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Major Article

Antibacterial effect and proposed mechanism of action of a topical surgical adhesive

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Background: Medical adhesives effectively hold closed approximated skin edges of wounds from surgical incisions, including punctures from minimally invasive surgery. In addition, they have been reported to be antibacterial against gram-positive bacteria.

Methods: Using membrane filtration to capture all organisms after contact with 2-octyl cyanoacrylate product for 3 minutes, we quantified the number of survivors. Controls were performed to rule out that the noted level of kill was caused by carryover product in the test system.

Results: We found that the product kills >7 logs of gram-positive and gram-negative bacteria. The mechanism of action for the antibacterial effect is described as a function of very low water content.

Conclusions: As an antibacterial agent, the risk of nosocomial infection is greatly diminished, and an uneventful clinical result is facilitated. Bacterial growth cannot occur in the formulation and on contact death rapidly ensues as cellular water diffuses from the cell into the product.

Anderson et al[1] comprehensively reported and discussed strategies to prevent surgical site infections (SSIs): "SSIs are common complications in acute care facilities. SSIs occur in 2%–5% of patients undergoing inpatient surgery. Approximately 160,000-300,000 SSIs occur each year in the United States. SSI is now the most common and most costly healthcare acquired infection . . ."

One strategy to prevent an SSI is to use surgical adhesives to close approximated topical wounds. Rushbrook et al[2] reported on the antimicrobial effectiveness of polymerized 2-octyl cyanoacrylate against staphylococci and streptococci bacteria. We have extended these findings using the same test system to other gram-positive bacteria, namely *Corynebacterium* spp and *Staphylococcus epidermidis*. Evidence of the antimicrobial effect was inferred by Rusbrook et al because they observed a zone of inhibition around the solidified polymer on a lawn of bacteria. The objective of our work was to test expand on that work and further to explain a plausible mechanism of action for the observed antibacterial effect.

Killing time studies are typically used to quantify the antimicrobial effect of test material. An optimal microbial test system accounts for all the challenge organisms at the start and conclusion of the contact time so the microbial reduction in log 10 can be calculated. Accordingly, rather than solely rely on zone of inhibition testing,[2] we also used membrane filtration methodology to capture all survivors. The mechanism of action by which products of this type can be expected to have antibacterial properties is discussed. Water activity is predictive of whether an organism can survive or grow in a material. Accordingly, we have measured the water activity and Karl Fischer value of the product and discuss its meaning in terms of how we think 2-octyl cyanoacrylate kills bacteria.

METHODS

Zone of inhibition of the product placed on a lawn of bacteria

Challenge organisms

The challenge organisms are as follows: *Staphylococcus epidermidis* (ATCC 14990; ATCC, Rockville, MD), *Staphylococcus aureus* (ATCC 6538; ATCC), *Corynebacterium pseudodiphtheriticum* (ATCC 10701; ATCC), Methicillin-resistant Staphylococcus aureus (ATCC 33591; ATCC), *Klebsiella pneumoniae* (ATCC 4352; ATCC), *Escherichia coli* (ATCC 8739; ATCC), and *Pseudomonas aeruginosa* (ATCC 9027; ATCC).

A lawn for each organism was created onto Tryptic Soy Agar (TSA) (Becton Dickinson, Franklin Lakes, NJ) by streaking each suspension onto a TSA agar plate with a sterile swab (1 TSA plate per organism). A 20 μL aliquot of SurgiSeal (Adhezion Biomedical, Reading,

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PA) was aseptically placed onto each lawn via micropipette. The plate was observed, and the time it takes for polymerization for each organism was measured. Incubation was at 30°C-35°C for 48 hours followed by observation of each plate for a zone of inhibition. A 20 µL aliquot of sterile purified water (USP PW) was aseptically placed onto each lawn via micropipette as a control.

Microbiologic efficacy using uninitiated 2-octyl cyanoacrylate–based product against the same challenge organisms

Preparation of challenge organisms

The inoculua used for the preparation of all the challenge organisms were freshly picked, isolated colonies that had been grown in TSA and incubated 30°C-35°C for 18-24 hours. The target challenge was $\geq 10^6$ colony forming units (cfu). A representative single isolated colony was aseptically harvested with a sterile inoculation loop and suspended into sterile purified water and counted by membrane filtration.

