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# *In vitro* Retention of Antimicrobial Activity of Ciprofloxacin-incorporated Central Venous Catheters

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*In vitro* ciprofloxacin (CFX)-release study and bioassay using microorganisms were performed to estimate the retention of the antimicrobial activity of the CFX-incorporated central venous catheters (CFX-CVCs). The release experiments were carried out under the optional CFX-release conditions to mimic the *in vivo* environment. The release of CFX experienced an initial burst followed by a slow and steady matrix-diffusion controlled release. The 1.0CP (polyurethane catheter containing 1.0% (w/w) of CFX) under dynamic condition showed a near zero-order CFX release profile, which is beneficial for the long-term antimicrobial activity. The modified Kirby-Bauer method was performed employing *S. aureus* and *E. coli* to evaluate the retention of antimicrobial activity of the catheters retrieved from the release experiments. The 1.0CP showed the long-term antimicrobial activity (≥ 21 days) against both *S. aureus* and *E. coli*. These results indicate that 1.0CP is useful as a long-term indwelling CVC.

**Key Words:** Central venous catheters (CVCs), Ciprofloxacin (CFX), Infections, Release profile, Long-term antimicrobial activity

## INTRODUCTION

The use of central venous catheters (CVCs) often suffers from serious infectious complications, even though CVCs are essential for the clinical management of the critically and chronically ill patients (Darouiche et al., 1999; Hannan et al., 1999). A number of technical measures and strategies have been investigated for preventing the CVC-related infections, including the use of maximal sterile-barrier precautions during catheter insertion (McGee and Gould, 2003), catheter coating with various antimicrobials (Elliott, 1999), catheter dressing with antimicrobial-impregnated sponges (Cicalini et al., 2004), and disinfection of catheter hubs (Mermel et al., 2001). Although some of the attempts described above have met with a partial success in preventing such infections, some important problems still remain unresolved. The emergence of antimicrobial resistant microorganisms and difficulty in maintaining the antimicrobial activity during the whole indwelling period of CVCs are considered as the most crucial problems. Particularly, the latter (retention of antimicrobial activity) is one of the most important criteria in determining the indwelling time of antimicrobial-impregnated CVCs, because cancer patients or other seriously ill patients frequently require prolonged use of CVCs (Raad and Hanna, 1999).

British standard (BS EN ISO 10993-1: 2003) formulated by British Standards Institute describes the category of medical devices based on the nature and duration of their contact with human body. According to this standard, CVCs are classified as one of the external communicating medical devices that belong to the category called 'prolonged exposure (duration of body contact; ≥24 hours ~ 30 days)'. Also, they are sub-classified as short-term CVCs (indwelling time; <10 days) and long-term CVCs (indwelling time; >10 days) (Cicalini et al., 2004). This indicates that the efficacy of antimicrobial CVCs for use as long-term CVCs should be maintained for at least 10 days or more.

In a previous study, we have already confirmed the antimicrobial activity of the catheters containing a synthetic fluoroquinolone antimicrobial agent (ciprofloxacin, CFX)

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against a majority of microorganisms causing catheter-related infections (Jeon and Kim, 2004). These catheters were manufactured by a matrix loading method (the direct incorporation of CFX into the polyurethane matrix). In this regard, it is expected that the CFX-incorporated polyurethane catheters (CPs) may be effective in preventing the infections associated with long-term indwelling CVCs. Therefore, we investigated the retention of antimicrobial activity of CPs as CVCs. *In vitro* release studies of CFX from the catheter under static and dynamic aqueous conditions were performed to predict the influence of CFX release environments on the retention of their antimicrobial activities. A bioassay was also performed to evaluate the retention of their antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.

## MATERIALS AND METHODS

### 1. Central venous catheters

Central venous catheters containing ciprofloxacin (CFX-CVCs) were manufactured by Sewoonmedical, Co. Ltd. (Korea). They were prepared by mixing the catheter raw material (polyurethane: PU, Tecoflex® EG-93A-B20, Noveon, USA) with antimicrobial agent (ciprofloxacin: CFX, Sanivar, Hongkong) followed by extrusion molding and sterilizing with ethylene oxide gas. Three different amounts of CFX were added to PU, i.e., 0.5%, 1.0% and 1.5% (w/w). These catheters were conveniently designated as 0.5CP, 1.0CP and 1.5CP, respectively. Standard CVC (CFX-free PU catheter) was used as a control sample. All catheters used in the present study were single-lumen CVCs (7.0 Fr., 20 cm long).

