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Biocompatibility evaluation of NiTi SMA stent via intervenient therapy

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Abstract: NiTi shape memory alloy (SMA) stent with film are the long-term implanted medical devices which could be used in human's organ, such as esophagus, bile duct, urethra-prostate and blood vessel, by intervene therapy. It is very important to have a good biocompatibility for implanted device. According to standard ISO 10993, we completed biocompatibility evaluation of NiTi stent that included following tests: cytotoxicity, sensitization, genotoxicity, hemolysis and acute systemic toxicity. The results of tests qualify the NiTi stent, and provide an optimistic conclusion for the eventual use of NiTi stent as implanted medical devices.

Key words: NiTi Stent; Biocompatibility; Intervenient therapy

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1 Introuction

NiTi stent are medical devices implanted into human body via intervenient therapy, which are woven out of NiTi memory alloy wire. NiTi stent for certain purposes are coated with silicon rubber and have long-term osculation with organs such as esophagus, bile duct, trachea, blood vessel, etc., which requires excellent biocompatibility as an important criterion to ensure their safe and effective clinical application. The International Organization for Standardization has established the ISO10993 series of standards[1] requiring a series of in vitro and in vivo safety tests before clinical application of any devices or materials implanted in human body to determine their biocompatibility. To have a full-scale and accurate biocompatibility evaluation for NiTi stent, we have accomplished the biocompatibility tests of NiTi stent with the assistance of Tianjin Center for Research and Inspection of Biological Material for Medical Application, which provides proofs for their safety and efficacy evaluation in clinical application.

2 Test item

The contact position with human body, time or features of use should be considered when selecting biological evaluation items of these devices or materials. According to ISO standards[1], tests have been conducted on cytotoxicity, acute systemic toxicity, hemolysis, sensitization and genotoxicity since NiTi stents contact tissue, mucous membrane surface and blood of human body with successive application period far

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exceeding 3 months.

3 Test methods and result

3.1 Cytotoxicity test

3.1.1 Mechanism

Method of agar bestows was used. Cell growth inhabitation, function alternation, cytolysis, apoptosis or other toxic reactions will occur when cell contacts poisonous substances. During toxic reactions, dyed agar medium will be decolorized and cytotoxicity should be determined through observation of decoloring area and cytolysis status within decoloring zone. Use eugonic L929 cells passage cultured for 48 h as cell strains[2].

3.1.2 Sample preparation

Taking 4 g NiTi alloy esophageal stent coated with silicon rubber, adding 20 mL RPMI1640 culture solution containing 15% foetal calf serum, extract solution was obtained after processing for 72 h at 50℃. During test, 0.1 mL extraction solution was taken and absorbed on 8 layers of 100mm round filter paper as a sample.

3.1.3 Control materials

Negative control materials: polyethylene film; Positive control material: polyethylene film containing tin; Extract medium control : RPMI1640 culture solution containing 15% foetal calf serum.

3.1.4 Test result

Test result is shown in Table 1.

Within control group, negative material reaction index is R=0/0 and positive material reaction index is R=2/2, which complies with ISO10993-5 standards. No cytolysis occurs in the test sample, reaction index R=0.5/0. NiTi coated esophageal stent has no cytotoxicity.

Table 1 Cytotoxicity test result of NiTi alloy coated esophageal stent

Group	Sample number	Status within zone(Z)	Cytolysis(L)	$R^{1)}$=Z/L
Negative sample	6	Cells under and around sample in good shape and color	no	R=0/0
Test sample	12	Cells under and around sample are light in color; all central cells decolorized; cells are in good shape; clear cell nucleus and membrane	no	R=0.5/0
Extract medium control sample	6	Cells under and around sample are light in color; all central cells decolorized; cells are in good shape; clear cell nucleus and membrane	no	R=0.5/0
Positive sample	6	All cells within 2mm in average under and around sample are decolorized.	20%	R=2/2

Note: 1)R represents reaction index, $R_{\text{test sample}}=R_{\text{extraction medium}}-R_{\text{test sample}}=0.5/0-0.5/0=0/0$

3.2 Acute systemic toxicity test

3.2.1 Method

The healthy white mice with 18-22 g body weight each were selected and randomly divided into sample group and control group, 5 in each group. Sample group was injected with sample extract solution 50 mL/kg into via tail vein with constant speed no exceeding 0.1 mL/s. Physiological saline with same volume and

batch number of was injected into via tail vein of control group under same condition with sample group. The general status, toxic signs and death number of two animal groups were observed and recorded within different time intervals of 0, 4, 24, 48 and 72 h. The body weights of two group mice were observed and recorded before injection and 24 h, 48 h and 72 h after injection.

