Materials Science and Engineering C 68 (2016) 221–228

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

A novel ureteral stent material with antibacterial and reducing encrustation properties

Jing Zhao [a,1], Zhiqiang Cao [b,1], Ling Ren [a], Shanshan Chen [a], Bingchun Zhang [a], Rui Liu [a], Ke Yang [a,*]

[a] Institute of Metal Research, Chinese Academy of Sciences, Shenyang 110016, China
[b] General Hospital of Shenyang Military Region, Shenyang 110840, China

ARTICLE INFO

Article history:
Received 14 February 2016
Received in revised form 26 April 2016
Accepted 29 April 2016
Available online 21 May 2016

Keywords:
Cu-bearing stainless steel
Antibacterial
Ureteral stent
Infection
Encrustation

ABSTRACT

Ureteral stents have been used to relieve ureterostenosis. Complications such as infection and encrustation occur in the long time of stent implantation, which is a clinical problem needs to be resolved. Indwelling ureteral stents have shown to develop microbial biofilm that may lead to recurrent infection and encrustation. This study was aiming to reduce those complications by using a novel material, Cu-bearing antibacterial stainless steel. The antibacterial performance, encrustation property, and biocompatibility were examined by SEM, image analysis, MTT and would healing. The *in vitro* immersion test showed that 316LCu-bearing stainless steel (316LCu-SS) not only inhibited proliferation of bacteria and formation of biofilm, but also had less encrustation deposition. Its antibacterial effectiveness against Escherichia coli reached to 92.7% in the artificial urine for 24 h and 90.3% in the human urine for 6 h. The encrustation surface coverage percentage was 30.2% by 12 weeks, which was nearly one half of NiTi alloy. The *in vitro* tests showed that 316LCu-SS had no toxicity, and promoted the migration of urethral epithelial cells.

1. Introduction

Ureterostenosis is a common problem in the urinary system of human body. The ureteral obstruction inevitably caused by benign and malignant tumors is challenging for urologists. Ureteral stenting serves as an important therapeutic option to alleviate the obstruction [1]. Various designs of ureteral stents have been reported, such as polyurethane tube, woven NiTi stent and nickel-chromium-cobalt stent [2].The double-J ureteral stent has been widely used in the endourology surgery. It connects the kidney and bladder, and goes inside of ureter. The aim of stenting is to adequately drain the upper urinary tracts while minimizing the hospitalization and the negative impact on the life quality [3]. However, infection and encrustation of the stent, which can cause significant morbidity, occur frequently because of its direct contact with the urine. Use of a kind of prevalent metal stent in clinic, *i.e.*, the NiTi stent, has reduced infection and encrustation by easier endothelialization, whereas it remains in body inevitably. Meanwhile, no matter double-J stent or NiTi stent, urinary tract infections (UTIs) and catheters encrustation still occur during the long time inserting, which needs to be resolved urgently [4–6].

Bacterial adherence to biomaterial surfaces is an important step in the pathogenesis of prosthetic infection [7]. The viability of bacteria which form biofilm against microbial agents at a concentration is 1000–1500 times higher than that planktonic cells in suspension [6]. The reason for UTIs is that bacteria enter the urinary tract, and then colonize and adhere to the ureteral stents through nonspecific interactions or *via* specific polysaccharide linkages [8]. UTIs are caused primarily by uropathogenic *Escherichia coli* (*E. coli*), which is the most common infectious bacteria in patients with foreign bodies. *E. coli* is responsible for >85% of all UTIs in statics [9]. Usually, infection is defined as the colonization number of organisms $\geq 10^5$ ml^{-1} in freshly voided urine [10]. Approximately 10–50% of patients develop UTIs in a short time catheterization (up to 7 days), and nearly 100% of patients with indwelling ureteral stent suffer a catheter associated UTIs for >30 days [11]. Although infections are generally asymptomatic, patients are at risk from a range of complications which make them more vulnerable than non-catheterized patients [12].