### 2. *In vitro* release of CFX from CFX-CVCs

*In vitro* release studies of CFX from CFX-CVCs (0.5CP, 1.0CP and 1.5CP) were performed by exposing the catheter segments to the release medium (10 mM phosphate-buffered saline, PBS, pH 7.2) under both static and dynamic conditions. Nomenclatures for CFX-CVCs under two different CFX-release conditions are listed in Table 1. The CVCs were aseptically sectioned into 1 cm segments in a biological safety cabinet using sterile forceps and scissors. The catheter segments were placed in a glass vial containing the release medium (3 catheter segments/1 ml of the release medium) and then the vial was tightly sealed to prevent evaporation of the release medium. The vial was wrapped with aluminum foil to prevent the plausible degradation of CFX as a result of exposure to light during the release experiments (Kwok et al., 1999). The release of CFX under dynamic condition was realized by placing the vials in a shaking incubator (SI-300R, Jeio Tech, Korea) operated at 120 rpm, while the static condition was provided by performing the release experiments in a static incubator (J-IB02, Jisico Co., Korea). All the release experiments were carried out at 37℃ for 21 days. To better mimic the *in vivo* situation where the catheters are in contact with a continuously renewing biological fluid, the release medium was continually removed and replaced with a fresh medium (the refreshments of the release medium were realized at 3 and 6 h after the start of the release experiment and then at 24 h intervals). At the predetermined time intervals, the release medium was taken for the quantitative measurement of the CFX content in the medium using a photometric method. The absorbance of the medium was measured by using an UV/VIS spectrophotometer (UV-1700, Shimadzu, Japan) at wavelength of 272 nm. The *in vitro* release profiles of CFX from CFX-CVCs were represented by:

(1) Cumulative amount (μg/cm) of CFX released from the catheter as a function of release time under static and dynamic conditions

(2) Cumulative release rate (%) of CFX as a function of release time under static and dynamic conditions

The ratio of burst effect also was calculated from the

**Table 1:** Nomenclature of ciprofloxacin-incorporated central venous catheters (CFX-CVCs)

| Nomenclature | CFX content (%, W/W) | CFX-release condition |
|---|---|---|
| 0.5CP-S | 0.5 | Static condition |
| 0.5CP-D | 0.5 | Dynamic condition |
| 1.0CP-S | 1.0 | Static condition |
| 1.0CP-D | 1.0 | Dynamic condition |
| 1.5CP-S | 1.5 | Static condition |
| 1.5CP-D | 1.5 | Dynamic condition |

# Abbreviations; CP, ciprofloxacin-incorporated polyurethane catheter; -S, static condition; -D, dynamic condition; CFX, ciprofloxacin

above CFX-release data to investigate the extent of the burst effect during the initial release phase in CFX-CVCs. The ratio of burst effect ($R_b$) was defined as $R_b$=A/B, where A is the cumulative amount of CFX released during the initial 24 hours, and B is that released from 48 to 120 hours after the inauguration of the release experiment (Schierholz et al., 1997). $R_b$ can also be regarded as the ratio of the initial burst release of CFX locating at the region near the surface of the catheter (A) to the matrix-diffusion-controlled release of CFX (B).

The catheter segments were also collected from the release medium at the predetermined time intervals, and then were completely dried and stored in the dark at -20℃ until the antimicrobial assay.

### 3. *In vitro* retention of antimicrobial activity

The retention of antibacterial activity of CFX-CVCs was assessed by the modified Kirby-Bauer technique (Schierholz et al., 2000; Basetti et al., 2001). *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used as test microorganisms. The strains were grown to log-phase in either brain heart infusion broth (BHIB) or trypticase soy broth (TSB). The final concentration of the log phase cells was adjusted to be $1\times10^8$ CFU/ml by diluting with Mueller-Hinton broth (MHB). The bacterial suspension was spread on Mueller-Hinton agar (MHA) plates using a cotton swab. The catheter segments retrieved from the release medium at 1, 3, 7, 14 and 21 days for the antimicrobial assays were placed vertically in the MHA plate. After the incubation for 24 h at 37℃, the growth inhibition zones created by the catheter segments were measured.

