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Effect of Silver Nitrate and Ofloxacine Blended Caprolactone-L-lactide Coatings on the Properties of Bioabsorbable Self-reinforced Polylactide Urospirals

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ACADEMIC DISSERTATION

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II Multanen M, Talja M, Hallanvuo S, Siitonen A, Välimaa T, Tammela TLJ, Seppälä J, Törmälä P: Bacterial adherence to ofloxacin-blended polylactone-coated self-reinforced L-lactic acid polymer urological stents. BJU Int (2000) 86: 966 - 9

III Multanen M, Talja M, Tammela TLJ, Seppälä J, Välimaa T, Järvi K, Törmälä P: Biocompatibility of silver nitrate and ofloxacine coated bioabsorbable SR-PLLA rods. Urol Res (2001) 29: 113 - 117

IV Multanen M, Tammela TLJ, Laurila M, Seppälä J, Talja M, Välimaa T, Törmälä P: Biocompatibility, encrustation and biodegradation of ofloxacine and silver nitrate coated poly-L-lactic acid stents in rabbit urethra. Urol Res (in press)

V Multanen M, Välimaa T, Tammela TLJ, Talja M, Seppälä J, Törmälä P: Encrustation of silver nitrate and ofloxacine blended caprolactone-L-lactide coated self-reinforced poly-L-lactic acid urinary tract stents in vitro. J Endourol (submitted)

2. INTRODUCTION

Fabian introduced in 1980 a spiral stent that pushed the prostatic lobes apart thus allowing urine flow easier (Fabian 1980). Since then, several permanent and transient stents have been used to relieve urinary obstruction. Permanent stents are intended to incorporate into the tissue, temporary sents will be removed after a certain period of time. A subgroup of transient stents, biodegradable stents, do not need to be removed because they degrade.

Biodegradable polymers have been used as surgical suture materials since 1960's (Kulkarni et al 1966). After that, various biodegradable suture materials and implants have been developed for surgical purposes. The development of biodegradable stents for urological purposes started in the late 1980's (Kemppainen et al 1993) and has led to an assortment of devices for use in experimental and clinical studies (Talja et al 1997). Biodegradable stents have been used clinically after visual laser ablation of prostate (VLAP) (Talja et al 1995, Petas et al 1997), transurethral microwave therapy (TUMT)(Dahlstrand et al 1997), free skin urethroplasty for recurrent bulbar urethral strictures (Oesterlink 1996), optical urethrotomy for recurrent urethral strictures (Isotalo et al 1998), interstitial laser coagulation of the prostate (Petas et al 2000), combined with finasteride in the treatment of acute urinary retention (Isotalo et al 2000) and after transurethral needle ablation of the prostate (TUNA) (Multanen et al 2000).

Stents have several problems, like migration, encrustation and infection (Nordling et al 1992, Williams et al 1993). Urinary tract infection occurred in 14 - 30 % of with a biodegradable prostatic stent after visual laser ablation of prostate (Talja et al 1995, Petas et al 1997). However, all these patients also had a suprapubic catheter, which probably increased the infection rate. Infection rates between 7.5 and 27 % have been reported in patients with postoperative ureteric double-J stents, in spite of antibiotic prophylaxis (Franco et al 1990, Reid et al 1992).

The key event in the pathogenesis of urinary tract infection is the ability of uropathogens to adhere to the urothelium or to the surface of urinary tract prosthetic devices. Within a few hours, adherent bacteria can aggregate, multiply and form biofilm matrices which, once surrounded by a dense glycocalyx, may become resistant to antimicrobial agents and constitute a reservoir of viable

micro-organisms (Costerton et al 1987, Reid and Sobel 1987, Anwar et al 1990, Denstedt et al 1998).

Encrustation can occur in both infected and sterile urine. The mechanism of encrustation in infected urine is identical to the formation of infected urinary stones. Urease-producing organisms, like Proteus mirabilis, Proteus vulgaris and Pseudomonas spp, elevate the urinary pH through the hydrolysis of urea. In alkaline environment magnesium and calcium precipitate readily out of solution in the form of magnesium ammonium phosphate and calcium apatite and form struvite encrustations (Gorman and Tunney 1997).

Bacterial adherence to urinary catheters can be inhibited by silver coating (Liedberg and Lundeberg1989). Cormio et al (1997) showed that adherence of uropathogens to biodegradable stents can be prevented by immersion in a suitable antibiotic solution. As we know, parenteral or per oral antibiotic prophylaxis is routinely used in clinical practice to prevent stent associated infections.

The aim of the present study was to investigate, whether bacterial adherence and encrustations to biodegradable urinary stents can be prevented by certain coatings. Considering the possible clinical use in the future, it was also essential to study the biocompatibility of these coated stents and possible effects of the coatings to biodegradation of the stents.

3. REVIEW OF THE LITERATURE

3.1. PROSTATIC STENTS

Fabian reported in 1980 the use of a stainless steel prostatic spiral stent to relieve infravesical obstruction due to benign prostatic hyperplasia. After that, several temporary or permanent (into the tissue incorporating) stents have been introduced (Milroy et al 1988, Nordling et al 1989, Oesterling 1993, Williams et al 1993, Kaplan et al 1993, Montorsi et al 1994, Kletscher et al 1994, Nissenkorn et al 1996). A subtype of temporary stents, biodegradable stents, have been used in numerous clinical studies and practice since the experimental study of Kemppainen et al (1993).

3.1.1. Temporary stents

Fabian's stent was a three-part stainless steel stent: the body was lying in the prostatic urethra with the proximal portion protruding into bladder, the neck at the sphincter area and the head in the bulbous urethra (Fabian 1980, 1984). Three commercially available prostatic metal stents (the stainless steel Urospiral® , the gold-plated stainless steel Prostakath® and the nickel-titanium Prostacoil®) , are based on the original (three-segment) Fabian stent configuration.(Yachia 1997) The fourth stent, the nickel titanium Memokath®, is a single-segment device. (Ellis and Gidlow 1996). Nickel titanium (nitinol) is a shape memory alloy (SMA) with the ability to change from one configuration to another at different temperatures (Mori et al 1994).

Nissenkorn et al (1996) introduced a polyurethane intraurethral catheter, the proximal end of which protrudes into the bladder and the distal end lies at the apex of the prostate. The Barnes stent® (Barnes and Yakubu 1998) is also a polyurethane device, which has a single retaining basket designed to sit at the verumontanum. Devonec and Dahlstrand (1998) used a Threstle catheter® to prevent urinary retention after transurethral microwave therapy (TUMT) of the prostate. This device consists of two silicone tubes connected by a thread.

Temporary stents have many side-effects, like migration, encrustation and infection. In a study of Nordling et al (1992) 75 out of 150 prostatic stents had to be removed for various reasons including proximal (42 stents) or distal (13

stents) migration.21 stents had calcifications. In a Finnish study of Ala-Opas et al (1993), 39 % of stents had to be removed. Chronic urinary tract infection reduced significantly the functional time of the spiral. However,Nissenkorn et al (1996) reported no urinary tract infections during two weeks stenting with intraurethral catheter after the VLAP procedure.

3.1.2. Permanent stents

Permanent stents are intended to incorporate into the tissue. Milroy et al (1988) reported the use of an endoscopically placed, self-retaining permanent urethral stent for thetreatment of recurrent bulbar urethral strictures. Urolume® (Wallstent) is a self-expanding tubular mesh woven of fine superalloy wire. The stent is placed under visual control. It is stable when expanded and the epithelium is assumed to cover the stent entirely (Oesterling et al 1994).