It has been confirmed that urine from normal individuals have the self-antibacterial and defensive activity, which is more greater at lower pH (between a range of 5.0–6.5) [13]. Urinary acidification has long been used as an aid in the treatment of urinary tract infection due to large amounts of bacteriostatic gas NO produced from acidification of nitrite. Unfortunately, once the urine is infected, the bacteria have the capacity of producing ammonia from urea leading to increasing urinary pH to levels that nitrite is not converted to toxic NO [14]. Moreover, the biofilm makes embedded bacteria less accessible to the human defense system, and significantly decreases antibiotic susceptibility, *i.e.*, even though the urine has the antibacterial ability, the

* Corresponding author at: #72 Wenhua Road, Shenyang 110016, China.
E-mail address: kyang@imr.ac.cn (K. Yang).
[1] Jing Zhao and Zhiqiang Cao equally contributed to this work.

http://dx.doi.org/10.1016/j.msec.2016.04.103
0928-4931/

bacteria adherent to the surface may still be alive. Besides, urine with infectious bacteria accumulated on the surface of implants is aggressive to human. Glucose in the urine of diabetic patients provides a better medium for multiplication of *E. coli* [15].

The antibacterial effect of copper (Cu) might be firstly used by Milharde in 1885. Cu as an alloying element added into the medical austenitic stainless steel has been studied in order to develop a novel kind of Cu-bearing medical stainless steel with function of inhibiting bacterial infection [16]. Continuous release of trace amount of Cu ions from the surface of Cu-bearing stainless steel (Cu-SS) should contribute to the antibacterial function. Some work has been done on the application of Cu-SS in the area of implantation, such as bone, coronary artery stent, and so on [17]. However, there is no study about Cu-SS for the application in the ureter. It is well known that the initial step for encrustation of any urinary drainage device (catheters, stents, nephrostomy tubes) is thought to be from bacterial colonization [18]. Cu-SS may have advantage on inhibiting encrustation and infection due to its strong antibacterial ability. The aim of this study is to focus on the *in vitro* anti-infection and inhibition of encrustation of this novel Cu-SS by means of antibacterial tests, observation on scanning electron microscope (SEM), Live/Dead stain and MTT assay, hoping to provide some experimental evidences for its possible application in urine system.

2. Materials and methods

2.1. Samples preparation

Antibacterial type 316LCu-bearing stainless steel (316LCu-SS with nominal composition of Fe-19Cr-14Ni-2Mo-4.5Cu, wt.%), medical grade 316L stainless steel (316LSS with nominal composition of Fe-19Cr-14Ni-2Mo, wt.%) and nitinol alloy (NiTi with nominal composition of 50Ni—50Ti, at.%) for experiments were prepared by normal metallurgical processes, in which 316LSS and NiTi were served as control groups in this study. All the samples were cut into sizes of Φ10 mm × 1 mm and 10 mm × 10 mm × 1 mm. The samples were mechanically polished sequentially with SiC papers of grits 180, 400, 800, 1200, 1500 and 2000, then ultrasonically cleaned with acetone and deionized water for 5 min at room temperature, and dried in air thoroughly. Each side of samples was sterilized under UV light for 30 min before experiments.

2.2. Artificial urine and human urine preparations

Artificial urine (AU) solution was prepared based on patent #7109035 [19], which was employed containing the inorganic constituents of human urine in comparable concentration. The concentration of the chemicals in the AU solution is listed in Table 1.

To prepare the AU solution, the above chemicals besides $CaCl_2$ were added into the deionized water, and the pH was adjusted to 6.4 with ammonium hydroxide or hydrochloric acid after chemicals dissolving completely. The solution was sterilized under the condition of 121 °C for 20 min and cooled to room temperature. Then, $CaCl_2$ was decontaminated by UV light radiation for 30 min and added into the sterilized solution. The AU solution was sonicated till completely dissolved and stored at 4 °C.

Table 1
Chemical concentration of the AU solution.

