BioCote®

Informationsunterlagen

Armstrong
Medical

BioCote®

Informationsunterlagen

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FAQ

Q. Was ist BioCote®?
A. BioCote® ist eine Silberionentechnologie, die in der Herstellung von AquaVENT® Beatmungssystemen verwendet wird, um dem Produkt antimikrobielle Eigenschaften zu verleihen. Die Silberionenpartikel werden während der Produktion von AquaVENT® gleichmäßig über das gesamte Polymer des Beatmungssystems verteilt.

Q. Warum Silber?
A. Silber- (Ag) antimikrobentechnologie ist sicher, natürlich und nachhaltig. Seit Jahrhunderten wird Silber wegen seiner schützenden Fähigkeiten eingesetzt. In der griechischen Antike wurden Silbergefäße benutzt, um Wasser frisch zu halten und chinesische Kaiser aßen mit Silberessstäbchen.
Ag leitet sich aus dem Lateinischen Argentum her, das „grau" beziehungsweise „glänzend" bedeutet.

Vor dem Einsatz von Antibiotika fand Silber in Krankenhäusern weite Verwendung, um Bakterien zu bekämpfen. Heutzutage wird es in vielen medizinischen Produkten eingesetzt, wie etwa Wundverbänden und Kathetern. Es hat sich gezeigt, dass sein Einsatz die Häufigkeit von Krankenhausinduzierten Infektionen reduziert.

Jüngste Fortschritte in der antimikrobiellen Technologie ermöglichen es, Silber alltäglichen Produkten während der Herstellung hinzuzufügen. Silber ist aufgrund seiner Wirksamkeit gegenüber einer Vielzahl von Mikroorganismen ein ideelles antimikrobielles Mittel.

Q. Wie funktioniert die Silberionentechnologie?
BioCote® antimikrobielle Technologie wird in der Form von Silberionen während des Spritzgussverfahrens bzw. des Extrudierens der Komponenten hinzugefügt. Die Silberionen sind dann auf den Oberflächen der Komponenten präsent und können sofort gegen kontaminierende Bakterien wirksam werden. Die Silberionen binden die Mikroben, die mit der Oberfläche in Berührung kommen und fügen ihnen irreparable Schäden zu. Dies stört ihre normale Zellfunktion, sodass sich die Mikroben nicht mehr vermehren können und schließlich absterben.

Q. Was zeichnet BioCote® von anderen antimikrobiellen Technologien aus?
A. BioCote® ist die einzige antimikrobielle Firma, die Umweltstudien durchgeführt hat, um nachzuweisen, dass mit BioCote geschützte Produkte im täglichen Einsatz genauso wirksam sind wie unter klinischen Bedingungen. BioCote® bietet sowohl mikrobiologische als auch regulatorische Unterstützung an. Der Zusatz von Biocote® bei AquaVENT® sorgt für eine antimikrobielle Wirksamkeit von >99.3%. (weitere Informationen auf S. 7)

Q. Sind Bakterien silberresistent?
A. Es gibt keine Beweise, dass BioCote® auf die gleiche Weise wie Antibitiotika funktioniert und es gibt bis dato daher keine Bakterien die gegenüber Biocote® resistent geworden sind

Q. Ist Biocote® gegen antibiotikaresistente Bakterien effektiv?
A. Ja, BioCote® ist derzeit gegen antibiotikaresistente Bakterien, einschließlich MRSA und Clostridium difficile, effektiv

Q. Ist BioCote® gegen den Influenza-A/H1N1-Virus («Schweinegrippe») effektiv?
A. Es wurde gezeigt, dass BioCote® infektiöse Viren, einschließlich H1N1, an den Oberflächen von behandelten Polymern deutlich reduziert

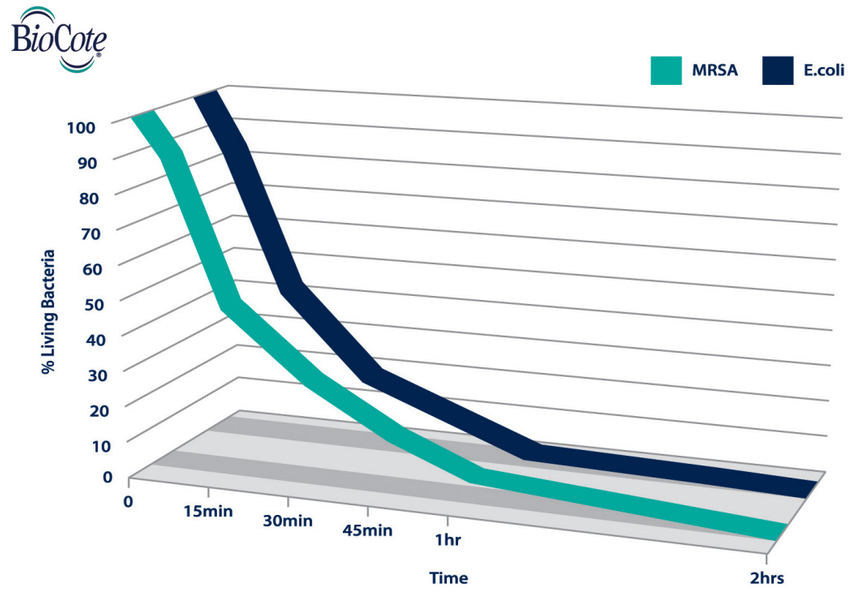
Q. Ist Silber gefahrlos?
A. Ja, Silber ist von natur aus gefahrlos und antimikrobiell

Q. Was ist die voraussichtliche Lebensdauer von mit BioCote® geschützten Produkten?
A. Produkte, die BioCote® enthalten, wurden beschleunigten Lebensdauertests unterzogen, die 25 Jahren entsprechen. Resultate zeigen eine Reduzierung der Zielzellen von mehr als 99,3%, jedoch beträgt die maximale Einsatzdauer der AquaVENT® Beatmungssysteme 7 Tage, in Übereinstimmung mit den Hersteller-Richtlinien und den örtlichen Vorschriften des Krankenhauses.

Q. Lösen sich die Silberionen vom Polymer?
A. BioCote® bleibt an der Oberfläche bis er von den Bakterien absorbiert wird. Es laugt nicht von der Oberfläche aus.