Gianturco® is a modification of Urolume® . It is a self-expanding stainless steel device assumed to be covered by epithelium. Gianturco® has greater spacing between the wires and less shortening when expanding (Guazzoni et al 1994). The titanium stent has excellent biocompatibility properties. It is expanded by inflating a balloon on the insertion catheter. (Kirby et al 1992, Kaplan et al 1995). The heat-expandable Memotherm® is made of nitinol wire woven to tubular mesh.(Ricciotti 1995).

The problems with permanent stents resemble those of temporary stents. In a study of Bajoria et al (1995), 14 stents out of 47 had to be removed during the follow-up of two years. The removal was due to stent migration or epithelial hyperplasia in the majority of cases. 15 patients out of 96 with a Urolume® had an urinary tract infection at 12 months follow-up. Seven stents were encrusted. (Williams et al 1993). Encrustation occurred, if the stent failed to be covered by the epithelium (Milroy and Chapple 1993).

3.1.3. Bioabsorbable stents

Biodegradable stents have been used since the experimental study of Kemppainen et al (1993) in several clinical studies. Self-reinforced polyglycolic acid (SR-PGA) spirals have been employed after VLAP (Talja et al 1995, Petas et al 1997), after TUMT (Dahlstrand et al 1997), in free skin urethroplasty for recurrent bulbar urethral strictures (Oesterlink 1996), after ILCP (Petas et al 2000) and after TUNA (Multanen et al 2000). Self-reinforced poly-L-lactic acid (SR-PLLA) stents have been used in the treatment of recurrent urethral strictures after optical urethrotomy and combined with finasteride in the treatment of acute urinary retention (Isotalo et al 1998, 2000).

The configuration of the stents resembles that of the original Fabian stent. The stent is pushed into the prostatic urethra with the tip of a cystoscope under direct vision using a ureteral catheter as a guide wire.

The problems of bioabsorbable stents differ a little from the problems encountered with other stents. Infection rates from 14 - 41 % have been reported with biodegradable stents after VLAP. However, all these patients had also a suprapubic catheter, which probably increased the infection rate (Talja et al 1995, Petas et al 1997) 2 stents out of 27 had migrated distally in a study of Petas (1997). Encrustation has not been reported.

3.2. BIODEGRADATION

The terminology varies depending on the author when the issue is the biodegradable materials. Terms like “bioabsorbable” and “bioresorbable” have been used in the same contexts. Generally “bioabsorption” means the degradation and metabolism of the material in vivo into small molecules and energy, ” biodegradation” morphological and chemical degradation of material in vivo. (Talja et al 1997).

The degradation of bioresorbable polymers proceeds via a random, bulk hydrolysis of the esther bonds in the polymer chains. At the early stage there is a decrease in the molecular weight of the polymer, although there is no change in the appearence of the implant. For example, SR-PLLA implants with initial molecular weight of 260 000 daltons (da) show an average molecular weight of 10 000 da after 36 weeks in the subcutis of the rabbit. When the molecular weight of the polymer goes below about 5 000 da, the implant disintegrates into debris, which triggers a nonspesific foerign body reaction. This includes macrophages, giants cells and leucocytes. When bioresorption is complete, the inflammatory reaction disappears, leaving only scar tissue. (Blasier et al 1997)

The bioresorption time of PLLA in soft tissue is about 12 months. (Niewenhuis 1992) In hydrolysis, PLA is depolymerized to lactic acid, which in turn is transformed to pyruvate by lactate dehydrogenase. Decarboxylation of pyruvate yields acetyl-CoA, which enters the tricarboxylic acid cycle, with water and carbon dioxide as end products.(Hollinger et al 1986)

The biodegradation of polymers is the sum of many factors and is accelerated by thepresence of residual monomers and oligomers in the polymer, the alkalinic pH of the surroundings, the reduction of crystallinity and orientation and certain enzymes (e.g. pronase, proteinase-K and bromeline). Also the site of implantation may have an influence on biodegradation with possible muscular movements and loads from outside (Talja et al 1997).

3.3. BIODEGRADABLE MATERIALS

Polyglycolic acid (PGA) with low molecular weight and no plastic properties was synthesized by Bischoff and Walden in 1893. PGA with a higher molecular weight was introduced by Higgins in 1954 (Higgins 1954). PGA does not form enantiomers. Polylactic acid (PLA) has two enantiomeric forms, the left-handed (L-lactide) and right-handed (D-lactide) molecules. The two enantiomers have different biodegradation rates (Cutright et al 1974). The left-handed molecule (PLLA) is widely used. The stereo-isomers can also be mixed in different ratios, for example PLA 96 contains L- and D-lactic acid in a ratio of 96:4. The physical properties of the copolymers of L- and D-lactic acid depend on the relative amounts of the chiral forms (Vert et al 1992). The biodegradation rate correlates with the relative amount of the right-handed molecule (Kulkarni et al 1971).

Kulkarni and coworkers reported the manufacturing of bioabsorbable PLA sutures in the 1960s (Kulkarni et al 1966). Since then, various bioabsorbable suture materials, such as PGA (Dexon®), copolymerate of PGA and PLA (Vicryl®), poly-p-dioxane (PDS®) and copolymerate of PGA and trimethylene carbonate have been widely used. The development of self-reinforced bioabsorbable implant materials led to a breakthrough for bone surgical applications (Törmälä 1992), and various fixation implants have been used with favourable results in orthopedics and traumatology (Mäkelä 1989, Hirvensalo 1990, Rokkanen et al 1985, 1992, 1996).

3.4. SELF-REINFORCED COMPOSITES

Partially crystalline, linear-chain biodegradable polymers show only modest mechanical strength values when manufactured with non-reinforcing techniques (Vainionpää et al 1989). Better mechanical properties can be achieved by self-reinforcing procedures, during which the molecular chains arrange themselves in parallel and form microfibrils in the polymer matrix. This re-arrangement can be accomplished by free drawing, die drawing, shearing and rolling techniques. Due to self-reinforcing, the strength and modulus values of SR-composites are significantly better than the corresponding values of nonreinforced materials. (Törmälä 1992).

3.5. CAPROLACTONE-LACTIDE COPOLYMERS

Polycaprolactone is a polyester that has unique properties: it is biodegradable and miscible with a variety of polymers and it crystallizes very readily. It lacks toxicity and has great permeability.(Hiljanen-Vainio et al 1996). The copolymers of caprolactone and lactic acid have been widely studied in recent years. Caprolactone and lactic acid can be mixed in various proportions, resulting in various physical properties. The copolymer used in our studies contained 95 mol-% caprolactone and 5 mol-% L-lactic acid. The molecular weight of the polymer was 107,000 g/mol. The melting temperature was 52°C, and glass transition temperature -60°C. For comparison, the melting temperature of poly-L-lactic acid is 175°C, and glass transition temperature 55°C (Hiljanen-Vainio et al 1996).

Coating a biodegradable urospiral with a caprolactone-L-lactide copolymer gives two important advantages: First, compounds, like antibacteric compounds can be mixed with the coating to achieve new properties. Second, the expansion properties of the urospiral can be refined. Since the coating is rigid and hard in cold, but soft and flexible in body temperature, a pre-molded caprolactone-L-lactide coated SR-PLLA urospiral can dilate rapidly in the urethra or in the ureter.