Component	Quantity (g)	Concentration (mmol · L^{-1})
NaCl	6.17	105
NaH_2PO_4	4.59	37
$Na_3C_6H_5O_7$	0.944	3.7
$MgSO_4$	0.463	3.9
$NaSO_4$	2.408	20
KCl	4.75	64
$Na_2C_2O_4$	0.043	0.3
$CaCl_2$	0.638	5.7

The human midstream urine (HU) samples used in the experiment were collected from six fasting healthy volunteers with no history of urinary tract infection. None of the volunteers were receiving antimicrobial agents and special diets. The samples were filtered through a cellulose acetate filter (0.45 μm pore size) and collected in glass containers that had been sterilized [20]. The urine was divided in tubes in 5-ml aliquots. Tubes with urine that were not used in test the same day were stored at −20 °C. The urine was stored at 4 °C until analysis was performed, with a minimum possible delay.

2.3. Bacterial strain and antibacterial test

A gram negative bacteria, *E. coli* (ATGG 25922), were obtained from the American Type Culture Collection. It was chosen as the model bacterium because it is the most prevalent specie in UTIs and the most clinically relevant. The strain was cultured with nutrient agar and grew in nutrient broth. In order to determine the antibacterial properties of samples under clinically relevant conditions, the bacteria growing overnight were diluted to 10^5 cfu/ml in the AU and HU solutions, respectively. All the samples (316LCu-SS, 316LSS and NiTi) were incubated directly with bacteria suspension (50 μl) in triplicate in a 24-well plate at 37 °C, along with blank reference (no sample) served as blank control. Then the samples were washed in centrifuge tubes with 1 ml 0.9% sodium chloride (NaCl) by shaking in a vortex generator for 1 min. Bacteria were counted at 24 h in the AU solution and 4, 6, 8, 24 h in the HU solution of incubations. 100 μl solution was diluted serially with 0.9% NaCl. After each dilution step, 50 μl were pipetted onto nutrition agar plates in duplicate incubating overnight at 37 °C. Afterward, the colonies were counted and back-extrapolated to the original volume. The antibacterial effectiveness was determined by following equation:

$$\text{Antibacterial effectiveness}\ (\%) = (A-B) \times 100\%/A$$

where B represents the bacterial count after contacting with experimental materials (316LCu-SS, 316LSS and NiTi), and A represents the blank group.

Biofilm formation assay was used to visualize bacteria that adhered to the surface of samples. Each sample was co-cultured with 1 ml bacterial suspension (AU solution) at the concentration of 5×10^8 cfu/ml for 24 h. Bacteria adhesion ability on samples in HU solution was evaluated by co-culturing with bacterial suspension at 2×10^5 cfu/ml for 4, 6, 8 and 24 h. Following this, samples were removed from the bacterial suspension to determine the number of adherent bacteria. Non-adherent microorganisms were removed by washing with 2 ml volume of AU solution for three times. The samples were prepared for SEM observation by fixation in 2.5% glutaraldehyde and dehydrated in a series of aqueous ethanol solutions (30%, 50%, 75%, 95% and 100%). Before observation, the surfaces of samples were gold-coated.

2.4. Live/Dead stain

Bacteria viability adhered on the surface of samples were measured by Live/Dead BacLight Bacterial Viability Kit (Invitrogen Molecular probes, Darmstadt, Germany). The combination of the two dyes distinguishes live and dead bacteria based on the membrane integrity. The green fluorochrome (SYTO 9) can penetrate intact membranes, while the larger red fluorochrome (propidium iodide) penetrates the only compromised bacterial walls of dead bacteria, resulting in red fluorescence by binding to the nuclear material of the bacteria.

The samples were washed with HU solution after co-culture with 1 ml suspension for 4, 6, 8 and 24 h, respectively. After that, the surface of samples were added 100 μl mixed stain solution (SYTO9: PI = 1:2) according to the manufacturer's instruction strictly and then analyzed on a confocal laser scanning microscopy (CLSM, C2 Plus, Nikon, Japan).