

In-vitro evaluation of antibacterial potential of cyanoacrylate tissue adhesives for intraoral wound closure

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Abstract

Background: Cyanoacrylate tissue adhesives have been used as a substitute to silk for intraoral wound closure. Placement of sutures provides a corridor for accumulation of microorganisms into tissue which leads to infection. Cyanoacrylate-based adhesives exhibit many properties of an ideal wound closure agent, minimizing the problems generated by suturing thread. The antimicrobial properties of cyanoacrylates have been extensively assessed in other fields of medicine. However, there is a dearth in the literature on the antibacterial effect of cyanoacrylates in oral environment against oral microflora. **Aim:** To assess the antibacterial properties of two commonly used formulations of cyanoacrylate tissue adhesives against oral pathogens. **Materials and Methods:** Iso-amyl cyanoacrylate and a blend of n-butyl and 2-Octyl cyanoacrylates were applied on sterile filter paper discs and placed on culture plates. Plates for aerobic & anaerobic bacterial cultures were incubated in blood agar & Brain-Heart infusion agar respectively. Following incubation period, the bacterial inhibitory halos were measured in millimeters. In order to evaluate the bactericidal efficacy, samples were collected from the inhibitory halos and re-cultured on new bacterial culture plates.

Antibacterial activity was assessed against five bacteria: *A.actinomycetemcomitans*, *P.gingivalis*, *T.forsythia*, *L.amylovorus* and *S.aureus*. Statistical analysis used: The data collected was analysed using Mann Whitney u test. **Results:** Cyanoacrylates demonstrated potent inhibitory effects against all test organisms. The zones of inhibition against gram positive bacteria were found to be larger than gram negative bacteria. The bactericidal activity of Iso amyl cyanoacrylate was found to be more potent than n-butyl + 2 octyl cyanoacrylate.

Conclusions: Due to its potent antibacterial properties, cyanoacrylate tissue adhesives can be considered as appealing alternatives to silk sutures for intraoral wound closure and help prevent postoperative.

Keywords: Cyanoacrylates, tissue adhesive, antibacterial, Periodontal Microflora, oral pathogens.

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Introduction

Oral cavity is colonized by more than 10^9 bacteria and contains a remarkably diverse microbiome, with more than 700 species reported (1). Chronic periodontitis is a common oral disease with diverse bacterial etiology. Oral and periodontal surgical sites being moist and favorable for accumulation and retention of microorganisms, are most susceptible to post-surgical infection and delayed wound healing. Bacterial colonization of sutures might lead to bacteremia and has been reported to contribute to post-surgical complications in dentoalveolar and periodontal surgeries (1).

Application of sutures requires passage of a foreign material through tissue which predisposes tissues to extreme reactivity. This also allows for the retention of microorganisms into the tissue which might lead to infection (2). Silk sutures sometimes exhibit the phenomenon of 'wicking' and can be a site of retention and ingress of bacteria.(3) Cyanoacrylates have been used as an alternative to braided silk, which is the most common suture material used for closure of oral wounds (3). Cyanoacrylates are tissue adhesives that were synthesized in 1959 by Coover et al. which demonstrate properties of an ideal wound closure agent. These are liquid monomers that polymerize on contact with wound moisture to form a solid bond. These solidified adhesives unite and hold the incised tissues stably, avoiding penetration of foreign bodies, thus promoting wound healing and vascularization(4,5). This favours clot stabilization and provides aesthetics to the surgical site (4).

An ideal surgical tissue adhesive must meet the following criteria: strong binding strength, ease of application, tissue biocompatibility, biodegradable and reasonable cost. Cyanoacrylates demonstrate most of these properties, giving them an edge over the conventional wound closing agents. These adhesives have strong adhesion to tissues in the presence of moisture, workable polymerization time, haemostasis, enhanced elimination of dead space, bacteriostatic ability, reduction in postoperative pain and biodegradability (6).

