Vancomycin Elution Kinetics from Porous Tantalum Metal

Pierre Sautet, Thibaut Mékidèche, Romain Guilhaumou, Matthew Abdel, Jean-Noël Argenson, Sebastien Parratte, Matthieu Ollivier

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Research Article

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Authors :

Pierre Sautet [1,4], Thibaut Mékidèche [2], Romain Guilhaumou [2], Matthew P. Abdel [3], Jean-Noël Argenson [1,4], Sébastien Parratte [1,4] , Matthieu Ollivier [1,4]

1: Institut for Locomotion, Center for arthritis surgery, Sainte-Marguerite hospital, Aix-Marseille University.

2: Departement of Clinical Pharmacology, Timone hospital, Aix-Marseille University.

3: Departement of orthopedic surgery, Mayo Clinic, Rochester.

4: Aix-Marseille Univ, CNRS, ISM, Inst Movement Sci, France

CORRESPONDING AUTHOR:

Sébastien Parratte

Adress : Institut for Locomotion, Center for Arthritis Surgery, Sainte-Marguerite Hospital, Aix-Marseille University.

Phone : +33491745012

E-mail: sebastien@parratte.fr

AUTHOR CONTRIBUTION STATEMENT

All authors were fully involved in the study and preparation of the manuscript, and each of them has read and concurs with the content in the final manuscript. Pierre Sautet and Thibaut Mékidèche had a substantial contribution to research design, analysis, and interpretation of data. They also drafted the manuscript. Romain Guilhaumou and Matthew P. Abdel had contribution to research design. Jean-Noël Argenson supervised the development of the study. Matthieu Ollivier supervised the development of the study, helped in data processing and analysis and revised the manuscript critically. Sebastien Parratte supervised the development of the study, the manuscript evaluation and acted as the corresponding author.

ABSTRACT

Revisions TKAs are being completed with uncemented constructs more frequently. We hypothesized that tantalum cones could be an efficient carrier of antibiotics in uncemented procedures. We aimed to compare the release of vancomycin between 1) tantalum and smooth stainless cylinders, 2) different concentrations of vancomycin and 3) different durations of bathing. Specifically designed tantalum cylinders were bathed in a vancomycin solution with various durations of baths. We investigated rinses between each interval as well as the dose of vancomycin. Vancomycin concentrations were determined in each group by fluorescence polarization immunoassay at different intervals (1 hour, days 1, 2, 3, 5). At 1 hour, the mean vancomycin concentration for the one-hour soaking group was 3172 µg/mL, whereas mean concentration for the smooth stainless steel group was 39.37 µg/mL ($p<0.001$). The rinsing group showed a significantly lower concentration at 1 hour and 1 day ($p<0.05$). The 2-gram vancomycin group showed no difference at days 1, 2 and 3 compared to the 1-hour group. The 5, 15 and 30-minute bathing groups showed significantly lower vancomycin concentrations at all-time points. All vancomycin concentrations at day 3 were superior to the minimal inhibitory concentration of *Staphyloccocus aureus*. The mean concentration of vancomycin depends on the material, duration of bathing, the rinsing effect, and the drug dose. Our in-vitro study is the first to show that porous tantalum cylinders allow antibiotic carriage and progressive release. If appearing in-vivo, in a similar extent, this intrinsic property might be useful to prevent and/or treat peri-prosthetic joint infection.

Key words: Porous tantalum; vancomycin; elution kinematic

INTRODUCTION

The demand for primary total knee arthroplasties (TKAs) is expected to grow by 673% to 3.48 million annual procedures.[1] From the initial hip or knee primary arthroplasty, there is a 30% to 45% likelihood that patients will undergo an arthroplasty in the contralateral cognate joint.[2] TKA revisions are also projected to increase by 601% from 2005 to 2030.[1] Periprosthetic joint infection (PJI) accounts for one of the most common reasons for revision.[3] Of these PJIs, *Staphylococcus aureus* (*S. aureus*) account for 51% of PJIs after TKA in United States, (US) and for 49% in Europe.[4] Percentage of methicillin-resistant *S. aureus* (MRSA) account for 48% of PJIs in the US.[4] Vancomycin, however, is effective against nearly all *Staphylococci* and according to the recent literature, vancomycin appears to be the least toxic antibiotic for the local environment.[5,6]

