



Cytotoxicity, blood compatibility and antimicrobial activity of two cyanoacrylate glues for surgical use

L. Montanaro^a, C.R. Arciola^a, E. Cenni^{b,*}, G. Ciapetti^b, F. Savioli^a,
F. Filippini^a, L.A. Barsanti^c

^aLaboratory for Biocompatibility Research on Implant Materials, Istituti Ortopedici Rizzoli, via di Barbiano 1/10, 40136 Bologna, Italy

^bDipartimento Putti, Istituti Ortopedici Rizzoli, Bologna, Italy

^cAssicurazione Qualità, General Enterprise Marketing, Viareggio, Italy

Received 10 February 1999; accepted 28 April 2000

Abstract

The biocompatibility of two cyanoacrylate surgical glues (Glubran and Glubran 2), supplied by General Enterprise Marketing, Viareggio, Lucca, Italy, was tested through cytotoxicity and blood compatibility tests and the evaluation of antimicrobial activity. Cytotoxicity and blood compatibility tests were performed on the polymerized glues. Using the neutral red uptake test, the extracts from Glubran and Glubran 2 after polymerization were non-toxic to L929 cells only when diluted 1 : 10 with culture medium. Glubran and Glubran 2 induced a significant decrease of activated partial thromboplastin time (APTT), which is favourable with regard to the desired haemostasis. The APTT shortening determines a haemostatic effect and therefore contribute to the tissue adhesion induced by the glues. Otherwise, no significant variation of prothrombin activity, fibrinogen, platelet number, total and differential leukocyte count was induced by the glues, which, in addition, did not show haemolytic effect. There was no difference between Glubran and Glubran 2 regarding haemocompatibility. The antimicrobial ability of the unpolymerized glues was tested onto *Bacillus subtilis* var. niger for 3 weeks: neither Glubran nor Glubran 2 were found effective in this respect. In conclusion, we can assume that cytotoxicity was severe with the undiluted glues, but was acceptable when glues were diluted. On the contrary, blood compatibility was acceptable for the intended use of the glues. No difference was found between Glubran and Glubran 2 after polymerization. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Cyanoacrylate; Surgical glue; Cytotoxicity; Blood compatibility; Antimicrobial activity

1. Introduction

Various haemostatic “means” have been used in surgery, including sutures, staples, clamps and application of biological agents, such as thrombin, fibrin and collagen glues, and synthetic agents, such as cyanoacrylate adhesives.

Cyanoacrylates have several advantages: they are easy and rapid to be applied, are relatively painless, eliminate the need for suture removal and for the external application provide an acceptable cosmetic result.

Surgical glues should have some essential requirements, such as adequate adhesive strength, polymeriz-

ation also in a moist environment, biocompatibility, gradual resorption without foreign-body response.

The use of cyanoacrylate tissue adhesives is well described in the literature for closure of skin wounds [1–5]. Owing to cyanoacrylate toxicity, their biocompatibility must be carefully evaluated before clinical application, in order to identify the less toxic compounds. Biocompatibility should be evaluated according to the EN 30993 standard. The aim of this study was the evaluation of cytotoxicity, blood compatibility and antimicrobial activity of two surgical glues (Glubran and Glubran 2).

Cytotoxicity testing of materials and/or devices is the very first step in the assessment of biological compatibility of devices: the standard EN 30993, part 5, gives guidelines to perform such tests [6]. The cytotoxic effect of aqueous extracts of Glubran and Glubran 2 surgical glues after polymerization was assayed onto L929 cells (ATCC, CCL1), using the neutral red uptake test.

*Corresponding author. Tel.: +39-51-6366748; fax: +39-51-6366869.

E-mail address: elisabetta.cenni@ior.it (E. Cenni).

Any test alone cannot predict blood compatibility, but a multiparametric approach with more tests investigating the different events of blood–material interaction is needed. So a number of tests were performed according to the standard EN 30993, part 4, which suggests the effect of devices to be evaluated on the plasmatic phase of coagulation, platelets, leukocytes and erythrocytes, respectively [7].

The plasmatic phase of coagulation was assessed by measuring the activated partial thromboplastin time (APTT) and prothrombin activity, and determining the fibrinogen concentration in the plasma after the contact with the two glues.

Platelet count permits to evaluate the adhesion of platelets to the test material.

The total and differential count of leukocytes permit to evaluate how the white blood cells are affected by the contact with artificial materials.

The effect of the test materials on erythrocytes was evaluated by the haemolysis test. After incubation of an extract of the material with a suspension of human red blood cells, the optical density of the supernatant was measured spectrophotometrically.

