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Biological safety evaluation of the modified urinary catheter

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ABSTRACT

The purpose of this study was to evaluate in vitro safety of the novel tosufloxacin (TOS)-treated catheters with the prolonged antimicrobial activity. The test samples of silicone latex catheter were prepared by the immobilization of TOS on chitosan (CHIT)-coated catheter by means of covalent bonds and non-covalent interactions. Each step of the modification process of catheter surface was observed using ATR–Fourier transform infrared spectroscopy. In vitro cytotoxicity of the modified and unmodified catheters was assessed by direct and indirect tests in accordance with ISO standards using green monkey kidney (GMK) cell line. The MTT, lactate dehydrogenase activity (LDH), WST-8, Sulforhodamine B (SRB) test results and microscopic observation clearly indicated that unmodified silicone latex catheters decrease cell metabolic activity, act as a cytotoxic agent causing cell lysis and induce cell death through necrotic or apoptotic process. We suggest that chitosan coat with TOS immobilized limits leaching of harmful agents from silicone latex material, which significantly enhances survivability of GMK cells and therefore is quite a good protection against the cytotoxic effect of this material.

1. Introduction

From a safety standpoint, the cytotoxicity determination based on various testing methods is a necessary step in the evaluation of material biocompatibility for biomedical applications [1]. Presently, safety evaluations are being standardized and recommended by standardization institutes like the International Organization for Standardization (ISO) [2]. The in vitro cytotoxicity tests conducted according to ISO are one of the most important studies for the preliminary biocompatibility assessment of new medical devices [3].

A well-known phenomenon in urological practice is the toxicity caused by the use of catheters, mostly latex catheters. The available literature based on cytotoxicity tests indicates that latex urinary catheters exhibit high toxicity [4–6].

Our previous studies have demonstrated that latex catheters modified with heparin and sparfloxacin show the antibacterial properties [7,8], as well as that the proposed modifications effectively reduce the toxicity of latex [9]. We decided to continue the experiments using other compound from fluoroquinolone group (tosufloxacin instead of sparfloxacin) and chitosan (CHIT) as coated polymer. We used CHIT (positively charged polysaccharide-type polymer) due to numerous advantages suitable for biomedical applications such as non-toxic and bacteriostatic properties, good biocompatibility, low antigenicity and rapid biodegradability [10,11]. However, it should be taken into account that some properties of chitosan, such as the average molecular weight and/or the degree of N-deacetylation may impact on the toxicity [12,13] and antitumor activity [14]. The presence of the free amino groups in the biopolymer chain of CHIT leads to the possibility of several chemical modifications, including the preparation of Schiff base with the active carbonyl compound [15].

Chitosan is one of the most popular biopolymers used in medicine, pharmacy and the related fields. The applications of chitosan are well demonstrated in medical area, especially as controlled drug delivery systems. Chitosan-based polyelectrolyte complexes with other natural polysaccharides such as alginate, hyaluronic acid, pectin or cellulose derivatives (in various forms: nanoparticles, microparticles, tablets, beads, gels, membranes) arouse the interest of investigators [16]. In the recent years, chitosan-based films as antibacterial protection have been often dedicated for medical use. Among various materials, the following deserve special attention: the polyurethane films modified by covalent immobilization of chitosan to obtain antibacterial activity [17], polysaccharide multilayer films composed of antimicrobial peptide, hyaluronic acid as polyanion, and chitosan as polycation that are active against both bacteria and yeasts [18], and polyethylene terephthalate films coated with chitosan and heparin by ozone-induced method to improve antithrombogenic properties [19]. However, there are few reports describing the modification of urological catheter with chitosan. In this area, the polyurethane urethral catheters coated with chitosan/poly(vinyl alcohol) blending hydrogel to minimize catheter-related bacterial infection should be mentioned [20]. In contrast to the existing reports on potential medical applications of chitosan films, we developed a new chitosan-based coating containing tosufloxacin (TOS) as antimicrobial substance to obtain biocompatible catheters with antimicrobial activity. Our previous report demonstrated that the novel TOS-treated catheters [21] (commercially available silicone latex catheters coated

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with CHIT hydrogel and then treated with TOS) show the antibacterial activity for at least 30 days [22].

The aim of this study was to detect the specific chemical groups in the modified catheter samples by FTIR surface characterization and to assess the biological safety of TOS-treated catheters by cytotoxicity testing.

2. Materials and methods

2.1. Preparation of TOS-treated catheter samples

The samples were prepared by immobilization of tosufloxacin (TOS) obtained from CHEMOS GmbH, Germany on commercial silicone latex catheters (Rusch, Germany) according to the patented procedure [21]. Briefly, the catheter specimens (1.5 mm thickness) measuring approximately 3 mm × 4 mm (direct-contact cytotoxicity tests) or 5 mm × 25 mm (indirect cytotoxicity tests) were coated with medium molecular weight chitosan (CHIT; degree of deacetylation: 75–85%, molecular weight: 190–310 kDa, from Sigma-Aldrich Chemicals) hydrogel (solution of CHIT in dilute aqueous acid) and activated using glutaraldehyde as a linker (POCh Gliwice, Poland). The activated specimens were subsequently linked with TOS to form a Schiff base in TOS-L (low — 0.1 mg/mL TOS concentration) and in TOS-H (high — 1.0 mg/mL TOS concentration) solutions. The TOS–CHIT treated surface was evaluated by ATR–Fourier transform infrared spectroscopy analysis. The resulting catheter specimens were then rinsed with water and subjected to the cytotoxicity tests. The amounts of TOS bound to the modified catheter surface via covalent bonds and non-covalent interactions were determined by high-pressure liquid chromatography (HPLC) method [22] and were equaled on average (mean ± SD) 0.1778 ± 0.0525 mg of TOS/piece for TOS-H-treated sample and 0.0334 ± 0.0069 mg of TOS/piece for TOS-L-treated sample (direct-contact cytotoxicity tests) or 1.8523 ± 0.5469 mg of TOS/piece for TOS-H-treated sample and 0.3484 ± 0.0723 mg of TOS/piece for TOS-L-treated sample (indirect cytotoxicity tests).

