Online Journal of Gynecology, Obstetrics and Maternity Care
ISSN: 2642-0163

Research Article *Volume 3 Issue 2*

Evaluation of Biocompatibility of Polymers and Cytotoxicity of Injection Drugs in Shelf Life by Assay MTT Method

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Received Date: January 23, 2020; **Published Date:** February 17, 2020

Abstract

According to international standards, the cytotoxicity test is one of the main controls for evaluating the biocompatibility of polymeric or elastomeric materials used in the manufacture of medical devices and pharmaceutical containers. These tests are required for substances that directly interact with cells and tissues within the body. This assay can be performed in both qualitative and quantitative ways using cell culture techniques and generally on the L929 cell line. In this study, the cytotoxic effects of polymeric containers of injectable products during their shelf life were evaluated by MTT assay. MTT Assay was performed as a quantitative colorimetric test in two phases: testing of polymeric raw materials and testing samples of serums that are produced in polymeric containers. The MTT assay can detect viability based on the rate of cell reduction activity. Then the results are examined by determining the optical absorption of the samples by ELISA Reader at 570 nm wavelength. If the results of the cytotoxicity test on the polymeric raw materials used in the production of serum containers are negative, they can be used safely and over time may lead to the effects of untreated cytotoxicity and migration of toxic components from the container's body into the solution has not occurred. Therefore, it can be concluded that this test is suitable as a complementary test to confirm the stability of the serums until the end of their expiration date.

Keywords: Cytotoxicity; Biocompatibility of Polymers; Polymeric Drug containers; MTT Assay

Abbreviations: ASTM: American Society for Testing and Materials; ISO: International Standard Association; FDA: Food and Drug Administration; HPLC: High Performance Liquid Chromatography; LDPEs: Lightweight Polyethylene Containers; WHO: World Health Organization.

Cell Culture

On the one hand, all manufacturer companies that produce medicines, serum, injectable products and medical equipment operate under the GMP international rules, due to the type of product manufactured. Among various drugs and injectable solutions are of particular importance due to their direct entry into the blood and contact with cells and tissues of the body and their quality control process is even more stringent [1].

On the other hand, containers used for these medicines

should be such as to protect the drug standard by the end of its expiration date and to prevent any chemical changes in the products.

These containers must be resistant to changes in environmental parameters such as light, temperature, pressure and humidity. In addition, the compounds used in these containers must be chemically neutral and do not react with the solution in the containers. Most importantly, over time, the compounds in the structure of these containers do not migrate into the drug solution. It is also significant to resist the penetration of microorganisms and other microbial agents [2]. One of the main methods used to study biocompatibility is cell culture. The extraordinary sensitivity of the cells to the toxic substances enables the examination of specific interactions in the cell and the conduct of numerous experiments. Relevant information and guidelines can be found in scientific sources or international standard institutes such as the American Society for Testing and Materials (ASTM), the International Standard Association (ISO), the US Food and Drug Administration (FDA), the Pharmacopoeia Books (USP, BP, JP, EP) and the Pharmaceutical Inspection Cooperation Organization (PIC/S) [1]. Nowadays, cell culture has become an essential technology in many fields of sciences and researches. It is also used as a tool to characterize the human genome and to describe the intracellular and intercellular pathways that regulate gene expression.

Replacement and improvement in organs and tissues that are damaged or malfunctioning are the goals of this science. Currently, the use of stem cells as cells capable of differentiating into various cell types, tissues and organs is one of the most important applications of cell culture [3]. Cell culture is a process in which cells grow under controlled conditions in a laboratory. Cell proliferation is carried out under similar conditions in the body at 37°C and in incubators with saturated humidity and 5% CO_2 and completely disinfected conditions. Cell culture is carried out in a separate room with controlled conditions in terms of air, temperature, humidity and personnel numbers [4].