While multiple solutions have been proposed to both prevent and treat PJI, the possibility to deliver local antibiotics through orthopedic implants remains a challenge. Antibiotic-loaded bone cement is frequently employed in the complex primary and many revision settings to mitigate the risk of PJI[7] given the fact that local antibiotics can be administered at much higher concentrations than achievable with parenteral antibiotics and without systemic toxicity.[8-13] Furthermore, antibiotic-loaded bone cement facilitates delivery of antibiotics to avascular areas of the joint that are inaccessible when using parenteral treatment alone.[14,15]

However, over the past decade, we have seen an increasing trend towards the utilization of uncemented metaphyseal fixation during revision TKAs.[16-18] While there are currently several products available on the market, one of the first was with tantalum femoral and tibial cones.[19-22] Tantalum is an exciting biomaterial given its low stiffness, high porosity (75-80%), high biocompatibility, and its ability to undergo osseointegration.[23,24] While some

have hypothesized that tantalum itself minimizes the risk of PJI given its topographic structure, recent *in vitro* study demonstrated that the tantalum alone has no intrinsic anti-microbial property.[25,26]

Based upon the wealth of data surrounding antibiotic-loaded bone cement in revision TKAs, combined with the significant trend towards uncemented metaphyseal fixation, we sought to determine the effectiveness and reliability of local antibiotics from tantalum in an effort to potentially prevent and treat PJIs in the revision setting. We hypothesized that in a clinician-directed fashion, tantalum cones could be an efficient and a reliable carrier of local antibiotics. In particular, we aimed to compare the release of vancomycin between 1) tantalum and smooth stainless steel cylinders, 2) different concentrations of vancomycin baths, and 3) different durations of vancomycin baths.

MATERIALS AND METHODS

Sterile and standardized (1 cm^3) tantalum cylinders (Trabecular Metal^{TM}; Zimmer-Biomet; Warsaw, IN) were specifically designed for this study to study the ability of vancomycin to be eluted in a clinician-directed fashion (similar to antibiotic-loaded bone cement).

Vancomycin Elution Properties

The first step involved evaluating the elution properties of vancomycin utilizing ten tantalum cylinders loaded with vancomycin via a standardized soaking process as described below. The vancomycin solution [50 mg/mL] was reconstituted by adding 20 mL of sterile saline (H_2O, pH 5.5, osmolarity: 0 mOsmol/L; Lavoisier, France) to 1 gram of dry vancomycin powder (Sandoz, France). Each tantalum cylinder was placed in an individual beaker and completely covered by the solution at room temperature for one hour. After this soaking

period, cylinders were withdrawn with sterile forceps and immersed in 5 mL of phosphate-buffered saline (PBS) at a pH 7.4.

To define the vancomycin elution properties, a 1.5 mL sample was taken from each cylinder after 1 hour. Thereafter, each experimental cylinder was immediately placed into a fresh 5 mL PBS solution bath. This process was repeated at day 1, day 2, day 3, and day 5 (Figure 1). Ten stainless smooth steel cylinders (all measuring $1cm^3$) were used as control samples, and followed the exact same protocol.

Vancomycin Baths Concentrations Change

After the above protocol was completed, the impact of rinsing, as well as modifications to vancomycin bath concentrations, was analyzed on both tantalum and smooth stainless steel cylinders. To evaluate the impact of rinsing, ten tantalum cylinders and ten smooth stainless steel cylinders were rinsed with 5 mL of PBS baths before each new PBS solution immersion occurred. To evaluate the vancomycin saturation threshold, ten tantalum cylinders and ten stainless steel cylinders were loaded in a bathing solution containing a concentration of vancomycin that was double the prior experiment (i.e. 2 grams diluted in 20-mL of sterile saline resulting in 100 mg/mL concentration).

Duration of Vancomycin Baths

To evaluate the impact of the bathing duration, three groups of ten tantalum cylinders and three groups of ten smooth stainless steel cylinders were soaked for three different durations: 5 minutes, 15 minutes, and 30 minutes. The vancomycin concentration of the bath was the same as the original 1-hour bathing group [50 mg/mL]. The remaining elution protocol was as described above, with comparisons made to the groups of cylinder soaked for 1 hour.