### 4. Statistical analysis

All data were expressed as the mean ± standard deviation (SD). The difference between two study groups was evaluated using Student's t-test and statistical significance was defined as a $P<0.05$.

## RESULTS AND DISCUSSION

*In vitro* release study of antimicrobials has been widely used to evaluate the long-term antimicrobial efficacy of a polymer matrix containing antimicrobials (Golomb and Shpigelman, 1991; Price et al., 1996; Schierholz et al., 2000; Kohnen et al., 2003). It is possible to predict the retention period of antimicrobial activity of the polymer matrix by measuring the amount of the antimicrobials released from the polymer matrix over time. However, exact prediction of *in vivo* release profiles of the antimicrobials by way of their *in vitro* release study is not easy because the *in vivo* release environment is different from the *in vitro* counterpart. Moreover, various *in vivo* physicochemical factors affect the release behavior of the antimicrobials. A simplified *in vivo* release condition, so called the 'perfect skin condition', has been conventionally used to settle these problems for the polymer matrix containing antimicrobials (Golomb and Shpigelman, 1991; Sezaki et al., 1998). This is a hypothetical release condition that mimics the release of the antimicrobials into human body (as a 'sink') from the polymer matrix (Sezaki et al., 1998). The perfect skin condition has also been used as a release condition to investigate the long-term-release profiles of antimicrobials from catheters bearing antimicrobials (Kohnen et al., 2003; Piozzi et al., 2004).

### 1. *In vitro* release of ciprofloxacin (CFX) from CFX-CVCs

Release of CFX from CFX-CVCs was investigated to predict the sustainability of their antimicrobial activity. The perfect sink condition was maintained by continually changing the release medium (10 mM PBS) at 37℃ during the whole experimental period. The perfect sink condition was chosen for mimicry of the *in vivo* situation where CFX-CVCs are in contact with a continuously renewing biological fluid. PBS is widely used as a diluent in the serological assays (Harlow et al., 1988) as well as the release medium in the studies of antimicrobial medical devices (Price et al., 1996; Schierholz et al., 2000) since PBS is an isotonic to the human body fluids. An experimental temperature also was adjusted to human core body temperature since the human body temperature is normally regulated between 35.8 and 37.2℃ (Wilson et al., 1991). That is why most of antimicrobial catheter-related experiments have been carried out at 37℃ (Smith et al., 1996; Kohnen et al., 2003).

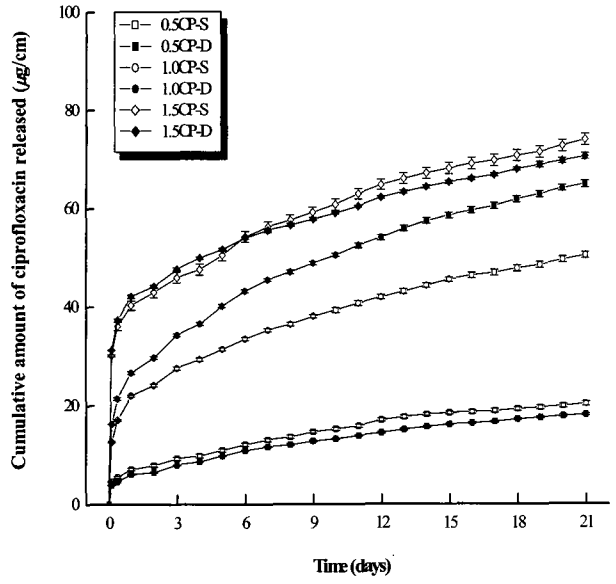


**Fig. 1.** Cumulative amount of ciprofloxacin released from the polyurethane catheters under static (ST) and dynamic conditions (DY).