3.6. BIOCOMPATIBILITY

All surgical implants must fulfil certain biocompatibility requirements. Since 14th June 1998 all medical devices for sale in European Union countries have to be labeled with a CE mark indicating fulfilment of the biocompatibility requirements.(Nevalainen 1998) The biological safety evaluation of medical devices is guided by the revised versions of the documents ISO 10993 and CEN 30993 (Biological evaluation of medical devices - Part I 1995). The selection and evaluation of any new material or device intended for use in humans requires a structured programme of assessment.

Biodegradable materials have proved their good biocompatibility as suture material in clinical use for about 30 years. Herrmann et al showed in 1970 that polyglycolic acid (PGA) evoked only minimal inflammatory response as suture material. The biodegradation of SR-PGA implants proceeds with cellular reactions comparable to the reactions of PGA sutures (Echeverria and Jimenez 1970). In bone surgery, the degrading implants disintegrate to small fragments which cause a non-spesific, mild foreign-body reaction. The debris particles are ingested by giant cells and macrophages (Törmälä 1992).

PLA implants cause only moderate and reversible tissue reactions which consist of the presence of macrophages, giant cells, phagocytic foam cells, lymphocytes, plasma cells, fibroblasts and histiocytes only in close contact to the implant. (Majola 1992). However also controversial observations have been made. Bergsma et al (1993) reported of symptomatic intermittent swelling three years after zygomatic fracture fixation with PLLA implants. A nonspesific foreign body reaction with crystalline-like PLLA material in the cytoplasm of various cells was seen. SR-PLLA spirals showed good biocompatibility in the anterior urethra, whereas stainless steel spirals evoked a marked inflammatory reaction (Kemppainen et al 1993).

3.7. BACTERIAL ADHERENCE

The adherence of uropathogens to the uroepithelium or to the surface of prosthetic devices is the key event in the pathogenesis of urinary tract infection (Costerton et al 1987, Reid and Sobel 1987). The bacteria which are not able to adhere, will not multiple and causean infection, because they will be washed out with urine.

The attachment of Escherichia coli is mediated by bacterial adhesins that are located either on the filamentous appendages called fimbriae or directly on the bacterial surface. The fimbriae are thread-like protein structures. Different adhesins are associated with different infections. S-fimbrial adhesins associate with serious neonatal infections and colonization factor antigens (CFA) adhesins with diarrhoeal diseases. P-fimbriae are thought to be the major virulence factor in urinary tract infections with E. coli. Bacteria may possess more than one type of fimbriae simultaneously or they may have no fimbriae (Siitonen 1994). Bacterial adherence to stents apparently correlates more with the properties of the bacteria (e. g. the presence of fimbriae) than with the properties of the stents (Petas et al 1998, Cormio et al 1996).

The adherent bacteria can aggregate, multiply and form biofilms, which are surrounded by polysaccharide matrices. These dense layers give bacteria prostection against antibiotics and thus biofilms constitute a reservoir of viable micro-organisms (Costerton et al 1987, Reid and Sobel 1987, Anwar et al 1990, Denstedt et al 1998). Urinary tract infections may occur, when fragments of biofilms break of and bacteria enter the bladder (Jumaa et al 1996).

Numerous kinds of efforts have been made to prevent bacterial adherence to foreign material in the urinary tract. Systemic antibiotic prophylaxis fails to prevent bacterial adherence and biofilm formation on ureteric stents, and infection rates between 7.5 and 27 % have been reported (Franco et al 1990, Reid et al 1992, Keane et al 1994). Silver coating of urinary catheters prevents

bacterial adherence to the catheters and reduces the amount of catheter associated urinary tract infections (Liedberg and Lundeberg 1989). Cormio et al (1997) showed that immersion in suitable antibiotic solution prevented bacterial adhesion onto bioabsorbable stents.

3.8. SILVER

Silver in various forms has a long history in medical therapy. The most well-known use is perhaps the Crede's prophylaxis against gonorrhoeal infections in the eyes of a newborn (Crede 1881). The silver ion has a broadspectrum activity against gram-positive, gram-negative, aerobic and anaerobic micro-organisms (Spadaro et al 1974).

In toxicological studies with zooplankton and rainbow trouts, silver or silver nitrate were not toxic in low concentrations (Hook and Fisher 2001, Guadagnolo et al 2001). Silver ions did not seem to exert any toxic effects on mammalian cells in a study of Berger et al (1976). In a study of Liedberg and Lundberg (1989) with mouse fibroblasts, silver was not found cytotoxic. Silver nitrate or silver sulphide coating increased the cytotoxicity of silicone catheters, but reduced the cytotoxicity of latex and teflon coated latex catheters. In a more recent study, silver nitrate was found to exert cytotoxic effects on human fibroblasts and endothelial cells which are related to the wound healing process (Hidalgo and Dominguez 1998). However, silver in different preparations has been found effective against burn wound infections in topical use, especially with antibiotic resistant bacteria (Tredget et al 1998, Wright et al 1998, Klasen 2000). In an experimental study of Liedberg et al (1989) silver coating reduced the inflammatory reaction caused by latex, teflon or even silicone catheter material in the urethra of a rat. Silver coating of indwelling urinary catheters inhibits the adherence and growth of both gram-positive and gram-negative bacteria(Liedberg and Lundeberg 1989, McLean et al 1993, Ahearn et al 2000) and the rate of catheter-associated urinary tract infections (Bologna et al 1999, Saint et al 2000). Silver coating of vascular prostheses and central venous catheters has reduced the bacterial colonization of these devices (Schierholz et al 1998, Gatter et al 1998). Silver nitrate has also been used intrapleurally in rabbits with favourable results to produce pleurodesis (Vargas et al 2000).

3.9. OFLOXACINE

Ofloxacine is one of the quinolones, whose mechanism of action is the inhibition of bacterial DNA gyrase. This inhibition leads to degradation of the bacterial DNA and to death of the cell. 80 - 90 % of the dose is secreted as such into urine

and good concentrations are achieved also in genitourinary tract tissues. Ofloxacine has a good therapeutic effect in vivo against most uropathogens, Neisseria gonorrhoeae and chlamydia trachomatis. However, enterococci are often resistant to ofloxacine in vitro. Anaerobic bacteria are usually resistant to ofloxacine (Naber et al 1998, Onrust et al 1998).

Ofloxacine is administered perorally or intravenously, and other routes have been studied relatively little. In an experimental study of Okahara et al (1995) ofloxacine impregnation of a vascular graft prevented effectively graft infection caused by Escherichia coli. In another study (Nie et al 1998) ofloxacin inpregnated DL-lactide-glycolide polymer implant proved to be effective in curing experimental Pseudomonas aeruginosa osteomyelitis in rabbits.