3.2.2 Sample preparation

2 g coated NiTi esophageal stent was crushed and put into an triangular flask, then added 13 mL+3 mL physiological saline to reach full immergence and extracted for 72 h at 50℃.

3.2.3 Control materials

Negative control: physiological saline; Test animal: Kunming white mice.

3.2.4 Result

The general status, toxic signs of sample and control animals for acute systemic toxicity test: within 72 h observation period, both sample and control animals were in good condition with increase of body weight, no toxic signs or death, seeing weight changes of sample and control animals in Table 2.

Table 2 Weight changes of animals

Weight changes of control animals					Weight changes of sample animals				
Animal number	Before injection/g	After injection/g			Animal number	Bbefore injection/g	After injection/g		
		24 h	48 h	72 h			24 h	48 h	72 h
1	20.0	19.5	22.0	24.0	1	19.5	20.5	22.0	24.0
2	18.5	19.5	20.0	21.0	2	19.0	20.0	20.5	21.0
3	18.5	19.0	21.0	22.0	3	18.0	19.0	21.0	22.0
4	17.0	19.0	20.1	20.5	4	20.0	19.0	20.0	20.5
5	19.0	20.5	20.5	21.5	5	20.0	20.5	21.0	21.5
X±S	18.6±1.08	19.2±0.76	20.7±0.84	21.8±1.35	X±S	19.3±0.84	19.8±0.76	20.9±0.74	21.8±1.35

3.2.5 Conclusion

Determination standard: the sample is deemed complying with test requirements if reaction of sample animal is not bigger than that of control animal within 72 h observation period. Conclusion: test result complies with determination standard, which indicates acute systematic toxicity test qualification of NiTi coated esophageal stent sample.

3.3 Hemolysis test

3.3.1 Mechanism

The hemolytic components of devices or materials during the contact with blood may lead to the destruction of red blood cells and the release of hemoglobin, thereby increase dissociative plasma hemoglobin and cause toxic side effects. Hemolytic activities in hemolysis test indicate toxicity of devices or materials. In recent years, hemolysis test has been recognized as a supplementary test to cytotoxicity evaluation. This test has adopted spectrophotometer chromometry[3].

3.3.2 Sample preparation

Taking 3 batches of NiTi coated esophageal stents, 30 g for each, after cleaning and dehydrating, stents were cut into 5mmX30mm strips and put into a conical flask. 10 mL physiological saline was added in it. 0.2 mL fresh diluted rabbit blood was added after water bathed it for 30 minutes at 37℃. Then it was shaked and water bathed for 60 minutes at 37℃; centrifuged for 5 minutes via centrifugal machine and supernatant fluid was taken out.

3.3.3 Conditions and method

Environment: 26℃, 50% humidity; Animal: healthy big-ear white rabbit; Reagent: physiological saline, antiplatelet agent and fresh diluted antiplatelet rabbit blood; Method: to determine absorbency of sample and control groups at 545nm wavelength with spectrophotometer

3.3.4 Result

Taking the average value of absorbencies of 3 flaskes both in sample and control groups, absorbency of negative control group should not exceed 0.03; seeing hemolysis test result of all groups in Table 3. Hemolytic rate (%) is adopted to represent hemolytic degree and the product is deemed qualified if the rate is lower than 5%.

Calculating formula:

$$\text{Hemolytic rate}=\frac{Dt-Dnc}{Dpc-Dnc}\times 100\%=\frac{0.013-0.006}{1.025-0.006}\times 100\%=0.69\%$$

Dt: test sample absorbency, Dnc: negative control absorbency, *Dpc*: positive control absorbency

Table 3 Absorbency determination result of all groups in hemolytic test

Group	Negativecontrol group	Positive control group	Test sample group
Absorbency	0.005, 0.007, 0.007	1.000, 1.008, 1.067	0.018, 0.006, 0.016
Average value	0.006	1.025	0.013

The NiTi coated esophageal stent with 0.69% hemolytic rate complies with standards requiring hemolytic rate not exceeding 5%. Hence, the test sample is qualified for hemolysis test.