The aim of the infectability test was the evaluation of the antimicrobial activity of Glubran on *Bacillus subtilis* var. niger, with regard to the hypothesis of an “antibacterial effect” of the glue, which was reported for other cyanoacrylates. It was demonstrated that *n*-2-butylcyanoacrylate (Histoacryl blue) had an antibacterial effect particularly on gram-positive organisms; in addition, the glue did not become contaminated after repeated use [8]. Our aim was to evaluate if Glubran and Glubran 2 inhibit the bacterial growth and this effect is unchanged during the usual storage.

2. Materials and methods

2.1. Materials

Two surgical glues for internal use (supplied by General Enterprise Marketing, Viareggio, Lucca, Italy) were tested: Glubran and Glubran 2. Glubran components were ethyl [2] cyanoacrylate (monomer), butyl acrylate (monomer) and methacryloxysulpholane (monomer). Glubran 2 was based on *n*-butyl [2] cyanoacrylate (monomer), methacryloxysulpholane (monomer).

Both glues were sterilized by filtration and stored at +4°C. The biocompatibility tests were performed on sterilized samples of glues. The procedure for sterilization was the same employed in the clinical use of the glues.

The glues polymerize in the presence of air, tissues and blood. The polymerization time of Glubran was 150–180 s; the polymerization time of Glubran 2 was faster (60–90 s).

Polytetrafluoroethylene (Goodfellow, 1.0 mm thick) as 3.0 cm² squares was used as negative control in the coagulation tests.

USP Reference Standard High-Density Polyethylene (USP, Rockville, MD, USA) was used as negative control in the haemolysis test.

Saline (0.9% NaCl) was used as negative control in the test with bacteria.

2.1.1. Extract preparation

Each glue was dropped onto one side of sterilized glass slides and flattened to a continuous layer < 0.5 mm thick. After polymerization, the glue films were covered with either minimum essential medium (MEM) or Dulbecco's modified phosphate-buffered saline (D-PBS), to provide extracts for cytotoxicity and blood compatibility tests, respectively. Material surface area/extracting vehicle ratio was 6 cm²/ml, according to ISO 10993, part 12 [9].

For cytotoxicity testing, cell-culture-treated polystyrene was extracted in MEM and used as negative control; the addition of phenol 0.64% provided the positive control.

For the haemolysis test the USP Reference Standard High-Density Polyethylene by USP (Rockville, MD) was extracted in D-PBS and tested as negative control.

The extraction was performed at 37 ± 2°C for 72 ± 2 h. The extracts, after centrifugation and filtration to eliminate any material and/or contamination, were aliquoted and stored at –70 ± 15°C until tested.

2.1.2. Preparation of the samples tested as a film

The samples were tested as a polymerized film in the following tests: APTT, prothrombin activity, fibrinogen, platelet, total and differential leucocyte count. One drop of each material was allowed to form a < 0.5 mm-thick layer on the inner surface of a siliconized tube and to polymerize. The material was tested after 24 h.

2.2. Cell cultures for cytotoxicity

L929 cells (ATCC, NCTC clone CCL1) were cultured in 75 cm² flasks in minimum essential medium with Earle's salts (MEM) added with fetal calf serum 10%, L-glutamine 2 mM, penicillin 100 UI/ml -streptomycin 100 µg/ml and non essential aminoacids for MEM 1%.

Cultures were maintained at 37 ± 0.5°C in 95% air/5% carbon dioxide atmosphere, with 95% relative humidity, and at confluence they were used for the test. For the experiments, the cells were seeded at 1 × 10⁴/well/0.2 ml in flat-bottomed 96-well microplates and maintained for 24 h.

Three to five replicates were seeded for each of the following: ‘fresh’ MEM, negative control, positive control and samples.

After 24 h, the medium was discarded and replaced with 0.2 ml of (i) freshly prepared MEM (control of cell

growth), (ii) MEM extracted in polystyrene flask (negative control), (iii) 0.64% phenol solution in polystyrene flask-extracted MEM (positive control) and (iv) extracts of Glubran and Glubran 2, undiluted and diluted 1:2 and 1:10 with MEM (samples).

Before addition to the wells, the extracts were supplemented as described for complete MEM and the microplates were re-incubated for 24 h.

2.3. Neutral red uptake assay

The neutral red solution was prepared using neutral red dye (CI 50040) tested for cell culture.

At each run the dye was diluted 50 µg/ml (final concentration) in complete MEM.