The catheters also were exposed to the aqueous environments with two different physical stimuli (static or dynamic conditions) to examine the influence of the release environment on the retention of their antimicrobial activity. The release of CFX from the catheter *in vivo* may be influenced by the variation in the physical factors such as central venous pressure (CVP) or blood flow velocity when the CFX-CVCs are placed in the central veins for a long period of time. Considering this possibility, both static and dynamic conditions were optionally chosen to give a relatively large interval of physical stimulation, although the present experimental conditions differ from the actual *in vivo* environment. In this study, the sensitivity of CFX-CVCs to the physical stimuli on the release of CFX was also investigated. The amount of CFX released from CFX-CVCs was determined by using the photometric method as previously described.

According to Fig. 1, the release of CFX from the catheters depended on the initial amount of the incorporated CFX. Relatively small amount of CFX (20.3±0.44 μg/cm) was released from 0.5CP-S under the static condition, while 50.4±0.63 μg/cm of CFX and 73.9±1.20 μg/cm of CFX were released from 1.0CP-S and 1.5CP-S respectively for the same period of time. The amount of CFX released from 1.5CP-D was 70.5±0.61 μg/cm under the dynamic condition for 21 days, while 64.8±0.66 μg/cm and 18.1±0.22 μg/cm of CFX were released from 1.0CP-D and 0.5CP-D, respectively. It is to be noted that the CFX-release behavior of 1.0CP-D was distinctly different from that of 1.0CP-S, indicating that the CFX-release from 1.0CPs (1.0CP-S and 1.0CP-D) depended significantly on the release environments. For 1.0CPs, the difference between the cumulative amount of the released CFX under dynamic condition and that under static condition became larger and larger as the release time increased. The difference for 1.0CPs was much larger than that of either 0.5CPs (1.0CP-S and 1.0CP-D) or 1.5CPs (1.0CP-S and 1.0CP-D).

Generally, it is difficult to evenly distribute the hydrophilic drugs into hydrophobic polymer matrix because they tend to form a drug clumps (Choi et al., 2001). Also, the formation of drug clumps increases with the increase of the initial drug loading ratio (Schierholz et al., 1997). These polymer-drug combinations with low compatibility tend to show the very slow release rates over time after the initial burst release of drugs from the polymer matrix, which is unfavorable for the long-term antimicrobial activity. The effective and sustained release of drugs from the polymer matrix is needed to resolve these problems and it can be achieved by a uniform distribution of drugs in the polymer matrix and appropriate initial drug loading amount.

As mentioned before, the CVC is one of the external communicating medical devices, part of which is usually exposed to the intravenous environment. The sensitivity of CFX-CVC to the contacting fluid flow is an important factor in predicting the long-term antimicrobial activity of the catheter, because the prevention of intravenous bloodstream infections is crucially important for prolonged use of CVC. Continuous release of sufficient amount of CFX under dynamic condition is prerequisite for an effective long-term antimicrobial activity of CVC. In this respect, the larger amount of CFX released from 1.0CPs under dynamic condition rather than under static one is beneficial for the long-term antimicrobial activity. However, the faster exhaustion of the CFX loaded in the catheter due to the larger amount of CFX released in the initial stage may reduce the sustainability of the antimicrobial activity of 1.0CPs. In order to clarify whether the 1.0CFX-PCs main-