For each sample, the concentration of vancomycin was analyzed following the same standardized protocol.[27] The samples were preserved at -40°C and defrosted before being assayed. Vancomycin concentrations were determined by fluorescence polarization immunoassay (Cobas Integra 400+; Roche Diagnostic, Mannheim, Germany).[28] Intra- and inter-day precision accuracy values were within 15%.[28] The limit of quantification was 0.76 µg/mL. The level of vancomycin was considered as bactericidal if the measured concentration was greater than the minimal inhibitory concentration (MIC) for the *Staphylococcus aureus* (i.e. MIC> 2 µg/mL).[29]

Statistical Analysis

The amount of antibiotic released from each cylinder at the different times of sampling, or for the different timing or concentration of bathing, was calculated by measuring the antibiotic concentration of each sample at each time point, and then multiplying by the constant volume of the chamber (i.e. 5 mL). To evaluate the vancomycin elution properties, the tantalum cylinders were compared with the smooth stainless steel cylinders at the different times using Wilcoxon non-parametric testing to estimate mean differences and related 95% confidence interval (CI).

To evaluate the effect of initial bath concentration , the one-hour tantalum cylinders with standard concentration ([50 mg/mL]) were compared with the rinsed group and with the double concentration ([100 mg/mL] group using also Wilcoxon non-parametric testing. To evaluate the effect of the duration of bathing, the eluted antibiotic concentrations were compared between the 1 hour, 5- minute, 15-minute, and 30-minute groups using also Wilcoxon non-parametric testing. Sample size was estimated with a post-hoc analysis based on our one-hour concentration analysis (mean value of 3171.9 ± 25 µg/mL). As such, our

study was able to distinguish difference >300 µg/mL between groups with a statistical power of 80%. We considered $p < 0.05$ as significant.

RESULTS

Vancomycin Elution Properties

The results of the vancomycin elution for the one-hour soaking process showed a 20.8 mg mean total antibiotic release of vancomycin, which represents 2.1% of total vancomycin present in the soaking bath (Table1). Initially, the tantalum cylinders showed a high release of the loaded vancomycin. The mean maximum concentration observed during the first hour was 3172 µg/mL (Figure 2). This concentration exponentially decreased (at day 3 = 23.12 µg/mL) until day 5, when the vancomycin concentration becomes undetectable. The mean vancomycin concentration of the control group with stainless steel cylinders was 39.37 µg/mL at one hour (mean difference of 3133 µg/mL with 95% CI of 3015−3250; $p<0.0001$). At only one day, vancomycin was completely undetectable in the stainless steel group.

Vancomycin Loading Concentrations

The amount of vancomycin released from the rinsed tantalum cylinders was lower than the amount released by cylinders that had not been rinsed before elution (Table 1). However, the difference decreased throughout the experiment and, at day 5, both rinsed and un-rinsed cylinders stopped releasing vancomycin. The amount of vancomycin released in the tantalum cylinder group with the initial double concentration of vancomycin ([100 mg/mL]) was significantly higher (24.72 mg) than the amount released by the tantalum cylinders (15.86 mg) loaded with the standard concentration of vancomycin ([50 mg/mL]) at one hour ($p < 0.0001$). Again, the difference decreased throughout the experiment and no vancomycin was detected

at day 5 in either group. At day 1, vancomycin was completely undetectable in the stainless steel group regardless of concentration change or rinsing process.

Impact of Loading Duration

The mean concentration of vancomycin in the tantalum cylinders was influenced by shorter bathing durations of 5, 15, and 30 minutes, respectively, as noted in the significantly lower concentrations of vancomycin elution when compared to the one-hour bathing group ($p < 0.001$ at all time points except group 5 minutes at 1 hour). At day 1, mean vancomycin concentrations were 258.07 μg/mL (bath of 5 minutes), 251.88 μg/mL (15 minutes), and 195.28 μg/mL (30 minutes) (Figure 2). The mean concentration for each group decreased exponentially thereafter. At day 1, vancomycin was completely undetectable in the stainless steel group regardless of bath duration ($p<0.001$)

DISCUSSION

While several surgeons have utilized antibiotic-loaded bone cement in high-risk primary TKAs, revision TKAs, and during a two-stage exchange protocol, the trend has been towards uncemented metaphyseal fixation for long-term biologic fixation. As such, we sought to determine an effective and a reliable delivery of local antibiotics through tantalum. In this *in vitro* investigation, we were able to show that tantalum cylinders have the ability to release vancomycin for four days in comparison to smooth stainless steel cylinders. Moreover, the concentration of eluted vancomycin was dependent on the duration of the bathing as well as the concentration of vancomycin in the bath.

