mm disks (Fisher Scientific, Burnaby, British Columbia) containing 500 mM of $CuCl_2$ were also used to identify inhibition zone diameter. Plates were incubated for 48 h at 37 °C and examined for growth. *Salmonella enteriditis* S9, S19, and S20 - strains with a minimal inhibitory concentration of 12 mM and a KB zone < 10 mm (obtained from Dr. Sadhana Ravishankar, University of Arizona) were used as Cu-resistant controls.

Materials Characterization: All measurements were done before, during, and after one year of use according to methods described by Bryce *et al*.[10] Mass loss analysis was used to determine the abrasion-corrosion rate using the ASTM G1–03 standard[11]. All coupons (45 of each formulation from benches and carts) were weighed before, during, and after one year using an analytical balance. After one year use, Cu coupons were soaked 1-3 minutes in an aqueous solution of 50% v/v hydrochloric acid (HCl, sp gr 1.19) and SS coupons were soaked for 1-3 minutes in an aqueous solution of 10% v/v nitric acid (HNO_3, sp gr 1.42) to remove their corrosion products and reweighed to calculate final mass loss. EDS was performed in spot analysis mode to monitor any chemical composition alteration of the near surface before and after one year use. Four coupons for each formulation were analyzed and at least ten spots on each carrier were measured.

Data Analysis: Data were analyzed by standard descriptive methods using GraphPad Prism™ (San Diego, California). Normal distribution within each SS control and Cu formulation coupon groups was assessed using the D'Agostino & Pearson normality test. Kruskal-Wallis and Dunn's post-tests were used to compare the median (CFU/cm^2) variation between groups. Wilcoxon Signed Rank Tests were used to compare the median bioburden of each formulation against the

recommended standard for surface-level cleanliness; this recommendation states that the total aerobic colony counts should not exceed < 2.5 to 5 CFU/cm^2 from hand-touch sites and for this study the standard was set at 2.5 CFU/cm accordingly[2,12,13]. Mass change, abrasion-corrosion rate were analyzed by repeated measure of ANOVA as appropriate.

RESULTS

A total of 720 coupons; 480 in clinical areas (120 of each SS control and Cu formulation) and 240 in laboratory benches (60 of each SS control and Cu formulations) were installed at the four hospitals. Over the course of one year, 104 coupons were lost from clinical areas; (21.7 %) 24 SS, 28 Integral, 25 Spray-on, and 27 from CIS coupons (36 at NYGH, 27 at MSH, 27 at BCCH, and 14 at VGH). One (0.4%) Integral coupon at BCCH was lost from the laboratory bench at BCCH. All hospitals used AHP disinfectants but some had different compositions. At NYGH Preempt™ (SKU: 100906585 Virox Technologies, ON, Canada) was used on laboratory benches, and Virox 5 RTU wipes (SKU: 53810, Virox Technologies, ON, Canada) with dodecylbenzene sulfonic acid at pH.1.75 was used to clean carts. At MSH Accel PREVention Wipes (SKU: 100906722, Diversy Canada Inc, ON, Canada) with dodecylbenzene sulfonic acid at pH.1.75 was used on laboratory benches and carts. At BCCH both the laboratory benches and carts were cleaned using Accel PREVention Wipes. At VGH, liquid Virox 5 RTU (SKU: 53808) containing hydroxyethylidene diphosphonic acid and dodecylbenzene sulfonic acid at pH 1.75 was used on laboratory benches and either Accel PREVention Wipes or INTERVention wipes (SKU: 100906585) with benzyl alcohol, potassium citrate, and dodecylbenzene sulfonic acid at pH 3 were used to clean carts.