Following incubation of cells for 24 h with the extracts, the supernatant was discarded: each well received 0.2 ml of neutral red solution in MEM and the microplate was re-incubated at $37 \pm 0.5^\circ\text{C}$ for 2 h. After discarding the dye in excess, each well was added with 0.1 ml of lysing solution. After gentle shaking of the microplate for 1 min, the colour intensity of each well was read at 540 nm wavelength with a spectrophotometer for microplates [10].

4–5 separate runs were performed on each sample.

2.4. Statistical evaluation of neutral red uptake test

Mean and standard deviation of the optical density (OD) values obtained for replicates of controls and samples were calculated for each different experimental run.

The percent viability of the sample (and of the positive control) was calculated versus the negative control; the value of the positive control was expected to be $\leq 50\%$, while the negative control, calculated versus the 'fresh' MEM value, was expected $\geq 80\%$.

The samples were considered as cytotoxic if the cell viability was $\geq 70\%$.

Percent viability of cells in the separate runs were averaged and given as final results, with negative controls set arbitrarily to 100.

2.5. Blood processing

Human venous blood was collected in siliconized tubes with a 9:1 blood/3.8% sodium citrate ratio and the tests were performed within 3 h since collection.

2.6. Plasma-material contact for APTT, PT and fibrinogen assay

Platelet-rich plasma (PRP) was obtained by centrifugation of blood at $120 \times g$ for 5 min at room temperature. One ml of PRP was put in contact, in siliconized vials, with the test materials or PTFE (negative control). One ml of PRP was put in a siliconized vial with no

material, to assess the effect of blood manipulation on APTT.

The samples were gently stirred for 30 min at room temperature. Then, the materials were removed and the PRP was centrifuged at $700 \times g$ for 15 min at room temperature to remove platelets. APTT, PT and fibrinogen assay were performed on the platelet poor plasma.

2.7. Blood-material contact for platelet and leukocyte count

The whole blood was put in contact, in siliconized tubes, with the test materials and PTFE. Another aliquot of whole blood was put in a siliconized tube with no material, to assess the effect of blood processing on platelet number. The samples were gently stirred for 30 min at room temperature.

2.8. Activated partial thromboplastin time

For the determination of APTT, micronized silice and phospholipids from rabbit brain (Automated APTT, Organon Teknika) and 0.025 M calcium chloride solution (Biomérieux) were used. The APTT ratio was determined by dividing the APTT of the sample by the APTT of a plasma pool. The pool was prepared using citrated blood from 15 healthy male donors and 15 healthy female donors. The pool was fractionated and the aliquots stored at $-70 \pm 15^\circ\text{C}$. In each experimental run, one aliquot was thawed at 37°C and the APTT measured within 2 h since thawing.

2.9. Prothrombin activity assay

A mixture of tissue thromboplastin from rabbit brain, calcium ions and buffer (Simplastin Excel S, Organon Teknika) was used for the determination of prothrombin time. The results were expressed as international normalized ratio. Prothrombin time ratio (PT ratio) was calculated by dividing the time of the sample by the time of the plasma pool. International normalized ratio was calculated using the international sensitivity index (ISI). The lot of thromboplastin used had an ISI value of 1.27. The international normalized ratio was calculated using the following formula: International normalized ratio = (PT ratio)^{ISI}

2.10. Fibrinogen assay

A commercial bovine thrombin (Fibriquik, Organon Teknika) was used. Calibration plasma, control plasma and samples were tested after 1:10 dilution with imidazole buffer. The clotting time of the samples was converted to fibrinogen concentration using a reference curve made with serial dilutions of a calibration plasma.

2.11. Platelet and leukocyte count

Immediately after contact with the materials, the blood was separated from materials. Platelet and leukocyte counts were performed by an electronic counter. A blood smear was prepared for each sample, stained with May-Grunwald–Giemsa and examined by optical microscopy ($100\times$). Two-hundred leukocytes were counted for each smear. The absolute values were calculated using the total leukocyte number and the relative percentage of each population.

2.12. Haemolysis test

Dulbecco's modified phosphate-buffered saline (D-PBS) was used to wash red blood cells, to prepare suspension and to determine the 'blank'. Sterile distilled water was used to cause 100% haemolysis. A sterile solution of saponin (Sigma) in saline (1.25 g/l) was used as positive control after dilution 1:10 with PBS.

Human venous blood was collected with 3.8% sodium citrate, diluted 1:5 with D-PBS and centrifuged for 5 min at $700\times g$ at room temperature. After discarding the supernatant, the procedure was repeated with the pelleted erythrocytes. The purified erythrocytes were diluted 1:9 with D-PBS and the suspension was used immediately.