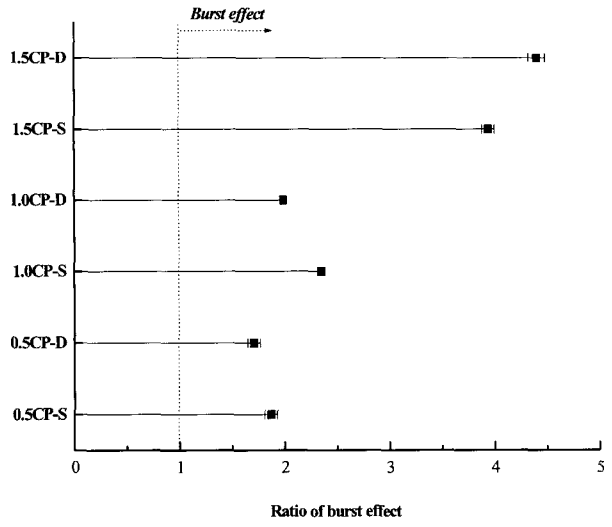


**Fig. 2.** The ratio of burst effect of the polyurethane catheters loaded with different amounts of ciprofloxacin.

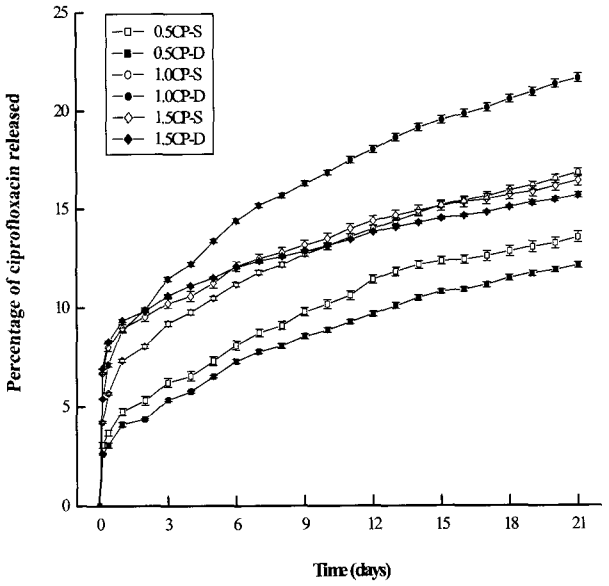


**Fig. 3.** *In vitro* release amount of ciprofloxacin expressed in terms of the percentage with respect to the initial loading of ciprofloxacin.

tain the antimicrobial activity longer than the other catheters (0.5CPs and 1.5CPs), the followings were additionally analyzed:

(1) The extent of the burst during the initial release phase

(2) The residual amount of CFX in the catheter after 21 days of the release experiment

(3) Time dependence of the release behavior of CFX

A significant burst effect was observed in all the CFX-CVCs under both static and dynamic conditions (Fig. 2). The ratio of burst effect ($R_b$) of 1.5CP-D was remarkably higher than that of the other catheters. $R_b$ of 1.5CP-D was 4.4±0.08, while that of 1.5CP-S was 3.9±0.06. In contrast, $R_b$ of 1.0CP-D was lower than 1.0CP-S (2.0±0.02 vs. 2.4 ±0.00, $P$<0.0001). 0.5CP-D also exhibited lower $R_b$ than 0.5CP-S (1.7±0.06 vs. 1.9±0.06, $P$=0.03602). Therefore, it can be said that the burst effect of CFX-CVCs becomes more pronounced with increase in the CFX loading. Schierholz et al. (1997) also demonstrated that the burst effect during the initial *in vitro* release period in polyurethane discs containing antimicrobials was more prominent as the loading of the antimicrobials increased. Examination by a scanning electron microscope (SEM) revealed that, at high contents of the antimicrobials, it was dispersed in the form of crystals, small granules and clumps in the polyurethane matrix. They argued that the weak compatibility between the polymeric matrix and the antimicrobials was responsible for the burst effect. Although the degree of the CFX distribution in the CFX-CVCs could not be clearly assessed by the SEM observations, the values of $R_b \geq 1$ obtained from the CFX-release experiments indicate the inhomogeneous distribution of CFX in the polyurethane catheters.

Fig. 3 demonstrates the amount of CFX released from the catheter in terms of percentage of the initially loaded amount of CFX. According to Table 2 and Fig. 3, the percentage of CFX released is nearly independent on the initial amount of incorporated CFX. The polymeric matrices containing antimicrobials which are manufactured by the matrix-loading method have conventionally two significant release steps of antimicrobials. The antimicrobials releases out fast in the initial stage due to the fraction locating at the region near the surface of the polymer matrix, and then the matrix-diffusion controlled slow and steady release follows afterwards (Schierholz et al., 1997). The two step release pattern was observed in 1.5CPs more remarkably than in the other catheters. These results disclose that a long-term antimicrobial activity of 1.5CPs can not be assured even though the amount of CFX released for 21 days was less than 16% of the initially loaded CFX (Fig. 3 and Table 2),