Microorganism burden and identification: The CFU/cm^2 values in the SS control and the Cu coupons were not normally distributed. Nonparametric test Kruskal-Wallis one-way analysis of variance showed significant variation of the medians of the microorganism burden between all formulations at all hospitals at 3 months (P<0.0001), 6 months (P=0.0026), and 12 months (P=0.0006). A statistically significant difference was found only at BCCH (P=0.0244) and at VGH (P=0.0005) at 9 months, but there were no variations overall (P=0.16622). Dunn`s multiple comparison post-test showed that the microorganism burden (CFU/cm^2) in the three Cu

formulations were significantly lower compared to SS control coupons for the initial 3 and 6 months at all hospitals. At month 12, the Integral product maintained significantly fewer CFU/cm^2 (median; min-max) (1; 0-56) compared to SS (2; 0-148). Spray-on and CIS Cu formulations had lower CFU/cm^2 (1; 0-101 and 1; 0-38) but these were not statistically significant. Wilcoxon Signed Rank Test was used to compare the median bacterial bioburden of each Cu formulation or SS against the standard[12] acceptable for bioburden threshold post-cleaning, of 2.5 CFU/cm^2. All the Cu formulations (P<0.0001) were significantly lower than the set value at months 3 and 6 (P=0.0452, P=0.0430). At month 12, Integral (P<0.0001) and Spray-on (P=0.0020) formulations but not SS (P=0.8396) or CIS (P=0.0625) bacterial bioburden were found to be significantly below the set value (Fig 2a) (Table 1). Microbial burden percentage reduction was observed in all Cu formulations at all-time points (Table 1). Importantly, Cu formulations installed in the microbiology laboratory benches at all hospitals had no significant difference in colony counts per coupon compared to SS controls. The colony counts were consistently below 2.5 CFU/cm^2 (P<0.0001) likely reflecting the increased frequency and compliance with cleaning (Fig 2b) (Table 2).

The total bacteria recovered (6192) were largely normal skin flora, with 5928 (95.7%) gram positive organisms including coagulase-negative *Staphylococcus, Bacillus* and *Micrococcus* spp. Only 156 (2.5%) were gram negative organisms and eight isolates (0.1%) were yeast. Potentially clinically significant isolates included 19 *S. aureus* isolated from all the formulations from bench and carts at NYGH and MSH, one *Streptococcus pneumoniae* from a laboratory bench at VGH and one *Pseudomonas aeruginosa* at NYGH recovered from a SS coupon.

Copper susceptibility test: A total of 442/1165 (38%) isolates were tested against varying concentrations of $CuCl_2$ on solidified media (371) and embedded disks (71) containing $CuCl_2$.

Only four isolates had a MIC greater than 12 mM: one *Kocuria kristinae* recovered from a CIS cart coupon, two *Candida parapsilosis* recovered from SS from a bench and a cart coupon and one *Cryptococcus diffluens* from an Integral cart coupon (Table 3). All 19 *S. aureus* isolates were susceptible to Cu.

Mass change and abrasion-corrosion rate: All Cu formulation and SS control coupons changed in mass over one year. (Table 4). With the exception of the integral product on MSH carts, all products experienced mass changes, either positive or negative, of less than 6% of their initial mass. Overall, the Spray-on Cu formulations had the largest mass change across all hospitals (a mass loss for NYGH and MSH and a mass gain at BCCH and VGH). The abrasion-corrosion rates were calculated after one year use; the Spray-on and Integral Cu formulations showed significantly different rates when compared to SS at all hospital sites (Table 5).

Energy-dispersive x-ray spectroscopy: EDS was performed to monitor alterations in the chemical composition after one year. The Integral and Spray-on formulations had Cu content as the main element and this did not change significantly over the year. However, for oxygen content there was a trend to decrease at the Toronto hospitals (NYGH and MSH) and an increase at the Vancouver hospitals (BCCH, VGH). For the CIS product, Cu content was variable in all coupons due to the multiphasic nature of the material[10] (Supplementary Table 1).

DISCUSSION

Self-sanitizing surfaces are a potential mitigation strategy to reduce environmental contamination, and Cu formulations in particular are being promoted. However little has been done to assess microbial bioburden or durability in the face of repeated exposure to cleaner/disinfectants from time zero of use and guidance as to the best application of these materials is sparse, with most articles suggesting only that it be applied to high-touch surfaces.[13,14,15,16]

