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Investigation of a rifampin, fusidic-acid and mupirocin releasing silicone catheter

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Abstract

After strict hygienical measures have been exhausted the use of plastic materials with antibacterial activity may reduce catheter related-bacterial colonization. An antimicrobial silicone catheter was investigated by HPLC-measurement, SEM, antimicrobial assays and standard biocompatibility tests. The modified catheter was highly biocompatible and the antimicrobial leaching non-toxic. The initially release rate was governed by the drug solubility in the 'sink' and surface loading ('burst effect'). The second continuous period depended on the drug velocity in the silicone matrix and was extended up to 100 days with a proportionality to $\sqrt{t}$ for each drug. Diffusion exponents were in range of 2×10^{-8} to 1×10^{-9} ($cm^2\ sec^{-1}$). The lower diffusion exponent of mupirocin was explained by its higher cohesion energy and lower physico-chemical compatibility with the embedding silicone. The antimicrobial drugs were in a molecular-dispersed state with the silicone-matrix, whereas superficially located crystals of the antibiotics covering the catheter surface could be demonstrated by SEM.

Keywords: Controlled release; Hemocompatibility; Silicone catheter; Diffusion coefficients; Antibiotics; Biocompatibility; Cohesion energy

1. Introduction

Polymer-associated infections are a major problem in modern medicine [1–5], especially CSF-infections in neurosurgery with an incidence reported of 1–39% [5–9]. Preoperative, postoperative and preventive topical application of antibiotics to the wound and to the valve of prosthetic devices minimized but did not prevent bacterial colonization of the shunt systems [7]. Therefore, eradication of the microorganisms (mostly coagulase negative staphylococci (CNS)) is seldom achieved without removal of the infected device, long-term antimicrobial chemotherapy, transient external drainage, and implantation of a new shunt system [5, 7]. Various attempts to prevent shunt infection have been made, including the use of prophylactic antibiotics and modifications of technique [7–9], but infectious complications remain, causing rising morbidity and mortality. Attempts have been made to make implants resistant to infections by various methods involving different adsorption techniques of antimicrobials onto the polymeric surfaces [10–21]. One major disadvantage of such a plastic material coated with currently available antibiotics is that these complexes are unstable with rapid desorption or leaching of the antibiotics [21–23].

Prevention of colonization of polymeric surfaces by continuous release of bactericidal, highly biocompatible antimicrobial substances incorporated into polymers, has been tried as a new promising approach [11, 24–30]. However, the safety of chlorhexidine–silver–sulfadiazine coated catheters remain contradictory due to the local toxic effects of chlorhexidin in blood and nervous tissues. Silver is a highly biocompatible metal but free silver ion concentrations being bactericidal are toxic to human cell cultures [31]. Detergent agents such as TDMAC (tri-dodecyl-methyl-ammonium-chloride) and benzalkonium-chloride used for catheter-coating induce hemolysis and are deleterious to nervous tissue.

As an improvement of the rifampin-loaded CSF-shunt, which has been shown to be absolutely infection-resistant to staphylococci in vitro and in vivo [24, 32], we incorporated a highly effective and biocompatible combination of antibiotics up to 10% (wt/wt) into the silicone material.

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2. Materials and methods

2.1. Materials

Rifampicin ($M_w = 823$, MMD, Darmstadt, Germany) is a competetive inhibitor of bacterial RNA-polymerase with a very good activity against Gram-positive microorganisms and a high physicochemical compatibility with the hydrophobic polydimethylsiloxane.

Mupirocin (pseudomonic-acid A, $M_w = 500$, Smithkline Beecham, Munich, Germany) has a novel chemical structure exhibiting a spectrum of activity to Gram-positive microorganism and Candida albicans. The mode of action is conferred by the inhibition of bacterial isoleucyl-*t*-RNA-transferase and the subsequent inhibition of RNA-synthesis. Mupirocin was applied to the insertion sides of the central venous catheters with a reduction of infected tips and in the prophylaxis for CSF-shunt infection [33].

Free fusidic acid ($M_w = 516$, Thomae, Biberach (Riss), Germany), a steroidal antibiotic is active against nearly all strains of *S. aureus* at inhibitory concentrations of 0.015–0.12 $\mu g\,ml^{-1}$.

The cerebrospinal-fluid shunt polymer (2 mm ∅, wall thickness 0.6 mm) was supplied by Fa.CORDIS, Haan, Germany. Its chemical composition is a polydimethylsiloxane network, filled with hydrophobized SiO_2 particles (30% (wt/wt)) and $BaSO_4$ (10% (wt/wt)) for X-ray detection.

2.2. Methods

2.2.1. Incorporation of the antibiotics into the polymeric materials

The solubility parameter (Hildebrand parameter) of the drugs was used to determine the most useful information for the mixing ability and thus compatibility of polymers with drugs. These parameters were calculated by reduced solubility and increments of molar attraction contributions [32, 34, 35]. Incorporation of the antibiotics into the polydimethylsiloxane matrix (5 wt% rifampicin, 1.7 wt% fusidic acid, 1.4 wt% mupirocin) by a diffusion-controlled process [32, 36] gave an antibiotic–polymer system having reproducibly defined surface- and matrix-loading relations [32].

The concept of the drug solubility in the macromolecules [36] seems to be useful to describe the physicochemical properties of drugs with respect to the compatibility with the polymeric matrix. We chose the concept of the solubility parameter δ (cohesion energy, cohesion density, Hansen parameter). The solubility parameter of rifampin, mupirocin and fusidic acid were calculated according to Fedor et al. [32, 34, 35]. With the following equations the cohesion energies of the homomorphs $F_{d,p,h}$ are added increment by increment and divided by the total molecular volume V:

$$\delta_d = (\sum F_d)/V. \quad (1)$$

The polar density δ_p was calculated by

$$\delta_p = (\sum F_P^2)^{0.5}/V \quad (2)$$

The cohesion energies of the hydrogen bonds calculation was performed by

$$\delta_H = (\sum F_z - U_H/V)^{0.5} \quad (3)$$

(F_z = increments of hydrogen bonds).