The result of our study regarding elution properties of tantalum demonstrated the ability to deliver vancomycin during four days in a liquid medium of PBS. The elution rate observed at

one-hour was the highest and the mean elution rate decreased exponentially thereafter (Figure 2). The high initial release was probably related to the release of the vancomycin trapped at the surface of the tantalum. This elution release profile is similar to the elution release profile of antibiotics from cement, calcium phosphate cement beads[27, 30]. In our study, a single 1-cm^3 tantalum cylinder was able to absorb and release at least 20 mg out of 1 gram of vancomycin. However, in the control group of stainless steel cylinders, only negligible traces of vancomycin were observed at 1 hour and none was detectable at 24 hours. To our knowledge, this study is the first study to report the vancomycin elution properties from tantalum cylinders, which limits the comparison with previously published studies. [27, 30]

The results of our study demonstrated that even rinsed, tantalum cylinders significantly delivered vancomycin during at least 4 days. Despite this rinsing effect, concentrations observed at day 3 were higher than the maximum MIC for *Staphylococcus aureus* (clinical breakpoint: 2 µg/mL)[29], which means that vancomycin could have therapeutic activity during at least 3 days even when rinsed. This observation is consistent with the hypothesis that the critical amount of vancomycin released is within the first hour and related to antibiotic molecules trapped at tantalum surface. This is clinically relevant since blood and routine sterile saline irrigation at the time of primary or revision TKAs will not mitigate the elution of vancomycin from the tantalum. In addition, the elution curve for the double-dose vancomycin group showed that concentrations were higher than the simple-dose group at one-hour only. The spaces between the interconnected pores of the tantalum cylinders might be saturated by a certain amount of vancomycin and the excess of vancomycin remains on the external surface.

The effect of the bathing duration on vancomycin elution was essential since a clinical application would require surgeons to apply an intraoperative bath to tantalum components during surgery. In the current study, one hour of bathing was used as the reference, but we also compared different bathing durations of 5, 15 and 30 minutes with the same vancomycin concentration. While no difference was found between 5, 15 and 30-minute groups, all were lower than the group that was bathed with the vancomycin solution for 1 hour. Importantly, however, the vancomycin concentration that eluted over 3 days was still over the MIC for *Staphylococcus aureus* in the 5, 15, and 30 minute bath groups.[29]

The major limitation of this study is that these *in vitro* findings may not replicate *in vivo* release of vancomycin. However, our study design was robust with different concentrations of vancomycin evaluated, various durations of bathing, and impact of rinsing investigation. Moreover, our study was similar to previously validated protocols.[27, 29-30] A second limitation includes the fact that we only evaluated one antibiotic- vancomycin. Several PJIs require other antibiotics to treat the infection, and future investigations in our lab are focused on similar studies with other antibiotics. Finally, we only evaluated a single clinically relevant biomaterial- tantalum. Clearly future investigations are needed studying other biomaterials that are frequently utilized in clinical practice.

In conclusion, the result of our study revealed the *in vitro* ability of vancomycin to be released from tantalum cylinders at a much higher dose than with smooth stainless steel cylinders. In addition, higher concentrations of vancomycin were released when the concentration of vancomycin was increased, the cylinders were not rinsed, and the duration of bathing was one hour. The results of this study are encouraging in the complex primary and most revision

settings where biologic fixation, particularly in the metaphysis, is increasingly utilized in these patients who are at high risk for periprosthetic joint infection.

REFERENCES

1. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am. 2007;89-4:780-5.

2. Sanders TL, Maradit Kremers H, Schleck CD, Larson DR, Berry DJ. Subsequent Total Joint Arthroplasty After Primary Total Knee or Hip Arthroplasty: A 40-Year Population-Based Study. J Bone Joint Surg Am. 2017 Mar 1;99(5):396-401.