Medical microbiology laboratory benches and clinical carts were specifically chosen for this evaluation as they reflect different levels of bioburden (laboratory benches having the potential for much higher concentrations of organisms), compliance with cleaning and disinfection (laboratories clean the benches before each break and the end of each shift as a minimum) and physical wear and tear. All the hospitals used AHP and the laboratories used the same biosafety protocols. It should be noted that although AHP 0.5% was the main ingredient, the different brands had additional components and variable pH values that might have impacted on the Cu and SS surfaces. Our results showed that the laboratory coupons that were frequently and thoroughly cleaned/disinfected had no difference in bacterial burden between the SS controls and the Cu formulations, suggesting Cu installation had little additional benefit. Conversely, Cu reduced bacterial bioburden significantly on the coupons installed in clinical areas, suggesting that it could be a useful mitigation strategy in areas where compliance with daily cleaning and disinfection may be sub-optimal. This is an important distinction – while others[13,14,18] have demonstrated reduction in contamination on room surfaces by 1-2 logs, they have not indicated where Cu formulation surfaces should be placed for optimum benefit. At the end of one year we observed that the Integral product conferred the greatest benefit in reducing bacterial burden in clinical areas.

Environmental hospital cultures of high touch surfaces have reported gram negative isolation rates of between 18.7% and 29.1% of total organisms.[19, 20] Gram positive organisms are more resistant to the bactericidal effect of Cu because of their thicker peptidoglycan wall and resistance to immediate membrane depolarization[21] and the predominance of these microorganism in sampling reflects this survival advantage. Our results confirm that Cu exerts its antimicrobial activity best in Gram negative organism as only 2.5% of the isolates samples were gram negative. Copper surfaces might best be used in areas where both cleaning is a challenge and gram negative organisms are more problematic/common, e.g. patient washrooms, endoscopy suites, ICUs.

Resistance to copper was infrequent with one yeast on the Integral product and one *K. kristinae* from a CIS coupon. These results are likely spurious as two SS coupons also revealed two *C. parapsilosis* isolates with high MICs. Cu exhibits multiple mechanisms of bacterial killing,[22] minimizing the likelihood of the development of resistance. The lack of observed resistance over one year in the four hospital environments (particularly the laboratory benches where exposure to resistant organisms is frequent) was reassuring.

The observed mass changes and corrosion rates were all quite low. With the exception of the integral product on MSH carts, all one-year mass changes were within 6% of the initial mass. The corrosion rates, as a result, were correspondingly low and not exceeding 0.4 mm/y. This is an acceptable corrosion rate by engineering standards and certainly acceptable for surfaces that are not structurally or mechanically important. The variation observed in terms of mass gain (BCCH and VGH) versus mass loss (NYGH and MSH) is likely due to different cleaning procedures or different environmental conditions that would have resulted in slight variations in the extent of surface oxidation, and thus the amount of oxygen associated with the corroded surface. This hypothesis is corroborated by the increased oxygen concentration (Supplementary Table 1) for the

British Columbia versus the Ontario hospitals. Products with higher copper corrosion rates might perform well as bactericidal surfaces;[23] this is correlated with the low bioburden found in the Integral and Spray-on formulation. While AHP was the only cleaner/disinfectant used in this study, hospitals need to consider the effect of other cleaner/disinfectant products.[10]

This study did not control for variables such as the number of individuals touching the gaskets, the use of gloves, the different AHP formulations, the daily location of the ward carts and rooms visited all of which could affect colony counts. The one-year duration and four hospital design hopefully minimized these variables and others that could impact colony counts such as temperature and relative humidity. Further studies that consider these variables and the economic impact of Cu installation would be helpful.

Cu formulation subjected to one year of clinical use demonstrated different degrees of antimicrobial activity, although corrosion rates and mass changes were acceptable. Clinical areas that might be suboptimally cleaned and/or areas where gram negative bacteria are predominant may experience the greatest benefits.

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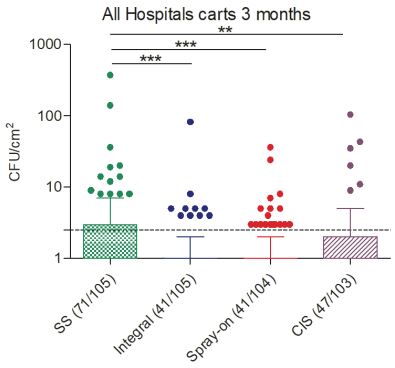
Fig 1. Examples of (A) cart gasket adhered to a phlebotomy cart handle on a clinical unit and (B) Medical Microbiology laboratory bench gasket. All gaskets contained stainless steel and copper formulations coupons ($1cm^2$) in triplicate (red circles).