The erythrocyte suspension was divided into different aliquots:

- one aliquot was incubated 1:5 with the extract of the test material,
- one aliquot was incubated 1:5 with the extract of the negative control,
- one aliquot was put in contact 20:1 with the saponin solution,
- one aliquot was put in contact 1:5 with distilled water,
- one aliquot was put in contact 1:5 with D-PBS (blank).

The samples were incubated at $+37 \pm 1^\circ\text{C}$ for 4 h with no agitation. After the incubation, the suspensions were centrifuged at $700\times g$ for 5 min and the optical density (OD) of the supernatants was read at 540 nm. The OD of the 'blank' was subtracted from the OD of samples and of suspension incubated with distilled water. Then, the percent of haemolysis was calculated, setting the OD of the suspension with distilled water as 100%. The samples were considered as haemolytic if the percent of haemolysis was $> 0.5\%$.

2.13. Statistical evaluation of blood compatibility tests

The results were expressed as arithmetic mean plus and minus standard deviation of the different experimental

runs. The effects of the materials were evaluated by the analysis of variance (ANOVA). The Bonferroni–Dunn multiple comparison test was applied to detect specific differences between the test materials and between the test materials (or controls) and the plasma (or blood) not incubated with materials. The p value < 0.005 was considered as statistically significant.

For the haemolysis test, the arithmetic mean and the standard deviation of the replicates were calculated.

2.14. Contact "material–*Bacillus subtilis*"

To verify the antimicrobial ability, five samples of Glubran and Glubran 2 were tested. During packaging of the fluid in plastic vials (1 ml/vial), a strip with 10^6 *Bacillus subtilis* var. niger was inserted into each vial (Biological Indicators spore strip, ATCC 9372, DID, Milan, Italy). These strips are usually employed for the control of the ethylene oxide sterilization. At the end of packaging, the strip contained in the vial was entirely covered by the glue. The contact duration was 3 weeks (21 days), that is the mean period of storage before use.

Five samples of sterile saline (0.9% NaCl), which were packed exactly as the glue and put into contact with a strip of *Bacillus subtilis* var. niger, were used as controls.

2.15. Seeding of the bacillus strips after the contact with the glue

After the 3-weeks contact, each vial of glue or saline (control) was opened and a small amount was dropped into two TSB bottles. Each strip was cut into two pieces, in order to put also the edge in contact with the broth. Both pieces were put in the same TSB broth. Each handling was done under sterile conditions in a laminar flow cabinet. All samples were incubated at 37°C .

2.16. Seeding on solid medium

After incubation, the broth cultures of the strips were seeded on TSA plates. All samples were seeded on five sides of the plate. Besides, a little amount of broth both from glue or saline cultures was shed directly on the plate.

Some drops from all broths were put onto a slide and observed by microscopy.

The cultures were incubated at 37°C for 48 h.

2.17. Biochemical identification of *Bacillus subtilis*

Biochemical identification of the colonies grown onto TSA plates was performed by API 50 system (Roche).

3. Results

3.1. Neutral red uptake assay

The neutral red uptake test was repeated in 4–5 separate runs and percent viability of cells were averaged. Results are reported in Table 1. The viability of L929 cells was strongly compromised when cells were exposed for 24 h to extracts from Glubran and Glubran 2, both undiluted and diluted 1:2. After 1:10 dilution of the extracts, the cells were quite unaffected, with a viability score higher than 70%. No substantial difference in the cytotoxic effect of the two extracts was found.

3.2. Activated partial thromboplastin time

Ten experiments were performed. Both Glubran and Glubran 2 after polymerization induced a significant decrease of APTT ratio (Table 2), compared to PRP not in contact with materials. PTFE did not determine any significant variation in APTT ratio, compared to PRP not in contact with materials. There was no significant difference in APTT ratio ($p = 0.0168$) among Glubran and Glubran 2 after polymerization.

3.3. Prothrombin activity

Ten experiments were performed. None of the two glues tested after polymerization induced any variation of the international normalized ratio (Table 3) in comparison to PRP not in contact with materials. There was no significant difference among Glubran and Glubran 2 ($p = 0.5095$).