The use of cyanoacrylate-based adhesives in periodontal surgery, has demonstrated ease and efficiency, minimizing the problems generated by suture thread. The antimicrobial properties of cyanoacrylate tissue adhesives have been extensively investigated in other fields of medicine (7,8) However, there is a dearth in the literature on the antibacterial effect of cyanoacrylates in oral environment against the oral microflora. The present study was undertaken with the aim of assessing antimicrobial properties of two commonly used formulations of cyanoacrylate tissue adhesives against oral pathogens.

We hypothesized that cyanoacrylate tissue adhesives express significant antibacterial properties against oral pathogens and the null hypothesis was that cyanoacrylate tissue adhesives would not possess significant antibacterial properties against oral pathogens.

Materials and Methods

Two cyanoacrylate tissue adhesives were studied in this in vitro study: n-butyl + 2-octyl cyanoacrylate (B+OC) (PeriAcryl®90-HV) and iso-amyl cyanoacrylate (AC) (VERIBOND®) [fig 1a & 1b]. Six microliters of cyanoacrylate adhesive was applied on standard filter-paper discs, using micropipettes under sterile conditions.



Figure 1. Materials used: figure1a. n -butyl + 2-octyl cyanoacrylate



figure1b. Iso Amyl 2- cyanoacrylate

Previously cultured anaerobic colonies of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* & *Tanarella forsythia* were incubated in Blood agar plates and aerobic colonies of *Lactobacillus amylovorus* and *Staphylococcus aureus* were incubated in Brain-Heart infusion agar. In each agar plate, a sterile blank disc without adhesive was placed in the center as a control. Six additional discs

with six microliters of adhesive on each disk were placed onto the cultures: three with polymerized (solidified) B+OC and three with polymerized AC. To achieve prior polymerization of cyanoacrylate, the adhesive-soaked discs were exposed to air under a sterile hood for 10 min before being placed on the cultures.

Plates for anaerobic and aerobic bacterial cultures were incubated at 37°C for 48 & 24 hours respectively. After the completion of incubation period, the bacterial inhibitory halos were measured in millimeters (Fig 2a-e). In order to evaluate whether the bacterial inhibitory halos were the result of mere bacteriostasis or actual bactericidal effects, samples were collected from the clear agar within the inhibitory halos and re-cultured on new bacterial culture plates. The new plates were incubated at 37°C and analyzed after 48 h for anaerobic bacteria & after 24h for aerobic bacterial cultures (Fig 3a-e). Finally, the bactericidal activity was measured by calculating the percentage of plates with no bacterial growth.

Mann Whitney U test was used to analyse the collected data.

Figure 2. Bacterial inhibitory halos against :

figure 2a. *Streptococcus aureus*

figure 2b. *Lactobacilli amylovorus*

figure 2c. *Tanerella forsythia*

figure 2d. *Porphyromonas gingivalis*

figure 2e. *Aggregatibacter actinomycetemcomitans*

With a blank disk in the center, B+OC on left side and AC right side.



figure 2a. *Streptococcus aureus*

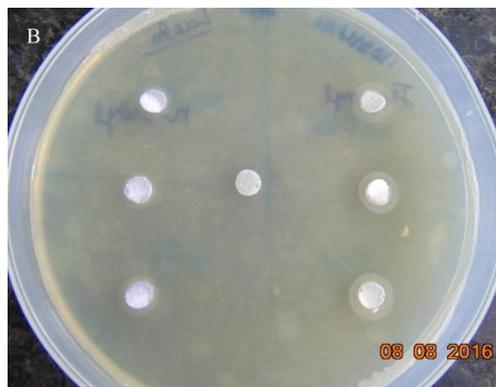


figure 2b. *Lactobacilli amylovorus*



figure 2c. *Tanerella forsythia*



figure 2d. *Porphyromonas gingivalis*

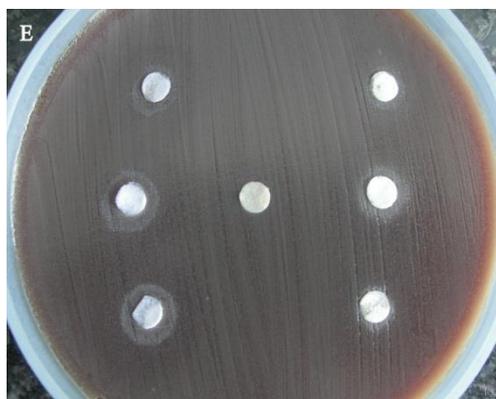


figure 2e. *Aggregatibacter actinomycetemcomitans* with a blank disk in the center, B+OC on left side and AC right side.