The partial cohesion energies δ_d, δ_p, δ_H were connected to the total cohesion energy:

$$\delta = (\delta_d^2 + \delta_p^2 + \delta_H^2)^{0.5}. \quad (4)$$

2.2.2. Measurement of drug release kinetics of the antibiotic-containing silicone shunt

To measure the drug release kinetics, short catheter pieces (1 cm length, 0.6 mm wall thickness, cut in length to get a film-like opened hollow cylinder) were eluted in 0.9% NaCl solution at room temperature (acceptor volume 10 ml, pH 7). Constant removing, replacing fresh solvent (every 3 h in early fractions released, after this every day) and stirring was performed to arrive approximately at the diffusional 'steady state' and nearly 'perfect sink conditions'. The elutions were investigated by HPLC. A high-performance liquid chromatogram two-pump system that could deliver a gradient flow was used (HP 1050). An RP_8 reverse-phase column (Nucleosil ET 200/8/4 5C8, Macherey-Nagel) and a pre-column (Nucleosil ET 200/8/4 C8 720005, Macherey-Nagel) was employed. The mobile phase was monitored with a Diode-Array-variable wavelength-UV-detector (HP 9000 Series 300) and data received by a microprocessor (HP 9000 Series 300). The mobile phase consisted of acetonitrile–MeOH–0.001 M sodium phosphate at 22°C. The solvent gradient started at a 3 : 1 : 6 ratio up to 8 : 1 : 1 with a flow rate of 1.5 ml. Pressure in the column varied starting from 190 to 150 bar. Rifampicin was detected at wavelengths of 254 and 450 nm, mupirocin at 220 nm and fusidic acid at 250 nm.

2.2.3. Calculation of diffusion coefficients

The diffusion coefficients of the diffusant migration were calculated by Eq. (6) [37, 38], when the short time approximation of fractional drug release followed Eq. (5) with $n = 0.5$:

$$\frac{M_t}{M_\infty} = kt^n \quad (5)$$

M_t/M_∞ is the relationship between the released amount at time t and the initial loading concentration of the device. k is an individual constant of each polymer/drug system. Equation (2) is a short time approximation of fractional drug release derived from the second Fickian

law, when $M_i/M_\infty < 0.6$:

$$M_i/M_\infty = \frac{4(D*t/\pi)^{1/2}}{\Delta} \quad (6)$$

M_i is the cumulative rate of the released drug and Δ the thickness of the sample. The diffusion coefficient in Eq. (6) is constant with a constant slope of M_i/M_∞; with following times of release ($M_i/M_\infty > 0.6$) the diffusivity decreases significantly with decreasing loading concentration [36].

2.2.4. Serial dilution or three-dimensional checker-board technique

The antibiotic concentrations to be tested ranged from 0.25 to twice the MIC. Every combination matching to each of these concentrations was given to the overnight cultures (10^4 CFU/ml *S. epidermidis* RP_{62}, KH_{11} and *S. aureus* 5aW 1136) achieving 36 concentrations for the threefold combination. Microtiter plates were incubated overnight and examined for growth pattern. Antimicrobial activity was exhibited at concentrations suppressing visible growth of bacteria during incubation. Each antibiotic in a combination producing the specified effect was expressed as a fraction of the concentration that produces the same effect when the antibiotic was used alone (fractional inhibitory concentration, FIC). Checker-board titration is interpreted by the sum of these fractions. For the sum ($\sum$ FIC) near 1, the combination acts indifferent, combinations $\ll 1$ exhibit synergistic effects and antagonistic cooperation is expected for a sum $\gg 1$ following Eq. [39, 40]:

$$\frac{[A]}{MIC_A} + \frac{[B]}{MIC_B} + \frac{[C]}{MIC_C} = FIC_A + FIC_B + FIC_C = \sum FIC \quad (7)$$

2.2.5. Measurement of bacterial adhesion to the antibiotic containing polydimethylsiloxanes

Antibiotic-loaded siloxane samples and unmodified ones were incubated for 3 h in phosphate-buffered saline (PBS) containing the test strain *S. aureus* 5aW 1136 (isolated from an infected implant). At the equilibrium of bacterial adhesion (2 h), the contaminated samples were transferred into the proliferation medium (Müller Hinton, OXOID UK) and incubated for periods of 24, 48 and 72 h at 37°C. After each incubation period, the bacteria were removed from the polymeric surfaces by ultrasonication (Branson sonifer, Danbury USA, 90 s, 150 W) and the number of detached viable bacteria was determined by a colony count method.

2.2.6. Scanning electron microscopy (SEM)

Observations were performed with a scanning electron microscope Jeol JSM T200 and the objects investigated were gold-sputtered with an SCD 303 (Fa. Balzers Union, Liechtenstein).

2.3. Biocompatibility testing

2.3.1. C_{3A}-Des-Arg-ELISA

The activation of the complement system was measured by a C_{3A}-ELISA (ProGen, Heidelberg, FRG). 1 cm silicone catheter, 1 cm modified silicone catheter and 1 cm sterilized, modified silicone catheter (γ-irradiation) were incubated in 1 ml fresh Na_2EDTA plasma for 5, 15, 30 and 60 min. A standard curve was employed by 1100, 560, 337 and 150 ng ml^{-1} C_{3A}. All samples were incubated for 1 h and o-nitrophenyldiamine was added. The reaction was stopped after 5 min with acetic acid and the substrate was measured photometrically at 496 nm.

2.3.2. Hemocompatibility (Hemolytic activity)

Silicone catheters (1 cm, silicone pure, modified silicone, modified silicone sterilized (γ)) were incubated in CH_{50}-Test-Tubes (Nobiment, CH_{50}-Test, FRG) containing heparinized serum and the supernatant fluid was measured photometrically at 405 nm.

3. Results

3.1. Three-dimensional isobologram

Figure 1 shows the idealized isobolograms depicting the possible interactions between antibiotics in combination. The theoretical definition of synergy requires the FIC index to be <1.0 (figure in the middle), the geometric definition is that the isobologram formed falls below the additive plane (left figure). Antagonistic combinations require FIC indices significantly higher than one.

In Fig. 2, the lowest concentration suppressing bacterial growth for each antibiotic in the combinations was expressed as the fractional inhibitory concentration (FIC). Most of the sums of inhibitory concentrations $\sum$FIC were smaller than one indicating synergistic action (points of the plane in the isobologram). The computer-generated three-dimensional isobologram for the isolate S. aureus SaW1136 sinks toward the origin (i.e. the fractional inhibitory concentration (FIC) for each drug approaches nearly 0) [40]. Antagonistic effects as shown by fractional sums significantly higher than one were not detected.