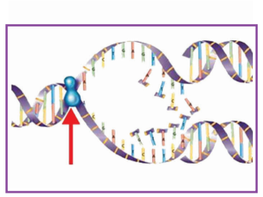
Q. Wie schnell wirkt BioCote® gegen Mikroben
A. Siehe Graphik unten

Q. Muss ich weiterhin einen Beatmungsfilter bzw. einen HME-Filter verwenden?
A. Ja. Obwohl BioCote® sowohl die internen als auch die externen Oberflächen des Beatmungssystems schützt, sollte ein angemessen validierter Filter verwendet werden, um den Patienten und das Anästhesiegerät bzw. den Ventilator vor gasgetragenen Krankheitserregern zu schützen



Wie funktioniert BioCote®?

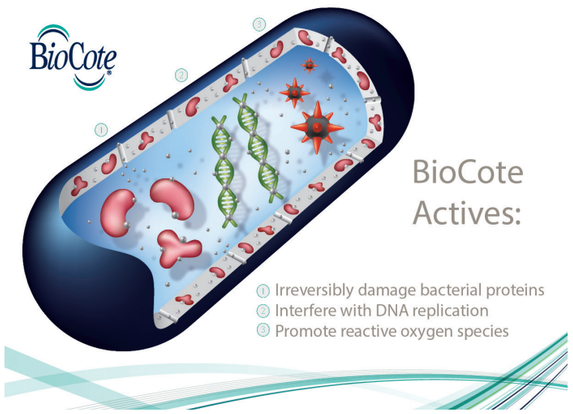
1. Silberionen docken sich an mikrobielle Proteine in der Zellwand und dem Zytoplasma an und unterbinden dadurch ihre normale Funktionsweise.



2. Silberionen hemmen diese Vermehrung der Keime, indem sie die Verdoppelung ihres Erbmaterials blockieren.

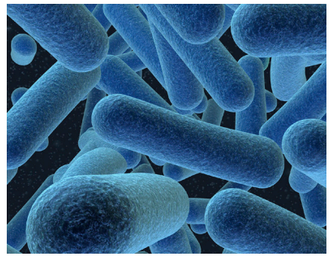
3. Es ist wissenschaftlich erwiesen, dass Silberionen die Bildung schädlicher Chemikalien, der sogenannten reaktiven Sauerstoffspezies (ROS), innerhalb von Mikroben begünstigen.

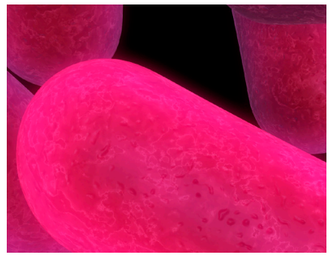
Die von den ROS ausgehenden Schäden sind ein maßgeblicher Auslöser des funktionellen Verfalls, ähnlich des Alterns, der zu einer weiteren Hemmung des Keimwachstums führt.

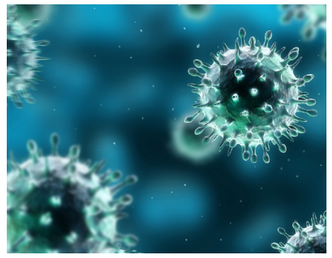


Die untersuchten Mikroben

Untersuchungen haben gezeigt, dass die antimikrobiellen Zusatzstoffe von BioCote® gegen eine Vielzahl von Krankheitserregern wirksam sind, wie z. B. Bakterien, Pilze und Viren. Einige der am häufigsten auftretenden Mikroben finden Sie untenstehend aufgelistet. Die Wirksamkeit kann je nach Mikrobenart variieren. Detailliertere Daten sind auf Wunsch verfügbar.







Bakterien

Acinetobacter baumanii
Bacillus subtilis
Campylobacter coli
Campylobacter jejuni
Clostridium difficile (excluding spore form)
E.coli
E.coli O157
Enterobacter aerogenes
Enterococcus faecalis
Escherichia coli ESBL
Legionella pneumophila
Listeria monocytogenes
MRSA
Pseudomonas aeruginosa
Salmonella enteritidis
Salmonella typhimurium
Shigella sp.
Staph aureus
Staph epidermidis
Streptococcus faecalis
Vancomycin resistenter Enterococcus

Pilze

Aspergillus niger
Aspergillus brasiliensis
Candida albicans
Penicillium sp.

Viren

Influenza A H1N1

Die untersuchten Mikroben

Armstrong Medical Ltd hat sich zum Ziel gesetzt, das Risiko von Krankenhausinfektionen zu reduzieren. Aus diesem Grund sind die beheizten AquaVENT® Beatmungsschlauchsysteme mit BioCote® ausgestattet; einem antimikrobiellen Additiv, das die Ausbreitung von Keimen zwischen Patienten und Anwendern eindämmt sobald übertragbare Keime in Kontakt mit dem Material des Beatmungssystems kommen. Die BioCote® Technologie in den beheizten AquaVENT® Beatmungsschlauchsystemen verwendet anorganische Silberionenpartikel, die gleichmäßig über das gesamte Polymer des Beatmungssystems verteilt sind.

Am 9. April 2013 hat unser Produktionspartner BioCote® eine Pressemitteilung zum Test ihrer antimikrobiellen Technologie gegen Carbapenem-resistente Enterobakterien (CRE) herausgegeben.

Der von einem unabhängigen Institut durchgeführte ISO 22196 Test zeigte eine Reduktion des Bakteriums Klebsiella pneumonia um mehr als 99,9%. BioCote® weist darauf hin, dass ihre Technologie nachweislich gegen eine Vielzahl von Bakterien und Pilzen wirksam ist. Mit den jüngsten veröffentlichten Ergebnissen steigt die Zahl der multiresistenten Bakterien, gegen die die BioCote® Technologie erwiesenermaßen wirksam ist auf vier Erreger:
1. MRSA (Methicillin-resistenter Staphylococcus aureus)
2. VRE (Vancomycin-resistenter Enterococcus)
3. ESBL (Extended Spectrum Beta Lactamase)
4. CRE (Carbapenem-resistente Enterobakterien, Klebsiella pneumoniae)

Für weitere Informationen zu dem vielfältigen Angebot an Armstrong Medical Produkten kontaktieren Sie bitte Ihren nationalen Vertriebspartner MedCare Visions GmbH unter +49 (0) 89 2000433-9 oder per Email: info@medcarevisions.de. Oder besuchen sie die BioCote® Homepage für weitere Informationen.