**Table 2.** Effect of increasing ciprofloxacin loadings on the ciprofloxacin release rates

| Time (days) | Percentage of ciprofloxacin released with respect to the initial loading of ciprofloxacin | | | | | |
|---|---|---|---|---|---|---|
| | Under static condition | | | Under dynamic condition | | |
| | 0.5CP-S | 1.0CP-S | 1.5CP-S | 0.5CP-D | 1.0CP-D | 1.5CP-D |
| 1 | 4.8±0.18 | 7.3±0.07 | 9.0±0.21 | 4.1±0.10 | 8.9±0.09 | 9.4±0.08 |
| 7 | 8.7±0.20 | 11.8±0.11 | 12.5±0.24 | 7.8±0.10 | 15.2±0.10 | 12.4±0.05 |
| 14 | 12.1±0.21 | 14.8±0.16 | 14.9±0.26 | 10.5±0.14 | 19.1±0.19 | 14.3±0.08 |
| 21 | 13.5±0.29 | 16.8±0.21 | 16.4±0.27 | 12.1±0.15 | 21.6±0.22 | 15.7±0.14 |

# Abbreviations; See Table 1.

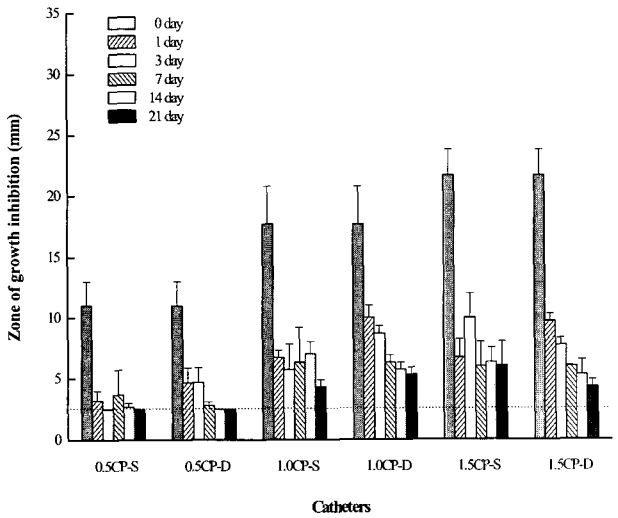


**Fig. 4.** *In vitro* retention of antimicrobial activity of the polyurethane catheters against *S. aureus* as expressed by the growth inhibition zone. The horizontal dotted line corresponds to the diameter of the catheter.

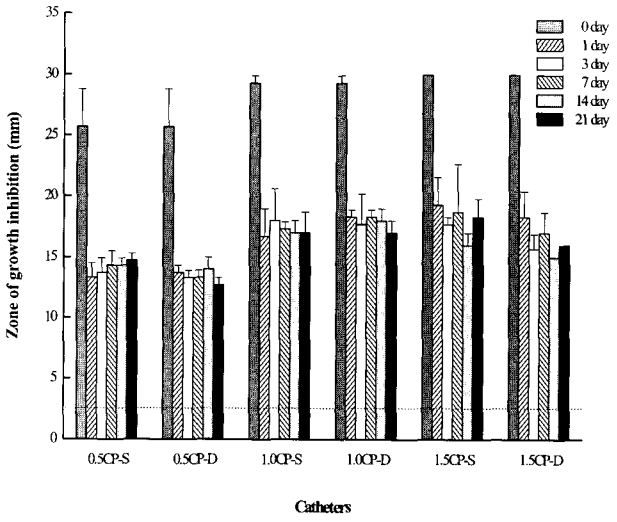


**Fig. 5.** *In vitro* retention of antimicrobial activity of the polyurethane catheters against *E. coli* as expressed by the growth inhibition zone. The horizontal dotted line corresponds to the diameter of the catheter.

because the released amount of CFX thereafter might not be sufficient to express an effective antimicrobial activity.