Similar results were achieved with the strains *S. epidermidis* RP_{62}, *S. epidermidis* KH_{11}, and *S. epidermidis* KH_6. Mutants of resistant strains to rifampin in combination did not emerge in either the checker-board or kill-rate experiments (not shown) indicating resistance prevention [40] by this combination.

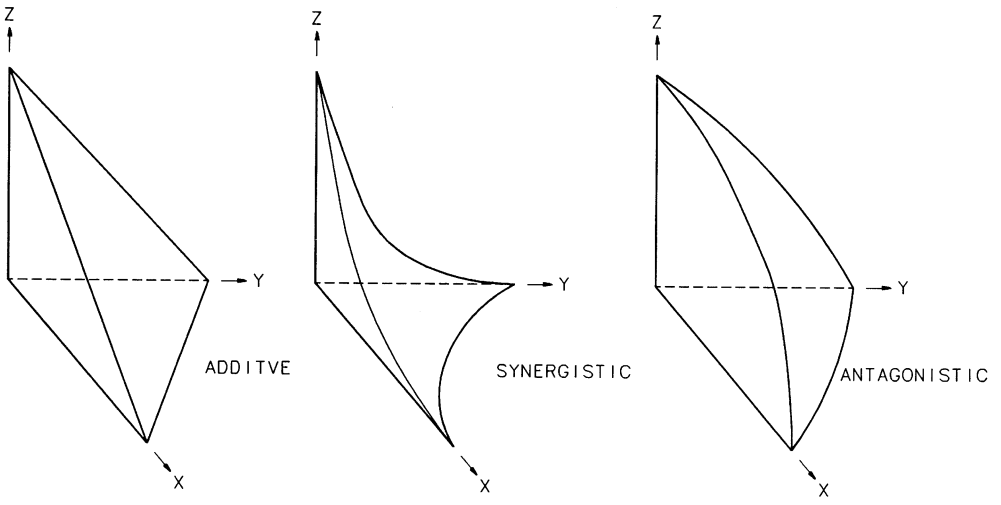


Fig. 1. Idealized isobolograms depicting three possible interactions (left: additive, middle: synergistic, right: antagonistic) between the FICs of three antibiotics (x, y, z) in combination.

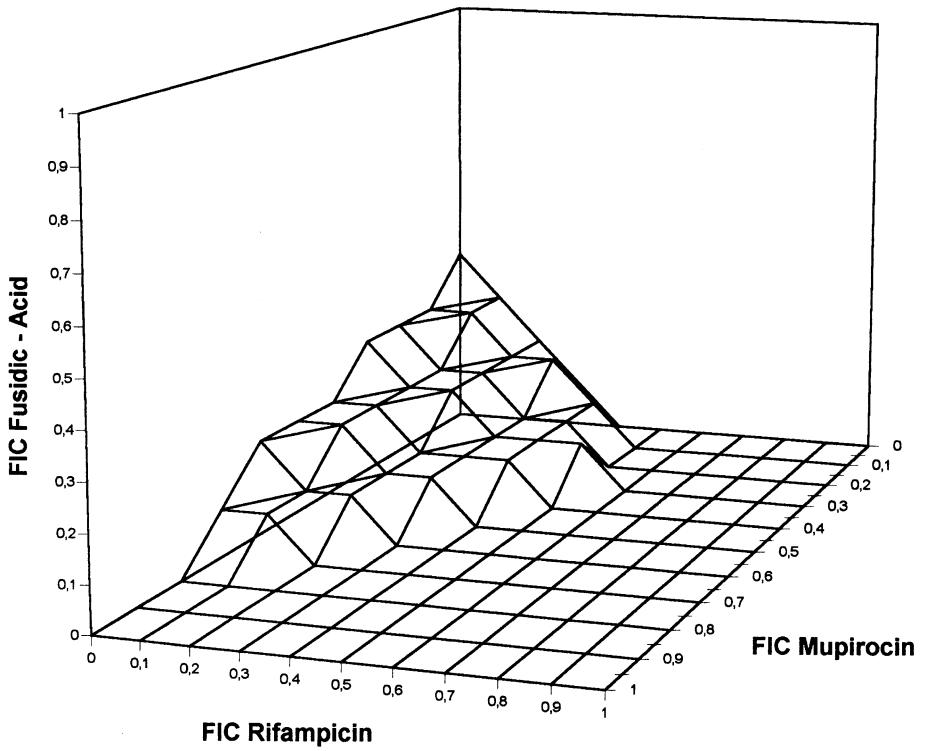


Fig. 2. The computer-generated three-dimensional isobologram of the checker-board titration. Fractional inhibitory concentrations (FICs) of rifampicin, fusidic acid and mupirocin do not significantly exceed 0.5 FIC showing synergistic cooperation in vitro.

3.2. Controlled release of rifampicin, mupirocin and fusidic acid from the silicone

Perfect sink conditions were used to determine controlled release of these antibiotics (Fig. 3). The release rate of each of these antibiotics showed two significant periods. The initial release rate, the so-called 'burst effect', depended on the mass, distribution and solubility of antibiotic crystals on the surface of the loaded polymer as revealed by a proportionality to $\log t$ in the first 24 h [41]. The second continuous period occurred after one day and was diffusion-controlled by the structure of the