Inoculation of the test material with clinically relevant microorganisms

The experiment was carried out on 20 replicates. A fresh isolated colony, as previously described, was picked with a sterile inoculation loop and transferred to a container containing approximately 0.35 mL of uninitiated product. After a 3-minute contact time, the entire contents of the inoculated sample of the product was transferred to 9.65 mL of sterile USP PW using a micropipette. Ten-fold serial dilutions were prepared. The final volume of tube 1 was 10.0 mL. The pH was taken with a pH strip, and the tube was immediately vortexed for approximately 15 seconds. Tube 1 was used to make the 10^{-1} dilution for tube 2. One mL from tube 2 was added to 9 mL sterile USP PW and vortexed. Additional 10-fold serial dilutions were made.

The number of surviving organisms in each tube was determined by membrane filtration (Table 1). Each dilution tube was membrane filtered through sterile 0.45-µm-Nalgene Filters (no. 145-2045; Thermo Fisher Scientific, Bridgewater, NJ) followed by 2 × 100 mL rinses with USP PW. The filter was placed onto TSA plates. The filtrate was also plated into TSA. The plates were incubated at 30°C-35°C for 48-72 hours, and the number of cfu present were counted and recorded. If survivors were present, a representative viable colony on the filter was confirmed to verify it was the same as the challenge organism using either VITEK (bioMérieux Inc., Cambridge, MA) identification or restreaking onto selective agar plate.

Residual carryover cyanoacrylate in the dilution tubes was ruled out by challenging the 10-fold serial dilution tubes with <100 cfu of each challenge organism. Filtration and incubation was as previously mentioned. A negative control substituted USP PW in place of the product. It determines the starting level of the inoculum used to challenge the product. The rest of the procedures previously listed were followed.

Water activity measurements

Measurements were made at 21°C with a Novasina LabTouch instrument (Novasina AG, Lachen, Switzerland). The water activity meter was calibrated using a 4-point calibration at the beginning of each day that the instrument was used. Each determination was made 5 times, and the average was recorded.

Measurement of the water content of the product sample by Karl Fischer analysis USP <921>, method I

The product was dissolved in a mixture of chloroform and methanol to determine the total water content of bound and unbound water.

RESULTS

A prominent zone of inhibition was observed against the 4-g positive bacteria tested (Fig 1).

The quantitative antibacterial effect against gram-positive and gram-negative organisms of uninitiated product is shown in Table 1.

The inoculum challenge used to challenge the product and negative control was in the range of 10^7-10^8 cfu. The negative control was USP PW. The membrane filtration counts were transformed to

Table 1
Antibacterial effect of the uninitiated product after a 3-minute contact time: detailed results versus *Pseudomonas aeruginosa*

Replicate No.	Log 10 survivors	Log 10 reduction	Neutralization challenge
1-19	0	8 – 0 = 8	+
20	1.48 = 1	8 – 1 = 7	+

P aeruginosa versus negative control	
Dilution	Count
10^{-4}	TNTC, TNTC
10^{-5}	>200, >200
10^{-6}	27, 58
10^{-7}	5, 3
cfu/10 mL	4.3×10^7
Log 10	7.63 = 8
Summary of kill against 7 clinically relevant organisms	Average (n = 20) log 10 reduction
Methicillin-resistant Staphylococcus aureus	8
Escherichia coli	8
P aeruginosa	8
Klebsiella pneumoniae	7
Staphylococcus epidermidis	7
Corynebacterium pseudodiphtheriticum	8
Staphylococcus aureus	8

NOTE. The filtrates were also plated and had no growth. The results for all organisms were equivalent as shown above. On challenge with <100 cfu, all dilution tubes were positive, meaning no carryover residual cyanoacrylate was present in the dilution tubes. The results for all the eight organisms tested were equivalent having ≥7 log kill.
+, positive; cfu, colony forming units; TNTC, too numerous to count.

Fig 1. Zone of inhibition. Bacteria included MRSA, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Corynebacterium pseudodiphtheriticum*, respectively. Polymer formation was complete after approximately 10 minutes.

Table 2
Water activity of the product in the presence of clinically relevant bacteria

Organism	Sample replicate no.	a_W reading at 21.5 ± 0.5°C
Escherichia coli	1	0.334
	2	0.326
	Average	0.330
Methicillin-resistant Staphylococcus aureus	1	0.319
	2	0.305
	Average	0.312
Staphylococcus epidermidis	1	0.334
	2	0.337
	Average	0.336
Pseudomonas aeruginosa	1	0.352
	2	0.343
	Average	0.348
Klebsiella pneumoniae	1	0.334
	2	0.332
	Average	0.333
Corynebacterium pseudodiphtheriticum	1	0.363
	2	0.344
	Average	0.354

Table 3
Percent water of product by Karl Fischer analysis

Sample weight (g)	Volume of Karl Fischer reagent (mL)	% water	Average % water
0.2718	0.081	0.16	0.16
0.1835	0.053	0.16	

log 10, and the kill was calculated relative to the negative control. After 3 minutes of contact time, the test samples of liquid, uninitiated, 2-octyl cyanoacrylate produced >7 log kill for *Escherichia coli*, Methicillin-resistant Staphylococcus aureus, *S epidermidis*, and *Corynebacterium* spp, with no survivors in any of the 20 replicates tested. *K pneumoniae*, *P aeruginosa*, and *S aureus* had a few survivors and produced on an average >7 log kill after 3 minutes of contact time. The water activity and Karl Fischer water level (Tables 2 and 3), respectively, are very low.