Legionella

Biocote® Limited (Wolverhampton, UK) hat jüngst das unabhängige Labor Industrial Microbiological Services Limited (Hants, UK) damit beauftragt, die Zusatzstoffe zu untersuchen, die bei Armstrong Medical für die Produktion von beheizten AquaVENT® Beatmungssystemen verwendet werden.

Der Test sollte die Wirksamkeit des Zusatzstoffes feststellen, indem untersucht wurde, inwieweit das Additiv die Anzahl des Bakteriums s. Legionella auf der Oberfläche des Beatmungsschlauchsystems reduziert.

Eine Verringerung von >99,9% (log 10-3) wurde nach 24 Stunden beobachtet. Dieses Ergebnis bestätigt die Wirksamkeit des Additivs unter Testbedingungen und ergänzt die schon zuvor bewiesene Wirkung gegenüber anderen Bakterien, inklusive MRSA, Escherichia Coli, Acinetobacter baumanii und Influenza A (H1N1).

Das Bakterium s. Legionella ist ein gramnegativer Keim, der über Wassertröpfchen verbreitet wird und bei anfälligen Personen durch Inhalation von Wasserteilchen als Auslöser für die Legionärskrankheit L. Pneumophilia fungiert. Menschen fortgeschrittenen Alters und Personen mit geschwächtem Immunsystem sind besonders gefährdet. Eine Übertragung von Mensch zu Mensch scheint soweit nicht möglich zu sein.
Jüngste Epidemien in England und Schottland führten zu Todesfällen stationärer Patienten der Universitätsklinik North Staffordshire sowie der Klinik NHS Lothian.
Auch wenn die Quelle dieser Ausbrüche Krankenhausextern zu sein scheint, sind die Krankenhäuser dennoch unweigerlich mit diesen Ausbrüchen konfrontiert und laufen vor allem durch Klimaanlagen und die Wasserversorgung ebenfalls Gefahr zur Brutstätte dieser Bakterien zu werden.

Durch unser Bestreben, das Risiko von Krankenhausinfektionen zu reduzieren sind unsere beheizten AquaVENT® Beatmungsschlauchsysteme mit dem antimikrobiellen Biocote® Additiv ausgestattet. Dadurch wird die Verbreitung von Bakterien zwischen Patienten und Anwendern im Falle eines Oberflächenkontakts der Bakterien mit dem Beatmungsschlauchsystem nahezu verhindert.
Die BioCote® Technologie in den beheizten AquaVENT® Beatmungsschlauchsystemen verwendet anorganische Silberionenpartikel, die gleichmäßig über das gesamte Polymer des Beatmungsschlauchsystems verteilt sind.

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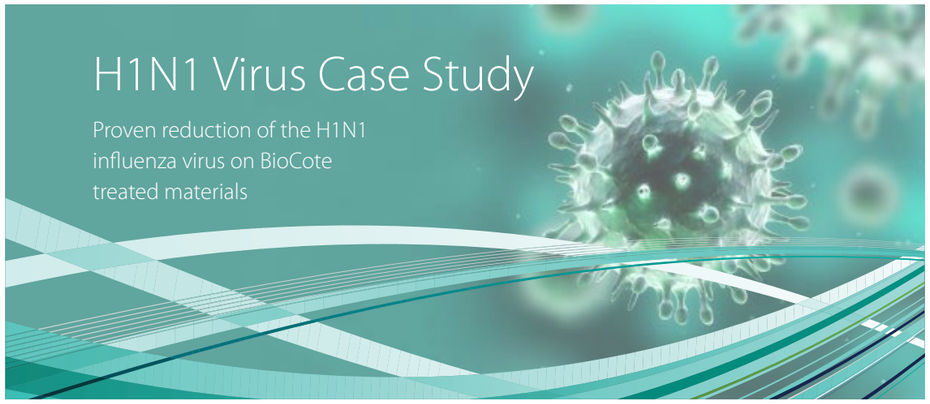

H1N1 Virus Case Study

Proven reduction of the H1N1 influenza virus on BioCote treated materials

Background

Outbreaks of influenza caused by the H1N1 virus are a repeated threat. The contagious H1N1 virus spreads effectively between people and, due to the widespread international travel, between countries.

July 2009 saw the beginning of the most recent global H1N1 influenza pandemic with around 30,000 confirmed cases reported in 74 countries although unconfirmed cases make this outbreak undoubtedly more significant. The economic impact of influenza can be huge; the World Health Organisation estimated an H1N1 pandemic could cost the UK economy over £70 billion so a measure with the potential to limit the spread of viral infection is worthy of including in an infection control strategy. The evidence described here suggests the application of BioCote® antiviral technology has the potential to complement strategies aimed at inhibiting the spread of viruses responsible for influenza illness.

Viruses cause human disease by infecting cells of the body. Viral disease can be averted if the virus is rendered noninfectious before it enters the body's cells and establishes an infection. Antiviral vaccines typically operate by converting the virus from an infectious to noninfectious form. This study quantified the conversion of influenza A H1N1 virus from an infectious to non-infectious form because of its exposure to BioCote® containing materials.

Aim

To understand how effective BioCote® approved silver ion antimicrobial technology is against influenza A H1N1 virus when incorporated into various manufacturing materials.

Method

Known amounts of infectious H1N1 virus (Fig.1) were added to the surface of a variety of materials commonly used for manufacturing that contained BioCote® approved antimicrobial silver ions; specifically, acrylonitrile butadiene styrene (ABS), polycarbonate (PC), thermoplastic polyurethane (TPU), polyvinyl chloride (PVC) and polybutylene terephthalate (PBT) polymers, laminated wood board and wet and powder paints. Exposures were left overnight after which the virus was recovered from the test materials. Viruses still able infect cells after exposure to BioCote® technology were counted using an immunological microplate plaque assay (Fig.2). Because controls were included in these experiments, the amount of virus inactivation directly attributable to the BioCote® silver technology was determined.