3.10. ENCRUSTATION

The process by which crystalloids and colloids adhere to biomaterial surfaces is referred to as "encrustation" (Choong and Whitfield 2000). All biomaterials that are currently used will become encrusted to some extent when exposed to urine. A biomaterial is defined as any substance, natural or synthetic, that is used in the treatment of a patient and that at some stage interfaces with tissue (Mardis and Kroeger 1988). Polyethylene, the first synthetic polymer used for construction of urological prostheses, has been abandoned by the majority of manufacturers because of its stiffness and its tendency to become brittle and break if left indwelling for extended periods. Silicone performs better, because it is nontoxic and more resistant to encrustation. However its flexibility and elasticity make it difficult to pass as a stent through narrow or tortuous ureters. Polyurethane partly combines the stiffness of polyethylene to the elasticity of silicone. Silicone-based copolymers, such as C-flex® (Concept Polymer Technologies, Clearwater, Florida, USA) and Silitek® (Medical Engineering Corporation, Racine, Wisconsin, USA), as well as olefinic block copolymers such as Percuflex® (Boston Scientific Corporation, Natick, Massachusetts, USA) are currently being used in stent manufacture. These biomaterials are softer than polyurethane and are easy to manoeuvre in the urinary tract (Sofer and Denstedt 2000).

One of the recent developments in the stent technology is the use of hydrogel coatings. These hydrophilic polyurethane polymers swell while retaining water within their polyanionic structure. Hydrogel coatings have a low coefficient of friction, which results in easier insertion of the stent and less discomfort for the patient (Candela and Bellman 1997). Kulik and Ikada studied platelet adhesion to nonionic and ionic hydrogels with different water contents and found that adhesion was lower to nonionic hydrogels than adhesion to conventional hydrophobic polymers like polyvinyl chloride, polyurethane and silicone (Kulik and Ikada 1996). The same may apply to microbial cell adhesion. In vitro studies

with poly (ethylene oxide)/polyurethane composite hydrogel (Aquavene®, Landmark, Menlo Care, Menlo Park, California) showed superior resistance to encrustation and intralumainal blockage over 24 weeks (Gorman et al 1998).

However, encrustation rates up to 58 - 76 % have been reported with ureteric stents indwelling for more than 12 weeks (El-Faqih et al 1991, Keane et al 1994). Encrustations increase with the duration of stenting: in the study of El-Faqih, only 6 % of stents were encrusted at six weeks. Encrustations can cause stent blockage,infective complications and trauma to urinary tract mucosa during removal of the stent. The development of encrustations depends on the composition of urine, possible presence of bacteria and the surface properties of the stent (Gorman 1995).

Supersaturation of urine is an important risk factor. Often these patients have also urinary lithiasis and should be encouraged to high fluid intake (Thomas 1993). An elevated urine pH was an important factor in catheter blockage with long-term indwelling catheters. (Burr et al 1993, 1995). Urinary stasis plays also a role: the bladder portion is affected more than the ureteral portion of a double-J stent (Keane 1994).

The mechanism of encrustation in infected urine is identical to the formation of infected urinary stones. Urease-producing bacteria, like Proteus mirabilis, Proteus vulgaris, Pseudomonas spp and Corynebacterium urealiticum, elevate the urinary pH through the hydrolysis of urea. In alkaline environment magnesium and calcium precipitate readily out of solution in the form of magnesium ammonium phosphate and calcium apatite and form struvite encrustations.(Gorman and Tunney 1997, Dominguez-Gil et al 1999). The encrustations harbour bacterial biofilm which can cause repeated episodes of bacteriuria (Gorman 1995).

Prevention of biofilm formation and bacterial colonization on the stents has been one strategy in avoiding encrustation. Clinical efficacy has been reported with the use of silver-hydrogel, minocycline, rifampin and nitrofurazone coatings on stents in the urinary tract (Darouiche R 2000). Silver coating of urinary catheters is known to inhibit the adherence of bacteria to urinary catheters (Liedberg 1989). Subramanyam et al reported the development of an antimicrobial coating, containing poly(hexamethylenebiguadine) and silver iodide, for use on urological devices (Subramanyam et al 2000). It has been shown that ciprofloxacin can adsorb onto stent materials and penetrate young < 24 h biofilms (Reid et al 1995) . This effect can be amplified by protamin sulphate (Soboh et al 1995).

Surface properties and structure of the stent or catheter may be the most important factors in encrustation in sterile urine (Gorman and Tunney 1997). Silicone catheter was superior to latex catheter in resisting encrustation (Bruce et

al 1974), presumably because of its smooth surface. In an in vitro study of Tunney et al (1996) increasing surface roughness resulted in increased amount of encrustation. The authors suggested that surface irregularities act as nucleation sites for crystal growth. El-Faqih et al (1991) noted that encrustation was greatest at the location of sideholes along the shaft of ureteral stents. Increased hydrophobicity decreases encrustation tendency in ureteral stents (Tunney et al 1996).

Biodegradable stents seem to encrustate less than metallic stents. In the study of Kemppainen et al (1993) there were fewer encrustations on SR-PLA stents than on stainless steel stents in the anterior urethra of rabbit. Petas et al (1998) found some encrustations on gold plated steel wire, but not on SR-PGA or SR-PLA stents after 2 weeks' incubation in artificial urine. The authors suggested that the lack of encrustation may be explained by the sloughing off of the surface of a biodegradable stent.

4. AIMS OF THE STUDY

Nosocomial urinary tract infections associated with indwelling catheters and urinary tract stents cause substantial morbidity and costs. The key event in the pathogenesis of a urinary tract infection is the adherence of uropathogens to the epithelium or foreign material in the urinary tract. In the present study we sought answers to the following questions:

1. Is it possible to prevent bacterial adherence to SR-PLLA urological stents bysilver nitrate blended caprolactone coating? (I)

2. Does ofloxacine blended caprolactone coating prevent the adherence of uropathogens to SR-PLLA urological stents? (II)

3. What is the biocompatibility of silver nitrate and ofloxacine coated SR-PLLA urological stents? (III)

4. What effects do silver nitrate and ofloxacine coatings have on thebiocompatibility, encrustation and biodegradation of SR-PLLA urospirals? (IV)

5. Are there differences in encrustation of silver nitrate and ofloxacine coated SR-PLLA stents compared to stainless steel stents during long-term incubation in artificial urine? Do silver nitrate or ofloxacine coatings have any effect to biodegradation of SR-PLLA stents during long-term incubation in artificial urine? (V)

5. MATERIAL AND METHODS

5.1. Polylactic acid stents

The biodegradable polymer poly-L-lactic acid was obtained from Purac Biochem b.v. (The Netherlands). The polymer was extruded into cylindrical rods, and self-reinforcing was made by die-drawing in the solid state. Caprolactone-L-lactide polymers were polymerized according to the method developed in Helsinki University of Technology, copolymer coating was blended with various concentrations of silver nitrate and ofloxacine and the SR-PLLA test material was coated by the blended copolymer coating in Bionx Implants Ltd, Tampere, Finland. The configuration of the stents resembled the original Fabian-type urospiral.

5.2. Bacterial adherence studies

5.2.1. Silver nitrate study (I)

Silver nitrate concentrations in the copolymer coating in the first study (I) were 10, 5, 2 and 0.5 weight-%. The thickness of the SR-PLLA wire was 1.1 mm and the coating 0.1 mm. The wire was cut exactly to 5 mm-long pieces after coating with the copolymer. SR-PLLA pieces with pure caprolactone-L-lactide coating were used as controls. The test material was preincubated in artificial urine for 1 h, 24 h, 1 week and 2 weeks at 37°C. The composition of artificial urine is in Table 1:

Table 1: The composition of artificial urine (A:B = 1:1)

A solution (g/l)		B solution (g/l)	
CaCl2 x 2H2O	1.765	NaHPO4 x H2O	6.800
Na2SO4	4.862	NaHPO4	0.869
MgSO4 x 7 H2O	1.143	Na3Cit x 2 H2O	1.168
NH4Cl	4.643	NaCl	13.545
KCl	12.130		

Each assay was performed in duplicate. For bacterial strains (Pseudomonas aeruginosa, Enterococcus faecalis, Proteus mirabilis and a strain of Escherichia coli, IH 13258) had been isolated from patients with urinary tract infections. A strain of Escherichia coli (IH 50797) had been isolated from stools of a healthy subject.