Review of Studies

Experiments were performed by MTT assay in the presence and absence of serum proteins. This polymer was highly toxic for both cell lines and its IC50 for HpeG2 cells was 60 µg/mL and for CCRF cells was 30 µg/mL. In the absence of serum proteins, poly-L- lysine toxicity was increased and IC50 was reduced to 25.5 µg/mL for adherent cells and 0.8µg/mL for suspension cells. However, other polymers such as poly-L-proline, polyethylene glycol, poly-L-glutamic acid, polyvinylpyrrolidone and dextran showed no toxicity. As a result, the MTT assay was identified as a relatively rapid initial response test to evaluate the cellular toxicity effect of the polymers. Furthermore, appropriate cell lines should be used to evaluate the compatibility of polymers and their toxicity effects [5].

Since a biomaterial should not cause any adverse reaction to the organism and endanger the patient's life, the cytotoxicity test is performed in vivo using a rabbit-based direct contact test and a cell culture assay (in vitro). Results for all tested samples showed no toxicity and skin irritation and were considered suitable for clinical application [6]. An investigation about the compatibility of plastics with pharmaceutical solutions was presented in 1999, which showed that no turbidity, discoloration or crystallization occurred during this time. Samples were collected at different time intervals and the content of the material was determined by high performance liquid chromatography (HPLC). At 4°C, no absorption was observed for glass containers and lightweight polyethylene containers (LDPEs).

At room temperature for LDPE a slight decrease in concentration due to adsorption was observed but this decrease was more pronounced in PVC bags. Dacarbazine and Melphalan, which were not material-dependent, also showed a slight decrease in concentration. In the rest of the cases there was no decrease in the drug content. It was thus found that the best stability conditions for these materials are in glass containers and then in lightweight polyethylene containers and then in PVC bags [7]. The MTT method has been used as a quantitative test of the latest version of ISO (ISO 10993-5: 2009) in relation to the biological evaluation of medical devices. While qualitative cytotoxic methods are useful for screening purposes, quantitative methods may be more appropriate for determining the cytotoxicity of substances.

In this technique, the yellow substance MTT is used to react with mitochondrial succinate dehydrogenase enzyme in living cells. This reaction occurs only in living cells and is performed after contact with the sample or control to determine the percentage of living cells. Based on the ISO standard, the Pacific BioLabs Institute recommends the MTT method to evaluate the results because of its high sensitivity and the use of a digital device (ELISA reader) [5].

Materials and Methods

Guidelines and protocols have been developed and are available to users to perform various cell-related tests. They are usually prepared in a standard format, according to international reputable sources such as USP and ISO and some credible articles. Therefore, all matters including entry and exit regulations, disinfection of surfaces and equipment, equipment operations, test procedures and the others are governed by standard rules.

1. It should always be observed when working in this laboratory.
2. All cell culture steps (except cell counts) should be performed under the laminar flow.
3. All tools must be disinfected by alcohol and placed on the laminar surface before starting work, and then the UV lamp will be lit for about 20'.
4. During the work, always use gloves and 70% alcohol to disinfect.
5. To prevent fungal and microbial contamination, health and safety considerations should be strictly observed.

In this study, the conventional cell culture assay was performed by using RPMI medium and L929 cell line. In the first phase, the cytotoxicity assay was performed quantitatively and based on the MTT assay technique on the polymeric raw materials, and in the second phase, the same method was performed on samples of products manufactured in polymeric containers that reached the end of their shelf life. Thus, the quality of the sera was compared at the time of production and expiration. The cells can be stored in a liquid nitrogen tank (-196 °C) by use of polypropylene cryo tubes for a long time on inactive form. They can be de-freezed when necessary and revive them according to the specified protocol [8].

In this process, two points are important to prevent cell damaging: speed of operation and compliance with sterile conditions. Because cells coming out of the nitrogen tank are very sensitive and many of them are destroyed during the regeneration phase. In this test, sample extraction was not performed and SWFI (sterile water for injection) as the sample, was added to the cell culture medium directly and its effect on cell growth and cell viability was investigated. Since no compound can be substituted for the culture medium and add into the cell medium alone, it is necessary to prepare a medium with a concentration of 2x at first, then add it to the sample in equal volumes. By doing so, the medium was obtained in a balanced concentration involve of sample containing, but without any ionic composition. This step was performed separately for the 7 samples in question. These samples were examined by MTT assay with negative control and positive control in a 96-well microplate, respectively.

