Original Article

Anti-Fungal Effect of N-Butyl Cyanoacrylate Glue in Ophthalmic Application: An Experimental Study

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Objective: To determine anti-fungal effect of butyl cyanoacrylate glue in vitro in common fungi related to corneal infections.

Materials and Methods: The authors transferred the following fungi; *Fusarium* spp. (2 isolates, no.1431 and no.2861), *Aspergillus flavus* (no.5573) and *Curvularia* spp. (no.1631) into Sabouraud dextrose agar plates. Then, a single drop of butyl cyanoacrylate glue was dropped on the center of studied plates in various volumes (5, 15, 25, and 35 µl), two copies for each condition. Anti-fungal activities were determined by measuring inhibition zones (inhibition zone diameter [IZD]) and the ratio between IZD and direct contact diameter [DCD] after 48-hour incubation at room temperature.

Results: Mean of IZD of all fungi at glue volumes of 5, 15, 25, and 35 µl were 10, 23, 22.75, and 26.86 mm, respectively. The maximum IZD was found in *A. flavus* 5573 at glue volume of 35 µl (36.50 mm) and the minimum IZD was found in *Curvularia* spp. 1631 at glue volume of 5 µl (0 mm). The IZD/DCD ratio were directly increased with higher glue volume in dose-dependent relationship. Every 1 µl of additional glue volume, increased IZD for 0.5 mm with R-square 0.78 (95% CI 0.30 to 1.31).

Conclusion: Butyl cyanoacrylate glue demonstrated anti-fungal effects in dose-dependent fashion in vitro experiment. The clinical application of glue for small fungal infectious corneal perforations might be considered as an optimal treatment, particularly for developing countries and areas with shortage of corneal tissue.

Keywords: N-butyl cyanoacrylate glue, Anti-fungal effect, Ophthalmic sealing, Synthetic adhesive compound, Corneal perforation

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N-butyl cyanoacrylate glue is a form of medical grade synthetic glue, which is commonly used in different areas of medicine mainly for sealing surgical wounds[1], fistulas[2], doing endovascular embolization[3], and for connecting parts of tissue together[4]. This particular glue is also widely used in the area of ophthalmology for similar purposes. The chemical structure of butyl cyanoacrylate composes of alkyl side chains containing 4 carbon ester and active double bond with oxygen. This active bond plays an important role in the polymerization phase. Regarding to several advantages of cyanoacrylate glue consisting of strong bonds, rapid polymerization, commercially available, low cost, and simple application and well tolerated, it is considered as a primary treatment for small corneal perforation (less than 3 mm) and descematocoele, especially in emergency situation[5]. The clinical application of cyanoacrylate glue for corneal sealing improves visual outcomes, reduces enucleation rate and also reduces unplanned tectonic keratoplasties[5,6].

Interestingly, Chen et al found that cyanoacrylate derivatives had bacteriostatic effects against gram positive organisms including *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Mycobacterium chelonae*, however the effects disappeared in gram negative organisms consisting of *Pseudomonas aeruginosa* and *Escherichia coli*[7]. The results imply that cyanoacrylate glue might be safe and has benefit for clinical use in cases of infectious corneal perforations that were caused by gram-positive and mycobacteria.

In developing countries, the incidence of fungal keratitis was pre-dominated, and in comparison to bacterial keratitis, fungal keratitis associated with larger infiltration or scar size and higher perforation rate than bacterial keratitis[8]. Cyanoacrylate glue also has been used to manage corneal perforations

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from fungal infections[9]. However, the effect of cyanoacrylate glue on fungal infection has not been explored. This study was designed to demonstrate the anti-fungal properties of butyl cyanoacrylate glue against the common causing keratitis fungi including *Fusarium* spp., *Aspergillus* spp. and *Curvularia* spp.

Materials and Methods

This experimental study was conducted at laboratory of microbiology department of Ramathibodi Hospital and was approved by the Ethics Committee of Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

One or two strains of each following fungi were grown at room temperature on Sabouraud dextrose agar [SDA] for five to seven days until conidia were produced: *Fusarium* spp. (two isolates, RAMA 1431 and RAMA 2861), *Aspergillus flavus* (one isolate, RAMA 5573), and *Curvularia* spp. (one isolate, RAMA 1631). These fungi were isolated from fungal keratitis patients who were admitted at Ramathibodi Hospital. Fungal spores were then collected by flushing each well with sterile distill water and the diluents were collected until reaching 0.5 on the McFarland scale. Next, they were inoculated by confluent streaking on SDA media.

