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Cytotoxicity Study of Different Disposable Medical Devices Extract Using MTT Assay

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Abstract

A large number of disposable medical devices (DMD) are being used every day. In this regard, one of the most important issues is the safety of these tools for patients. In this study, the cytotoxicity of different DMD from different brands was investigated. Cytotoxicity of extracts of DMD including intravenous transfusion sets (IVTS), intravenous infusion set (Microset) and syringe were studied using L-929 cells. Alterations in cell morphology and viability were evaluated using light microscopy and the MTT assay, respectively. The findings of the study demonstrated that 20 percent of IVTS samples, 10 percent of microset samples and 5 percent of syringe samples were cytotoxic. In this regard, four cytotoxic IVTS induced about 95%, 88%, 84% and 79% lethality in treated L929 cells. Also, two cytotoxic microset samples induced 74% and 65% lethality in treated L929 cells and a cytotoxic syringe sample induced 71% lethality in treated cells. Microscopic evaluation showed that the treated cells with the toxic samples were damaged and there was no normal morphology and cell growth. It is concluded that some DMD are toxic and as a result they could be hazardous to human health. Therefore,before clinical use of these medical devices, their biocompatibility must be evaluated by certified laboratories.

Keywords Disposable medical devices, cytotoxicity, MTT, L-929 cells

Introduction

Medical devices are widely used in different clinical measures. These devices have direct and indirect contact with the tissues and cells of the body. Therefore, they should be biocompatible to avoid harmful impacts in the body [1-2]. These medical tools are composed of different type of polymers and other components and are generally considered safe if they are manufactured from high quality primary materials, but in some occasions are not safe for patients when they are not biocompatible. However, manufacturers do not demonstrate the components and the production process of their products and it could be tough to determine their elements [3-4]. It is known that polyvinyl chloride (PVC) is the main material for producing of DMD. Plasticizers are added to polymers for making it flexible because naturally PVC is not flexible. In addition, synthetic polymers, latex and bisphenols are other materials which are used for DMD manufacture. However, some of these materials are hazardous to human health [3-5-6]. It has been considered that if the manufacturer of DMD does not use primary biomaterials with high quality, final products would be harmful for patients because these materials will be released into the body and induce various types of health problems. Particularly, intravenous infusion sets which have prolonged contact with the patient [7]. Fortunately, there are reliable international standards

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Fig 1. IVTS (A), intravenous infusion set (Microset) (B) and syringe (C).Preparation and extraction

for DMD that have been published by ISO to ensure safety of DMD for clinical use [8-10]. One of the most valuable assays which has been considered in ISO standard part 5 for the evaluation of biocompatibility of DMD is cytotoxicity because it has high sensitivity though it is complicated assay. With this assay, there is no need to use animals, as a result, this assay supports animal rights. In this test, cells are used to evaluate the toxicity of DMD. For this purpose, cell growth, and morphological alterations are analyzed following cell exposure with extracts of medical devices [8-11-12]. Therefore, the aim of the present study was to evaluate and compare cytotoxicity of different brands of different medical devices extract including intravenous transfusion sets, intravenous infusion set (Microset) and syringe using MTT assay in L929 cell line. Up to our knowledge, this is the first study about the cytotoxicity of DMD extract in Iran.

Materials and Methods

Materials

Biomedical devices including intravenous transfusion sets (20 brands), intravenous infusion set (Microset) (20 brands) and syringe (20 brands) were prepared. ZDBC (Zinc N, N-dibutyldithiocarbamate) film were used as positive controls as indicated in the ISO standard 10993 part 5. This material was selected as positive control because it has ability to induce a cytotoxicity. HDP (high-density polyethylene) film was used as negative control because it has no ability to induce cytotoxic response [20]. These films prepared from (Food and Drug Safety Center, Hatano Research Institute).

Aseptic dried biomedical devices were extracted according to the ISO 10993-12 standard [9]. Briefly, different parts of IVTS (spike, tube, flashball, needle and drift chamber), Microset (tube, filter and chambers) and syringe (plunger, barrel and needle) were separated under aseptic condition and they were cut into small pieces of 1.25 cm2 and immersed in serum - free α-MEM and then, they were incubated at 37°C in a humidified atmosphere with 5% CO2 for 24 h (9).

Cell Culture

The L-929 cells were purchased from the Cell Bank of Pasteur Institute (Iran). The cell line was cultured in a minimal essential medium (α-MEM medium), supplemented with fetal bovine serum (10%, v/v) and antibiotics (penicillin and streptomycin 1%) at 37°C in a 5% CO2 atmosphere with 95% humidity. The cells were seeded in a flask and examined microscopically with an inverted microscope equipped with cameras (Hund, Germany). The cell count was monitored with a hemocytometer (Germany).

