In vitro biocompatibility characterization of Glubran 2® modified cyanoacrylate surgical glue

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Introduction

Various haemostatic and adhesive means have been used in surgery, including sutures, staples, clamps and application of biological agents, such as fibrin and collagen glues, and synthetic agents, such as cyanoacrylate adhesives. Between these cyanoacrylates have several advantages over standard wound closure devices: they are easy and rapid to be applied, eliminate the need for suture removal and are painless avoiding the use of local anaesthetics. They are liquid monomers that polymerize on contact with living tissue surfaces in an exothermic reaction creating a strong yet flexible film that bonds the apposed wound edges. Surgical glues should have some essential requirements, e.g. adequate adhesive strength, polymerisation in a moist environment, biocompatibility, gradual resorption without foreign-body response and, therefore, due to cyanoacrylates potential toxicity, their biocompatibility must be carefully evaluated before clinical application, in order to identify and, eventually, minimize toxic compounds.

The aim of this study was to investigate the biocompatibility of a modified cyanoacrylate surgical glue named GLUBRAN 2®, supplied by GEM (Viareggio, LU, Italy) particularly through cytotoxicity, cytostaticity, genotoxicity and apoptosis induction tests.

Materials and Methods

Biocompatibility tests were performed *in vitro* on GLUBRAN 2® polymerised in two different ways: as films and irregularly shaped porous low-density materials (ISPL-DM). GLUBRAN 2® extracts were obtained incubating 3 cm^2 of film surface area and 0,1 g of ISPL-DM per ml of extraction vehicle, according to guidelines of ISO 10993 - part 5. The appropriate culture medium was used as extraction vehicle and the extraction was performed in dynamic condition (40 RPM) at 37°C for 72 h. The culture medium in the same conditions was used as negative control. The extracts were tested undiluted, diluted 1:2 and 1:10 with the culture medium.

Cytotoxicity was evaluated using the lactate dehydrogenase (LDH) test after 72 h contact between extracts and mouse fibroblast L929 (ATCC, CCL-1).

Cytostaticity was evaluated using the 5-bromo-deoxyuridine (BrdU) incorporation in L929 replicating cells after 48 h contact with extracts.

The proliferation rate of survival cells after 48 h contact with extracts was assessed by tetrazolium salt (MTT) reduction test on L929 fibroblasts. The MTT test was repeated at 2, 4 and 6 days after extracts replacing with fresh medium.

The apoptosis induction was estimated using Annexin-V and Propidium Iodide (PI) staining by flow cytometry on human peripheral blood mononuclear cells (PBMCs) after an exposure time of 24 h with extracts.

The Single Cell Gel Electrophoresis (SCGE) Comet assay was employed as genotoxicity test on chinese hamster lung fibroblast V79 (ATCC, CCL-93) and human lymphocytes after 5 h incubation with extracts.

Results

The viability of L929 fibroblasts, assessed by LDH assay, was strongly compromised when cells were exposed to undiluted extracts from film. After 1:2 and 1:10 dilution of the extracts, the cells were quite unaffected, with a viability score higher than 70% respect to the control. Concerning ISPL-DM, only the diluted 1:10 extracts resulted not cytotoxic. About the cytostaticity, the film extracts reduced the cell proliferation at 90% when undiluted and diluted 1:2 and at 40% when diluted 1:10. The ISPL-DM extracts, both undiluted and diluted, affected significantly the proliferation respect to the control. The MTT test showed that, when the extracts are employed diluted 1:10, the survival cells start again to proliferate up to confluence. In the apoptosis evaluation, about 90% of cells were positively stained with Annexin-V and PI when undiluted extract were employed. Only the extracts diluted 1:10 induced a low degree of apoptosis (about 40% $AnnexinV^+/PI^+$). The SCGE Comet assay showed that the undiluted glue extracts did not induce genotoxic damage.

Conclusion

The cytotoxicity and apoptosis induction of GLUBRAN 2® was marked when the undiluted glue was used, but it was considered acceptable when it was diluted. On the contrary, the cell proliferation was inhibited also with extract diluted up to 1:10. This appeared to be a temporary effect as demonstrated by the cell proliferation rate which increased over time as showed by the MTT test. This effect seems not to depend on damages at DNA level as shown by the negative outcome of the Comet assay. It has been evidenced that the cytotoxic effect showed in this *in vitro* study by the GLUBRAN 2® glue is due to some formaldehyde release during the extraction procedure; however, the cytotoxicity effect showed by the undiluted extracts may be minimized in clinical applications where the formaldehyde release can be diluted *in situ* by the biological fluids of the body. This moderate toxicity does not affect the several advantages of GLUBRAN 2® glue, concerning especially the rapid polimerization also in a moist environment, as it has been reported in a wide variety of clinical and surgical applications for multiple types of wounds.

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