Figure 2. Colony forming units (CFU) per coupon (cm^2) comparing Stainless Steel (SS) and three Cu formulation: Integral, Spray-on, and CIS collected every 3 months during 1 year on (A) carts and (B) microbiology laboratory benches in four hospitals. Box plot represent the values of SS control and Cu. Horizontal line in box is the median, boxes extremities 1-4 quartiles, whiskers 25%-75% percentiles. * denotes $p < 0.05$, ** $p < 0.01$, ***denotes $p < 0.001$. Dashed lines indicate a 2.5 CFU/cm^2 bioburden threshold considered to be an acceptable value post-cleaning. *(see next page)*

A


All Hospitals carts 3 months
CFU/cm2
1000
100
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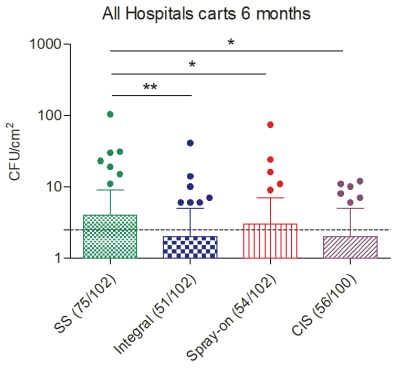
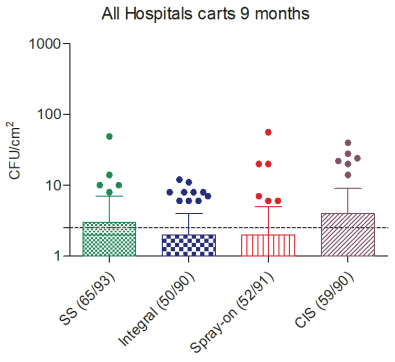
1
**
***
***
SS (71/105)
Integral (41/105)
Spray-on (41/104)
CIS (47/103)


All Hospitals carts 6 months
CFU/cm2
1000
100
10
1
*
*
**
SS (75/102)
Integral (51/102)
Spray-on (54/102)
CIS (56/100)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CFU/cm2
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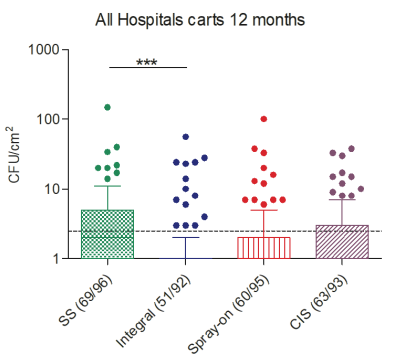
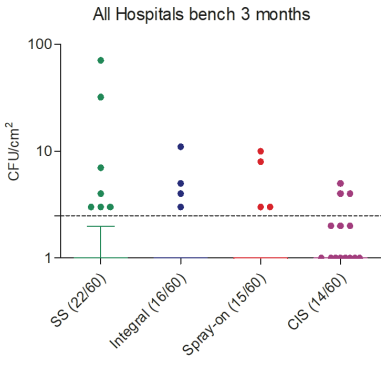
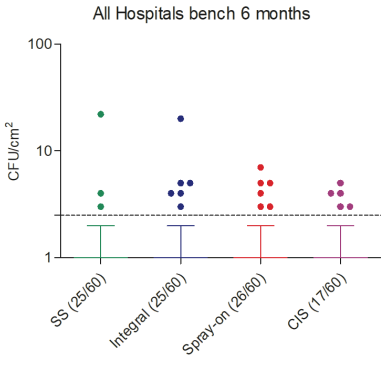
100
10
1
SS (65/93)
Integral (50/90)
Spray-on (52/91)
CIS (59/90)


All Hospitals carts 12 months
CFU/cm2
1000
100
10
1
***
SS (69/96)
Integral (51/92)
Spray-on (60/95)
CIS (63/93)