DISCUSSION

Other authors[2] have reported the antimicrobial effectiveness of polymerized 2-octyl cyanoacrylate against *Staphylococcus* spp and *Streptococcus* spp bacteria and surmised it be because of the strong electronegative charge of the cyanoacrylate monomer preferentially acting on the positively charged cell wall of gram-positive bacteria. Our findings are new because they were obtained by application of the uninitiated 2-octyl cyanoacrylate onto the lawn of challenge bacteria at neutral pH. We confirm the noted antibacterial effect and additionally report that *Corynebacterium* spp is also highly sensitive in our experimental design.

We note that the zone of inhibition for the gram-positive bacteria is obvious extending outward from the polymerized product. A limitation of the zone of inhibition methodology is that the antibacterial effect against the gram-negative bacteria, directly beneath polymerized product, if any, is not detectable. Therefore, it was important to overcome this limitation by using a test wherein the antibacterial effect is not obscured by the polymer.

A membrane filtration killing time test was used for this purpose. Accordingly, the challenge bacteria were added directly to the uninitiated liquid product for 3 minutes. The monomeric 2-octyl cyanoacrylate was prevented from polymerizing with USP PW at pH 6.1, and any surviving organisms were captured on a membrane filter.

The log reduction was calculated by subtracting the number of recovered cfu on the negative control membrane filter from the number of cfu recovered after a 3-minute challenge with the product. We confirmed that no organisms escaped detection by also plating the entire filtrate. To rule out false-negative results caused by residual monomeric 2-octyl cyanoacrylate, low numbers of each organism were shown to survive in the dilution system.

Our findings support the conclusion that the observed antibacterial effect found in our killing time membrane filtration test system is caused by the uninitiated monomeric form of 2-octyl cyanoacrylate and is not explained by electrostatic charge effects postulated in the zone of inhibition methodology. Furthermore, the antibacterial effect is broader than previously reported because both gram-negative and gram-positive bacteria are substantially killed in 3 minutes.

Water activity (a_w) is the partial vapor pressure of water in a substance divided by the standard state partial vapor pressure of water.[3] The standard state is most often defined as the partial vapor pressure of pure water at the same temperature. Using this particular definition, pure distilled water has a water activity of exactly 1. The concept of water activity was first described by Scott[4] when he showed that microorganisms had a limiting water activity level for growth. The concept is widely applied in the food industry and cosmetic industry. Now, a_w levels in drug ingredients are official.[5] Karl Fischer titration is a classic USP[6] titration method in analytical chemistry that uses colorimetric or volumetric titration to determine trace amounts of water in a sample. Karl Fisher analysis found only trace water content in agreement with the a_w results. Therefore, as appropriate, both water activity and Karl Fisher analysis can be used to predict the antibacterial effect against vegetative organisms.

Bacteria usually require at least 0.91 a_w to exist.[3,5] The product tested has a very low a_w of approximately 0.3 and only 0.16% water. Therefore, our results are explained by diffusion that is expected as the water from the cell migrates into the 2-octyl cyanoacrylate–based product leaving the cell barren. Death occurs because all biologic life depends on a hydrous environment for all synthesis and replication processes to operate. Accordingly, this is the proposed mechanism of action. Other physical effects, such as morphologic alteration and cellular destruction, caused by the product may also play a role in the antibacterial effect.

The bactericidal results reported here are therefore expected as a function of the product's main ingredient, 2-octyl cyanoacrylate, having a very low water activity. At 0.3 it is well below the 0.61 level for the most resistant organism and substantially below the USP published value of 0.91. Accordingly, the appropriate employment of cyanoacrylate-based products should be considered as part of the health care industry's strategy against SSIs. The results are similar to a microbial sealant composed from octyl and butyl cyanoacrylate.[7]

CONCLUSIONS

In addition to being a surgical adhesive used to close approximated wounds, 2-octyl cyanoacrylate rapidly kills bacteria known to cause nosocomial infection. The antibacterial effect is explained by the fact that by diffusion cells lose water essential for life.

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