Figure 3. Bactericidal activity against :
figure3a. *Streptococcus aureus*
figure3b. *Lactobacilli amylovorus*
figure3c. *Tanerella forsythia*
figure3d. *Porphyromonas gingivalis*
figure3e. *Aggregatibacter actinomycetemcomitans*
 with B+OC on left side and AC on right side.



figure3a. *Streptococcus aureus*



figure3b. *Lactobacilli amylovorus*



figure3c. *Tanerella forsythia*



figure3d. *Porphyromonas gingivalis*

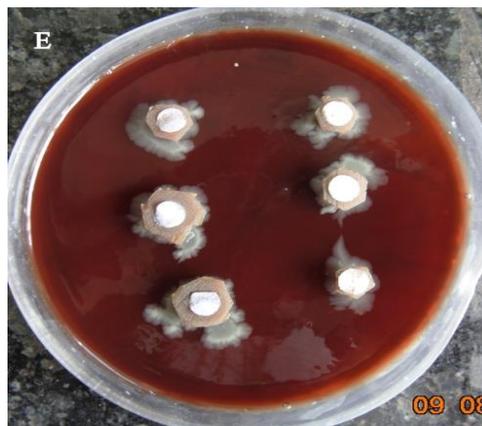


figure3e. *Aggregatibacter actinomycetemcomitans*
 with B+OC on left side and AC on right side.

Results

Table.1 shows the mean and standard deviation (SD) of the inhibitory halos (mm) for B+OC and AC for each microorganism studied. Under the conditions employed, both formulations of cyanoacrylate had a potent inhibitory effect against all test organisms. The diameter of inhibition zone ranged from 8 – 22 millimetres. The antibacterial efficacy of the two formulations was comparable (graph 1). A the test microorganisms, the zones of inhibition against Gram positive bacteria (*Staphylococcus aureus* & *Lactobacillus amylovorus*) were found to be larger than the Gram negative bacteria (*Aggregatibacter actinomycetemcomitans*, *Tanerella forsythia* & *Porphyromonas gingivalis*).

The bactericidal activity of AC ranged from 0% to 100% whereas B+OC showed bactericidal activity of upto 50% against the tested organisms (graph2). The bactericidal activity of AC was found to be more potent than B+OC against *P.gingivalis* and *T.forsythia*. Amongst the tested microorganisms, both formulations of cyanoacrylate showed maximum bactericidal activity against *P.gingivalis* and least against *L.amylovorus*.

Table 1 Individually the formulations showed potent inhibitory effect against all test organisms. Comparison between the groups did not reveal statistically significant results.

Mean and standard deviation (SD) of inhibitory halos (mm), comparing n-butyl + 2 octyl cyanoacrylate with Iso amyl cyanoacrylate against the microorganisms in question					
Bacteria	n-butyl + 2 octyl cyanoacrylate		Iso amyl cyanoacrylate		p-value
	Mean	SD	Mean	SD	
<i>S.aureus</i>	13.33	(±4.041)	14.33	(±1.154)	0.73
<i>L.amylovorus</i>	19.66	(±0.577)	21.33	(±1.154)	0.56
<i>A.actinomycetemcomitans</i>	12.33	(±0.577)	9	(±1.000)	0.75
<i>P.gingivalis</i>	9	(± 0.000)	9	(± 0.000)	0.32
<i>T.forsythia</i>	11	(±1.000)	9	(±1.000)	0.25