B


All Hospitals bench 3 months
CFU/cm2
100
10
1
SS (22/60)
Integral (16/60)
Spray-on (15/60)
CIS (14/60)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CFU/cm2
100
10
1
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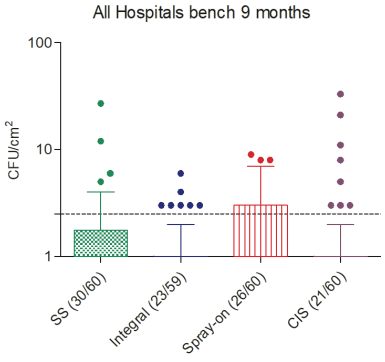
Integral (25/60)
Spray-on (26/60)
CIS (17/60)


All Hospitals bench 9 months
CFU/cm2
100
10
1
SS (30/60)
Integral (23/59)
Spray-on (26/60)
CIS (21/60)

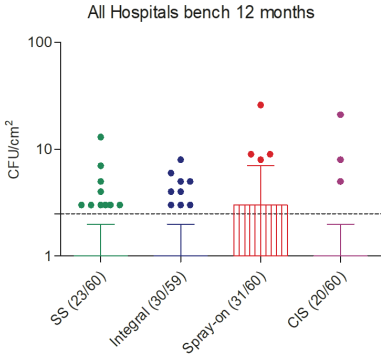

All Hospitals bench 12 months
CFU/cm2
100
10
1
SS (23/60)
Integral (30/59)
Spray-on (31/60)
CIS (20/60)

Table 1. SS and Cu formulation growing bacteria (CFU/cm^2) over 1 year in use in 4 hospitals installed on carts.

Products	Contaminated coupons / Total coupons (%)	# CFU	CFU/cm^2		Difference in rank sum	Wilcoxon signed rank *P* value	% Red[b]
			Mean	Median (min, max)			
month 3 (*P*<0.0001)[a]							
SS	71/105 (68)	805	7.6	1 (0, 372)		0.0452	
Integral	41/102 (39)	161	1.5	0 (0, 8)	***	<0.0001	80.3
Spray-on	41/101 (39)	150	1.4	1 (0, 24)	***	<0.0001	81.3
CIS	47/100 (46)	309	3	1 (0, 104)	**	<0.0001	60.5
month 6 (*P*=0.0026)[a]							
SS	75/102 (74)	411	4	1 (0, 104)		0.0430	
Integral	51/102 (50)	179	1.7	0.5 (0, 41)	**	<0.0001	57.5
Spray-on	54/102 (53)	259	2.5	1 (0, 74)	*	<0.0001	37.5
CIS	56/100 (56)	154	1.5	1 (0, 12)	*	<0.0001	62.5
month 9 (*P*=0.1622)[a]							
SS	65/93 (70)	248	2.7	1 (0, 49)		0.0067	
Integral	50/90 (56)	161	1.8	1 (0, 12)	*ns*	<0.0001	33.3
Spray-on	52/91 (57)	218	2.4	1 (0, 56)	*ns*	<0.0001	11.1
CIS	59/90 (66)	311	3.5	1 (0, 40)	*ns*	0.1541	-29.6
month 12 (*P*=0.006)[a]							
SS	69/96 (72)	530	5.5	2 (0, 148)		0.8396	
Integral	51/92 (55)	256	2.8	1 (0, 56)	***	<0.0001	49.1
Spray-on	60/95 (63)	350	3.6	1 (0, 101)	*ns*	0.0020	32.7
CIS	63/93 (67)	324	3.4	1 (0, 38)	*ns*	0.0625	36.4

[a]Median variation as calculated by Kruskal-Wallis test. Difference in rank sum was calculated using Dunn's multiple comparison post-test: * denotes $p < 0.05$, ** $p < 0.01$, ***denotes $p < 0.001$ significant difference between of SS versus Cu formulations, *ns* denotes no significant difference. Wilcoxon signed rank test *P* values comparing the median of each SS or Cu formulations against the standard for surface-level cleanliness subsequent to terminal cleaning 2.5 CFU/cm^2. [b]Percentage (%) Reduction was calculated: (SS mean - Cu formulation mean / SS mean) *100. SS: Stainless Steel. CIS: Composite impregnate surface.

Table 2. SS and Cu formulation growing bacteria (CFU/cm^2) over 1 year in use in 4 hospitals installed on laboratory benches.