The percentage of CFX released was the highest for 1.0 CP-D among the tested catheters. Under dynamic condition, 21.6% of the initially loaded CFX was released from 1.0 CFX-PC within 21 days. A fast exhaustion of CFX may be undesirable for the long-term antimicrobial efficacy of the catheter. However, 1.0CP-D always showed steeper slope in the plot of the percentage of CFX released as a function of time than the other catheters during the whole release experiment. Moreover it showed a near zero-order release profile, indicating that the most effective release of CFX into the release medium was achieved during the whole experimental period. The zero-order release profile is more suitable for a long-lasting antimicrobial efficacy than higher-order release profile, because a continuous release rate is required to keep the concentration of antimicrobials at constant level in the release medium (Sezaki et al., 1998). However, the zero-order release profiles are limitedly observed only in the polymeric matrices with a specific release rate-controlling system (Sezaki et al., 1998).

### 2. *In vitro* retention of antimicrobial activity

The above in vitro release study of CFX predicts that 1.0CP-D provides a long-term antimicrobial activity. The modified Kirby-Bauer method was employed to assure the retention of antimicrobial activity of the catheter. The modified Kirby-Bauer technique is widely used to assess the antimicrobial spectrum, to determine the retention of antimicrobial activity and to predict the clinical efficacy of the catheters with antimicrobial activity (Gaonkar and Modak, 2003). The bioassay measures the microbial growth inhi-

bition zone, because the growth inhibition zone formed around the catheter containing antimicrobials reflects the activity of the antimicrobials (Piozzi et al., 2004). The catheter segments retrieved from the release medium (10 mM PBS) at 1, 3, 5, 7, 14 and 21 days were subjected to the bioassay.

Fig. 4 and Fig. 5 demonstrate the growth inhibition zone diameter of CFX-CVCs against *S. aureus* and *E. coli*, respectively. The control sample did not show any growth inhibition zone, saying that the polymer matrix, residual organic solvents and other additives used in the catheter manufacturing process were inert to the antimicrobial activity. The antimicrobial activity of CFX-CVCs against *S. aureus* was remarkably reduced after 1 day of the release and the growth inhibition zone diameter was decreased by 44~71% due to the burst release of CFX from the catheter in the early stage. 0.5CP-S already lost its antimicrobial activity against *S. aureus* after 1 day of the release and 0.5CP-D showed no zone of growth inhibition after 7 days of the release. 1.0CPs and 1.5CPs had a relatively longer retention period of the antimicrobial activity than 0.5CPs, and 1.0 CP-S and 1.0CP-D manifested respectively 4.3±0.6 and 5.3±0.6 mm of the growth inhibition zone even after 21 days of the release. The reduction of growth inhibition zone diameter over time is ascribed to the gradual exhaustion of CFX.

*E. coli* was more sensitive to the antimicrobials than *S. aureus*, and the growth inhibition zone diameter was much larger. Virgin CFX-CVCs formed 26~30 mm of the growth inhibition zone against *E. coli*, while 11~22 mm of the growth inhibition zone was formed when *S. aureus* was tested instead of *E. coli*. Moreover, all the catheters including 0.5CPs still showed a considerable antimicrobial activity against *E. coli* even after 21 days of the release. These results are not unexpected, because MIC value of CFX against *E. coli* (0.0075 μg/ml) is much smaller than that of *S. aureus* (0.25 μg/ml).

1.0CPs possessed almost the same level of the antimicrobial activity as 1.5CPs against both *S. aureus* and *E. coli*, indicating that the incorporation of 1.0% (w/w) of CFX into the polyurethane matrix was sufficient for the long-term antimicrobial activity. Loading of less amount of CFX to the catheter is beneficial, because of the saving of the cost-intensive CFX, the prevention of CFX overuse in the initial stage of the intake and the mitigation of the reduction in the mechanical properties provoked by the CFX loading (Gorman and Jones, 2002).

Long-term retention of antimicrobial activity of the CVCs containing antimicrobials is an important factor in the treatment of the patients who require prolonged use of CVCs. The 1.0CP-D showed not only a near zero-order CFX release profile but also the long-term antimicrobial activity ($\geq$ 21 days) against both *S. aureus* and *E. coli*. Since 1.0CPs possessed almost the same level of the antimicrobial activity as 1.5CPs as well as the sustained antimicrobial activities, 1.0CP can be said to be an effective catheter in preventing the microbial infections associated with long-term indwelling CVC.

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