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Elasticity and safety of alkoxyethyl cyanoacrylate tissue adhesives

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ABSTRACT

Cyanoacrylate glues are easily applied to wounds with good cosmetic results. However, they tend to be brittle and can induce local tissue toxicity. A series of cyanoacrylate monomers with a flexible ether linkage and varying side-chain lengths was synthesized and characterized for potential use as tissue adhesives. The effect of side-chain length on synthesis yield, physical and mechanical properties, formaldehyde generation, cytotoxicity in vitro and biocompatibility in vivo were examined. The incorporation of etheric oxygen allowed the production of flexible monomers with good adhesive strength. Monomers with longer side-chains were found to have less toxicity both in vitro and in vivo. Polymerized hexoxyethyl cyanoacrylate was more elastic than its commercially available and widely used alkyl analog 2-octyl cyanoacrylate, without compromising biocompatibility.

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1. Introduction

For surgical adhesives to be attractive alternatives to sutures and staples they should allow rapid adhesion and maintain strong and close apposition of wound edges for a sufficient time. Ideally surgical adhesives should not elicit a vigorous inflammatory response and should be biodegradable with minimal tissue toxicity [1]. α-Cyanoacrylates (CA) possess some of these properties and can be applied in medicine and dentistry with little discomfort and with good cosmetic results [2]. However, the use of commonly available CA adhesives, particularly within tissues, is limited by two major concerns. First, tissue toxicity, including necrosis, occurs in the immediate vicinity of the CAs, and is attributed to by-products such as cyanoacetate and formaldehyde [3], insufficient tissue vascularization [4], and the exothermic nature of the reaction [5]. Secondly, CA polymers are hard and brittle and may have insufficient flexibility for the dynamic nature of in vivo conditions [6]. Consequently, CAs are currently contraindicated for high tension wounds [7] and are only used in external or temporary applications, such as skin closure [8,9] and repair of corneal perforations [4]. The objective of this study was to develop CA adhesives that have better elastic properties without compromising biocompatibility.

Our hypothesis was that CA monomers containing etheric oxygen could produce polymers with superior elasticity, while the use of longer carbon side-chains could mitigate the toxicity. The incorporation of etheric oxygen could improve the elastic properties because of the absence of hydrogen atoms on the etheric oxygen (asterisk in Fig. 1) facilitates chain rotation and consequently polymer flexibility [10,11]. It has also been suggested that tissue injury due to cyanoacrylates occurs in part because of the poor elasticity of the polymerized glue [12]. Improving the elastic properties could therefore improve tissue reaction. Toxicity is believed to be reduced by the longer alkyl side groups, which slow degradation and therefore decrease the accumulation of toxic by-products [13–15].

To produce a potential surgical adhesive with improved physical properties and reduced toxicity we have developed and characterized a range of ethylene glycol alkyl ether monomers with increasing side-chain lengths. The mechanical strengths of the resulting polymers were assessed, as was their cytotoxicity in vitro and biocompatibility in vivo.

2. Materials and methods

2.1. Chemicals

Cyanoacetic acid was purchased from Alfa Aesar (99% pure, Ward Hill, MA). Ethylene glycol hexyl ether was purchased from TCI America (Portland, OR). All other ethylene glycol ethers,

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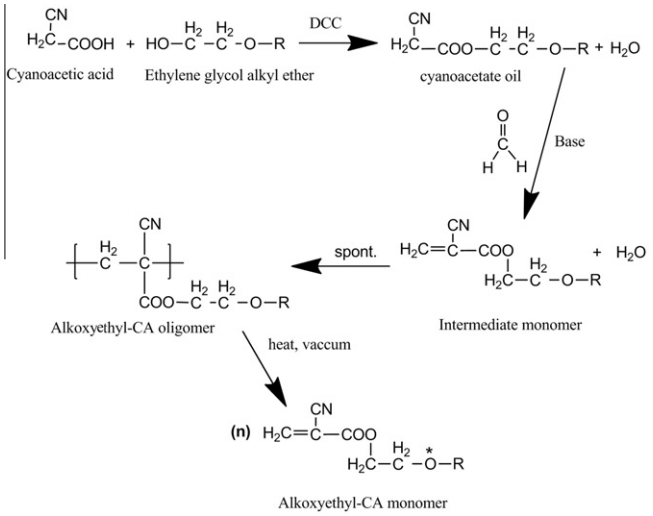

Fig. 1. The synthesis of alkoxyethyl-CA monomers. The etheric oxygen in the final product is indicated by an asterisk.

phosphorus pentoxide, hydroquinone, dicyclohexylcarbodiimide (DCC), paraformaldehyde (91–99% pure), piperidine, *p*-toluenesulfonic acid, dioctyl phthalate and Dulbecco's phosphate-buffered saline (DPBS) were purchased from Sigma–Aldrich (St Louis, MO). Benzene, methanol and tetrahydrofuran (THF) were OmniSolv grade from EMD Chemicals (Gibbstown, NJ) and were used as received. 2-Octyl-CA (Dermabond®) and *n*-butyl-CA (Vetbond®) were purchased from Ethicon Inc. (Somerville, NJ) and 3M (St Paul, MN), respectively.