2.2. Fourier transform infrared spectroscopy (FTIR) characterization

FTIR with transmittance mode was used to characterize the presence of specific chemical groups in the tested catheter samples. FTIR spectra were obtained in the range of wavenumber from 4000 to 600 cm^{-1} during 32 scans, with 4 cm^{-1} resolution using a Thermo Scientific Nicolet 8700A spectrometer equipped with versatile Attenuated Total Reflectance (ATR) sampling accessory.

2.3. Materials for in vitro tests

Minimum essential medium eagle (MEM), penicillin–streptomycin, 0.25% trypsin–EDTA solution, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), Cell Counting Kit-8 (WST-8) containing (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, sodium salt) as reagent, lactate dehydrogenase (LDH) cytotoxicity kit, Sulforhodamine B, Trizma base, sodium dodecyl sulfate (SDS), Hoechst 33342 and propidium iodide stains were purchased from Sigma-Aldrich Chemicals. Fetal bovine serum (FBS) was supplied by PAA Laboratories and phosphate buffered saline (PBS) was from BIOMED Serum and Vaccine Production Plant (Lublin, Poland). Hydrochloric acid (HCl), acetic acid, and trichloroacetic acid (TCA) were purchased from POCH (Gliwice, Poland).

2.4. Cell culture experiments

In vitro experiments were carried out using green monkey kidney cell line (GMK) obtained from BIOMED Serum and Vaccine Production Plant (Lublin, Poland). Cells were cultured in MEM supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and maintained at 37 °C in a humidified atmosphere of 5% carbon dioxide and 95% air.

2.5. Evaluation of cytotoxicity

Cytotoxicity of modified and unmodified catheters was evaluated by direct and indirect methods in accordance with ISO 10993-5 [3]. The following variants were tested: 1 — silicone latex catheter (the untreated sample), 2 — CHIT-coated catheter sample, 3 — TOS-L-treated catheter sample, 4 — TOS-H-treated catheter sample.

2.5.1. Direct-contact cytotoxicity tests

Before the experiment, catheter specimens were cut into small pieces approximately 3 mm × 4 mm × 1.5 mm. GMK cells were seeded in 24-multiwell plates in 500 μL of the complete culture medium at a concentration of 6 × 10^4 cells/well and cultured for 24 h at 37 °C to near confluence. Then the growth medium was removed and 500 μL of fresh medium supplemented with 2% FBS was added. Subsequently, individual specimens of the test material were carefully placed directly on the cell monolayer covering approximately one tenth of the cell layer surface [3]. Cells cultured in wells with polystyrene pieces served as controls. After 24 h of incubation at 37 °C, the culture medium and pieces of the samples were removed with great care and 2 colorimetric assays (MTT, LDH) were performed to evaluate cytotoxicity of catheters. Metabolic activity of GMK cells was assessed by MTT test as described earlier [9]. The results of MTT test were expressed as the percentage of OD values obtained with the control cells. Toxicity of catheters was determined using LDH cytotoxicity kit in accordance with the manufacturer procedure. LDH release test allows for membrane damage assessment via measurement of LDH activity released into the medium. MTT and LDH assays were repeated in 2 separate experiments in quadruplicate. Moreover, live/dead fluorescence staining was applied to estimate the cytotoxicity effect of the catheters on GMK cells. Hoechst 33342 dye stains nucleic acids (blue fluorescence) of all cells in population whereas propidium iodide stains nucleic acids of necrotic cells and cells at the middle and late apoptotic stages (pink/red fluorescence). Briefly, after removing of the catheter samples, cell layers were rinsed with PBS and 500 μL per well of Hoechst 33342 solution (1 μg/mL prepared in PBS buffer) was added. Cells were stained for 15 min, Hoechst solution was removed and cell layers were rinsed with PBS. Then 500 μL per well of propidium iodide solution (2 μg/mL prepared in PBS buffer) was added. GMK cells were stained for 10 min and observed under laser scanning microscope, using two-dimensional scan technique (Olympus Fluoview IV81 equipped with FV1000 laser scanner).

2.5.2. Indirect cytotoxicity tests

The cytotoxicity of the modified and unmodified catheters was also evaluated indirectly by means of fluid extracts. Extracts were obtained by immersing the test materials in a complete culture medium supplemented with 2% FBS under standard conditions: 24 h, at 37 °C in a humidified atmosphere of 5% carbon dioxide and 95% air [3]. The ratio between the sample surface area and the volume of the extraction vehicle was 1.25 cm^2/mL. Before the extraction, catheters were cut into small pieces approximately 5 mm × 25 mm in order to enhance submersion in the medium. Polystyrene extract served as negative control of cytotoxicity. GMK cells were seeded in 96-multiwell plates in 100 μL of the complete culture medium at a concentration of 2 × 10^4 cells/well and cultured for 24 h at 37 °C to near confluence. Then, the growth medium was replaced with appropriate extracts. After 24 and 48 h of incubation at 37 °C, WST-8 (evaluation of cell metabolic activity via measurement of mitochondrial dehydrogenases activity) and Sulforhodamine B (cytotoxicity assessment via measuring of total biomass by staining cellular proteins with the Sulforhodamine B dye) colorimetric assays were performed to evaluate cell viability. WST-8 test was carried out using cell counting WST-8 kit in accordance with the manufacturer procedure. In order to perform Sulforhodamine B