Table 1
Mean and standard deviation of percent viability of L929 cells in neutral red uptake test

	No. of runs	Percent viability
Growth control	4	96.2 ± 5.1
Negative control	4	100
Positive control	4	19.9 ± 11.4
Undiluted extract of Glubran	4	16.2 ± 5.6
Extract of Glubran diluted 1:2 with MEM	4	17.2 ± 5.1
Extract of Glubran diluted 1:10 with MEM	4	78.5 ± 7.0
Growth control	5	104.0 ± 5.7
Negative control	5	100
Positive control	5	7.9 ± 0.8
Undiluted extract of Glubran 2	5	7.4 ± 1.1
Extract of Glubran 2 diluted 1:2 with MEM	5	7.1 ± 1.5
Extract of Glubran 2 diluted 1:10 with MEM	5	81.7 ± 12.2

Table 2
Mean and standard deviation of APTT ratio after contact with test materials

	No. of tests	APTT ratio	<i>p</i>
PRP not in contact with materials	10	1.333 ± 0.079	—
PTFE	10	1.302 ± 0.092	0.3912
Glubran	10	1.074 ± 0.051	< 0.0001
Glubran 2	10	1.163 ± 0.097	< 0.0001

Table 3
Mean and standard deviation of international normalized ratio after contact with test materials

	No. of tests	International normalized ratio	<i>p</i>
PRP not in contact with materials	10	1.009 ± 0.076	—
PTFE	9	1.000 ± 0.059	0.7606
Glubran	10	1.036 ± 0.038	0.3498
Glubran 2	10	1.055 ± 0.063	0.1146

Table 4
Mean and standard deviation of fibrinogen after contact with test materials

	No. of tests	Fibrinogen (mg/dl)	<i>p</i>
PRP not in contact with materials	10	434.9 ± 55.2	—
PTFE	10	442.2 ± 66.9	0.7820
Glubran	10	442.8 ± 58.7	0.7646
Glubran 2	10	431.2 ± 61.5	0.8877

3.4. Fibrinogen assay

Ten different experiments were performed. None of the two glues tested after polymerization induced any variation of the fibrinogen, in comparison to PRP not in contact with materials (Table 4). There was no significant difference among Glubran and Glubran 2 ($p = 0.6597$).

3.5. Platelet number

Twelve experiments were performed. None of the two glues tested after polymerization induced any variation of platelet number, in comparison to the whole blood not in contact with materials (Table 5). There was no significant difference among Glubran and Glubran 2 ($p = 0.5953$).

3.6. Total and differential leucocyte counting

Twelve experiments were performed. None of the two glues tested after polymerization induced any variation

Table 5
Mean and standard deviation of platelet number after contact with test materials

	No. of tests	Platelet no. ($\times 10^3/\mu\text{l}$)	<i>p</i>
Whole blood with no material	8	148.8 \pm 40.4	—
PTFE	8	144.2 \pm 56.0	0.8471
Glubran	12	147.7 \pm 48.9	0.9572
Glubran 2	12	158.2 \pm 57.6	0.6732

of total and differential leukocyte number, in comparison to whole blood not in contact with materials (Table 6). There was no significant difference among Glubran and Glubran 2 (leukocyte no: $p = 0.7855$; neutrophil no: $p = 0.8314$; eosinophil no: $p = 0.5741$; basophil no: $p = 0.4270$; lymphocyte no: $p = 0.8532$; monocyte no: $p = 0.5449$).

3.7. Haemolysis test

Three tests were performed. Both extracts of Glubran and Glubran 2 induced a percent haemolysis less than 0.5%; therefore we can assume that the two glues had no haemolytic effect on the human red cell suspension. The percent of haemolysis was similar for Glubran and Glubran 2 (Table 7).

3.8. Antimicrobial capacity

3.8.1. Broth examination

The broth cultures were observed every day for 14 days. Since the first day, the cultures from the strips which had been in contact with the glue showed turbidity, indicating the presence of bacteria, while the cultures from the glue near strips were clear until the end of incubation. All control cultures, both from strips and saline, had striking turbidity from the beginning of the experiment.

3.8.2. TSA plates examination

In all TSA plates seeded with the broth cultures of strips, a single type of round and white-greyish colonies grew. No growth was demonstrable on the plates shed

with the broth cultures of the glues. The growth was diffuse on the plates shed with the broth cultures of the saline.

3.8.3. *Bacillus subtilis* identification

After incubation, a population formed by long, thin, moving bacilli was observed at the microscopic examination of the broth. The population was identified biochemically as *Bacillus subtilis*.

3.8.4. Examination of broths and plates seeded with the glue surrounding the strips

Neither broth turbidity nor colony growth on solid medium was observed for the cultures of the glue surrounding the strips. This demonstrates the ability of the glue to inhibit the spread of contamination with *Bacillus subtilis*, even when seeded at high concentration. However, this observation cannot be applied to other microbial contamination without further analysis, because we tested exclusively *Bacillus subtilis*. However, the ability of the glues to hamper the spread of a high microbial concentration suggests that both the glues could hamper even the spread of a spontaneous 'bioburden'.