A 50-microl aliquot of bacterial suspension was added to a test tube containing 1 ml artificial urine and the two stent pieces. To calculate the amount of bacteria added (inoculum dose), 100-microl samples of bacterial suspension were plated on cystine-lactose. electrolyte-deficient (CLED) agar plates after serial 1:10 dilutions of bacterial suspensions. The amount was expressed as colony forming units (CFU) per millilitre. After 3 h of incubation with mild shaking , the stent pieces were removed from the test tube and rinsed four times with phosphate buffered saline (PBS). The adherent bacteria were thereafter detached with a water-bath sonicator. The amount of detached bacteria was calculated as CFU/ml after the 1:10 dilution series were plated onto CLED agar plates. The effect of inoculum dose was taken into account by dividing the CFU of detached bacteria by the CFU of the inoculum dose. A control series without bacterial inoculation was performed parallelly.

To see whether the stent could inhibit bacterial growth during the 3 h incubation period, remaining bacteria were calculated as CFU/ml after a 1:10 dilution series. The ratio of CFU of the remaining bacteria to CFU of the inoculum dose was calculated.

SPSS for Windows 8.0 was used as the statistical package and Friedman's nonparametric test was used to estimate the differences in bacterial adherence, with statistical significance level at $p<0.05$.

5.2.2. Ofloxacine study (II)

Ofloxacine was obtained from Hoechst-Marion-Roussel (Romainville, France). Ofloxacine concentrations in the copolymer coating were 5, 2 and 0.5 weight-%. Pure copolymer coated SR-PLLA stent pieces were used as controls. The design of the study was identical to the silver nitrate study.

5.3. Rabbit muscle biocompatibility study (III)

The study was performed according to the recommendations of ISO 10993 - 6. The caprolactone-L-lactide copolymer coating was blended with three different concentrations (10, 5 or 2 weight-%) of silver nitrate or two concentrations of ofloxacine (5 or 2 weight-%). The coated 1.1 mm thick SR-PLLA wire was cut

into 12.5 mm pieces. Stent pieces with pure copolymer coating were used as controls. Silicone rods served as negative and organotin positive PVC rods (from Portex Ltd, Kent,UK) as positive controls.

25 New Zealand White male rabbits were used as experimental animals. The animals were anesthesized with medetomidine hydrochloride (Domitor®) 0.3 mg/kg and ketamine hydrochloride (Ketalar®) 15 mg/kg intramuscularly. A dorsal midline incision was made. The test material pieces were implanted into the dorsal muscles via a 2.0 mm i.v. cannula. The implantation sites were marked in the fascia with nonabsorbable sutures, and the skin was closed with resorbable running suture. There were 8 pieces of each test material randomly implanted, with each type of material in at least three rabbits.

Eight rabbits were sacrified after 1 week, eight after 1 month and eight after 6 months by giving an overdose of pentobarbital sodium (Mebunat®) intravenously. The test material was excised and fixed in formalin. After paraffin embedding, routine haematoxylin-eosin staining was performed and the tissue reactions assessed. The tissue reactions (acute and chronic inflammatory changes, eosinophilic reactions, fibrosis, foreign body reaction, muscle necrosis around the implant, necrosis facing the implant and calcific depositions) were scored semiquantitavely.

SPSS for Windows 8.0 was used as statistical package and Kruskall-Wallis test was used to estimate the differences in tissue reactions, with statistical significance level at $p< 0.05$.

5.4. Rabbit urethral biocompatibility, encrustation and biodegradation study (IV)

The caprolactone-L-lactide copolymer coating was blended with 10 weight-% of silver nitrate or 5 weight-% of ofloxacine. Pure copolymer coating served as a control. The SR-PLLA stents resembled the configuration of the original Fabian stent, with outer diameter of 4 mm and length of 4 cm. The thickness of the SR-PLLA wire was 0.7 mm and that of the copolymer coating 0.1 mm.

18 New Zealand White male rabbits were used as experimental animals. They were divided into six groups (A - F), with three rabbits in each group. The animals were anesthesized as in study III. The stents were pushed into the prostatic urethra by using a 14 Ch cystoscope. No antibiotic prophylaxis was given. Groups A and D got silver nitrate stents, groups B and E ofloxacine stents and groups C and F stents with pure copolymer coating. The stents were fixed into the urethral wall by a nonresorbable suture through the distal ring of the stent. Groups A - C were sacrificed after 1 month and groups D - F after 6

months by giving an overdose of pentobarbital sodium (Mebunat®) intravenously.

Three urethral tissue blocks per animal were excised and fixed in 10 % formalin. Two blocks had been in contact with the stent, the most distal had not. After paraffin embedding, routine haematoxylin eosin staining was performed and tissue reactions were scored semiquantitavely. The histological parameters assessed included the presence of acute and chronic inflammatory cell reaction and tissue fibrosis. Also urethral epithelial erosions were analyzed.

The stents were removed, rinsed with saline, cut into three pieces and the pieces fixed in 2 % glutaraldehyde. Thereafter the specimens were dehydrated in ethanol, dried in a critical point dryer according to the recommendations of Andersson (1951) and coated with 200 Å thick gold layer in a Jeol Fine Coat Ion Sputter JFC-1100 (1.2 kV, 5 - 10 mA, 6 min). Scanning electron microscopic (SEM) analysis was performed from ten randomly selected areas per each stent (DSM 962, Zeiss, Germany). The analyzed parameters were: loosening of coating, crystals (magnification 100x), bacteria, inflammatory cells and biofilm on the stent (magnification 2000x). The relative areas of the parameters were estimated and the means were calculated. No statistical analysis was performed because of the small number of tissue specimens and stent pieces.

5.5. Artificial urine incubation study (V)

The SR-PLLA wire was coated with caprolactone-L-lactide copolymer or copolymer blended with 10, 5 weight-% silver nitrate or 5 weight-% of ofloxacine. The wire diameter was 1.1 mm and the thickness of the coating 0.1 mm. Gold plated stainless steel stents (Prostakath®) served as controls. The wires were cut into 2 mm long pieces, which were incubated in sterile artificial urine for 4, 8, 12 and 24 weeks. There was one piece of each stent material for each incubation period. After incubation, the stents were rinsed with saline and fixed in 2 % glutaraldehyde. Thereafter the specimens were dehydrated in ethanol cascade, dried in a critical point dryer and with gold like in the previous study. Scanning electron microscopic analysis with a magnification of 100x from three randomly selected areas was performed. Each picture was divided in 200 000 pixels and the area of encrustation in pixels was assessed. To get the relative area of encrustations, the area of encrustations in pixels was divided by 200 000 pixels. Statistics was not calculated because of the small number of stent pieces.