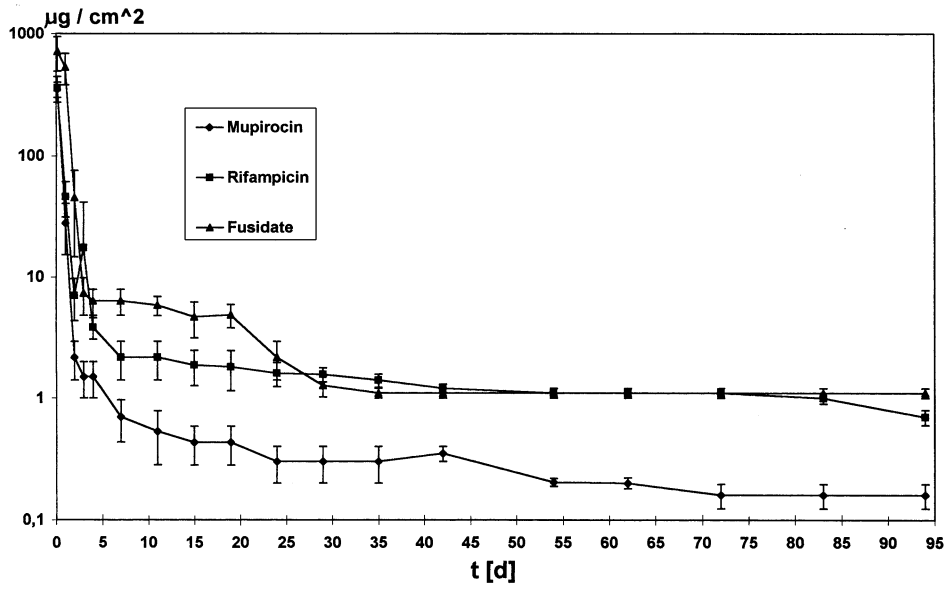


Fig. 3. Dynamic release of the antibiotic-loaded shunt reaching nearly 'perfect sink conditions' measured per day. Release pattern of each antimicrobial out of the silicone matrix is governed by Fickian diffusion processes after the 'burst effect'.

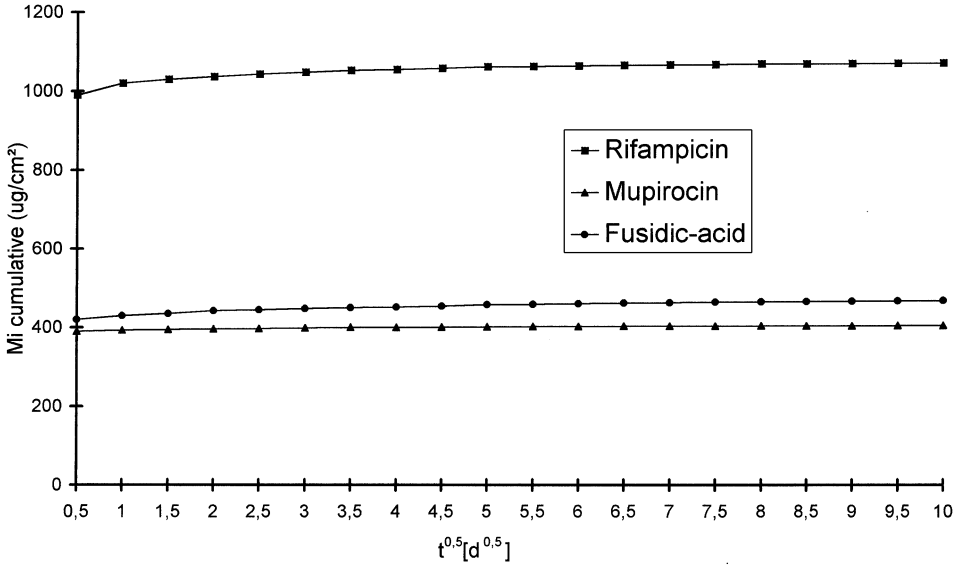


Fig. 4. Proportionality of cumulative release of rifampin to $t^{1/2}$ in the matrix-controlled period of the antibiotic-loaded silicone.

elastomeric matrix of the filled polydimethylsiloxane [32] and proportional to $\sqrt{t}$ (Fig. 4). Antimicrobial delivery was prolonged up to 100 days in the range 1–10 $\mu g\,cm^{-2}\,d^{-1}$.

The diffusion exponents calculated by Eq. (6) were in the range 2×10^{-8} to 1×10^{-9} $cm^2\,s^{-1}$. (Table 1).

Table 1
Diffusion exponents D ($cm^2\,sec^{-1}$) for rifampicin, mupirocin and fusidic acid in the polydimethyl–siloxan matrix

	Rifampicin	Fusidic acid	Mupirocin
D ($cm^2\,sec^{-1}$)	2×10^{-8}	3.1×10^{-8}	1.2×10^{-9}

3.3. Solubility parameter (cohesion energy)

A higher cohesion energy was calculated [1–4] for mupirocin ($\delta = 25.55\ MPa^{1/2}$) in comparison to rifampin ($\delta = 20.76\ MPa^{1/2}$) [36] and fusidic acid ($\delta = 15.71\ MPa^{1/2}$). The lower compatibility of the more hydrophilic mupirocin with the lipophilic silicone ($\delta = 19.38\ MPa^{1/2}$) [36] and the lower solubility in the

Table 2
Cohesion energies (solubility parameter δ) of mupirocin derived from different homomorphs and their partial parameter δ_d, δ_p, δ_H

Structure units	M ($g\,mol^{-1}$)	V ($cm^3\,mol^{-1}$)	F_d ($(J\,cm^3)^{0.5}\,mol^{-1}$)	F_p ($(J\,cm^3)^{0.5}\,mol^{-1}$)	δ_H^2 ($J\,mol^{-1}$)
3*CH_3	15	31.7	3*420	0	0
10*CH_2	14	16.6	10*270	0	0
8*>CH	13	−1	8*80	0	0
–O–	16	3.8	100	401	1467
3*OH	17	10.5	3*210	500	9770
cyclohexyl-		13.5	190	0	0
=C<	12	−5.7	45	70	143
CH=	131	2.4	223	70	143
O>	28	10	291	769	978
COOH	45	27.8	530	420	4900
COO–Ester	44	8.2	670	510	2550
Divided by mol volume:	Σ: 351.6	Σ: 7279	3740	39 493	
			Σ: 20.68	10.63	112.32
With Refs. [1–3]			$\delta_d = 20.68$	$\delta_p = 20.68$	$\delta_H = 10.59$
With Refs. [4]			$\delta = 25.55\ M\ Pa^{1/2}$		

Table 3
Adhesion assay of not modified and antimicrobial-loaded shunts under static and dynamic conditions (flow 2000 ml Müller-Hinton Bouillon/d)

(CFU/cm^2)	t (d)				
	1	2	3	4	14
Static assay					
Not modified	2×10^6	3.1×10^7	5×10^9	8×10^9	
Modified	1×10^5	8×10^3	<1	<1	
Dynamic assay					
Not modified	1×10^6	3×10^7	5×10^8	1×10^9	1×10^9
Modified	5×10^5	2×10^2	15	<1	<1

silicone matrix as well is a possible explanation for the lower diffusivity and the narrow loading.