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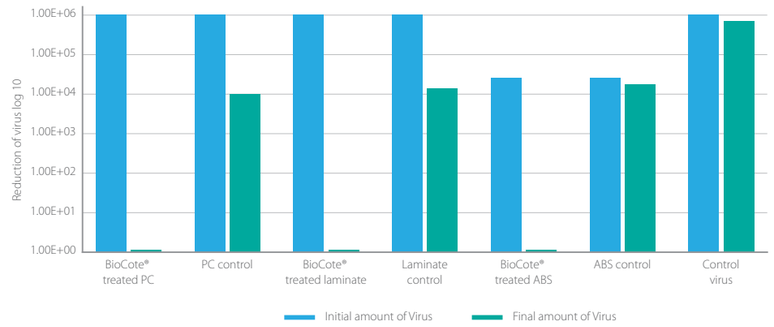
Results

All BioCote® containing materials demonstrated significant antiviral activity compared to untreated and/or virus controls.

A selection of reductions in numbers of infectious H1N1 virus because of exposure to treated materials is presented in Fig.3 alongside corresponding reductions by untreated controls. The survival of the H1N1 virus under test conditions not exposed to any material was also determined.



Results for Laminate

>99.99%

reduction in viable H1N1 virus particles on BioCote treated laminate

Results for PC

>99.99%

reduction in viable H1N1 virus particles on BioCote treated PC

Conclusions

BioCote® approved silver ion technology is effective at significantly reducing numbers of infectious influenza A H1N1 virus. Antiviral activity was demonstrated by BioCote® containing ABS, PC, TPU, PVC and PBT polymers, laminated board and wet and powder paints.

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Merkblatt über die BioCote® Antimikrobielle Technologie

Die BioCote-Technologie zeichnet sich durch eine Reihe von Vorteilen aus, denn sie schützt Oberflächen gegen Mikroben und ihre potenziell negative Auswirkung auf ein Produkt. Die Pluspunkte der BioCote-Technologie sind:

Als 'antimikrobiell' bezeichnet man die Eigenschaft, das Wachstum und den Befall durch Mikroben, Bakterien und Schimmelpilzen zu verhindern.

1. **Sie erhöht die hygienischen Eigenschaften der Produkte**, die aller Wahrscheinlichkeit nach von krank machenden Mikroben kolonisiert sind.
2. **Sie hält ein Produkt länger frisch**, falls es aller Wahrscheinlichkeit nach von geruchs- oder fleckenbildenden Mikroben kolonisiert werden kann.
3. **Sie verlängert die Nutzungsdauer eines Produkts**, sofern es aller Wahrscheinlichkeit nach von Mikroben kolonisiert werden kann, die seine Oberfläche beeinträchtigen könnten.

BioCote: die antimikrobielle Marke, der Sie vertrauen können

Die BioCote-Marke:

- Ist ein anerkanntes Qualitätszeichen
- Steht für überlegene Produktleistung
- Ist auf Produkten weltweit vertreten

BioCote ist der einzige antimikrobielle Anbieter der Welt:

- Der für Leistungsfähigkeit globale Maßstäbe setzt
- Der den Nachweis erbringt, dass Kundenprodukte wirklich antimikrobiell sind
- Der die internationalen HACCP-Grundsätze erfüllt
- Der die Leistungsfähigkeit dieser Technologie in der reellen Welt nachweist

Die BioCote-Marke genießt globale Anerkennung, denn sie garantiert eine überlegene antimikrobielle Leistungsfähigkeit. Das BioCote-Warenzeichen ist das Merkmal einer Marke, der Sie vertrauen können.

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BioCote reduziert das Mikrobenniveau auf einer Oberfläche

Was kann die BioCote-Technologie erzielen?

Die BioCote-Technologie stattet ein Produkt mit lang anhaltendem Schutz aus, indem es eine Oberfläche erzeugt, auf der die Mikroben nicht überleben können.

Ein niedrigeres Bakterienniveau bedeutet nicht nur bessere Hygiene sowie reduzierte Flecken- und Geruchsbildung, sondern verhindert auch den vorzeitigen Materialabbau.

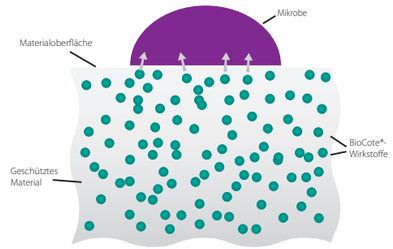
BioCote-Technologie:

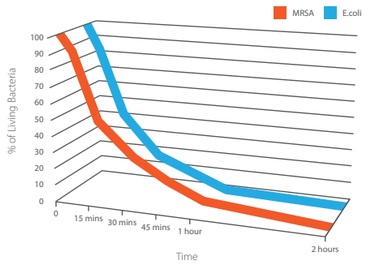
- Entfaltet eine nachgewiesene Wirkung gegen ein ganzes Spektrum von Bakerien, Schimmelpilzen und den H1N1-Virus
- Reduziert Mikroben um bis zu 99,99 %
- Signifikante Verminderung innerhalb von 15 Minuten
- Eine Verminderung um bis zu 99,5 % binnen nur 2 Stunden
- Kontinuierliche Wirkung für die voraussichtliche Nutzungsdauer des Produkts

Wie funktioniert die BioCote®-Technologie?

Die BioCote-Technologie schützt Produkte, indem der Wirkstoff eine Verbindung mit den Mikroben eingeht, die ihre Oberfläche kontaminieren. Mikroben können nicht überleben, wenn sie der BioCote®-Technologie ausgesetzt sind.

Die BioCote-Technologie ermöglicht auf ideale Weise, die Produktoberfläche zwischen den Reinigungszyklen hygienischer zu halten.





Die BioCote-Technologie:

- Bringt die Vermehrung von Zellen zum Stillstand
- Beschädigt die Zellwand
- Stört die Energieerzeugung und andere zelluläre Funktionen

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Hospital Case Study

Proven reduction in bacterial contamination in a real-life hospital environment

Background

The control of healthcare-associated infections (HCAIs) remains a priority for healthcare providers, who are employing a combination of infection prevention and control strategies, including hand hygiene, cleaning, training and the adoption of new technologies, to tackle the problem.