6. RESULTS

6.1. Bacterial adherence studies

6.1.1. Silver nitrate study (I)

Silver nitrate blended copolymer coating reduced bacterial adherence to SR-PLLA stent pieces in vitro, and the prevention of bacterial adherence correlated with the concentration of silver nitrate ($p<0.001$, Friedman's test). With the exception of Enterococcus faecalis, the 10 and 5 weight-% coatings could nearly totally prevent bacterial adherence to stent pieces. The weakest concentration seemed to promote bacterial adherence in some cases (Pseudomonas aeruginosa and Escherichia coli IH 50797). The preincubation time in artificial urine between one hour and two weeks did not have any substantial effect on bacterial adherence.

Silver nitrate inhibited bacterial growth in ambient urine, and the inhibitory effect increased as a function of silver nitrate concentration ($P<0.001$, Friedman's test) and preincubation time (from one hour to two weeks).

6.1.2. Ofloxacine study (II)

Ofloxacine blended caprolactone-L-lactide copolymer coating prevented bacterial adherence to SR-PLLA stents in vitro almost totally, with the exception of Enterococcus faecalis. The preventive effect correlated closely ($p< 0.001$, Friedman's test) to ofloxacin concentration in the coating. The length of preincubation time between one hour and two weeks did not have any substantial effect on the adherence.

Ofloxacine inhibited bacterial growth in the test tube solution, and the inhibitory effect correlated significantly ($p<0.001$, Friedman's test) with the ofloxacin concentration in the coating. The length of preincubation time from one hour to two weeks did not have effect on bacterial growth inhibition.

6.2. Rabbit muscle biocompatibility study (III)

The tissue reactions were most marked at one month after implantation. Caprolactone-L-lactide copolymer coated SR-PLLA showed good biocompatibility, in fact, sometimes it caused less tissue reactions than the negative control. 10 weight-% silver showed marked tissue toxicity. The other silver nitrate and ofloxacine coatings seemed to possess better biocompatibility properties, but usually little worse than the negative control.

At 6 months after implantation there were generally no tissue reactions in test samples, however mild to moderate foreign body reactions were seen. The reactions due to implantation trauma, like muscle necrosis and fibrosis, dominated at one week after implantation.Acute and chronic inflammatory changes were sparse.

Statistical significance (Kruskall-Wallis test) was reached at one month with eosinophilic and muscle necrosis reactions and at 6 months with fibrosis and foreign body reaction. However, the role of eosinophilic reactions seems to be of minor importance, because also silicone caused marked eosinophilic reactions. The fibrosis reactions at six months can also be neglected, because all the reactions were mild.

6.3. Rabbit urethral biocombatibility, encrustation and biodegradation study (IV)

Both silver nitrate and ofloxacine blended copolymer coated SR-PLLA spirals caused less tissue reactions than SR-PLLA spirals with pure copolymer coating at one month after stent application, and only minimal epithelial erosions were seen in all test groups at the sites in contact with the stent.

At six months the stents had unexpectedly degraded, and only two rabbits had stent pieces, both with pure copolymer coating, in their urethras. The rest of the animals had voided out the stent pieces between one and six months after insertion. However, the planned tissue samples were taken. Again silver nitrate and ofloxacine blended copolymer coatings caused less tissue reactions than pure copolymer coating. Of course, this can partly be caused by the fact that these stents were not in situ and the possible tissue reactions had time to heal. Epithelial erosions were not seen in any of the animals.

SEM analysis of the stents at one month after insertion revealed that silver nitrate blended copolymer coating prevented biofilm formation and accumulation of bacteria and inflammatory cells. Crystal formation was sparse

on silver nitrate blended coating, whereas it was more marked on both ofloxacine blended and pure copolymer coating. The same applied to biofilm formation and the presence of bacteria and inflammatory cells. The loosening of the ofloxacine and pure caprolactone coating was obviously one factor contributing to this.

The two stents remaining at six months after insertion had both pure copolymer coating. The biofilm formation was extensive, there were masses of inflammatory cells and bacteria and also heavy encrustations were seen on degraded stent pieces. The biodegradation rate had been surprisingly rapid, especially with the blended coatings.

6.4. Artificial urine incubation study (V)

The differencies in encrustation were not substantial after incubation periods up to 12 weeks. After that, the ofloxacine blended caprolactone coated stents got clearly more encrusted than the other stents, which had encrustations on 2 - 3 % of their surface. There were no signs of degradation or loosening of coating with the bioabsorbable stents after the 24 weeks' incubation.

7. DISCUSSION

7.1. Bacterial adherence (I and II)

Factors influencing the initial attachment of bacteria to a biomaterial include electrostatic and hydrophobic interactions; ionic strength, osmolality and pH of urine; urine concentration of urea, creatinine and proteins; surface properties of the biomaterial and bacterial surface components (Denstedt et al 1998). In the study of Petas et al (1998), bacterial adherence correlated more to the bacterial strain than to the material of the stent. The interactions between bacteria and polymers are very complex and not easily generalized by simple rules and schemes. It has been suggested that dissolution of the underlying material which the bacteria have been attached to during degradation, results in the release of bacteria. Multiple adhesive points keep the bacteria on the surface, while individual contact points may dissolve and afterwards reform on freshly exposed polymer (Stickler 1996) The type of bacteria and possible presence of fimbriae thus greatly influence the adhesion process.

It is known that the ability of uropathogens to adhere to the uroepithelium or to the surface of prosthetic devices is the key event in the pathogenesis of urinay tact infection (Costerton et al 1987, Reid and Sobel 1987). Within a few hours, adherent bacteria can aggregate, multiply and form biofilm matrices, which, once surrounded by a dense glycocalyx, may become resistant to antimicrobial agents and constitute a reservoir of viable micro-organisms (Denstedt et al 1998). Bacteria are able to adhere to ureteric stents and form biofilms in vivo despite systemic antibiotic prophylaxis (Keane et al 1994).

Silver coating inhibits the adherence of bacteria to catheters (Liedberg 1989, McLean et al 1993, Ahearn et al 2000) and reduces the count of catheter-associated urinary tract infections (Bologna et al 1999, Saint et al 2000). Cormio et al (1997) found that immersion of biodegradable stents in suitable antibiotic solutions prevented the adherence of bacteria on these stents. Ofloxacine coatings have not been studied in this respect. On the other hand, we knew that it is possible to mix various compounds to the caprolactone-L-lactide copolymer coating of bioabsorbable stents (Hiljanen-Vainio et al 1996) thus presumably creating a long-term preventing effect on bacterial adherence. Originally this copolymer coating was designed to modify the expansion properties of biodegradable stents.

We found that both silver nitrate and ofloxacine blended copolymer coatings prevented the adherence of bacteria to SR-PLLA stent pieces in vitro. The prevention effect correlated with the concentration of the antibacterial agent in the copolymer coating. However, sparse concentrations (0.5 wt-%) of silver nitrate or ofloxacine paradoxically promoted bacterial adherence with some bacterial strains, which may be due to relatively rougher surface of the blended coatings.

It must be kept in mind that there was no coating at the both ends of the SR-PLLA stent pieces, because the cutting was performed after the coating. If also the ends had been coated, there would probably have been greater differences in bacterial adhrerence to stents with blended and non-blended coatings.

Enterococcus faecalis remained a problem to both coatings. Presumably the strain in our study was not susceptible to either silver nitrate or ofloxacine, and thus neither prevention of bacterial adherence to stents nor inhibition of bacterial growth during the 3-hour incubation period were seen.