3.4. Adhesion assay

Table 3 depicts the effectiveness of modified silicone devices containing high amounts of rifampicin (5% wt/wt), fusidate (2.5% wt/wt), mupirocin (2.5% wt/wt) in the stationary adhesion assay. Sterility was achieved after 24 h in contrast to the conventional shunts. In a second perfusion experiment (2.000 ml flow day^{-1}), sterility of antimicrobial shunts was achieved for 14 days, respectively (Table 3).

3.5. Biocompatibility testing

C_{3A}-Des-Arg-ELISA: In all samples a slight increase could be shown after 1 h incubation within a physiological range of 200–270 ng ml^{-1} C_{3A}. The coated shunt induced no significant activation of the complement system.

3.6. Hemolysis test

C_9-induced hemolysis of erythrocytes is directly proportional to the activity of the overall complement. The hemolysis rate for modified catheters as well as for unmodified silicone shunts ranged from 88 to 115% of the initial hemolysis rate of the serum. The modified shunts do not induce hemolysis.

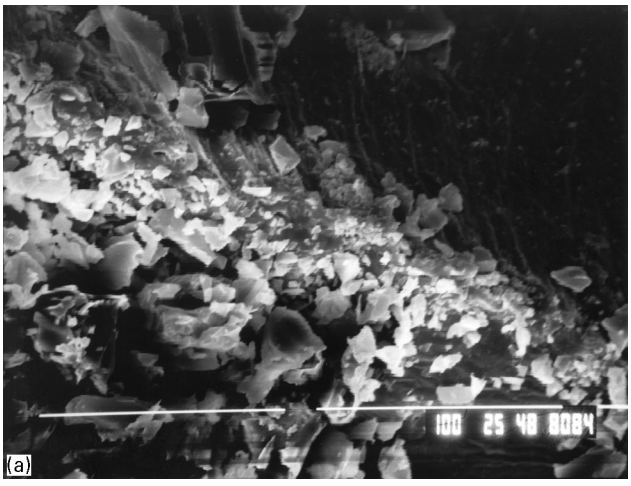
3.7. Scanning electron microscopy

In contrast to the mixture of drug crystals located on the surface (Fig. 5a), a monolithic, molecular dispersed distribution in the polymeric bulk could be demonstrated (Fig. 5b). After 24 h controlled delivery in the sink, the polymer surface appears smooth as the conventional silicone material (Fig. 5c).

4. Discussion

4.1. Choice of antimicrobial substances

Several criteria were taken into consideration to select the antimicrobials used in this study. The first was that they should be capable of molecular migration through cross-linked silicone elastomer. An index of this was their cohesion energies [32, 34, 35]. Similarity of cohesion energies is a prerequisite for incorporation of fine dispersed drugs into a polymeric matrix and for a high drug rate of diffusion out of the matrix during implantation [36]. Antimicrobials as aminoglycosides and ciprofloxacin



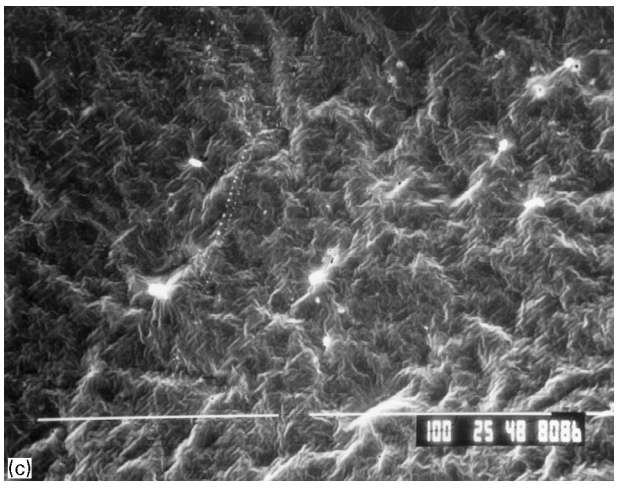


Fig. 5. (a) Scanning electron micrograph (SEM) of the drug-loaded silicone. Note the crystals of the antibiotics on the surface. (b) SEM of the drug-loaded silicone bulk. The antimicrobials are in a molecular dispersed state, the small nodes in the matrix typically represent radiopaque $BaSO_4$-particles. SEM of non-modified silicone catheters gave an identical bulk morphology (not shown). (c) SEM of the modified silicone catheter surface after one day immersion in phosphate buffer. The superifically located crystals are dissolved completely, the silicone surface is in the same morphological state as compared to the conventional silicone shunt.

were excluded being not compatible with the shunt material. The second criterion was that they should be active against most strains of staphylococci, enterobacteriaceae and candida. Neither clindamycin, nor β-lactam antibiotics or fluorochinolones as ciprofloxacin or sparfloxacin possess adequate activity against methicillin-resistant staphylococci, a major cause of catheter-associated infections. Rifampicin, highly compatible with polydimethylsilioxanes, offers advantages in treatment of staphylococcal abcesses [42], osteomyelitis [43, 44], foreign-body infection [45], endocarditis and disseminated infections [39, 46–47]. Most coagulase negative staphylococci isolated from colonized shunts are susceptible to rifampicin, and the majority are susceptible to mupirocin and/or fusidic acid. Enterobacteriaceae in general are susceptible to rifampicin and mupirocin has been shown to be active against *candida* in burns [49]. The third reason was that they should not have been known for a significant risk of hypersensitivity or toxicity when administered systemically in humans. Penicillin derivates, chlorhexidine and silver-sulfadiazine [31, 50, 51] were excluded for these reasons. The fourth criterion was that the antibiotics should be sufficiently stable to allow sterilization by γ-irridation or treatment with ethylenoxide. After γ-irridation, no drug degradation could be determined by HPLC (unpublished data). In general, antibiotics as β-lactams, aminoglycosides and gyrase inhibitors, used for therapy of bloodstream infections should be preserved for systemic application.