Discussion and Conclusion

First Polypropylene Test

The results of the microplate optical absorption measurements in the first polypropylene evaluation are presented below and the corresponding graphs are plotted using Excel software. Thus, after selecting 3 suitable numbers from 6 wells for negative control, their average was determined and recorded. Then, in each column of 5 concentrations of extract, 3 optimum numbers of optical absorption were determined and their mean was recorded. Finally, to determine cell viability, the mean absorbance of each concentration was divided by the mean of negative control (Table 1).

PP: Test - 1 (97.09.04)						
		1	2	3	4	5
Cell No.	Negative	Dilution:	Dilution:	Dilution:	Dilution:	Dilution:
	Control	1	2-Jan	4-Jan	8-Jan	16-Jan
1	0.252	0.217	0.23	0.225	0.238	0.24
2	0.265	0.21	0.219	0.24	0.253	0.248
3	0.262	0.195	0.222	0.235	0.232	0.25
Average:	0.26	0.207	0.224	0.233	0.241	0.246
80%		Viability = Dilution Ave / Neg. Control Ave				
		86%	90%	93%	95%	

Table 1: Optical Absorption Rate, Mean and Survival Percentage at Extract Concentration (Test 1).

According to ISO standard (ISO 10993-5), the sample has a toxic effect if the viability of extract- treated cells is less than 70% negative control (blank), or in other words, cell death is more than 30%. Therefore, to be considered non-Cytotoxic, the cell viability must be equal to or greater than 70%. In addition, the viability of the cells under the 50% concentration of the extract should be similar to or greater than the 100% concentration of the extract, otherwise the test should be repeated [9].

According to Figure 1, the results showed that the lower the extract concentration, the better the cell growth and natural proliferation. Thus, at the lowest concentration of 1:16 (sample number 5), the viability of the cells is 95%, indicating almost normal growth conditions, and at the highest concentration, 100% of the extract, the cell viability

is 80% of the initial rate. Be it. Given that the cell death rate is within the acceptable range of the ISO standard, the amount of compounds released from the polypropylene sample did not have a significant toxic effect on L929 cells and was considered a non-toxic polymer [10,11].

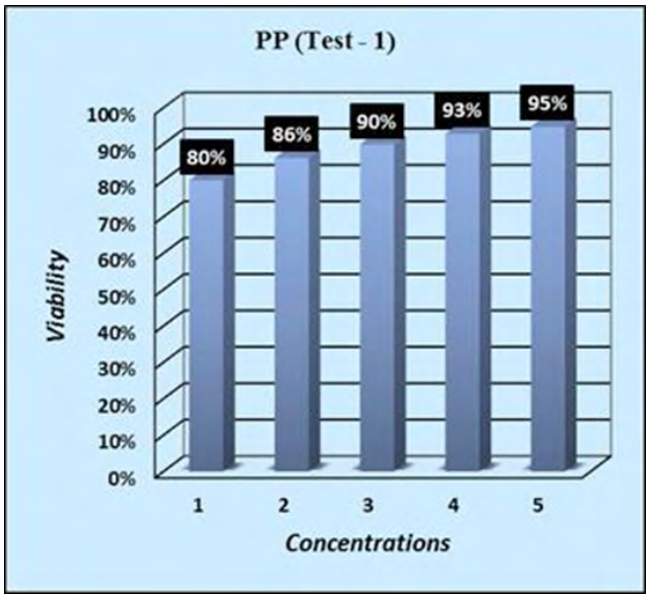

Figure 1: Cell viability in terms of extract concentration (Test 1).

Second Polypropylene Test

As in the first test, the results of microplate optical absorption were recorded and 3 suitable wells from 6 wells for negative control and extract concentrations were selected and their mean was determined (Table 2). Then, pre-test was performed to determine cell viability and plot using Excel software.