Products	Contaminated coupons / Total coupons (%)	# CFU	CFU/cm^2		Difference in rank sum	Wilcoxon signed rank P value	% Red[b]
			Mean	Median (min, max)			
month 3 (*P*= 0.3181)[a]							
SS	22/60 (37)	140	2.3	0 (0, 71)		<0.0001	
Integral	16/60 (27)	35	0.6	0 (0, 11)	*ns*	<0.0001	75.0
Spray-on	15/60 (25)	35	0.6	1 (0, 10)	*ns*	<0.0001	75.0
CIS	14/60 (23)	27	0.5	1 (0, 5)	*ns*	<0.0001	80.7
month 6 (*P*=0.2809)[a]							
SS	25/60 (42)	56	0.9	1 (0, 4)		<0.0001	
Integral	25/60 (42)	64	1.1	0.5 (0, 20)	*ns*	<0.0001	-14.3
Spray-on	26/60 (43)	54	0.9	1 (0, 7)	*ns*	<0.0001	3.6
CIS	17/60 (28)	32	0.5	1 (0, 4)	*ns*	<0.0001	42.9
month 9 (*P*=0.3489)[a]							
SS	30/60 (50)	95	1.6	2 (0, 27)		<0.0001	
Integral	23/59 (39)	44	0.7	1 (0, 6)	*ns*	<0.0001	52.9
Spray-on	26/60 (43)	95	1.6	1 (0, 9)	*ns*	0.0012	0.0
CIS	21/60 (43)	105	1.8	1 (0, 33)	*ns*	<0.0001	-10.5
month 12 (*P*=0.0070)[a]							
SS	23/60 (38)	64	1.1	2 (0, 148)		<0.0001	
Integral	30/59 (51)	54	0.9	1 (0, 56)	*ns*	<0.0001	14.2
Spray-on	31/60 (52)	93	1.6	1 (0, 101)	*ns*	0.0123	-45.3
CIS	20/60 (33)	46	0.8	1 (0, 38)	*ns*	<0.0001	28.1

[a]Median variation as calculated by Kruskal-Wallis test. Difference in rank sum was calculated using Dunn's multiple comparison post-test: *ns* denotes no significant difference between SS versus Cu formulations. Wilcoxon signed rank test *P* values comparing the median of each SS or Cu formulations against the standard for surface-level cleanliness subsequent to terminal cleaning 2.5 CFU/cm^2. [b]Percentage (%) Reduction was calculated: (SS mean - Cu formulation mean / SS mean) *100. SS: Stainless Steel. CIS: Composite impregnate surface.

Table 3. Susceptibility to $CuCl_2$: Stainless Steel and Cu formulations

Organism	Tested / Total org	MIC (mM CuCl2)						
		2	4	6	8	10	12	14
Gram positive								
CNS	68/406	10	28	19	11	0	0	0
Staphylococcus aureus	19/19	6	5	8	0	0	0	0
Cocci	55/229	6	12	24	11	1	1	0
Rod spore forming	168/359	33	64	59	12	0	0	0
Rod non-spore forming	20/34	2	5	9	4	0	0	0
Gram negative								
Cocci	28/34	7	8	10	3	0	0	0
Rod	10/10	2	0	3	4	1	0	0
Yeast								
Candida, Cryptococcus	3/3	0	0	0	0	0	3	0
TOTAL	442/1165	66	122	132	45	2	4	0

MIC results represent a stratified sample; 16.7 % CNS (Coagulase-negative staphylococci), all *Staphylococcus aureus*, 24 % GP (Gram positive) Cocci, 46.7 % GP Rod Spore forming, 59% GP Rod Non-spore forming, 82% GN (Gram negative) Cocci and all GN Rods. An additional 71 isolates evaluated by disc diffusion method (18 CNS and 53 GP Rod Spore forming) were all susceptible.

Table 4. Percentage (%) mass change during one-year use.