Synthetic adhesive compound, N-butyl-2-cyanoacrylate (GlueStitch®, British Columbia, Canada) was used in this study. Once after inoculating fungi on SDA as described, a single drop of 5, 15, 25, and 35 µl of N-butyl cyanoacrylate glue was placed at the center of each culture plate and subsequently incubated for 48 hours at room temperature before analysis. Two copies of culture plates in each glue volume were done for each fungus.

Anti-fungal activities of glue were evaluated by measuring inhibition zone diameter [IZD] (mm) on the plates by single technician at 48 hours after incubation. IZD referred to the maximum diameter of halo area at the center of the culture plate. The authors also measured glue diameter on each plate and defined as a direct contact zone diameter [DCD] (mm) as shown in Figure 1. To demonstrate dose-dependent correlation, the authors calculate the ratio of IZD and DCD. The correlation between volume of butyl cyanoacrylate glue and IZD/DCD ratio were analyzed by using linear regression analysis. All of analysis was performed by Stata version 14.2. The Statistically significant was assigned at p-value <0.05.

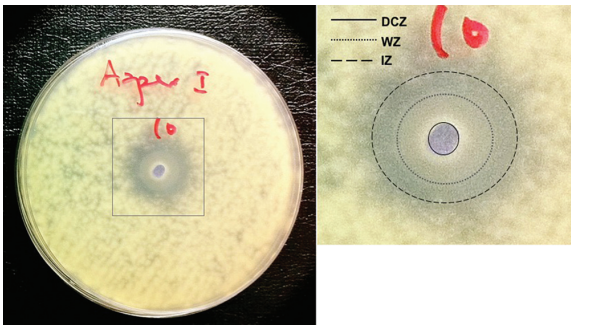

Figure 1. Three visible zones on experimental plate; 1) direct contact zone [DCZ] was the area that directly contacted by butyl cyanoacrylate glue, 2) whitening zone [WZ] was the area that supposed to be the area of glue diffusion in culture media before complete solidification, and 3) inhibitory zone [IZ] was the clear area without visible fungal colonies.

Results

Of all 32 studied plates from four different glue volumes on four different fungi, with two copies for each condition, mean of IZD of all fungi at N-butyl cyanoacrylate glue volumes of 5, 15, 25, and 35 µl were 10, 23, 22.75 and 26.86 mm, respectively. The maximum IZD was found in *A. flavus* 5573 at glue volume of 35 µl and the minimum IZD was found in *Curvularia* spp. 1631 at glue volume of 5 µl (0 mm) as shown in Table 1.

Table 2 demonstrated the correlation between glue volume and IZD/DCD ratio. The IZD/DCD ratio were dependently increased with higher glue volume (Figure 2). Every 1 µl of increasing glue volume, resulted in increasing IZD 0.5 mm with R-square 0.78, 95% CI -0.30 to 1.31. However, the authors could not

Table 1. Direct contact diameter [DCD] (mm) and inhibition zone diameter [IZD] (mm) of cyanoacrylate glue at volume of 5, 15, 25, and 35 µl

Fungi, isolation no.	DCD (mm), mean ± SD (n = 2)				IZD (mm), mean ± SD (n = 2)			
	5 µl	15 µl	25 µl	35 µl	5 µl	15 µl	25 µl	35 µl
Aspergillus flavus, 5573	4.00 (0.00)	7.00 (0.00)	7.50 (0.71)	9.00 (0.00)	16.00 (1.40)	30.00 (0.00)	28.50 (0.71)	36.50 (0.71)
Fusarium spp., 2861	4.25 (0.35)	7.00 (0.00)	8.00 (0.00)	8.00 (0.00)	15.00 (1.41)	34.00 (1.41)	29.50 (2.12)	34.00 (2.83)
Fusarium spp., 1431	4.00 (0.00)	5.75 (0.35)	7.50 (0.71)	8.50 (0.71)	9.00 (0.00)	12.00 (0.00)	16.00 (0.00)	17.00 (0.00)
Curvularia spp., 1631	5.00 (0.00)	5.75 (0.35)	7.00 (0.00)	8.50 (0.71)	0.00 (0.00)	16.00 (2.83)	17.00 (2.83)	20.00 (2.83)
Average	4.31	6.37	7.50	8.50	10.00	23.00	22.75	26.86

Table 2. Ratio between mean direct inhibition zone diameter [IZD] (mm) and mean contact diameter [DCD] (mm) of cyanoacrylate glue at volume of 5, 15, 25, and 35 µl

Fungi, isolation no.	Mean IZD/mean DCD			
	5 µl	15 µl	25 µl	35 µl
Aspergillus flavus, 5573	4.00	4.29	3.80	4.05
Fusarium spp., 2861	3.53	4.86	3.69	4.25
Fusarium spp., 1431	2.25	2.09	2.13	2.00
Curvularia spp., 1631	0.00	2.78	2.43	2.35
Average	2.44	3.43	3.01	3.16

find statistically significant increasing of IZD when the glue volume was over 15 µl. Therefore, the most effective volume in our studied was 15 µl.