PP: Test - 2 (97.09.11)

		1	2	3	4	5
Cell No.	**Negative**	**Dilution:**	**Dilution:**	**Dilution:**	**Dilution:**	**Dilution:**
	Control	**1**	**2-Jan**	**4-Jan**	**8-Jan**	**16-Jan**
1	0.252	0.202	0.229	0.245	0.23	0.242
2	0.25	0.212	0.222	0.238	0.249	0.251
3	0.264	0.193	0.216	0.22	0.239	0.236
Average:	0.255	0.202	0.222	0.234	0.239	0.243
79%		Viability =	Dilution Ave	/ Neg. Con	trol Ave	
		87%	92%	94%	95%	

Table 2: Optical Absorption Rate, Average and Survival Percentage at Extract Concentration (Test 2)

According to Figure 2, the results of this test showed that the viability of the cells in the main extract concentration (sample 1) was 79% and in the dilute state (sample 5) 95%. In other words, the concentration of the extract was inversely correlated with cell growth and survival, and in the worst case, the amount of compounds released from the polymer sample resulted in 21% cell death. Therefore, at this stage, the test material was found to have no toxic effects.

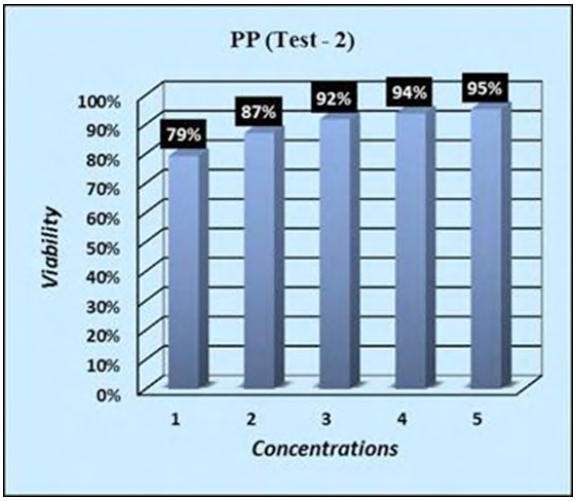

Figure 2: Cell viability by concentration of extract (Test 2)

PP: Test			- 3 (97.09.19)			
Cell No.	**Negative**	**Dilution:**	**Dilution:**	**Dilution:**	**Dilution:**	**Dilution:**
	Control	**1**	**2-Jan**	**4-Jan**	**8-Jan**	**16-Jan**
1	0.24	0.211	0.212	0.207	0.222	0.254
2	0.256	0.188	0.205	0.235	0.243	0.245
3	0.26	0.193	0.218	0.217	0.239	0.228
Average	0.252	0.197	0.212	0.22	0.235	0.242
78%		Viability =	Dilution Ave	/ Neg. Con	trol Ave	
		84%	87%	93%	96%	

Table 3: Optical Absorption Rate, Mean and Survival Percentage at Extract Concentration (Test 3).

Third Polypropylene Test

The procedure was repeated for the previous two tests. The results and the mean of three appropriate numbers of negative control and extract concentrations were selected and their mean was determined (Table 4).

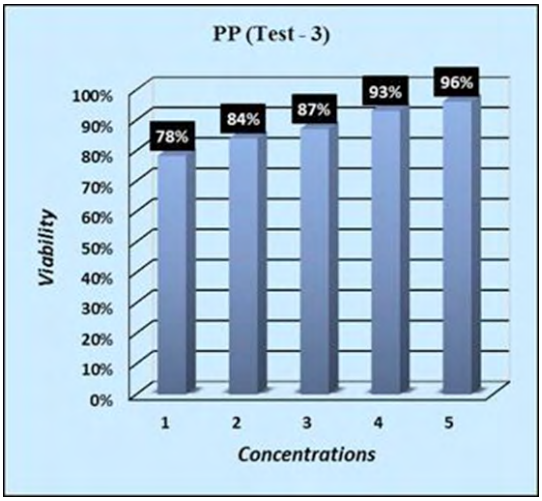

Figure 3: Cell viability in terms of extract concentration (Test 3).

Then, as in the previous tests, the cell viability and the corresponding chart were obtained by Excel software. The results of this test showed cell viability between 78% and 96%, which was within acceptable range and no cytotoxic effect was detected in the sample as in the previous two tests. As shown in Figure 4-3, the effect of the main extract on cell death was 22% and the effect of the lowest concentration was 4%. Therefore, the viability of the cells was standardized.