It was very interesting that both silver nitrate and ofloxacine blended co-polymer coatings could inhibit the growth of bacteria (except E. faecalis) during the 3-hour incubation period. This will presumably happen in vivo also, because there is a microenvironment of urine in the prostatic urethra as the stent pushes the prostatic lobes apart.

7.2. Biocompatibility (III and IV)

Medical devices should be analysed for long-term safety by spesific animal tissue implantation tests. Rabbit was chosen to a test animal, because it has a life expectancy suitable for the test period and muscular tissue amenable to multiple implantations. Caprolactone-L-lactide coated SR-PLLA stent had shown no acute cytotoxicity in the study of Isotalo et al (1998) and was biocompatible in the rabbit muscle implantation test (Isotalo et al 1999).

A nonspesific foreign body reaction was practically the only tissue reaction at 6 months after implantation, regardless of the coating type. There was often black pigment in the macrophages lying on the rods with 10 and 5 weight percent silver nitrate coating. This was presumably due to the presence of silver compounds, which were thus ingested, transported from the muscle and later excreted from the body. Silver ions did not seem to exert any toxic effects on mammalian cells in an early study of Berger et al (1976). Liedberg et al (1989) found in a cell culture study with mouse fibroblasts that silver coating of catheters is not cytotoxic, but silver nitrate and silver sulphate are. However,

silver nitrate and silver sulphate coating decreased slightly the toxicity of latex and teflon.

The pathologist was sometimes unable to score the tissue reaction because some of the rods had been implanted to fascia instead of muscle. It must also be kept in mind that tissue reactions at urothelial surfaces may differ from those in muscle.

As was expected, caprolactone-L-lactide coated SR-PLLA was biocompatible. Also ofloxacine blended coatings and silver nitrate blended coatings up to 5 weight-% proved to be fairly biocompatible.10 weight-% silver nitrate showed marked tissue toxicity in the rabbit muscle biocompatibility study.

In the study of Kemppainen et al (1993) the SR-PLLA stents were macroscopically eliminated from the rabbit urethra in 14 months. The implantation into the urethral wall was achieved by coating the stent with a layer of small molecule D-lactide. We did not see any implantation, and the biodegradation was unexpectedly fast. It seems that caprolactone-L-lactide accelerates the biodegradation. It may may even be concluded that blending silver nitrate or ofloxacine to the colopoymer coating still shortens the biodegradation time. It is known that degradation of bioabsorbable polymers may be accelerated by the presence of residual monomers and oligomers in the polymer and the small chain polymer coating of the core polymer (Kemppainen et al 1993, Talja et al 1997). The activity of pelvic floor muscles may also have contributed to the unexpectedly rapid degradation of the stents.

As a divergence to the rabbit muscle biocompatibility study, 10 weight-% silver nitrate coating did not show more tissue toxicity than other coatings in the rabbit urethral biocompatibility, encrustation and biodegradation study. There were no signs of silver particles in the urethral wall. In the rabbit muscle biocompatibility study, silver compounds could be seen in the macrophages lying adjacent to the silver nitrate coated SR-PLLA rods.

The tissue reactions seen in sites in contact to the stent were mostly weak or moderate in semiquantitative analysis, and could at least partly be explained by the muscular activity around the stent. However, the unexpectedly rapid degradation of the stents makes the interpretation of the tissue reactions somewhat difficult.

7.3. Encrustation (IV and V)

The process by which crystalloids and colloids adhere to biomaterial surfaces is referred to as “encrustation” (Choong and Whitfield 2000). Encrustation can occur both in sterile and infected urine, but in sterile urine encrustation depends mostly on the composition of urine and the surface properties of the stent (Gorman and Tunney 1997).

It has been been assumed that because of continuous hydrolyzation and sloughing off of the surface, biodegradable stents are partly protected from getting encrusted (Kemppainen et al 1993, Petas et al 1998) The present study did not confirm this finding, caprolactone-L-lactide coated stent pieces were heavily encrusted at six moths after insertion. The encrustation may be caused by the properties of the caprolactone coating. Another explanation is that caprolactone-L-lactide coating itself reduced the stability of SR-PLLA, accelerated the biodegradation and promoted thus encrustation on the surface of the degraded polymer, since the rough surface of the degraded polymer acted as nucleation sites for encrustation.

We had problems with the stability of the ofloxacine blended coating from the beginning of the study. This coating seemed to loosen too early, causing the accumulation of bacteria and inflammatory cells on the nuded areas of stents. Moreover, the ofloxacine blended coating was rough due to the blending method (ofloxacine was added to liquid caprolactone as a small particle powder).

Silver nitrate blended caprolactone-L-lactide copolymer coating prevented the formation of crystals and biofilm. Silver nitrate could prevent also the accumulation of bacteria, as could be expected after our bacterial adherence study. This eliminated at least partly the activity of urea splitters and contributed to decreased struvite encrustation formation. Based on the results of our earlier in vitro study, the ofloxacine blended coating was presumably also effective in preventing the activity of urea splitters, but the roughness and the loosening of the coating promoted encrustation.

We did not use any antibiotic prophylaxis in this study, because it seems very unprobable that any prophylactic antibiotic could have prevented the adherence of bacteria to the stent in the colonized distal urethra. As we know, infection rates between 7.5 – 27 % have been reported in patients with postoperative ureteric double-J stents, despite of antibiotic prophylaxis (Franco et al 1990, Reid et al 1992, Keane et al 1994).

With the exception of ofloxacine blended copolymer coating, we found that encrustation was not a substantial problem in sterile urine, because usually less than 5 % of the surface of the stents were encrusted. On the contrary to the study of Petas et al (1998), bioabsorbable stents were not less prone to get encrusted

than gold plated stainless steel. The explanation is probably that caprolactone-L-lactide has different surface characteristics than uncoated SR-PGA or SR-PLA.

The encrustation behaviour of ofloxacine blended copolymer coating can at least partly be explained by the roughness of the coating. It is probable that this tendency to encrustation can be diminished by developing the blending method. On the other hand, the difference in encrustation between ofloxacine blended and other coatings was seen only after 12 weeks, so theoretically ofloxacine coating could perhaps be used on biodegradable materials with shorter degradation times, for example SR-PGA or SR-PLGA. It was interesting that in vitro ofloxacine blended coating seemed stable up to 24 weeks'incubation period: there were no signs of loosening of the coating.

Encrustation of urinary tract stents is a major problem in vivo. All biomaterials currently used in urinary tract will become encrusted at least to some extent, which can cause significant morbidity: discomfort, bleeding, stent blockage and persistent urinary tract infections.Encrustation rates of up to 58 - 76 % have been reported with ureteric stents indwelling for more than 12 weeks (El-Faqih et al 1991, Keane et al 1994). It is well-known that a proportion of this encrustation is caused by the activity of urea-splitters, like Proteus mirabilis, Proteus vulgaris, Pseudomonas spp and Corynebacterium urealyticum (Tunney et al 1999, Dominguez-Gil et al 1999). Since both silver nitrate and ofloxacine coatings decrease bacterial adherence to SR-PLLA stents (studies I and II), they could both theoretically decrease the tendency to encrustation of bioabsorbable stents in vivo. This may be true, but the manufacturing method of the ofloxacine blended coating should be developed, since it could not prevent the accumulation of bacteria and encrustations on a SR-PLLA stent in vivo (study IV). It was also encrusted more than the other stent materials in a long term in vitro test in sterile urine (study V). On the other hand, silver nitrate blended coating encrusted less in the present in vitro test and seemed to prove its efficacy also in vivo (study IV). This may give possibilities to prevent stent associated infections and stent encrustation also in clinical practice. However, its efficacy and safety in humans needs to be studied.