4.2. Controlled release pattern

The long period of slow release was chosen in order to ensure protection over the operative and immediate postoperative period. Controlled release from this matrix reservoir was typical for monolithically distributed drugs [41, 52] as shown previously for rifampicin [32]. Drug release through these intermolecular spaces between the macromolecules opened by relaxation processes is known to be governed by a Fickian diffusion mechanism with a square-root-of-time dependence at early times [37, 38] (Fig. 4). Properties such as molecular weight of the drugs were not the most influencing factors in controlling release from the silicone. Proportionality of the diffusants flux to molecular weight is in the range $\sqrt{[\mathrm{g\,mol^{-1}}]}$ and $1/\sqrt[3]{[\mathrm{g\,mol^{-1}}]}$. The rate-limiting step of diffusion was assumed to be the solubility of the antibiotics with the polymer [37, 38, 41, 52]. More than 50% of residual antibiotics were found after a three months release in the examined drug-polymer-alloy.

A large part of the burst effect could be removed simply by cleaning the catheter surface. However, a major part of catheter-associated infection in early times is due to contamination of skin bacteria from the host or physician during the insertion procedure. It might be assumed that most of the initially adherent bacteria are

detached and killed by the initial high delivery of the superficially located antibiotics.

4.3. Pharmacokinetical and pharmacodynamical considerations

The total concentration of antimicrobials in the modified shunts has been found to be orders of magnitudes less than a single therapeutic dose, and this is released slowly over several weeks. For this reason, major organ toxicity such as hepatic dysfunction is very unlikely. A further possible complication is the local accumulation of antimicrobials in the tissues immediately adjacent to the catheter with local toxicity or irritation. Previous animal toxicological studies have not shown any such local or systemic irritation or toxicity caused by rifampin [13]. Attempts to decrease the concentration of the most potent antimicrobial (rifampicin) led to a failure of protection for the target period [31]. Regarding the superior antistaphylococcal activity of rifampin to mupirocin and fusidic acid, most of the antimicrobial efficacy and the major part of the antimicrobial loading was performed with rifampin. The loading capacity with mupirocin (1.4% wt/wt) was limited by its lower solubility (higher hydrophilicity: $\delta = 25.55\ \mathrm{MPa}^{1/2}$, $\delta_p = 20.68\ \mathrm{MPa}^{1/2}$, $\delta_H = 10.59\ \mathrm{MPa}^{1/2}$) with the silicone matrix ($\delta = 19.38\ \mathrm{MPa}^{1/2}$).

4.4. Drug-dependent adverse reactions

Any coated catheter may elicit an allergic/immune response. The use of muscle relaxants in the ICU is believed to be the main source of the anaphylactoid reaction. Cross reactions between quarternary ammonium groups containing benzalkonium-coated catheters and sensitized patients should be considered. Patients sensitized to penicilin (IG_E mediated) can trigger anaphylactoid reactions (1%). The risk of severe circulation collapse is calculated to be 1 : 50 000. Chlorhexidine sensitization is a well-known phenomenon due to the frequent use of this disinfectant in urology. Following the growing number of anaphylactic reactions in Japan, the silver–sulfadiazine–chlorhexidine (SSC)-catheters were banned in 1997. Detergent agents such as TDMAC (tri-dodecyl-methyl-ammonium-chloride) and benzalkonium-chloride work by diffusing into cytoplasma membranes and have hemolytic activity. Silver is a highly biocompatible metal but free silver ion concentrations acting as bactericidal are toxic to human cell cultures [31]. We checked different concentrations of rifampin, mupirocin and fusidic acid up to 300 $\mu g\ ml^{-1}$ in a mouse-fibroblast agar layer test and observed no significant proliferation inhibition (unpublished data). The substances are unlikely to induce mutagenicity or sensibilization reactions. For use of this catheter modification as a long-term venous access (Hickman- or Broviac-catheter), the hemocompatiblity and device safety has to be evaluated more intensively in animal experiments.

4.5. Topical use of antimicrobial combinations—emergence of bacterial resistance?

Development of resistance in the clinical use of the modified shunt might become a problem. The internal hydrocephalus-shunt is not exposed continuously to skin flora, the external drainage is exposed to the skin. Resistance could develop at the entry site of all types of silicone catheters. Since mutations occur with a freqency of about 10^{-6} to 10^{-9}, one can predict that drug resistance is most likely to emerge with high bacteria inocula. In contrast, it is extremly rare to retrieve more than 10^4 cfu from infected catheters. The fear for inducing mycobacterial resistance to rifampin-coated shunts is unlikely to materialize. In case of topical delivery from a coated catheter to blood and subcutaneous tissue, maximum amounts of release can be calculated to be $<0.1\%$ as compared to systemic administration. Resistance pressure by antimicrobial doses with magnitudes of the order below sub-inhibitory concentrations is not very probable. Resistance transfer from staphylococci to mycobacteria has not been observed clinically. Attempts to develop bacterial mutations on gradient plates with RFM (rifampicin, fusidic-acid, mupirocin)-combination failed (results not shown), resistant strains could neither be determined in the checker-board experiment nor in the killing experiment, indicating the ability of the combination to prevent the development of resistance [40]. However, combination of rifampin with trimethoprim failed in several killing experiments due to the detection of resistant staphylococci (unpublished data). During a clinical trial with a rifampin–minocyclin-coated short-term central venous catheter, no resistant staphylococci could be determined [53], supporting our suggestion, that carefully developed antimicrobial combinations incorporated in such devices as CVCs may prevent occurrence of resistant bacteria.

4.6. Previous attemps of catheter modification

Various antibiotics were bonded to catheters using benzalkonium chloride or tridodecylmethylammonium chloride (TDMAC) [16–21, 45]. This method has the disadvantage of short duration of activity, not exceeding two days [22, 23], leading to clinical failure [21]. Benzalkonium chloride, TDMAC and chlorhexidine cannot be used in contact with sensitive nervous tissue because of their high toxicity and deleterious effects to cells [50, 51]. The incorporation of the disinfectant trichlorohydroxyphenylether (Irgasan DP 300, Ciba Geigy) mixed into thermoplastics has similar adverse effects [24].

Bayston et al. developed an impregnation method claiming prolonged activity of the processed shunts [10].