Phase II: End-Product Tests

The first test of distilled water: After testing on polymeric raw materials used in the manufacture of injectable drug containers, the products in these containers were also evaluated by MTT assay. Samples consisted of 7 vials of 5 mL polypropylene vial and content of sterile distilled water (SWFI). The first one, produced in January 1977, was at the beginning of the expiry date, and the last one, produced in January 2015, was at the end of its expiration date. Three consecutive tests were performed on distilled water in the vials and the results were recorded as before. In the order mentioned above, 3 appropriate numbers were selected from 6 replicates for negative control and samples and plotted using Excel software, cell viability and their charts, which is visible in Table 4 [8].

SWFI: Test - 1 (97.10.24)								
		1-Jan	2-Jan	3-Jan	4-Jan	5	6	7
Cell No.	Negative	Jun	Jul	Jun	Jul	Jun	Jul	Jun
	Control	2018	2018	2017	2017	2016	2016	2015
1	0.259	0.272	0.242	0.25	0.247	0.225	0.221	0.215
2	0.28	0.26	0.267	0.246	0.229	0.241	0.215	0.205
3	0.269	0.252	0.258	0.239	0.236	0.239	0.219	0.212
Average:	0.269	0.261	0.256	0.245	0.237	0.235	0.218	0.211
97%		Viability = Dilution Ave / Neg. Control Ave						
		95%	91%	88%	87%	81%	78%	

Table 4: Optical Absorption Results, Mean and Survival Percentage from Production Time to Product Expiration (Test 4).

According to Figure 4, the results showed that the viability of cells under Sample 1 (fresh product) was 97%, and the addition of this solution to the culture medium did not alter the normal process of cell growth. On the other hand, cell growth in the medium containing the expired product (sample 7) decreased significantly and reached 78%. It was also observed at 6-month intervals from production to mild expiration with a decrease in cell survival. Therefore, with the shelf life of the product for 3 years the effects of migration of cytotoxic compounds from the polymeric body into the solution were seen but within the acceptable ISO standard [6].

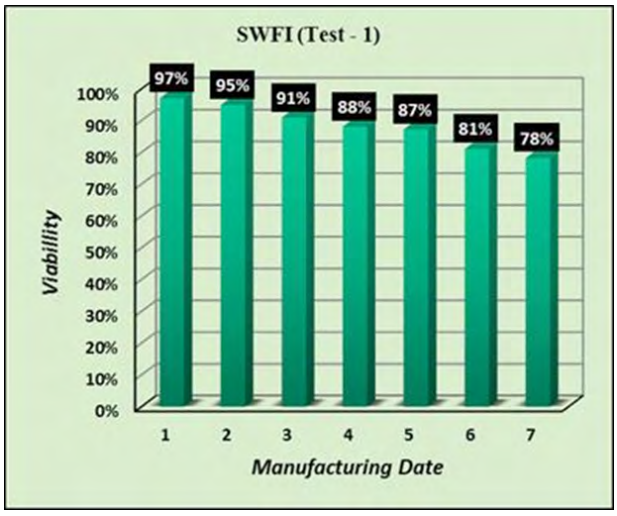

Figure 4: Cell viability by product production time (Test 4).

Second test of distilled water: After measuring the microplate optical absorption, the obtained numbers were recorded and by the pre-test method, three suitable negative control numbers and samples were selected and their mean was determined (Table 5). Excel software was then used to determine cell viability and plot.

SWFI: Test - 2 (97.11.01)								
		1	2	3	4	5	6	7
Cell No.	Negative	Jun	jul	Jun	jul	Jun	jul	Jun
	Control	2018	2018	2017	2017	2016	2016	2015
1	0.289	0.261	0.251	0.261	0.246	0.234	0.226	0.218
2	0.274	0.273	0.28	0.24	0.258	0.25	0.21	0.2
3	0.272	0.258	0.247	0.253	0.239	0.227	0.224	0.209
Average:	0.278	26%	26%	25%	25%	24%	0.22	0.209
95%		Viability = Dilution Ave / Neg. Control Ave						
		93%	90%	89%	85%	79%	75%	

Table 5: Optical Absorption Results, Mean and Survival Percentage from Production Time to Product Expiration (Test 5).