8. CONCLUSIONS

The following conlusions can be drawn on the basis of the present study:

1. Silver nitrate blended caprolactone-L-lactide copolymer coating prevents the adherence of some uropathogens (except Enterococcus faecalis) to SR-PLLA stents. The preventive effect correlates with the concentration of silver nitrate in the copolymer coating. Silver nitrate blended copolymer coating inhibits the growth of some uropathogens (except Enterococcus faecalis) in the microenvironment of the stent.

2. Ofloxacine blended caprolactone-L-lactide copolymer coating prevents the adherence of some uropathogens (except Enterococcus faecalis) to SR-PLLA stents. The preventive effect correlates with the concentration of ofloxacine in the copolymer coating. Also ofloxacine blended copolymer coating inhibits the growth of some uropathogens (except Enterococcus faecalis) in the microenvironment of the stent.

3. The biocompatibility of caprolactone-L-lactide copolymer coated SR-PLLA rod is good in the rabbit muscle implantation test. Blending the coating with silver nitrate or ofloxacine up to five weight-% does not compromize the good biocombatibility properties.

4. Silver nitrate or ofloxacine blended caprolactone-L-lactide coated SR-PLLA urospirals have good biocompatibility properties in the rabbit urethra. Silver nitrate blended copolymer coating could prevent accumulation of bacteria, encrustations and biofilm on the surface of the stent.

5. Pure or silver nitrate blended caprolactone-L-lactide coated SR-PLLA stents are not prone to get more encrusted than gold-plated stainless steel stents in long-term in vitro incubation. The manufacturing method of the ofloxacine blended copolymer coating needs to be developed.

9. SUMMARY

Encrustation and stent associated bacterial infections are a major problem with urinary tract stents. The bioabsorbable stents are not devoid of these problems. The aims of the present study were firstly to evaluate the effects of silver nitrate and ofloxacine blended coatings on bacterial adherence to bioabsorbable stents, secondly to study the biocompatibility of these coated stents and thirdly to investigate the effects of these coatings on encrustation and biodegradation of the stents in vitro and in vivo.

Caprolactone-L-lactide was blended with 10, 5, 2 or 0.5 weight-% of silver nitrate or 5, 2 or 0.5 weight-% ofloxacine. SR-PLLA stent pieces were coated with silver nitrate or ofloxacine blended copolymer, and bacterial adherence of Pseudomonas aeruginosa, Enterococcus faecalis, Proteus mirabilis and two strains of Escherichia coli to the coated stent pieces was studied. Stent pieces coated with pure copolymer coating were used as controls. The stents were incubated with bacterial suspension for three hours. Thereafter the adherent bacteria were detached from the stent surfaces and plated on CLED agar plates. Their amount was expressed as CFU. To see, whether silver nitrate or ofloxacine coatings could inhibit bacterial growth during the three hours' incubation period in the incubate, incubate was plated on CLED agar plates and the amount of bacteria counted as CFU.

With the exception of Enterococcus faecalis, silver nitrate blended copolymer coating reduced bacterial adherence to SR-PLLA stent pieces. The prevention of bacterial adherence correlated with the silver nitrate concentration in the coating. The preincubation of stents in artificial urine between 1 h and two weeks before the bacterial inoculation did not have any substantial effect on bacterial adherence.The inhibitory effect increased as a function of silver nitrate concentration and preincubation time (from 1 to 2 weeks).

Ofloxacin blended coating prevented effectively the adherence of bacteria to SR-PLLA stent pieces and the preventive effect correlated to the ofloxacin concentration in the coating. Only the highest concentration of ofloxacin could sligthly prevent the adherence of enterococcus faecalis. Ofloxacin blended coating could also prevent bacterial growth in the test tube during the three hours' incubation period and this preventive effect correlated with the ofloxacin concentration in the coating.

The biocompatibility of SR-PLLA stent pieces coated with silver nitrate or ofloxacine blended caprolactone-L-lactide- copolymer or pure copolymer was evaluated with muscle implantation test in 25 New Zealand White male rabbits. Silicone rods were used as negative and organotin positive PVC rods as positive controls. The test material pieces were implanted to dorsal muscles of the animals. Eight animals were sacrificed after one week, eight after one month and eight after 6 months, and the test material was harvested with a 5 mm margin of muscle. Tissue reactions were assessed semiquantitavely according to the updated Sydney system. The biocompatibility of SR-PLLA stents coated with pure copolymer or copolymer blended with silver nitrate or ofloxacine up to 5 weight-% was good.

Silver nitrate (10 weight-%) or ofloxacine (5 weight-%) blended caprolactone-L-lactide or pure copolymer coated SR-PLLA stents were implanted into the posterior urethras of 18 male New Zealand White male rabbits to study the biocompatibility, encrustation and biodegradation properties. The animals were sacrificed after one or after six months. Urethral tissue reactions were assessed and SEM-analysis of the stents was performed. Stents with silver nitrate and ofloxacine blended coating caused less tissue reaction than the stents with pure copolymer coating. However, the stents had degraded faster than was expected and only two rabbits with pure copolymer coated stents had stent pieces left in their urethras after six months' follow-up. The silver nitrate blended copolymer coating prevented biofilm and crystal formation and accumulation of bacteria and inflammatory cells. The loosening of the coating was a moderate problem with ofloxacine blended and pure copolymer coating.

We studied the encrustation properties of silver nitrate (10 or 5 weight -%) or ofloxacine (5 weight-%) blended caprolactone-L-lactide coated or pure copolymer coated SR-PLLA stents during 24 weeks' incubation in sterile artificial urine. Gold plated stainless steel stents (Prostakath®) served as controls. The encrustation of the stent material was assessed by SEM with a magnification of 100 x after incubation of 4, 8, 12 and 24 weeks. Usually less than 5 % of the surface of the the stents was coated by encrustations. The encrustation rate of the stents was almost equal with the exception of ofloxacine blended copolymer coated stents which got encrusted more than the other stents after incubation periods of 12 weeks. Neither silver nitrate blended copolymer coated nor pure copolymer coated SR-PLLA stents gathered less encrustations than gold plated stainless steel stents.

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12. ABBREVIATIONS

CE	Comite Europeen
CEN	Comite Europeen de Normalisation
CFU	colony forming units
CLED	cystine-lactose-electrolyte-deficient
da	dalton
ESWL	extracorporeal shock wave lithotripsy
ICS	International Continence Society
ILCP	Interstitial laser coagulation of the prostate
ISO	International Standardization Organization
IUC	intraurethral catheter
PDS	poly-p-dioxane
PGA	polyglycolic acid
PGLA	polyglycolic lactic acid
PLA	polylactic acid
PLLA	poly-L-lactic acid
PVC	polyvinyl chloride
SEM	scanning electron microscope
SR	self-reinforced
TUMT	transurethral microwave therapy
TUNA	transurethral needle ablation of the prostate
VLAP	visual laser ablation of the prostate
Å	ångström