4. Discussion

One of the most severe problems of the surgical use of cyanoacrylate is their toxicity: the methyl, ethyl and alkyl homologues have been reported to be cytotoxic [11,12]. The toxicity has been associated with the heat of polymerization and presence of unreacted monomer [13]. In general, it has been found that as the alkyl group on the cyanoacrylate becomes longer, the tissue reaction decreases [14].

Other cyanoacrylates showed a better biocompatibility. In vivo studies on 3-methoxybutylcyanoacrylate demonstrated a moderate tissue reaction [15]. Also 10% poly(tri(oxyethylene)oxalate/methoxypropyl cyanoacrylate is a promising glue and probably well tolerated [16].

Good results were obtained with *n*-ethyl-2-cyanoacrylate and *n*-isobutyl-2-cyanoacrylate in different clinical applications. They were used in orthopaedics for fracture osteosynthesis [17], in gastroenterology for oesophagus

Table 6
Mean \pm standard deviation ($\times 10^3/\mu\text{l}$) and significance of total and differential leucocyte number after contact with test materials

	Whole blood with no material (test no. 8)	PTFE (test no.9)	<i>p</i>	Glubran (test no.12)	<i>p</i>	Glubran 2 (test no.12)	<i>p</i>
Total no.	5.169 \pm 2.069	5.456 \pm 2.053	0.7748	5.729 \pm 2.020	0.5523	5.500 \pm 2.047	0.7251
Neutrophils	2.635 \pm 1.537	2.901 \pm 1.402	0.6865	3.245 \pm 1.263	0.3262	3.127 \pm 1.302	0.4274
Eosinophils	0.096 \pm 0.062	0.050 \pm 0.043	0.3262	0.128 \pm 0.134	0.4612	0.106 \pm 0.115	0.8138
Basophils	0 \pm 0	0.002 \pm 0.006	0.6117	0.003 \pm 0.010	0.4772	0 \pm 0	—
Lymphocytes	2.312 \pm 0.506	2.335 \pm 0.638	0.9493	2.122 \pm 0.757	0.5644	2.067 \pm 0.758	0.4588
Monocytes	0.125 \pm 0.107	0.168 \pm 0.125	0.4972	0.231 \pm 0.151	0.0759	0.199 \pm 0.136	0.2107

Table 7
Mean and standard deviation of the percent of haemolysis after contact with test materials

Sample	No. of tests	Percent of haemolysis
Saponin	3	3.287 ± 0.841
Polyethylene	3	0.010 ± 0.017
Glubran	3	0.060 ± 0.104
Glubran 2	3	0.067 ± 0.115

varix treatment [18], in dentistry and stomatology, in maxillo-facial surgery and in vascular surgery [19].

In this study the biocompatibility of two surgical glues, Glubran and Glubran 2, was tested in vitro. Both are tissue adhesives that polymerize rapidly in a moist environment. The higher polymerization speed of Glubran 2 compared to Glubran, due to the different chemical composition, is an advantage in the surgical application as it ensures a more efficient adhesion between the edges of the wound.

A multiparametric evaluation of biocompatibility of these materials is needed: their good adhesive properties suggest many surgical applications, but biological compatibility is fundamental.

The assay for cytotoxicity was based on the uptake of a solution of the vital dye neutral red.

Through an active process requiring energy the neutral red crosses the cell membrane to be stored into the lysosomes of viable cells, which thereafter are stained red. If the cell membrane is damaged by any toxic substance, neutral red is not endocytosed nor retained within the cell: the higher the damage to the cells, the less the uptake of neutral red. In our protocol, after the treatment with the glue extracts, the cells are allowed to engulf neutral red. Then, a lysing solution is added to release the intracellular dye: by measuring the absorbance of the released dye, a reliable index of the number of viable cells in the sample is obtained [20].

From our experience with a variety of materials, a value ≤ 70 for cell viability after challenge with biomaterials provides a reasonable threshold to discriminate between toxic (< 70) and non-toxic (> 70) materials.

In our hands, the extracts obtained from Glubran and Glubran 2 after polymerization were toxic to cells; after dilution 1:10 with medium no toxicity was observed for both glues, which were well tolerated by cells and allowed for a viability of more than 70% vs. control cells.

Other authors too have observed that isobutyl-cyanoacrylate was less cytotoxic [13]. They have ascribed the cytotoxic effect of cyanoacrylates to formaldehyde release: the compounds that release less residues during cell culture should be less cytotoxic.

As far as blood compatibility was concerned, both Glubran and Glubran 2 after polymerization induced decrease in APTT compared to PTFE and unexposed plasma (not in contact with materials).

Neither Glubran nor Glubran 2 after polymerization induced any significant variation of prothrombin activity and fibrinogen, compared to unexposed plasma.