Discussion

The adhesive property of cyanoacrylate was first recognized in the late 1950s (Coover et al, 1959). The initial shorter chain cyanoacrylates were found to cause inflammatory reactions and were replaced with longer chain formulations (2-octyl, n-butyl & iso-amyl cyanoacrylates) of the new generation adhesives which polymerize by an exothermic reaction when they come in contact with moisture, leading to a strong & flexible bond (8,9). It is indicated in passively approximated wound edges following surgical incisions and thoroughly cleansed trauma-induced lacerations.(10)

The antimicrobial property of cyanoacrylate-based tissue adhesives has been demonstrated in several fields of medicine such as Ophthalmology (7,11,12), Dermatology (8,13) and Orthopedics (14). The adhesive acts as an effective barrier to microbial penetration by Gram-positive and Gram-negative organisms (15) Clinical studies have demonstrated reduced infection rates associated with its use (15)

In periodontal surgical practice, the adhesive is applied to flap margins in non-polymerized form, which then polymerizes. Polymerization may occur by anionic or zwitter-ionic interactions by hydroxide or amine groups presented in the body, ultimately resulting in strong chains holding the two tissue surfaces together (16). In the current research methodology polymerized form was tested for the antimicrobial efficacy. Cyanoacrylate polymer decomposed to produce cyanoacetate and formaldehyde which diffused out producing inhibition halos even in a polymerized state. This research methodology was in accordance with the investigations carried out by Chen WL et al. and Romero IL et al (7,17)

In accordance with the results of the present study, the authors established that cyanoacrylate-based tissue

adhesive formulations possessed potent antibacterial activity against all the microorganisms tested. The antibacterial efficacy of the two formulations was equivalent (table 1). Amongst the microorganisms, cyanoacrylates showed greater inhibition of Gram-positive bacteria over gram-negative bacteria. This property can be attributed to the strong electronegative charge on the cyanoacrylate monomer that reacts with the positively charged carbohydrate capsule of Gram-positive organisms (18).

Antibacterial efficacy can be due to either bacteriostatic or bactericidal effects. The formulations showed bactericidal activity against all the organisms tested, except *L.amylovorus*. This shows that the antibacterial efficacy of cyanoacrylates against *L.amylovorus* is predominantly bacteriostatic. Also, both formulations of cyanoacrylates showed maximum bactericidal activity against *P.gingivalis* followed by *S.aureus* & *T.forsythia*. Authors suggested that the bactericidal activity could be attributed to the susceptibility of these microorganisms to degradation products such as cyanoacetate and formaldehyde. Amongst the formulations, bactericidal activity of AC was found to be more potent than B+OC as depicted from graph 2. The higher degree of bactericidal activity of AC could be related to its short polymer chain length, which is also responsible for a higher rate of degradation and release of toxic components to the bacterial cells (17,19,20)

The present study demonstrated that cyanoacrylate tissue adhesives are an appealing alternative to silk sutures for the closure of oral wounds and surgically dissected flap margins due to its antibacterial properties. The limitations of the study include inability to assess the MIC 90 (antimicrobial concentration that inhibits growth of 90% of the microorganisms) for these materials. This restriction is

due to the fact that it is not possible to use different concentrations of cyanoacrylate adhesives as they polymerize when in contact with water. Moreover, antibacterial properties of these materials should be evaluated against other periodontal pathogens as well.

Conclusion

Nowadays, cyanoacrylate tissue adhesives are being frequently used in routine clinical practice. The higher cost of these adhesives needs to be weighed against the benefits that these materials provide over silk sutures. The current research showed a potent antibacterial activity of cyanoacrylates against oral pathogens. This property may be exploited in flap closure after periodontal surgical procedures.

In terms of development, cyanoacrylates could be ideal substrates for incorporation of analgesics and antibiotics for sustained release. Further studies are necessary to determine binding and release kinetics of these substances to cyanoacrylates as a carrier or delivery device. Further research could also be directed towards evaluating the antibacterial properties of cyanoacrylate tissue adhesives by conducting thorough in-vivo studies.

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