As a result, a wide range of infection control products and technologies are emerging on the market, including antimicrobial technology.

BioCote Ltd works with equipment manufacturers, engineering silver ion technology into a variety of healthcare related products, helping them to resist the growth of bacteria and mould on their surface. Silver is an ideal antimicrobial agent because it has a high efficacy against a wide range of medically-important microorganisms and is regarded as non-toxic. For the NHS to employ new technologies and products they need to show a demonstrable ability to contribute positively to infection control. The use of any product that claims it has antimicrobial efficacy should be supported by a robust evidence-base.

Aim

A pilot study, conducted at the Heart of England NHS Foundation Trust, investigated to what extent BioCote® antimicrobial products can reduce microbial contamination in a healthcare environment.

In laboratory tests, BioCote® antimicrobial materials regularly demonstrate reductions in counts of E. coli and S.aureus greater than 95%, compared with untreated samples. The aim of this study was to determine to what degree this high level of antimicrobial efficacy could be achieved in a real-life hospital environment.

Method

Two outpatient units provided the environments for this 18 month pilot study. Unit A was refurbished with BioCote® treated products including blinds, tiles, door handles, sack holders and light switches and also a number of untreated products. A similar, refurbished outpatient ward containing untreated items (unit B), served as a control. Both outpatient units were similar in terms of volume of people, layout and floor-surface area and were subjected to standard cleaning practice. Both were allowed to function for 12 months before swabbing commenced.

Swabs were collected over a five month period from BioCote® treated and untreated products in both outpatient units. Swabs were processed for total counts of viable bacteria and results expressed as average counts of colony-forming units (CFUs).

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Results

Table 1 shows that CFU counts from BioCote®-treated products in unit A were between 62% and 98% lower than from comparable, untreated products in unit B.

The products used in the trial were manufactured from a variety of materials e.g plastics and fabrics. CFU counts from these different materials were also compared and are shown at the bottom of Table 1. CFU counts from BioCote® treated materials were between 70% (fabrics) to 99% (laminates) lower than untreated equivalents.

CFU counts from BioCote® treated products in unit A were compared with CFU counts from untreated products in both unit A and unit B. CFU counts on untreated products in unit A were also compared to untreated products in unit B.

This three way comparison is shown in Figure 1 and provides the following results:

- The average CFU count from any BioCote® treated product was 95.8% lower than that from any untreated product in unit B.
- The average CFU count from any BioCote® treated product was 92.6% lower than that from any untreated product in the same environment (unit A)
- The average CFU count from any untreated product in unit A was 43.5% lower than that from any untreated product in unit B.

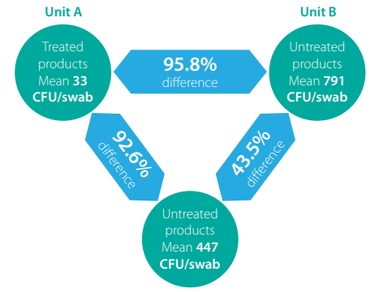
Table 1

% reduction of CFU counts from BioCote®-treated products / materials in unit A compared to nontreated products / materials in unit B

Product	% Reduction
Door	98%
Door handle	89%
Electrical Switch	95%
Curtains / blinds	73%
Chair	93%
Treatment Couch	62%
Sign	75%
Waste Bin	84%
Tiles	90%
Material	**% Reduction**
Powder Coating	94%
Plastic	98%
Wood Lacquer	98%
Fabric	70%
Laminate	99%

Figure 1

Inter-site comparison of average CFU counts from BioCote® treated and untreated products in units A and B.



Discussion

Results suggest that BioCote® antimicrobial products will demonstrate the same high level of antimicrobial e cacy in a real-life environment as seen in laboratory tests, e.g an average bacterial reduction of 95.8%.

In addition to the e ect of standard cleaning, BioCote® antimicrobial products showed sustained reductions in bacterial counts, compared to untreated products. Because BioCote® technology does not wear out or wipe off surfaces it can provide a continuous decontamination effect. Treated products can complement cleaning practices, helping to continually reduce levels of bacteria on surfaces and in the wider healthcare environment.

Bacterial contamination on untreated products in unit A was on average 43.5% lower compared with untreated products in unit B. This suggests that a reduction in bacteria on BioCote® antimicrobial surfaces results in lower numbers of bacteria on other surfaces because there are fewer bacteria being transferred. Using a number of antimicrobial objects in a healthcare environment may therefore help the decontamination of the wider environment.

Conclusions

This study, first published in the Journal of Infection Prevention1, highlights the ability of BioCote®-treated antimicrobial products to reduce levels of bacteria contaminating healthcare settings.

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Armstrong Medical

Die Beständigkeit des antimikrobiellen Silberadditivs von BioCote® in den Beatmungsschläuchen von Armstrong Medical während der aktiven Befeuchtung.

Das unabhängige Labor Wardell Armstrong (Truro, UK) analysierte Proben kondensierten Wassers, dass aus einem Aktiv befeuchteten Patientenschlauchsystem, wie es im neonatalen Bereich verwendet wird, entnommen wurde. Das Schlauchsystem beinhaltete das Silberadditiv (Ag) von Biocote® im Verteilungsverhältnis von 1,5% des Polymergewichts das zur Herstellung der Schläuche verwendet wird. Die Analyse sollte feststellen, inwieweit sich das Additiv von den Schläuchen lösen kann und Teil des inhalatorischen Gasflusses wird, sowohl in Gasform, als auch im Wasserdampf.

Die Analyse des Wasserkondensats erschien als der beste Indikator für die Beständigkeit des Ag Additivs in den Schläuchen. Zusammen mit den Testtemperaturen simulierte die Untersuchungsdauer von 10 Tagen die exakten Bedingungen des klinischen Gebrauchs. Unsere Beatmungssysteme sind, je nach Ausstattung des Beatmungsschlauchsystems, zunächst für den Einsatz von 7 bis 14 Tage nach Erstgebrauch vorgesehen.