From the results of this test, it was found that the effect of the newly produced product (sample 1) on cell death was negligible and about 3%, which can be neglected, as it is also possible in normal cell growth in pure culture medium. There is the same drop in cell density. Subsequently, a slight decrease in cell viability was observed every 6 months with increasing product interval from production date, respectively. In the latest specimen that has reached the expiration date, the lowest cell viability and, in other words, the highest damage and cell death were recorded. Cell viability was determined to be 78% by Sample No. 7, which was standard accepted (Figure 5). Therefore, during the 3-year shelf-life of injectable distilled water in the polymer vial (shelf life of the samples), a decrease in cell density and viability was observed, but was not considered to be cytotoxic by the ISO standard, meaning that the amount of toxic constituents From the polymer vial body to the released solution was within the permissible range [12,13].

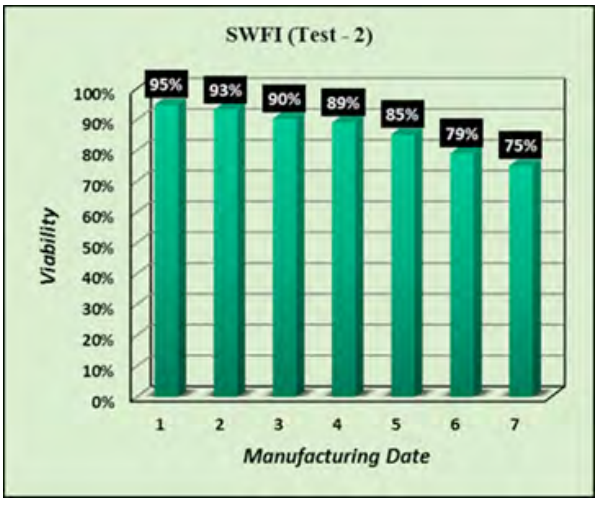

Figure 5: Cell viability in terms of product production time (Test 5)

Third test of distilled water: The results of optical absorption determination of wells were recorded and, as in the previous tests, 3 suitable numbers out of 6 negative control and samples were selected and their mean was calculated (Table 5). Then, cell viability was determined using Excel software and the chart was plotted.

SWFI: Test - 3 (97.11.07)						
		1	2	3	4	5
Cell No.	Negative	Jun	Jul	Jun	Jul	Jun
	Control	2018	2018	2017	2017	2016
1	0.269	0.256	0.233	0.224	0.22	0.208
2	0.257	0.24	0.248	0.24	0.229	0.224
3	0.251	24%	23%	23%	24%	0.217
Average:	0.259	24%	24%	23%	23%	22%

Table 6: Optical Absorption Results, Average and Survival Percentage from Production Time to Product Expiration (Test 6).

The results of this test, according to Figure 6, showed that only 6% of cell density in the medium containing the newly produced product decreased and no significant disruption of cell growth was observed. In the worst case scenario, cell viability in the medium containing sample 7 (expired product) reached 74%. Thus, during the shelf life of this product, little effect was observed on the release of cytotoxic compounds from the vial body and into the solution but was within the permissible range according to the ISO standard. Figures 4-6- Cell viability by product production time (Test 6) [14].

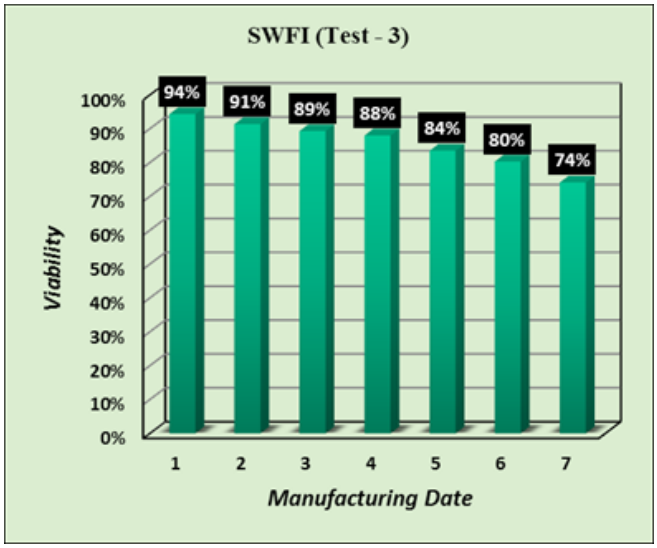

Figure 6: Cell viability by product production time (Test 6).