No significant reduction in platelet and leukocyte number was caused by the glues compared to whole blood not in contact with materials.

The same behaviour was observed for the different leukocyte populations.

Moreover, no significant difference was found between the two glues, as far as the blood compatibility tests are concerned.

The extracts of the two glues, after polymerization, had no haemolytic effect on the human red cell suspension; the haemolysis percent was negligible and similar for the extracts of Glubran and Glubran 2.

Therefore, the only alteration of blood compatibility tests was the decrease of APTT, that was similar for the two glues. The APTT shortening determined a haemostatic effect and therefore contributed to the tissue adhesion induced by the glues.

Other cyanoacrylates too have a haemostatic effect. The comparison between oxidized cellulose, fibrin glue and isobutyl-2-cyanoacrylate showed that the last had a better haemostatic effect in the anastomosis with PTFE grafts [21]. The haemostatic effect of isobutyl-2-cyanoacrylate was attributed to the stimulation of the synthesis of thromboxane, that induces platelet aggregation [22]. We did not observe a decrease in platelet number for none of the two glues. However, for technical reasons, neither thromboxane nor release reaction markers were determined.

The evaluation of the antimicrobial ability demonstrated that the samples of surgical glue did not inhibit bacterial growth from the strips of *Bacillus subtilis*, but hampered the spread of contamination. In fact, bacterial colonies grew only in the cultures from the strips, but they were not observed in the cultures of the glue surrounding the strip into the vial. However, this observation cannot be applied to other microbial contamination, because the test method was applied only to *Bacillus subtilis*.

Other authors have demonstrated that ethylcyanoacrylate had bacteriostatic effect [19]. However, the test method was different: the authors dropped the glue directly onto agar-blood plates previously seeded with *Bacillus subtilis* and *Staphylococcus aureus*: the inhibition of bacterial growth was observed after 24 h. Besides, the number of bacteria seeded was smaller than our concentration of *Bacillus subtilis*, that was chosen deliberately high.

Other authors tested the in vitro biocompatibility of *n*-butyl-2-cyanoacrylate and *n*-ethyl-2-cyanoacrylate.

n-butyl-2-cyanoacrylate was evaluated on Wister rats, by inserting an intestinal loop, in order to test its use for sutures in abdominal surgery. Histological examination demonstrated a low toxicity and a good adhesive effect

[23]. Good results were obtained also with other experimental implants of intestinal loops [24]. **Comparative clinical observations demonstrated that *n*-butyl-2-cyanoacrylate induced better epithelialization, less marked scar formation and less local inflammation than silk sutures [25].**

Palmieri et al. [19], by in vivo testing *n*-ethyl-2-cyanoacrylate and *n*-isobutyl-2-cyanoacrylate, observed a good histocompatibility. They suggested that cyanoacrylate toxicity was essentially due to the initial dehydration of the tissues. When cyanoacrylates were fully polymerized they did not induce any tissue reaction, with the exception of a slow and gradual fragment removal from macrophages [19].

5. Conclusion

In conclusion, Glubran and Glubran 2 after polymerization

- are toxic to L929 cells, using the neutral red uptake test. Only after dilution 1:10 with culture medium toxicity is acceptable;
- induce a significant decrease of activated partial thromboplastin time (APTT);
- do not induce any significant variation of prothrombin activity, fibrinogen concentration, platelet number, total and differential leukocyte number;
- do not have haemolytic effect.

Therefore, we can assume that cytotoxicity was severe with the undiluted glues, was acceptable when glues were diluted. On the contrary, blood compatibility was acceptable for the intended use of the glues.

No difference was found between Glubran and Glubran 2 after polymerization.

Neither Glubran nor Glubran 2 inhibit bacterial growth from the strips of *Bacillus subtilis* var. *niger*, but can hamper the spread of contamination of a high number of bacteria introduced into the test materials.

Acknowledgements

This research is supported by grants from Istituti Ortopedici Rizzoli, 'Ricerca Corrente'.

The Authors thank Mr. Branchetti and Mr. Antonuccio (General Enterprise Marketing, Viareggio, Italy) for providing test materials.