They chose very small amounts of antibiotics incorporated in superficial layers of the material. In an in vitro experiment, shunts with a discrete superficial antimicrobial coating did not withstand considerable microbial challenge [32]. Chlorhexidine–silver–sulfadiazine coating of central venous catheters has been considered as the most efficacious coating despite the high toxicity of the disinfectant. However, recent clinical study results of such coated catheters did not show significant differences between coated and noncoated catheters [55–59]. A minocycline–rifampin coating has been shown to be efficacious in vitro and in vivo [26, 53]. However, the TDMAC spacer-group for the antibiotics does not bind efficiently to hydrophobic polymers such as silicone because of its amphophilic nature (higher cohesion energy).

Previously we could show in a bacterial infection model that implantation of rifampin-loaded contaminated devices (9% Rifampin in silicone) in the cerebrospinal ventricles of rabbits withstand successfully bacterial colonization without any clinical sign of foreign-body infection [24, 32]. Further animal studies with this broad-spectrum of antimicrobial shunts are warranted for assessing device safety and efficacy.

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References

[1] Christensen GD. The sticky problem of staphylococcus epidermidis sepsis. Hosp Practice 1993;30:27–36.
[2] Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ. Bacterial biofilms in nature and disease. Ann Rev Microbiol 1987;41:435–64.
[3] Maki DG, Weise CE, Sarafini HW. A semiquantitative culture method for identifying intravenous catheter-related infection. N Engl J Med 1977;296:1305–9.
[4] McLean RJ, Nickel JC. Bacterial colonization behaviour: a new virulence strategy in urinary infections? Med Hyp 1991;36(3):269–72.
[5] Bayston R. Bacteriological examination of removed cerebrospinal fluid shunts. J Clin Path 1983;36:987.
[6] Fitzgerald R, Connelly B. An operative technique to reduce valve colonisation. Z. Kinderchir 1984;39(Suppl II):107–9.
[7] Haines SJ. Systematic antibiotic prophylaxis in neurological surgery. Neurosurg 1980;6:355–61.
[8] Tabara Z, Forrest DM. Colonisation of CSF shunts: preventive measures. Z Kinderchir 1982;37:156–8.
[9] Wald S, McLaurin RL. Cerebrospinal fluid antibiotic levels during treatment of shunt infections. J Neurosurg 1980;52:41–6.
[10] Bayston R. Prevention of hydrocephalus shunt catheter colonization in vitro by impregnation with antimicrobials. J Neurolog Neurosurg Psych 1989;52:605.
[11] Bach A, Böhrer H, Motsch J, Martin E, Geiss HK, Sonntag HG. Prevention of bacterial colonization of intravenous catheters by antiseptic impregnation of polyurethane polymers. J Antimicr Chem 1994;3:969–78.
[12] Dumitriu S, Popa MI, Haulacia I, Cringu A, Stratone A. Bioactive polymers 61. synthesis and characterization of some retard antibiotics. Colloid Polym Sci 1989;267:595–9.
[13] Jansen B, Kristinsson KG, Jansen S, Peters G, Pulverer G. In-vitro efficacy of a central venous catheter complexed with iodine to prevent bacterial colonization. J Antimicr Chem 1992;30: 135–9.
[14] Jansen B, Jansen S, Peters G, Pulverer G. In-vitro efficacy of a central venous catheter (Hydrocath) loaded with teicoplanin to prevent bacterial colonization. J Hosp Inf 1992;22:93–107.
[15] Jansen B, Rinck M, Wolbring P, Strohmeier A, Jahns T. In vitro evaluation of the antimicrobial efficacy and biocompatibility of a silver-coated central venous catheter. J Biomat Appl 1994; 9:55–67.
[16] Kamal GD, Pfaller MA, Rempe LE, Jebson RJR. Reduced intravascular catheter infection by antibiotic bonding. A prospective, randomized, controlled trial. JAMA 1991;265:2364–8.
[17] Sherertz RJ, Carruth WA, Hampton AA, Byron MP, Solomon DD. Efficacy of antibiotic-coated catheters in preventing subcutaneous *staphylococcus aureus* infection in rabbits. Inf Dis 1993;167:98–106.
[18] Sherertz RJ, Forman DM, Solomon DD. Efficacy of dicloxacillin-coated polyurethane catheters in preventing subcutaneous *Staphylococcus aureus* infection in mice. Antimicr Ag Chem 1989; 1174–8.
[19] Tebbs SE, Elliott TSJ. A novel antimicrobial central venous catheter impregnated with benzalkonium chloride. J Antimicr Chem 1993;31:261–71.
[20] Tebbs, SE, Elliott TSJ. Modification of central venous catheter polymers to prevent in vitro microbial colonisation. Eur J Clin Microbiol Infect Dis 1994;13:111–7.
[21] Trooskin SZ, Harvey RA, Lennard TW, Greco RS. Failure of demonstrated clinical efficacy of antibiotic-bonded continuous ambulatory peritoneal dialysis (CAPD) catheters. Perit Dial Int 1990;10(1):57–9.
[22] Mermel LA, Stolz SM, Maki DG. Surface antimicrobial activity of heparin-bonded and antiseptic-impregnated vascular catheters. J Inf Dis 1993;167:920–4.
[23] Sampath LA, Chowdhury N, Caraos L, Modak SM. Infection resistance of surface modified catheters with either short-lived or prolonged activity. J Hosp Inf 1995;30:201–10.
[24] Hampl J, Schierholz J, Jansen B, Aschoff A. In vitro and in vivo efficacy of rifampin loaded silicone catheter in prevention of CSF-shunt infections. Acta Neurochirurg 1995;133:147–52.
[25] Olanoff LS, Anderson JM, Jones RD. Sustained release of gentamicin from prosthetic heart valves. Trans Am Soc Art Intern Organs 1979;25:334–8.
[26] Raad I, Hachem, Daroniche R, Hachem R, Bodey GP. The broad spectrum activity and efficacy of catheters coated with minocycline and rifampicin. J Inf Dis 1996;173:418–24.
[27] Schierholz JM, Steinhauser H, Rump AFE, Pulverer G. Controlled release of antibiotics from polyurethanes: morphological and structural features. Biomaterials 1997;18(12):839–44.
[28] Schierholz JM, Jansen B, Steinhauser H, Peters G, Schuhmacher-Perdreau F, Pulverer G. Drug release from antibiotic-containing polyurethanes. New Polymeric Mater 1991; 3:61–72.
[29] Schierholz JM, Rump AFE, Pulverer G. Drug delivery concepts for efficacious prevention of foreign-body infections. Zbl Bakt 1996;284:390–401.
[30] Solomon DD, Sherertz RJ. Antibiotic releasing polymers. J Control Release 1987:343–52.
[31] Hollinger AM. Toxicological aspects of topical silver pharmaceuticals. Crit Rev Tox 1996;26(2):255–60.
[32] Schierholz JM, Jansen B, Jaenicke L, Pulverer G. In vitro efficacy of an antibiotic releasing silicone ventricle catheter to prevent shunt infection. Biomaterials 1994;15:996–1000.