Vefahrensweise zur Herstellung von Wasserkondensatproben zur Analyse

- Design des Beatmungssystems: AquaVent® Neo beheiztes Beatmungssystem zur nCPAP Applikation bei Frühgeborenen mit einem Gewicht >24. Gestationswoche
- Gassfluss (Luft- und Sauerstoffgemisch bei 30% O2, Flussrate von 10L/min und 3cmH2O PEEP)
- Wasserversorgung erfolgte über steriles pyrogenfreies Aqua Dest über die Befeuchterkammer des beheizten Beatmungsschlauchsystems
- Der Befeuchter wurde auf den Intubationsmodus gesetzt, um 37°C in der Heizkammer und 40°C am Patientenende zu erhalten. Beachten Sie bitte, dass der Atemgasbefeuchter im klinischen Einsatz ggfs. auf den „Maskenmodus“ eingestellt wird, um 31°C in der Heizkammer und 34°C am Patientenende zu erhalten.
- >6mL tägliche Mengenentnahme an Wasserkondensat von einem Sammelpunkt im unbeheizten Teil des Beatmungssystems, proximal zum Patienteninterface über 10 aufeinanderfolgende Tage
- Wasserproben wurden einzeln auf Silberbestandteile analysiert, zur Kontrolle wurde steriles, pyrogenfreies Aqua Dest. verwendet

Die Analyse wurde vom unabhängigen Labor Wardell Armstrong durchgeführt und aufgezeichnet.

Zusammenfassung der Ergebnisse
Der Wardell Armstrong Bericht 0115 vom 20. Mai 2013 bestätigt, dass das Vorkommen von Ag von Tag 1 bis inklusive Tag 10, auf oder unterhalb der Nachweisgrenze war und nicht höher war, als der Ag Anteil der analysierten Kontrollprobe.

Die Daten zeigen kein, beziehungsweise nur ein vernachlässigbares Potential der Anreicherung von Ag im Inhalationswasserdampf während der klinischen Simulation.

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Analytical Report

Client Biocote Limited

Sample: Condensed Water Samples

Sample Received: 16 May 2013 ***Report Number:*** 0115

Biocote Ref	*WAI lsn*	*Description*	*Ag (mg/l)*	*Ag (mmol/l)*
90/091	0969	Control	0.01	0.0002
90/092	0970	Day 1	<0.01	<0.0002
90/093	0971	Day 2	0.01	0.0002
90/094	0972	Day 3	<0.01	<0.0002
90/095	0973	Day 4	<0.01	<0.0002
90/096	0974	Day 5	0.01	0.0002
90/097	0975	Day 6	<0.01	<0.0002
90/098	0976	Day 7	0.01	0.0002
90/099	0977	Day 8	0.01	0.0002
90/100	0978	Day 9	<0.01	<0.0002
90/101	0979	Day 10	<0.01	<0.0002

Key of Abbreviations

< - Less than the detection limit
mg/l - Milligrams per litre
mmol/l- Millimoles per litre
lsn - Laboratory sample number

Signed:..... ***Date:*** 20 May 2013

Andrea E Semmens MRSC
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Live/dead imaging of *Pseudomonas* species on BioCote silver ion treated Armstrong Medical products

Abstract

Antibacterial performance and associated marketing claims are often anchored in international standards results such as ISO22196:2011 'Measurement of antibacterial activity on plastics and other non-porous surfaces'.

Good performance to this standard can be considered the first step in taking an antimicrobial product to market. However, percentage and LOG_{10} reductions may need additional explanation and context when an understanding of the potential of antibacterial or antimicrobial properties are made available to interested parties who perhaps do not have a scientific or technical background.

With this goal in mind, we investigated how we might demonstrate antibacterial performance via other means. In collaboration with Birmingham University Technology Hub Imaging Core, it was determined that current methods for wide field epi-fluorescent microscopy with a live/dead staining system could image a number of microorganisms on replicated material, technically equivalent to Armstrong Medical's AquaVENT® Breathing circuits.

We assessed Armstrong Material's antibacterial performance via ISO22196:2011 and achieved excellent levels of reduction of test organisms: summarized in the tables below.

Reductions against Control

Organism	% Reduction of treated material, compared with control
Pseudomonas aeruginosa	99.67%
Escherichia coli	>99.99%
Staphylococcus aureus (MRSA)	>99.89

The above table displays efficacy of silver ion treated material as percentages when compared with control or untreated material. Bacteria were inoculated onto treated and untreated material and allowed to grow for 24 hours. The reported percentage difference is the reduction of organism numbers on the treated material when compared with any growth on the control, during the same time period.

Reductions against initial

Organism	% Reduction of treated material, compared with the initial population
Pseudomonas aeruginosa	97.46%
Escherichia coli	>99.91%
Staphylococcus aureus (MRSA)	>99.93%

The above table displays the efficacy of the silver ion treated material when compared with the initial or starting population loaded onto the tested sample. Test organisms were inoculated onto treated material and allowed to grow for 24 hours. Reported percentage difference is the reduction of bacteria following exposure to the treated surface, when compared with the number of organisms initially loaded onto the sample.

Imaging of *Pseudomonas aeruginosa* on control and silver ion containing material was successful, and images are contained within this report. Clear distinction between silver ion treated and control material was observed, with cell counts of approximately 93.7% dead on the treated sample, compared with 57% on control material.

Imaging performed by Dr. Robert K Shaw, Imaging Specialist, Technology Hub Imaging Core, College of Medical and Dental Sciences, University of Birmingham.

Introduction

Silver ion technology

Silver belongs to the oligodynamic group of metals, those having a toxic effect on microorganisms at relatively low concentrations. This general mechanism is common amongst heavy and/or noble metals such as silver, zinc, copper and lead. Oligodynamic metals have similar effects on microorganisms; however the primary mode of action of each metal may be different. For example, copper ions are often reported to cause damage to cell membranes but do not cause damage to DNA (Christophe Espírito Santo *et al.*, 2012). Synergy is displayed between silver and copper ions, with an increase in efficacy demonstrated against pathogenic bacteria (Huang *et al.*, 2008).

Silver and its ionic state

In order for silver to display antimicrobial properties, it must be in its ionized form (Lok *et al.*, 2007; Rai *et al.*, 2009). Silver in its non-ionized form is inert (Guggenbichler *et al.*, 1999); however any contact with moisture will result in the formation of silver ions. Environmental moisture is considered sufficient for efficacy (Radheshkumar and Munstedt, 2005), thus any silver containing compound or additive in contact with moisture will display antimicrobial properties. The speed by which silver ions are released will depend on the antimicrobial compound.