At the glue volume of 15 µl, *Fusarium* spp. 2861 showed the highest level of IZD followed by, *A. flavus* 5573, *Fusarium* spp. 1431, and *Curvularia* spp. 1631 as demonstrated in Figure 3.

Discussion

For over 40 years since the first report of using cyanoacrylate adhesive on human eye by Webster et al in 1968[10], there are plenty of studies emerging with various indications. The most common application is sealing corneal perforation followed by descematoceles, leaking blebs, stromal thinning, wound leaks, exposure keratopathy, cataract wound closure, scleral buckle attachment, temporary tarsorrhaphy, punctal occlusion, blepharoplasty, and skin closure after DCR[5,11]. However, the studies on biological properties of cyanoacrylate glue are limited.

From this study, the authors reported a novel property of butyl cyanoacrylate glue. The authors proved that N-butyl cyanoacrylate glue had anti-fungal property against four different isolates of filamentous fungi; *Aspergillus*, *Fusarium*, and *Curvularia*. The authors explored the anti-fungal effect in various glue volumes and found the correlation between volume of glue and inhibition zone diameter. When the authors applied larger amount of glue on the plate, the larger inhibition zone was observed as "dose-dependent phenomenon". From linear regression analysis, every 1 µl of increasing glue volume, resulted in increasing IZD 0.5 mm and this correlation can explain 78% of all data. However, the authors found no statistical significance. The glue volume of more than 15 µl trend to produce steady inhibition zone diameters. In vivo situation, it has been estimated that 4 to 8 µl of cyanoacrylate glue is enough for covering deep corneal ulcer[12]. In combination with our study results, the optimal volume of applied glue that contained in anti-

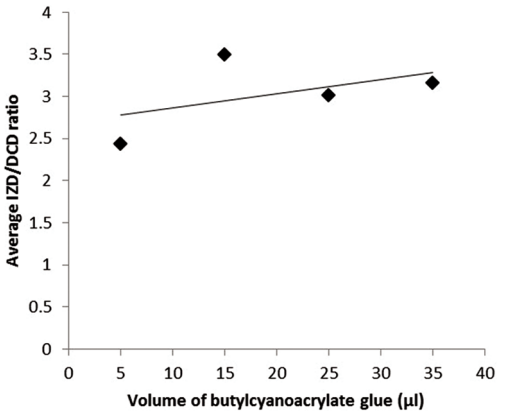

Figure 2. Relationship between average inhibition zone diameter [IZD]/direct contact zone diameter [DCD] and butyl cyanoacrylate glue volumes (µl).

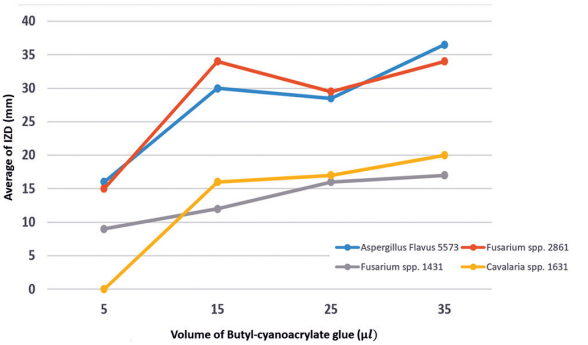

Figure 3. Relationship between IZD (mm) and butyl cyanoacrylate glue volumes (µl) of all fungi.

fungal effect in dose dependent fashion may range from 5 to 15 µl. As the authors expected, different fungi and different isolates from the same genus showed different IZD which is similar to the previous researches that studied on the bacteriostatic effects of cyanoacrylate glues[7,13-15].

By considering IZD, the authors found that *Aspergillus* and *Fusarium* were more susceptible to cyanoacrylate glue than *Curvularia*. The authors are unable to conclude whether butyl cyanoacrylate glue has fungistatic or fungicidal effect by using dilution technique due to rapid polymerization of glue after contact with water or weak base. Additionally, the authors decided not to do further subcultures from inhibition zones for testing fungicidal effect because in contrast to bacteria, fungi have spores and mycelia that can easily spread on the broth surface. Therefore, there was a possibility to produce false positive result that would have been difficult to interpret. Although the mechanism of anti-fungal effects of butyl cyanoacrylate

glue has not been proposed before, the authors hypothesize that the effect is derived from cytotoxicity of by-products releasing during glue polymerization. The toxic derivatives comprise of formaldehyde and unreacted cyanoacrylate monomers similar to what is postulated to play a role in the bacteriostatic effect[7,14,16]. Furthermore, cell wall of fungi does not compose of lipopolysaccharide layer, which is suspicious as a resistant factor for glue to attach on and to bind with free amino and/or hydroxyl groups in cell wall of gram-negative bacteria[14].