3. Abdel MP, Ledford CK, Kobic A, Taunton MJ, Hanssen AD. Contemporary failure aetiologies of the primary, posterior-stabilised total knee arthroplasty. Bone Joint J. 2017 May;99-B(5):647-652.

4. Aggarwal VK, Bakhshi H, Ecker NU, Parvizi J, Gehrke T, Kendoff D. Organism profile in periprosthetic joint infection: pathogens differ at two arthroplasty infection referral centers in Europe and in the United States. J Knee Surg. 2014 Oct;27(5):399-406.

5. Edin ML, Miclau T, Lester GE, Lindsey RW, Dahners LE. Effect of cefazolin and vancomycin on osteoblasts in vitro. Clin Orthop Relat Res. 1996 Dec;(333):245-51.

6. Antoci V Jr, Adams CS, Hickok NJ, Shaprio IM, Parvizi J. Antibiotics for local delivery systems cause skeletal cell toxicity in vitro. Clin Orthop Relat Res. 2007 Sep;462:200-6.

7. Hanssen AD, Osmon DR, Patel R. Local antibiotic delivery systems: where are we and where are we going? Clin Orthop Relat Res. 2005-437:111-4.

8. Duncan CP, Masri BA. The role of antibiotic-loaded cement in the treatment of an infection after a hip replacement. Instructional course lectures. 1995;44:305-13.

9. Seyral P, Zannier A, Argenson JN, Raoult D. The release in vitro of vancomycin and tobramycin from acrylic bone cement. J Antimicrob Chemother. 1994;33-2:337-9.

10. Adams K, Couch L, Cierny G, Calhoun J, Mader JT. In vitro and in vivo evaluation of antibiotic diffusion from antibiotic-impregnated polymethylmethacrylate beads. Clin Orthop Relat Res. 1992-278:244-52.

11. Gonzalez Della Valle A, Bostrom M, Brause B, Harney C, Salvati EA. Effective bactericidal activity of tobramycin and vancomycin eluted from acrylic bone cement. Acta Orthop Scand. 2001;72-3:237-40.

12. Nelson CL, Griffin FM, Harrison BH, Cooper RE. In vitro elution characteristics of commercially and noncommercially prepared antibiotic PMMA beads. Clin Orthop Relat Res. 1992-284:303-9.

13. Sterling GJ, Crawford S, Potter JH, Koerbin G, Crawford R. The pharmacokinetics of Simplex-tobramycin bone cement. J Bone Joint Surg Br. 2003;85-5:646-9.

14. Hanssen AD, Spangehl MJ. Practical applications of antibiotic-loaded bone cement for treatment of infected joint replacements. Clin Orthop Relat Res. 2004-427:79-85.

15. Cierny G, 3rd. Infected tibial nonunions (1981-1995). The evolution of change. Clin Orthop Relat Res. 1999-360:97-105.

16. Meneghini RM, Lewallen DG, Hanssen AD. Use of porous tantalum metaphyseal cones for severe tibial bone loss during revision total knee replacement. J Bone Joint Surg Am. 2008;90-1:78-84.

17. Girerd D, Parratte S, Lunebourg A, Boureau F, Ollivier M, Pasquier G, Putman S, Migaud H, Argenson JN. Total knee arthroplasty revision with trabecular tantalum cones: Preliminary retrospective study of 51 patients from two centres with a minimal 2-year follow-up. Orthop Traumatol Surg Res. 2016 Jun;102(4):429-33.

18. Kamath AF, Lewallen DG, Hanssen AD. Porous tantalum metaphyseal cones for severe tibial bone loss in revision knee arthroplasty: a five to nine-year follow-up. J Bone Joint Surg Am. 2015 Feb 4;97(3):216-23.

19. Howard JL, Kudera J, Lewallen DG, Hanssen AD. Early results of the use of tantalum femoral cones for revision total knee arthroplasty. J Bone Joint Surg Am. 2011 Mar 2;93(5):478-84

20. Potter GD 3rd, Abdel MP, Lewallen DG, Hanssen AD. Midterm Results of Porous Tantalum Femoral Cones in Revision Total Knee Arthroplasty. J Bone Joint Surg Am. 2016 Aug 3;98(15):1286-91.