[33] Hill RLR, Casewell MW. Reduction in the colonization of central venous cannulae by mupirocin. J Hosp Inf 1991;19(Suppl B): 47–57.

[34] Bustamate PA, Parera, Selles E. Applications of the multicomponent parameter in pharmaceutical liquid formulation. An R Acad Farm 1983;49:221.

[35] Martin A, Mauger J. The curious solubility of phenobarbital: how to use the solubility parameters. Am J Pharm 1988;52:68.

[36] Schierholz JM. Physico-chemical properties of a rifampicin-releasing polydimethyl-siloxane shunt. Biomat 1997;18(8): 635–41.

[37] Peppas NA, Lustig SR. The role of crosslinks, entanglements and relaxations of the macromolecular carrier in the diffusional release of biological active materials. Ann New York Acad Sci 1984;5:26–41.

[38] Ritger PL, Peppas NA. A simple equation for description of solute release I. Fickian and non-Fickian release from swellable devices in the form of slabs, spheres and cylinders or discs. J Contr Rel 1987;5:23–36.

[39] Bayer AS, Morrison JO. Disparity between timed-kill and chekerboard methods for determination of in vitro bacterial interactions of vancomycin plus rifampicin versus methicillin-susceptible and -resistent *Staphylococcus aureus*. Antimicrob Ag Chem 1988; 26:220–3.

[40] Carlton BC, Brown B. Clinical laboratory testing for antimicrobial resistence. In: Manual of methods for general bacteriology, New York: American Society of Microbiology 1981:229–40.

[41] Chien YW, Lambert HJ. Controlled drug release from polymeric devices II: Differentiation between partition-controlled and matrix-controlled drug release mechanisms. J Pharm Sci 1974;63 (4):515–9.

[42] Mandell GL, Vest TK. Killing of intraleukocytic *Staphylococcus aureus* by rifampicin: in vitro and in vivo studies. J Infect Dis 1972;125:486–90.

[43] Norden CW. Experimental chronic staphylococcal osteomyelitis in rabbits: treatment with rifampicin alone and in combination with other antimicrobial agents. Rev Infect Dis 1983;5(Suppl. 3): 491–4.

[44] Norden CW, Fierer J, Bryant RE. The Chronic Staphylococcal Osteomyelitis Study Group. Chronic staphylococcal osteomyelitis: treatment with regimes containing rifampicin. Rev Infect Dis 1983;5(Suppl 3):495–501.

[45] Tshefu K, Zimmerli W, Waldvogel FA. Short-term administration of rifampicin in the prevention or eradication of infection due to foreign bodies. Rev Infect Dis 1983;5(Suppl 3):474–80.

[46] Karchmer AW, Archer GL, Dismukes WE. Rifampicin treatment of prosthetic valve endocarditis due to Staphylococcus epidermidis. Rev Infect Dis 1983;5(Suppl 3):543–8.

[47] Kobasa WD, Kaye KL, Shapiro T, Kaye D. Therapy for experimental endocarditis due to *Staphylococcus epidermidis*. Rev Infect Dis 1983;5(Suppl 3):533–7.

[48] Zak O, Scheld WM, Sande MA. Rifampicin in experimental endocarditis due to *Staphylococcus aureus* in rabbits. Rev Infect Dis 1983;5(Suppl 3):481–90.

[49] Vizcaino-Alcaide MJ, Herruzo-Cabrera R, Rey-Calero J. Efficacy of a broad-spectrum antibiotic (mupirocin) in and in vitro model of infected skin. Burns 1993;19(5):392–5.

[50] Gabler WL, Bullock WW, Creamer HR. Chlorhexidine: not a drug for all reasons. JODA 1987;56(4):24–5.

[51] Reynolds JEF. Martindale—The extra pharmacopoeia, 30th ed., Vol. 1412. London: Pharmaceutical Press, 1993:788–90.

[52] Roseman TR. Release of steroids from a silicone polymer. J Pharm Sci 1979;61(1):46–50.

[53] Raad I, Darouiche R, Dupuis J, Abi-Sais D, Gabrielli A, Hachem R, Wall M, Harris R. Jones J, Buzaid A, Robertson C, Shenaq S, Curling P, Burke T, Ericsson C. Central venous catheters coated with minocycline and rifampin for the prevention of catheter-related colonization and bloodstream infections. Ann Int Med 1998;15:1–12.

[54] Kingston D, Seal DV, Hill ID. Self-disinfection plastics for intravenous catheters and prosthetic inserts. J Hyg 1986;96:185–98.

[55] Ciresi DL, Albrecht RM, Volkers PA, Scholten DJ. Failure of antiseptic bonding to prevent central venous catheter-related infection and sepsis. Am Surg 1996;62:641–5.

[56] Pemberton LB, Ross V, Cuddy P, Kremer H, Fessler T, McGurh E. No difference in catheter sepsis between standard and antiseptic central venous catheters. Arch Surg 1996;272/23:1819–20.

[57] Logghe C, Van Ossel Ch, D'Hoore W, Ezzedine H, Wauters G, Haxhe JJ. Evaluation of chlorhexidine and silver-sulfadiazine impregnated cenetral venous catheters for the prevention of bloodstream infection in leukaemic patients: a randomized controlled trial. J Hosp Inf 1997;37:145–56.

[58] Heard SO, Wagle M, Vijayakumar E, Doern G. The influence of central venous catheters coated with chlorhexidine/silver sulfadiazine on catheter-related infections. Crit Care Med 1997:25(1).

[59] Ellis ME, Rhydderch D, Zwaan, F Guy ML, Baillie. F. High incidence of line-related infection and mechanical failure of an antiseptic impregnated central venous catheter in highly immunocompromised patients. Scand J Infect Dis 1996;28:91–3.