The authors noticed the faint-whitening zone surrounding the glue that was dropped on the center of all culture plates (Figure 1). This finding was possibly caused by the diffusion of the glue through the media before complete polymerization. Cyanoacrylate glue polymerizes within 1 to 2 second after contact with water or weak base, then take approximately 10 to 20 seconds to complete the reaction[5,11]. The whitening zone was found only in vitro situation and was previously reported from Romeo et al[13] and Chen et al[7].

GlueStitch® in our study was a pure butyl cyanoacrylate without special additives such as plasticizers, accelerators, thickeners, stabilizers, or primers. The authors concluded that the anti-fungal effects were entirely generated from butyl cyanoacrylate. However, different commercial preparations may include some additives, then their anti-fungal properties might be variable. It has been known that sulfur dioxide, which the compound contains as a stabilizer, apparently enhances antibacterial property[14]. As the authors know, the smaller side chain length derivatives contain, the faster they polymerize and the higher amount of toxic substance they produce[16]. There are still the knowledge gap for further studies on this topic in different commercial butyl cyanoacrylate glues as well as different side-chain cyanoacrylate derivatives.

Although cyanoacrylate glue has not been approved by Food and Drug Administration [FDA] for small-sized corneal perforation, there are several evidences supporting safety and efficacy of the glue for that specific indication[9,5,17]. Sharma et al demonstrated the successful outcome after using butyl cyanoacrylate glue in fungal keratitis was as high as 83.3% (five of six cases)[9]. Concern issues in infectious corneal perforations include, 1) the underlying tissue that always be necrotic and this may impede glue from building a strong attachment, 2) the colonization of microorganisms from extended-schedule wearing contact lens, 3) the drug penetration of topical eye drops after glue application, which would unavoidably decrease anatomical barriers, and 4) the difficulty to observe clinical progressions under the opaque glue. Several studies reported secondary infection after glue applications of which most of them were bacteria in nature such as *S. aureus*, Haemophilus influenzae, and few of them caused by fungi[5,9,18,19]. Other minor side effects of cyanoacrylate glue applications are giant papillary conjunctivitis[20], foreign body sensation, corneal inflammation, stromal neovascularization, and anterior chamber inflammation, which vary upon individual sensitivity, amount of utilized glue, type of glue, and site of application[7,15]. Despite all drawbacks, the benefits of the adhesives always outweigh, particularly for emergency situations or in areas with shortage of corneal tissues.

In conclusion, butyl cyanoacrylate glue in optimal volume contains anti-fungal property in vitro experiment against common filamentous fungi including *Aspergillus*, *Fusarium*, and *Curvularia*. However, the in vivo effect may vary on several factors consisting of severity of infection, fungal strains, application volume, and technique. Continuous development of new derivatives with or without additives to minimize drawbacks and enhance further benefits of cyanoacrylate glue would be promising for ophthalmologists to comfortably implement the glue for patients with infectious corneal perforations.

What is already known on this topic?

N-butyl cyanoacrylate glue is a form of medical grade synthetic glue, which is commonly used in different areas of medicine mainly for sealing surgical wounds, fistulas, doing endovascular embolization, and for connecting parts of tissue together. Cyanoacrylate derivatives have demonstrated bacteriostatics effect against gram positive organisms including *S. aureus*, *S. pneumoniae*, and *M. chelonae*. The results imply that cyanoacrylate glue is safe and has benefit for clinical use especially in cases of gram positive and mycobacteria infectious in corneal perforations. Recently, cyanoacrylate glue also has been used to manage corneal perforations from fungal infections. However, the effect of cyanoacrylate glue on fungal infection has not been explored to anti-fungal effect properties such as IZD, DCD, and the ratio between IZD and DCD to apply this information into a clinical practice.

What this study adds?

This study was designed to demonstrate the anti-fungal properties of butyl cyanoacrylate glue

against the common causing keratitis fungi including *Fusarium* spp., *Aspergillus* spp., and *Curvularia* spp., demonstration of the optimal volume of synthetic glue that we can be use safety in dose dependent fashion, and correlate among volume of N-butyl cyanoacrylate glue and anti-fungal effect in vitro by measuring inhibition zones (IZD), DCD, and the ratio between IZD and DCD.

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Potential conflicts of interest

The authors declare no conflict of interest.

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