21. Bobyn JD, Poggie RA, Krygier JJ, Lewallen DG, Hanssen AD, Lewis RJ, Unger AS, O'Keefe TJ, Christie MJ, Nasser S, Wood JE, Stulberg SD, Tanzer M. Clinical validation of a structural porous tantalum biomaterial for adult reconstruction. J Bone Joint Surg Am 2004;86-A Suppl 2:123-9.

22. Wegrzyn J, Kaufman KR, Hanssen AD, Lewallen DG. Performance of Porous Tantalum vs. Titanium Cup in Total Hip Arthroplasty: Randomized Trial with Minimum 10-Year Follow-Up. J Arthroplasty. 2015 Jun;30(6):1008-13.

23. Cohen R. A porous tantalum trabecular metal: basic science. Am J Orthop (Belle Mead NJ) 2002;31-4:216-7.

24. Welldon KJ, Atkins GJ, Howie DW, Findlay DM. Primary human osteoblasts grow into porous tantalum and maintain an osteoblastic phenotype. J Biomed Mater Res. 2008;84-3:691-701.

25.Tokarski AT, Novack TA, Parvizi J. Is tantalum protective against infection in revision total hip arthroplasty? Bone Joint J. 2015 Jan;97-B(1):45-9.

26. Harrison PL, Harrison T, Stockley I, Smith TJ. Does tantalum exhibit any intrinsic antimicrobial or antibiofilm properties? Bone Joint J. 2017 Sep;99-B(9):1153-1156

27. Penner MJ, Masri BA, Duncan CP. Elution characteristics of vancomycin and tobramycin combined in acrylic bone-cement. J Arthroplasty. 1996 Dec;11(8):939-44.

28. Domke I, Cremer P, Huchtemann M. Therapeutic drug monitoring on COBAS INTEGRA 400--evaluation results. Clin Lab. 2000;46(9-10):509-15.

29. European committee on antimicrobial susceptibility testing. Recommandations 2017.

30. Sasaki T, Ishibashi Y, Katano H, Nagumo A, Toh S. In vitro elution of vancomycin from calcium phosphate cement. J Arthroplasty. 2005 Dec;20(8):1055-9.

31. Amin TJ, Lamping JW, Hendricks KJ, McIff TE. Increasing the elution of vancomycin from high-dose antibiotic-loaded bone cement: a novel preparation technique. J Bone Joint Surg Am. 2012 Nov 7;94(21):1946-51.

FIGURES' LEGEND

Figure 1. Elution protocol of vancomycin concentration as determined by immunoassay at each samples interval. M cylinder: Metal cylinder

Figure 2. Elution pharmacokinetic of vancomycin from tantalum cylinders. Concentration of antibiotic released is express in μg/mL.

Cylinder treatment	Number of samples	**Amount of vancomycin released (mg)**				
		1 hour	24 hours	48 hours	72 hours	5 days
Unrinsed	10	15.86 ± 1.26	4.60 ± 0.78	0.20 ± 0.02	0.12 ± 0.02	ND
Rinsed	10	5.33 ± 0.72	2.33 ± 0.45	0.12 ± 0.02	0.11 ± 0.02	ND
P		<0.0001	0.02	0.3	0.5	/
Double-dose loading	10	24.72 ± 2.59	4.97 ± 0.92	0.30 ± 0.04	0.13± 0.03	ND
P		<0.0001	0.5	0.4	0.7	/

Table. Amount of vancomycin released from 1 cm^3 Tantalum cylinder at each sampling interval. Values are expressed as mean ± SD. ND: Not Detectable