	Carts (mean SD)				Bench (mean SD)			
Products	SS	Integral	Spray-on	CIS	SS	Integral	Spray-on	CIS
Months				NYGH				
3	-0.36	-0.09	-3.76	-0.02	-0.20	-0.52	-3.01	-0.61
	-0.53	0.13	1.78	1.51	0.65	0.15	1.22	1.51
12	-0.47	-0.39	-4.25	-0.46	-0.45	-1.26	-3.67	-1.08
	0.54	0.14	1.80	1.50	0.68	0.41	1.23	1.12
				MSH				
3	-0.21	-12.54	-1.50	0.10	-1.04	0.11	-3.50	-0.35
	2.04	0.82	1.65	1.33	0.88	0.22	1.11	1.09
12	-0.64	-12.90	-1.88	-0.25	-1.23	-0.23	-3.83	-0.78
	1.90	0.17	1.66	1.44	0.86	0.24	1.08	1.38
				BCCH				
3	1.00	-0.19	3.46	-0.15	0.99	-0.11	3.58	-0.07
	0.46	0.23	1.71	0.94	0.88	0.13	1.14	1.20
12	0.77	-0.48	3.46	-0.41	0.74	-0.34	3.29	-0.26
	0.48	0.26	1.70	0.86	0.86	0.14	1.17	1.14
				VGH				
3	0.52	-0.13	3.35	-0.05	0.16	0.47	3.04	0.28
	0.53	0.21	1.99	1.20	0.65	0.17	1.28	1.34
12	0.29	-0.56	2.83	-0.36	-0.08	-0.40	2.14	-0.39
	0.51	0.25	1.74	1.12	0.65	0.46	1.33	1.40

Percentage Mass change (%) was calculated: Mass (at time point) - Mass (initial) / Mass (initial)*100. The 12-month time point presented here is for cleaned samples (after removal of corrossion). SD, standard deviation. Initial mass (g) mean; SD for SS: 2.43; 0.01, for Integral: 2.66; 0.0, Spray-on: 2.54; 0.05 and for CIS: 0.56; 0.0). Data represent individual measurements up to 15 coupons from benches and 27 from carts for each metal.

Table 5. Average Abrasion-corrosion rate (mm/y) after one-year use.

Products	Carts	SD	Bench	SD
	NYGH			
SS	0.015	0.02	0.014	0.02
Integral	0.012	0.00	0.039	0.01
Spray-on	0.133***	0.06	0.113***	0.04
CIS	0.014	0.05	0.030	0.03
	MSH			
SS	0.011	0.03	0.038	0.03
Integral	0.402***	0.01	0.007*	0.01
Spray-on	0.054**	0.05	0.120***	0.03
CIS	0.004	0.04	0.024	0.04
	BCCH			
SS	-0.027	0.01	-0.022	0.03
Integral	0.015***	0.01	0.011**	0.00
Spray-on	-0.097***	0.05	-0.109***	0.03
CIS	0.013***	0.02	0.007*	0.04
	VGH			
SS	0.000	0.02	0.002	0.02
Integral	0.016	0.01	0.013	0.01
Spray-on	-0.071***	0.04	-0.067***	0.04
CIS	0.010	0.04	0.012	0.04

Abrasion-corrosion rate after one year (mm/y: millimeters per year) of use of Stainless steel (SS) and three Copper formulations: Integral, Spray-on and CIS coupons (1 cm × 1 cm × 0.312 cm) installed in laboratory benches and carts in four hospitals across Canada. Data represent individual measurements up to 15 coupons from benches and 27 from carts for each metal. The abrasion-corrosion rate was calculated using the following formula[11]: Corrosion rate (mm/y) = (K × W)/ (A × t × D) where: K is a constant equal to 8.76×10^4, t is the exposure time in hours (i.e. 365 days × 24 hours = 8760 hours), A is the geometrical surface area in cm^2 (1cm × 1cm) that was exposed to the corrosive environment (i.e. disinfectant solutions, bacteria), W is the mass loss in g, and D is density in g/cm^3. Density was calculated as initial sample mass divided by initial sample volume (1 cm × 1 cm × 0.312 cm). Density for the spray-on and CIS materials

was assumed to be a uniform physical property and no special consideration of the porosity or coating thickness was attempted. Data represent individual measurements up to 15 coupons from benches and 27 from carts for each metal. SD, standard deviation. * denotes $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ significant difference between Cu formulations versus stainless steel control as measured by one-way analysis of variance with Dunnett's multiple comparisons test.