References

- [1] Quinn J, Wells G, Sutcliffe T, Jarmuske M, Maw J, Stiell I, Johns P. A randomized trial comparing octylcyanoacrylate tissue adhesive and sutures in the management of lacerations. *JAMA* 1997;277:1527–30.
- [2] Qureshi A, Drew PJ, Duthie GS, Roberts AC, Monson JR. N-butyl cyanoacrylate adhesive for skin closure of abdominal wounds: preliminary results. *Ann R Coll Surg Engl* 1997;79:414–5.
- [3] Liebelt EL. Current concepts in laceration repair. *Curr Opin Pediatr* 1997;9:459–64.
- [4] Adoni A, Anteby E. The use of Histoacryl for episiotomy repair. *Br J Obstet Gynaecol* 1991;98:476–8.
- [5] Messi G, Marchi AG. Evaluation of skin laceration repair by tissue adhesive in the pediatric emergency room. *Panminerva Med* 1992;34:77–80.
- [6] EN 30993 standard. Biological compatibility of medical devices — Part 5: tests for cytotoxicity: in vitro methods, 1994.
- [7] EN 30993 standard. Biological evaluation of medical devices — Part 4: selection of tests for interactions with blood, 1993.
- [8] Quinn JV, Osmond MH, Yurack JA, Moir PJ. N-2-butylcyanoacrylate: risk of bacterial contamination with an appraisal of its antimicrobial effects. *J Emerg Med* 1995;13:581–5.
- [9] EN ISO 10993 standard. Biological evaluation of medical devices — Part 12: sample preparation and reference materials, 1996.
- [10] Hansen MB, Nielsen SE, Berg K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J Immunol Methods* 1989;119:203–10.
- [11] Zumpano BJ, Jacobs LR, Hall JB, Margolis G, Sachs Jr, E. Bioadhesive and histotoxic properties of ethyl-2-cyanoacrylate. *Surg Neurol* 1982;18:452–7.
- [12] Papatheophanis FJ. Cytotoxicity of alkyl-2-cyanoacrylate adhesives. *J Biomed Mater Res* 1989;23:661–8.
- [13] Tseng YC, Tabata Y, Hyon SH, Ikada Y. In vitro toxicity test of 2-cyanoacrylate polymers by cell culture method. *J Biomed Mater Res* 1990;24:1335–67.
- [14] Matsumoto T, Aron A. "Sankyo" Japanese tissue adhesive in surgery of internal organs. In: Matsumoto T, editor. *Tissue adhesives in surgery*. Flushing, NY, USA: Medical Examination Publishing Co, 1972. p. 5–8.
- [15] Henderson AM, Stephenson M. 3-Methoxybutylcyanoacrylate: evaluation of biocompatibility and bioresorption. *Biomaterials* 1992;13:1077–84.
- [16] Linden Jr CL, Shalaby SW. Performance of modified cyanoacrylate composition as tissue adhesive for soft and hard tissues. *J Biomed Mater Res* 1997;38:348–55.
- [17] Capasso G, Bentivoglio G, Testa V, Maffulli N. Osteosynthesis with an adhesive in skeletal fractures: an experimental study. *Arch Putti Chir Organi Mov* 1991;39:309–14.
- [18] D'Imperio N, Piemontese A, Baroncini D, Billi P, Borioni D, Dal Monte PP, Borrello P. Evaluation of undiluted N-butyl-2-cyanoacrylate in the endoscopic treatment of upper gastrointestinal tract varices. *Endoscopy* 1996;28:239–43.
- [19] Palmieri B. *Attualità sui collanti biologici ad uso clinico: I cianoacrilati*. Continental Medical Group Trieste, 1994.
- [20] Ciapetti G, Granchi D, Verri E, Savarino L, Cavedagna D, Pizzoferrato A. Application of a combination of neutral red and amido black staining for rapid, reliable cytotoxicity testing of biomaterials. *Biomaterials* 1996;13:1259–64.
- [21] Barbalinardo RJ, Citrin P, Franco CD, Hobson RW, Vitrebond TM. Comparison of isobutyl 2-cyanoacrylate glue, fibrin adhesive, and oxidized regenerated cellulose for control of needle hole bleeding from polytetrafluoroethylene vascular prostheses. *J Vasc Surg* 1986;4:220–3.
- [22] Papatheofanis FJ. Prothrombotic cytotoxicity of cyanoacrylate tissue adhesive. *J Surg Res* 1989;47:309–12.
- [23] Tebala GD, Ceriati F, Miani N, Nori S, Piantelli M, Ceriati E, Cotroneo A. Polymeric glues in intestinal surgery. *Eur Rev Med Pharmacol Sci* 1994;16:13–20.
- [24] Tebala GD, Ceriati F, Ceriati E, Vecchioli A, Nori S. The use of cyanoacrylate tissue adhesive in high-risk intestinal anastomoses. *Epn J Surg* 1995;25:1069–72.
- [25] Giray CB, Atasaver A, Durgun B, Araz K. Clinical and electron microscope comparison of silk sutures and n-butyl-2-cyanoacrylate in human mucosa. *Aust Dent J* 1997;42:255–8.