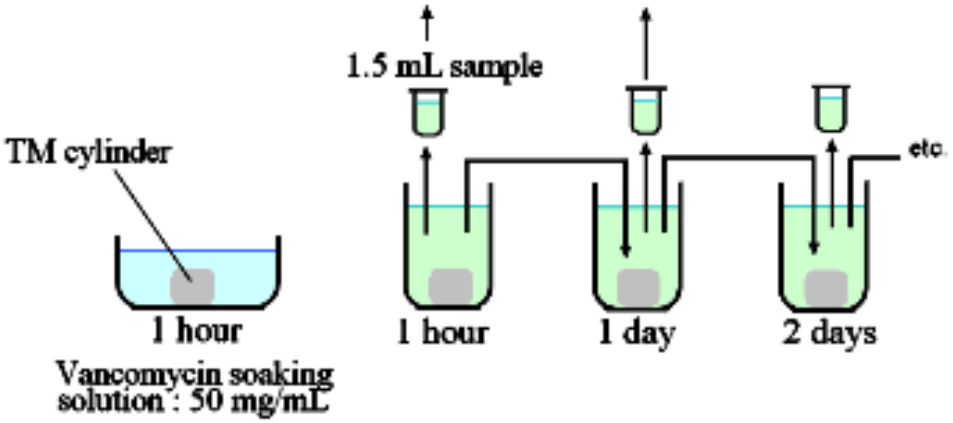
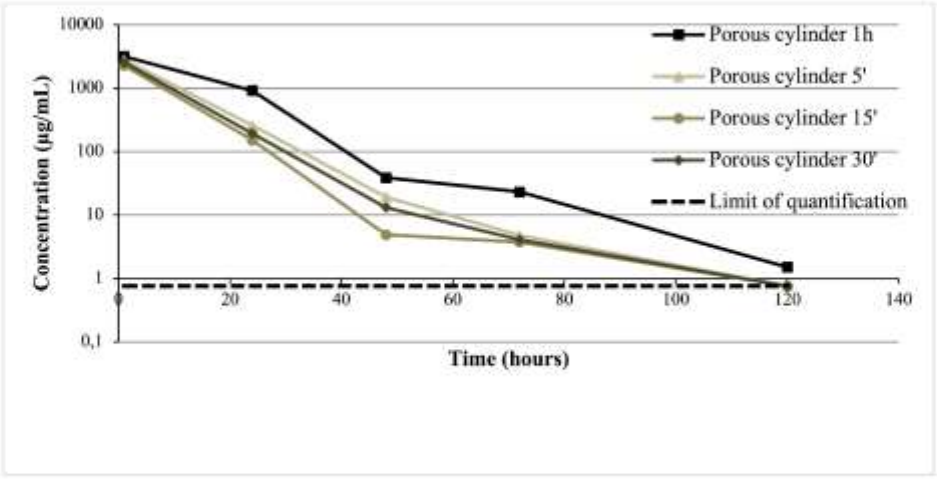

1.5 mL sample
TM cylinder
etc.
1 hour
1 hour
1 day
2 days
Vancomycin soaking solution : 50 mg/mL

Figure 1



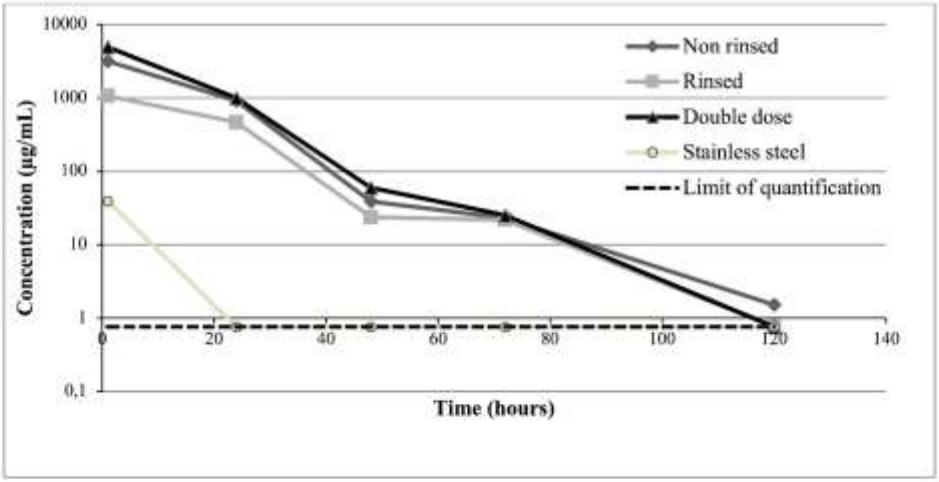


Figure 2