2.2. Synthesis

Cyanoacetate esters were synthesized by condensation between cyanoacetic acid and a suitable alcohol followed by Knoevenagel reaction [16] (Fig. 1). In a typical reaction (here relating to hexoxyethyl-CA) a mixture of 0.6 mol ethylene glycol hexyl ether and 0.6 mol cyanoacetic acid were stirred in 1000 ml of THF and maintained at 5–10 °C, then 0.6 mol DCC in 500 ml of THF was added in a dropwise manner. The resulting suspension was filtered to remove the dicyclohexylurea and evaporated using a rotary evaporator. After 12 h the crude oil was filtered again. Fractional distillation at reduced pressure through a short Vigreux column gave the final cyanoacetate oil.

0.5 mol paraformaldehyde and 0.3 ml of piperidine were placed in a three-necked glass flask and dissolved in 120 ml of methanol. A Dean–Stark trap combined with a reflux condenser, a thermometer, and a 500 ml separatory funnel were attached to the flask. The mixture was heated to 70 °C and 0.5 mol of cyanoacetate oil was added slowly while maintaining the boiling temperature. Then the heat was increased and the methanol removed via a Dean–Stark trap. Once about half of the methanol had been collected 100 ml of benzene was slowly added for azeotropic distillation. Of note, for industrial production, where heterogeneous azeotropic distillation columns are commonly used, alternative solvents such as toluene, ethanol, cyclohexane or a mixture of ethyl methyl ketone and hexane may be used [17,18]. After all the methanol and the entire theoretical amount of water (9 ml) were collected in the trap *p*-toluenesulfonic acid (0.6 g) was added to the mixture to neutralize the piperidine catalyst. The plastisizer dioctyl phthalate was then added (10 ml) and the solution was placed in a 0.6 mm Hg vacuum at 80 °C for solvent removal. 0.25 g hydroquinone and 2 g phosphorus pentoxide were added and the flask was connected to a short path distillation unit with a 100 ml receiver flask containing 0.125 g hydroquinone and 1 g phosphorus pentoxide. Sulfur dioxide gas was carefully introduced and a 740 torr vacuum was applied. The temperature was increased until depolymerization occurred (around 160 °C), as evident by the accumulation of droplets in the receiving flask. Repeated vacuum distillations and phosphorus pentoxide/hydroquinone additions to inhibit spontaneous polymerization were performed until a high purity product was achieved. The monomers were stored at 4 °C in the presence of *p*-toluenesulfonic acid.

2.3. Analysis of synthesized monomers

The chemical structures of the monomers were determined by ^{1}H NMR using a Varian Mercury (Palo Alto, CA) 300 MHz spectrometer at 25 °C in $CDCl_3$. Purity was determined by gas chromatography–mass spectrometry (GC–MS) (Agilent 5973 N, Little Falls, DE) with a temperature ramp from 100 to 350 °C at a heating rate of 10 °C min^{-1}. Monomer solution (300 p.p.m. in THF, 1 μl injection volume) was used for analysis. The hydrophilicity of the monomers was characterized by measuring the contact angle by the sessile drop method [19]. 5 μl of each monomer was dropped on a hydrophobic natural rubber latex wafer [20] (VWR, MA) and the contact angle images were recorded using a goniometer equipped with video capture (VCA-2000, AST Inc., NJ). Each reported contact angle measurement represents an average value of at least six separate drops.

The peak temperatures generated by CA bulk polymerization were monitored by a temperature recording system equipped with a thermocouple wire (Fluke 51-2, Fluke, MA). The wire was placed in a preheated (37 °C) 96-well plate, then 200 μl of test monomer was inserted and 10 μl of 0.1 N NaOH was added. Each reported peak temperature represents an average value of six separate measurements.

2.4. Release of formaldehyde

10 μl of glue monomer were placed at the center of a 24-well culture plate. Monomers were allowed to polymerize for 24 h at room temperature. The resulting film was submerged in 1 ml of phosphate-buffered saline (PBS) and incubated at 37 °C. At predetermined time points the PBS was removed for analysis and replaced with fresh medium. The analysis consisted of measurement of the formaldehyde concentration using a fluorometric detection kit (Assay Designs, Ann Arbor, MI). The results for each sample were averaged (n = 4).

2.5. Mechanical testing

Mechanical tests were conducted using an Instron universal testing machine provided with a load cell of 500 N (model 5542, load cell model 2530-416, 0.125 N resolution or 0.25% of load, Instron Corp., Canton, MA) at a cross-head speed of 10 mm min^{-1} (ASTM method 0897-49 [2]). The test machine was controlled by Merlin 1999 operating system software v. 22031 (Richardson, TX), which provides all the test set-up, control and analysis functions. Experiments were first performed using aluminum specimens (Ted Pella Inc., Redding, CA) with 6.25 mm slotted heads and 1 cm pins. 5 μl of each monomer were applied to one of the two specimens and the second gently laid on top. The specimens were held together with clips for 12 h to insure monomer curing. The probe was withdrawn from the upper moving crimp at a rate of 0.1 mm min^{-1}. The peak detachment force (N) was recorded as a function of extension diagram. The modulus was determined from the slope of the stress plotted against the applied strain. Each test trial consisted of eight replicate measurements.

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