Cytotoxic Evaluation

Cell viability was evaluated using the MTT assay. In this colorimetric assay, mitochondrial dehydrogenases convert MTT into formazan crystals in living cells. For cytotoxicity analysis, exponentially growing L-929 cells were collected and cultured (1 × 104 cells/well) in a 96-well microtiter plate and then incubated for 24 hours in a 5% CO2 atmosphere before treatment. The cells were treated with 100µL devices extract when their confluency reached up to 70%. After treatment for 24 hours, 50 µL of MTT solution (5 mg/mL) was added to each well and incubated at 37°C for two hours. Then, the supernatants were removed, and 50 µL of isopropanol was added to each well to solubilize the formed formazan crystals. Absorbance was read at 570 nm, using a plate spectrophotometer (BioTek, USA).

Statistical Analysis

Data are presented as mean ± SEM. The mean values of all parameters were compared between the groups, using one-way ANOVA, followed by Tukey's post-hoc test. Data were analyzed with SPSS version 19, and $P < 0.05$ was considered statistically significant.

Table 1. Cell lethality percent of toxic samples in L929 cells using the MTT assay. P ≤ 0.05 versus control group.

	control	IVTS	IVTS	IVTS	IVTS	microset	microset	syringe
Cell lethality (%)	0	88 ± 3*	95 ± 4.5*	79 ± 3.1*	84 ± 3.4*	65 ± 2.4*	74 ± 2.9*	71 ± 3.7*

Results

Cytotoxic Activity

The L929 cells were treated with extracts of different medical devices for 24 hours. The findings of our study demonstrated that 4 samples of twenty IVTS samples, 2 samples of twenty microset samples and one sample of twenty syringe samples were cytotoxic. In addition, the highest cell lethality rate was in the group of IVTS samples and the lowest cell lethality rate was in the group of syringe samples. In this regard, the highest lethality in treated cells was 95 ± 4.5 percent and the lowest lethality was 79 ± 3.1 percent for toxic IVTS. Meanwhile, the lowest lethality was about 65 ± 2.4 percent for one of the two microset among all of the samples (table 1). All in all, mean of viability of treated cells with extracts of syringe samples (88.65 ± 4.1) was more compared to the viability of treated cells with extracts of microset samples (81.1 ± 3.9) and IVTS samples (72.9 ± 3.7) (figure 2).

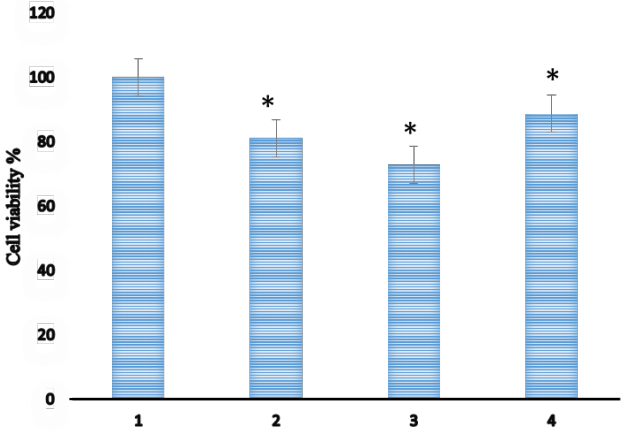

Fig 2. The outcomes of the cell viability analysis using the MTT assay: L-929 cells incubated with (1) control (2) the extracts of microset samples (3), the extracts of the IVTS samples (4) the extracts of syringe samples

Microscopic examinations

Figure 3 shows the photomicrographs of L929 fibroblast cells treated with the controls, IVTS, microset and syringe extracts. Light microscopic analysis of cell morphology demonstrated that the cells had been grown in the control group indicating no cytotoxicity. L929 mouse