Results

Based on the results of three tests on polymeric raw materials (polypropylene) and three tests on distilled water produced and stored in vials of this polymer, it can be concluded that over the life span The shelf-life of this injectable product contains substances with a cytotoxic effect on the structure of this polymer, which has been slowly introduced into the solution for 3 years. However, the amounts of these compounds and their cellular damage have been approved and approved. Because according to ISO standard (ISO 10993-5), if the cell viability is more than 70% under test material it is considered non-Cytotoxic. Since the results of the tests show that the cell survival rate is higher than this, the polypropylene tested confirms the lack of toxicity [15].

- It is initially recommended that these tests comply with GLP regulations. Then, in describing the toxicity test as one of the biocompatibility assessment methods, it is stated that it involves exposure of one or two cell lines to the extract extracted from the test material. He goes on to classify these tests and briefly describes each test based on several references including ISO, USP and Screening using the two L929 and MRC-5 cell lines:
- Agarose Overlay Test: Cells are coated with a permeable agar layer. The solid sample is disk- shaped and triplicate

on the agar surface and the response is checked 24 hours later.

- Membrane Extraction Test: Solids are extracted in cell culture medium and their effect on cells. Response time is 48 hours.
- Growth Inhibition Assay: Solids are extracted in cell culture medium and serially diluted. The extracts were then placed in cell containers for 72 hours. Finally, the protein content of the samples was determined using the Lowry method and then the growth inhibition percentage for the samples were calculated.
- Direct Contact Test: The specimens are placed directly on the surface of the cell layer and 24 hours later the test result is checked.
- Genetic Toxicity or Mutagenicity Test: This test is performed according to ISO 10993-3 to evaluate the ability of a material to cause mutations and genetic damage. All substances that have been in contact with cells and body tissues for a long time, such as implants, should be evaluated in this way.
- Hemocompatibility Tests: The most important test in this group is the hemolysis test, which examines the ability of the red blood cells to be eliminated by substances and their extracts. This test should be performed on all substances such as artificial veins that directly contact the bloodstream and blood cells.
- At the end of this article is a table listing all the biocompatibility tests appropriate to the shelf life of the body as well as a member of the body that will be in contact with the biomaterial. The following table also lists the types of methods and the type and amount of samples required for each test. These tables are designed according to FDA G95-1 and ISO 10993: 2009 standards [16].

In addition, medical equipment has been grouped into three groups in terms of the length of time the body has been in contact [9].

- Up to 24 hours: Short Term
- More than 24 hours to 30 days: Prolonged
- More than 30 days: Permanent

Based on reputable pharmaceutical and medical references such as Pharmacopoeia (USP), BP, JP, EP), US Food and Drug Administration (FDA), US Food and Drug Administration (ASTM), International Standards Organization (ISO), and the World Health Organization (WHO) All biomaterials (plastics, elastomers and polymers) that are clinically designed and manufactured for short or long-term exposure to cells and tissues of the body must undergo biocompatibility tests to assess the effects of toxicity.

And their clinical complications. These tests are divided into several groups depending on the type of material and their application, such as: cytotoxicity tests, allergy tests, systemic toxicity test, blood compatibility test. Cytotoxicity test is considered as one of the most important biocompatibility tests for medical materials and equipment and is required prior to application of these materials and equipment. The test is performed in three ways, by type and sex of biomaterial: agar diffusion, direct contact and extraction. Therefore polymers used in therapeutic branches can only be used if they are negative for these tests [17].