BioCote technology is based on silver ions held within a phosphate glass matrix. Ionic release from phosphate glasses and zeolites (as ion exchangers) can be considered relatively controlled when compared with nanosilver particles or elemental silver (Nagy *et al*., 2011). This is also true for silver based medicinal creams and/or lotions which can be tailored to have fast or slow ionic release

depending on the target application of the medicine.

Ions entering the cell

The silver cation may gain entry to the cell via transmembrane proteins that normally function to transport ions other than silver ions. Transmembrane proteins such as CopB-ATPase from *Enterococcus hirae* have been shown to transport silver ions although its putative function is a copper transporter (Solioz and Odermatt, 1995). The CUS silver and copper ion efflux transporters are also able to transport silver and copper ions in *E. coli* and other organisms (Mealman *et al.*, 2012).

DNA association

Klueh (2000) proposed the silver cations may enter the microbial cell and intercalate between the purine and pyrimidine base pairs. This intercalation disrupts the hydrogen bonding between the two anti-parallel strands and denatures the DNA molecule, suggesting no interaction with phosphate groups.

The significance of any DNA interaction by the silver cation when considering all other potential biocidal mechanisms is unclear, although interaction between silver ions has been reported multiple times (Izatt *et al.*, 1971; Rahn *et al.*, 1973; Thurman *et al.*, 1989; Zavriev *et al.*, 1979; Woo kyung jung, *et al.*, 2008).

Silver ions binding and denaturing proteins

Silver ions bind to thiol groups (-SH) in proteins which can lead to deactivation of enzymes. Silver forms stable S-Ag bonds with thiol-containing compounds in the cell membrane that are involved in transmembrane energy generation and ion transport (Klueh *et al.*, 2000). Amino acids such as cysteine and other thiol group containing compounds neutralize the activity of silver ions and their effectiveness against bacteria (Liau *et al.*, 1997). In contrast, disulphide bond containing amino acids and non-sulphur containing amino acids and were unable to neutralize silvers antibacterial activity. These and other findings strongly imply the interactions of silver ions and thiol groups in enzymes play an essential role in silver's antimicrobial activity although other cellular components such as hydrogen bonding may also be involved (Furr *et al.*, 1994). Silver ions are also implicated in the release of potassium ions from microorganisms, further suggesting interactions between enzymes and proteins associated with cytoplasmic or plasma membranes contribute to the biocidal activity of silver (Feng at al., 2000; Miller *et al.*, 1957; Schreurs and Rosenberg, 1982).

Protein interaction

Silver ions take part in catalytic oxidation reactions that result in the formation of disulfide bonds (R-S-S-R). This occurs via reactions between oxygen molecules in the cell and hydrogen atoms of thiol groups, water is released as a product and two thiol groups become covalently bonded to one another through a disulfide bond (Davies and Etris, 1997).

The silver-catalyzed formation of disulfide bonds can lead to changes in protein structure and the inactivation of key enzymes, such as those needed for cellular respiration (Davies and Etris, 1997). A number of proteins such as the 30S ribosomal subunit protein, succinyl coenzyme A synthetase, maltose transporter (MalK), and fructose bisphosphate adolase were identified as having decreased expression when treated with a 900 ppb Ag+ solution (Yamanaka *et al.*, 2005)

Recent investigation into silvers biocidal mode of action

Morones-Ramirez *et al., (*2013) reported a number of experiments detailing potential mechanisms by which silver ions exert antibacterial action, centering on the production of free radicals and their downstream effects. A brief summary of these mechanisms is shown below. It is worth noting that the author reports several incidences where silver ions have re-potentiated antibiotics against *E. coli* which had previously displayed resistance.

- General increased hydroxyl radical production.
- Increased membrane permeability.
- Disruption of internal and external iron homeostasis.
- Disruption of the electron transport chain (ECT).
- Disruption of the TCA (tricarboxylic acid) cycle.
- Morphological damage viewable via Electron Microscopy.
- Induction of aggregated and mis-folded proteins.
- Potentiation of antibiotics to resistant strains.

Methods

Antibacterial analysis

Control and silver ion containing material was assessed via the international standard ISO22196:2011 (Measurement of antibacterial activity on plastics and other non-porous surfaces). Assessment of the material was performed against three organisms, *Escherichia coli*, *Staphylococcus aeureus* and *Pseudomonas aeruginosa.* Briefly, three samples of control and three samples of treated material were prepared for each organism. Samples were then inoculated with known quantities of test organism and incubated at 37°C for 24 hours. After incubation remaining cells were washed from the surface of the assessed material, diluted as appropriate and enumerated on agar plates. Results were then expressed as percentage and LOG_{10} reductions against initial (total number of organisms loaded onto the material) and the control (against organisms recovered from non-treated, control material).

Fluorescence microscopy imaging

Preparation of samples

Polyethylene was dosed with BioCote antimicrobial masterbatch at appropriate rate and plaques manufactured. Plaques were cut into 10mm x 10mm squares using a hacksaw. These squares were filed to remove edges and burrs. Approximately 25 silver ion containing and 25 control squares were generated.

Growth of test organisms and inoculation

Test bacteria were grown in overnight broth culture and diluted to 1:100 in Luria Broth. Cultures were incubated under agitation for 2 hours at 37°C.

Treated and untreated polyethylene samples were attached to the bases of 12 well multiwall plates and test bacteria applied. These samples were then incubated for a further 3 hours at 37°C. Samples were washed by inverting onto water wells for 2 minutes. Bacteria on samples were then stained with Baclight kit Live/dead kit (Life Technologies) for 15 minutes at room temperature. Samples were then washed in water for two minutes to remove excess stain.

For cell staining, solution B was used from the Molecular probes kit L7007 at 1.50 dilution, the viable cells are stained green with 1.67mM Cyto9 and dead cells (red) with 18.3mM propidium Iodide.

Cells were mounted with the supplied mounting oil under a square coverslip and imaged on a Leica DMRE widefield epi-fluorescence microscope with a 100x plan apo oil immersion objective.