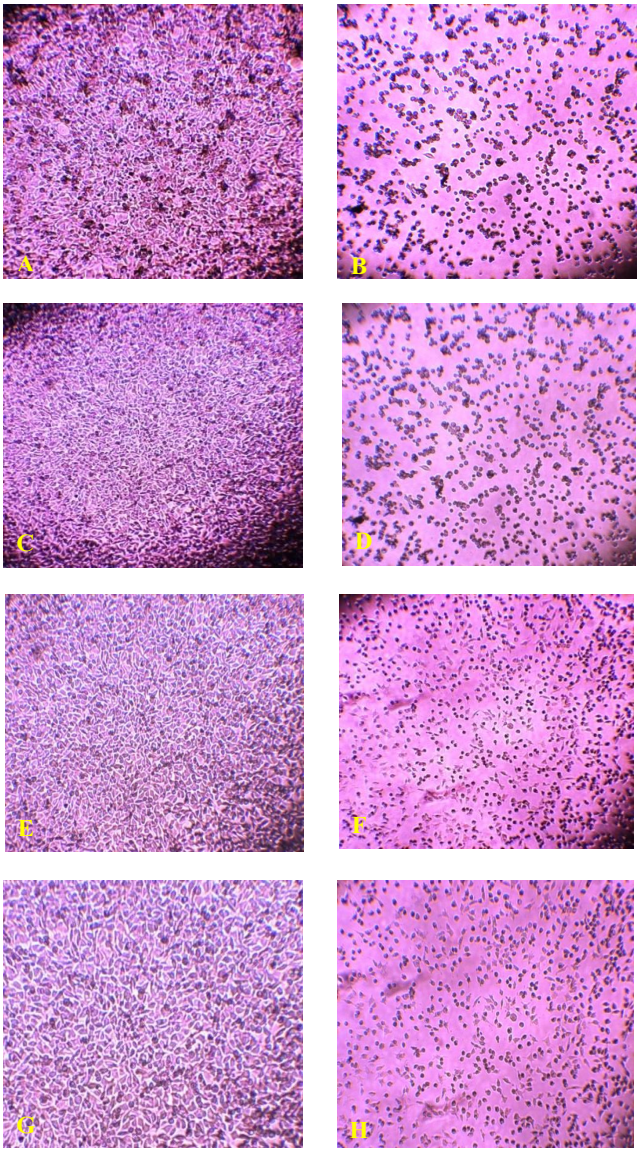

Fig 3. Cytotoxicity of IVTS, microset and syringe extracts on L-929 cells after 24 hours exposure. (A) Negative control (B) Positive control (C) Normal cells exposed to the non-toxic IVTS extract (D) Cells exposed to the toxic IVTS extract (E) Normal cells exposed to non-toxic microset extract (F) Damaged cells exposed to the toxic microset extract (G) Normal cells exposed to non-toxic IVTS extract (H) Damaged cells exposed to the toxic syringe extract.

fibroblasts are large, spindle-shaped, adherent cells growing as a confluent monolayer. It could be observed that the morphology of L929 cells was the same for non-toxic samples and the control group.
On the other hand, results from the microscopic analyses revealed that there were some differences in the L929 cells between toxic samples and control. Affected cells were damaged and died and there were no normal morphology and cell growth. In addition, significant elevated rounded cells which were suspended in the medium were observed in the treated cells with the toxic samples.

Discussion

Safety of DMD must evaluate with biocompatibility assays before using them in patients. Cytotoxicity test such as MTT assay is one the most reliable assays which performed in our study [11, 13]. The mechanism of MTT reduction is not well understood, but it is believed to involve NADH or similar reducing molecules which transfer electrons to MTT [13)]. In this investigation, we surveyed the safety of different DMD including intravenous transfusion sets, microset and syringe from different brands. The results of the present study demonstrated that 20 percent of IVTS samples, 10 percent of microset samples and 5 percent of syringe samples were not safe for clinical use. IVTS have different parts which are produced from different raw materials. These biomaterials must be medical graded, but some manufacturers use low quality medical grade raw materials for producing their products which is illegal. This medical tool has different parts including spike, solution filter, drip chamber, tube, roller clamp, flashball and needle. Different raw materials such as synthetic polymers including (polyurethane, polyethylene, polypropylene, polystyrene, polyester, polycarbonate, polyvinyl chloride), natural rubber latex (NRL, latex), plasticizers, bisphenols, phthalate plasticizers and so forth are used for production of these parts [3-14-16). However, it has been reported that some of these biomaterials have adverse effects for human health such as synthetic polymers which are used for manufacturing of DMD could induce inflammation or some plasticizers which are carcinogenic [3-17]. In this study, we also exposed extracts of different parts of toxic IVTS with L-929 cells (data not shown). Our toxicity analysis demonstrated that the latex part (flashball) is the toxic and different parts were not toxic. Hence, the latex part could be toxic for patients who receive medication by IVTS. Drewa et al demonstrated that latex could be highly cytotoxic in primary cultured rabbit urothelial cells (PRUC) [18]. In addition, Cormio et al reported that the latex of the catheter and latex gloves is cytotoxic in green monkey kidney (GMK) cells [19]. As a result, for safety of patients, latex part should be removed and another safe material should be substituted or for reducing toxicity of latex, the surface of flashball should be coated with biocompatible materials.
We also evaluated extract of different parts of toxic microset brands and a toxic syringe. We observed that there were no difference between different parts of these medical devices and all parts have equal toxicity. Therefore, it is clear that the quality of biomaterials which are used for manufacturing of these devices were not standard. Another reason for safety reduction of such medical devices could be inappropriate storage condition such as high temperature or humidity for a long time [3]. Particularly, DMD which are imported into our country and they are maintained for a long time in a harsh condition until release to the market.Another reason for manufacturing the unsafe final product is that raw materials are transformed chemically into the unsafe materials prior inappropriate manufacturing process [3]. Another hypothesis for negative effects of plastic medical devices extract on cells is that some metals such as Cd, Fe, Pb, Zn and so forth which are presented in final plastic medical products are released following extraction process from these devices. These metals could be cytotoxic and inhibit cell growth [20]. Pant et al also reported that 6 brands of IVTS were cytotoxic and caused a significant decline (56%) in survival rate following 36 h exposure with cells [2 which is in agreement with our results.

Conclusions

All in all, we conclude that some DMD brands are not appropriate for clinical use because they are toxic. Therefore, we strongly believe that before clinical use of these medical devices such as IVTS, microset and syringe, their biocompatibility must be evaluated by certified laboratories. In general, we suggest that different DMD including catether, angiocath, cannula and so forth should be evaluated for their biocompatibility.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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