For example, in the case of drug containers made of polymeric materials such as polyethylene and polypropylene, the polymeric raw materials are tested for cytotoxicity. If the test response is negative, it can be concluded that the migration of compounds with the toxicity effect from the body of the dishes into the solution does not occur. Therefore, these drugs are stable in their shelf life and do not adversely affect the growth and proliferation of body cells.

This research is based on valid sources and standards to prove the quality of injectable products and their durability over the production period until expiration. Since no research has been conducted in Iran to evaluate the toxicity of polymeric drug at the end of its shelf life, this study is recent and for the first time. The main focus of this research is the stability of injectable products to the end of their shelf life and the investigation of the migration of toxic compounds from the polymeric body of the containers into the solutions by the end of the shelf life.

Therefore, it can be inferred that if the tests are negative, injectable drugs such as sera produced in polymeric containers until the end of the expiry date are negative and safe for cytotoxicity. In other words, the cytotoxicity testing of polymeric containers, along with the required tests of the Ministry of Health (chemical and microbial analysis of the solutions inside these containers), can be performed on samples that have reached expiration date. The end result of these tests confirms the quality and stability of these products from the beginning of production to the expiry date (shelf life of the drug) [18].

Study Limitations

The purpose of this study is to ensure that the polymeric containers of the drugs have not cytotoxic compounds, which can lead to undesirable effects on the patient's body and even cell degeneration. Additionally, it is aimed at the stability of the products and their quality from production to end of the shelf life. According to GMP regulations, in pharmaceutical companies, a few numbers of all products keep in the stability warehouse until the expiration date. Therefore, the samples which are chose should be available in sufficient

amount in the warehouse and sampling will be possible in 6 months periods. In addition, as the normal conditions of the culture medium should not change, an ion- free product must be selected so that inappropriate conditions for cell growth will not create. Then, sterile water for injection as a suitable candidate was selected.

References

1. Experimental in-wiki tests.
2. Experiments inside the wiki. Retrieved
3. Hassanzadeh, Shapour, Bonyadi, Farzaneh (2016) Animal cell culture principles and methods. Urmia University of Urmia.
4. Khorramizadeh, Mohammad Reza, Falak, Reza (2009) The Basics and Basics of Cell Culture Techniques. Tehran: Tehran University of Medical Sciences.
5. Rogero S, Malmonge S, Lugão A, Ikeda T, Miyamaru L, et al. (2003) Biocompatibility Study of Polymeric Biomaterials. Artificial Organs 27(5): 424-427.
6. Pusnik M, Imeri M, Deppierraz G, Bruinink A, Zinn M (2016) The agar diffusion scratch assay-A novel method to assess the bioactive and cytotoxic potential of new materials and compounds. Scientific Reports, 6: 20854.
7. Beitz D, Bertsch T, Hannak D, Schrammel W, Einberger C, et al. (1999) Compatibility of plastics with cytotoxic drug solutions — comparison of polyethylene with other container materials. International Journal of Pharmaceutics 185(1): 113-121.
8. Vidal MNP, Granjeiro JM (2017) Cytotoxicity Tests for Evaluating Medical Devices: An Alert for the Development of Biotechnology Health Products. Biomedical Science and Engineering, 10(9): 431-443.
9. Biocompatibility Testing.
10. Li W, Zhou J, Xu Y (2015) Study of the in vitro cytotoxicity testing of medical devices. Biomedical Reports, 3(5): 617-620.
11. Aslantürk ÖS. (2017) In Vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages. Intechopen, chapter 1.
12. ISO 10993-5, Biological Evaluation of Medical Devices: Test for in Vitro Cytotoxicity.
13. ISO 10993-12, Practical Guide: Sample Preparation and Reference Materials.
14. USP. >87< Biological Reactivity Tests, In Vitro.
15. USP-NF. <1031> The Biocompatibility of Materials Used in Drug Containers, Medical Devices, and Implants.
16. Medical Device Testing Guide. (2011).
17. ATCC® 30-1011K, XTT Cell Proliferation Assay Kit, Instruction Manual, Copyright © 2011.
18. ASTM [International Standards Book-F813]. Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices 13.01.