Results

Antibacterial ISO22196:2011

A summary of the results of antibacterial analysis are shown below in Table 1. Broad spectrum antibacterial performance can be considered validated due to efficacy against standard test organisms *E. coli* and *S. aureus,* as representatives of Gram negative and Gram positive bacteria respectively. Good efficacy was also demonstrated against the target organism in this application, *Pseudomonas aeruginosa*.

Species	LOG_{10} Reduction (control)	% Reduction (control)	LOG_{10} Reduction (initial)	% Reduction (initial)
P. aeruginosa	2.48	99.67%	1.59	97.46%
E. coli	≥ 3.94	≥ 99.99%	≥ 3.03	≥ 99.91%
*S. aureus (*MRSA*)*	≥ 2.98	≥ 99.89%	≥ 3.13	≥ 99.93%

Table 1. ISO22196:2011 antibacterial performance of BioCote treated material against three test organisms, P. aeruginosa, E. coli, S. aureus (MRSA). Results show log_{10} and percentage reductions against control (reduction when compared with organisms recovered from non-treated control material) and initial (reduction of the total number of organisms loaded onto the material).

Fluorescence microscopy imaging

Figure 1 displays the results of the imaging of *Pseudomonas aeruginosa* on control (left) and silver ion containing (right) polyethylene plaques. Green indicates living cells, stained with Cyto9, whilst red shows dead, stained with propidium Iodide.

Of the three organisms tested, *Pseudomonas* was able to adhere to the surface sufficiently for imaging in the 3 hour time frame.

Via the use of ImageJ, a public domain imaging tool, and the cell counter plugin we approximated in the control sample field a total of 91 cells, of which 27 (57%) were dead. In contrast, the treated sample displayed approximately 209 cells of which 196 (93.37%) were dead.

Discussion

We were then able to successfully demonstrate via microscopy, the antibacterial properties of silver ion treated polyethylene, technically equivalent to Armstrong Medical AquaVENT® material. Significant differences between silver biocide treated and control material was observed, demonstrating comparable levels of efficacy to the ISO22196:2011 results.

It was reported during the imaging that although every attempt had been made to provide flat, burr free surfaces for imaging, due to the non-uniform surface of the sawn polyethylene square raised edges still caused problems in adherence of the coverslip to the plastics surface.

Feedback from the University of Birmingham's Technology Hub Imaging Core outlined technical challenges in imaging bacteria on material of this type. Dr. Shaw commented that in order to reliably and consistently image similar material significant time would need to be invested in developing a robust method, of both sample preparation of microscopy technique. It was suggested this could form the basis of an MSc research project.

Pseudomonas aeruginosa was the sole organism to adhere sufficiently to the polymer surface, allowing imaging. It is believed that after three hours of growth the organism had initiated biofilm formation, which aided or possibly allowed adherence to the necessary levels for visualization. Both enteropathogenic *E. coli* and *Salmonella* strains used in this work failed to adhere sufficiently for imaging.

To achieve good a representative image a balance was sought between contact or incubation time of test organism and substrate, and the desire to demonstrate a timely bactericidal action. The three hour incubation time of the test organism and material has presumably allowed *P. aeruginosa* time to adhere (possibly via the formation of a biofilm), prior to accumulating sufficient cellular damage via the biocidal action of silver, resulting in the death of the cell and accumulating propidium Iodide stain.

The required balance of adherence, growth and required time for biocidal effect should be taken into account when considering the required times to visualize dead cells on treated material. BioCote has demonstrated that significant effects can be observed within 15 minutes (approximately 80%) with reductions of approximately 99.5% observed after 2 hours under laboratory conditions.

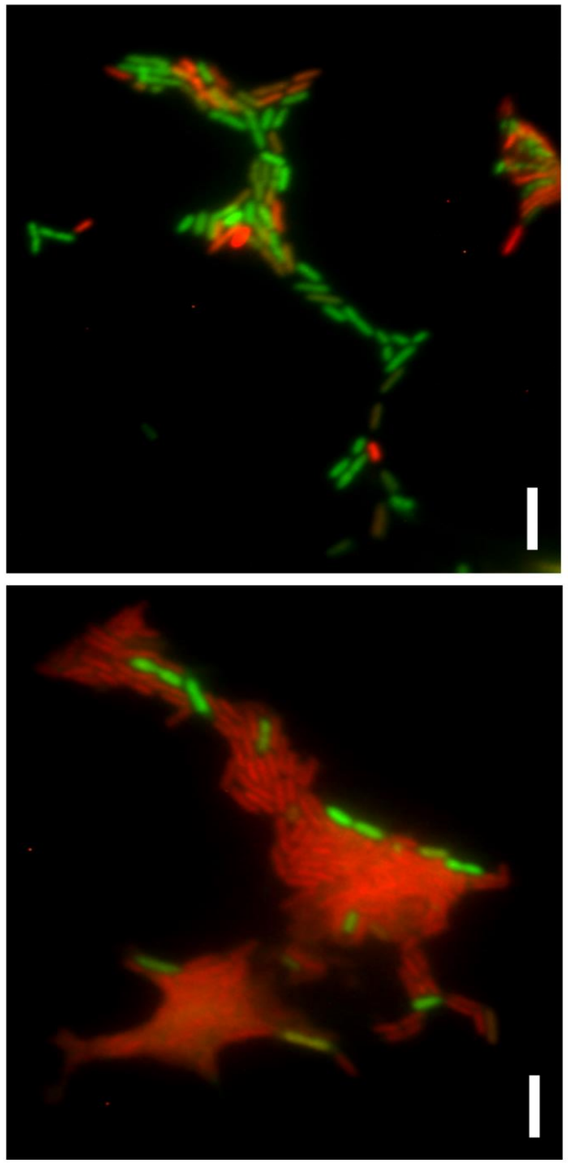

Figure 1. *Pseudomonas aeruginosa* imaged on a Leica DMRE widefield epi-flouresecnce microscope with a 100x plan apo oil immersion objective. Solution B was used from the Molecular probes kit L7007 at 1.50 dilution, the viable cells are stained green with 1.67mM Cyto9 and dead cells,(red), with 18.3mM propidium Iodide. The left hand image is control material without biocide; approximately 57% of cells are red stained. The right hand image, silver ion biocide treated, displays approximately 93.7% dead cells (stained red).

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