3.4 Sensitization test

3.4.1 Mechanism

According to immunoreaction classification, this test is a type 4 allergic reaction usually taking steps of induces-stimulate. In induce phase, foreign antigen will enter body and combine with protein to cause T cell sensitization; allergic reaction will appear in stimulate phase after sensitized lymphocytes contact the same antibody again: local skin erythema, edema and other inflammatory symptoms will appear.

3.4.2 Sample preparation

The extract solution was prepared using NiTi memory alloy slice sample 0.5-1.0 mm in thickness and Freund complete adjuvant.

3.4.3 Test animal and control materials

Test animal: healthy white guinea-pig with 300-500 g in weight, 30 at least, and 10 in test group, negative control group and positive control group respectively; Negative material: physiological saline; Positive material: 5% formaldehyde solution.

3.4.4 Sensitization toxicity determination

The grading of sensitization was shown in Table 4: one or more test animals, grade 3 sensitization; three or more test animals, grade 2 sensitization; five or more test animals, grade 1 sensitization; total number of test animal with grade 1 and 2 sensitization is 4 or more; any case of above 4 may indicate sensitization toxicity of test sample. Otherwise, test sample is free of sensitization toxicity. NiTi sample is free of sensitization toxicity without above mentioned status.

Table 4 Grading of sensitization

Grade	0(−)	1(±)	2(+)	3(++)
Evaluation	No reaction	Slight swelling and redness	Medium level diffused swelling and redness	Heavy redness, swelling and ulceration

3.5 Genotoxicity test

3.5.1 Mechanism

Salmonella typhimurium reverse mutation test(Ames test); the test has adopted mutant Salmonella typhimurium strains(histidine auxotroph) and tested the potency of chemical substance to induce reverse mutation under activation of mammal hepatic microsomal enzyme. It can determine potential toxicity of devices and materials in terms of genetic toxicology and genic level, predict cancer and mutation potency of materials in short term and evaluate its long-term biological safety.

3.5.2 Sample preparation and strains

The extract solution was prepared using NiTi memory alloy slice sample and physiological saline, ethanol, ethyl acetate, etc. as solvent.

Strains: 4 histidine auxotroph Salmonella typhimurium—TA97, TA98, TA100 and TA102, deeply freezed in fresh culture solution for storage.

3.5.3 Metabolic activator preparation

The metabolic activator S9 was prepared with ratliver according to standard methods, and S9 mixture was prepared via determination with test dose. Cultivate and identify Salmonella strain and determine mutation induction potency of test sample.

3.5.4 Test procedure

The mutation induction test was performed on a group of Salmonella typhimurium histidine auxotroph strains(his-). Mutation inducer of Salmonella typhimurium may change his- strain into his+ strain directly or via metabolic activation. The test strains were contacted with extract solution with or without metabolic activation and inoculate on lowest glucose agar plate. The numbers of colony with reverse mutation were recorded after a proper cultivation period and compared with spontaneous reverse mutation colony numbers of blank control and solvent control to determine whether the sample or its extract solution has mutagenicity on Salmonella typhimurium under the test conditions.

3.5.5 Result

(1) Determination standard: it is recorded of reverse mutation colony numbers of each utensil of test and control groups in Salmonella typhimurium reverse mutation test. The average value and standard deviation ($X \pm SD$) are used to represent reverse mutation colony number of each test group. It should be deemed positive in mutagenicity when reverse mutation colony number of test sample is over two times of that of negative control group with dose-effect relation or several repeatable dosage points. Otherwise, it should be deemed as negative.

(2) Conclusion: the metabolic activation material in NiTi alloy sample test hasn't led to colony reverse mutation. It can thus be determined according to standards that S9mix is sterile. Reverse mutation colony number of sample extract solution is approximate to spontaneous reverse mutation colony number of negative control group. It is then determined as non Salmonella typhimurium mutation inducer.

4 Conclusion

This quantitative in vitro test (cytotoxicity, acute systemic toxicity, hemolysis, sensitization, and

genotoxicity,) provide an optimistic results for eventual use of NiTi stent as implanted medical devices.

References

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[3] ISO Standard 10993－11:1993, 医疗器械生物学评价(第11部分:全身毒性试验)[S].