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Ciprofloxacin-Releasing Bioabsorbable Polymer Is Superior to Titanium in Preventing *Staphylococcus epidermidis* Attachment and Biofilm Formation *In Vitro*

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Abstract: Antibiotic coating systems have been successfully used to prevent bacterial attachment and biofilm formation. Our purpose was to evaluate whether bioabsorbable polylactide-co-glycolide (PLGA) 80/20 on its own, and PLGA together with ciprofloxacin (PLGA+C) have any advantages over titanium in preventing *Staphylococcus epidermidis* attachment and biofilm formation *in vitro*. Cylindrical specimens of titanium, PLGA, and PLGA+C in triplicate were examined for *S. epidermidis* ATCC 35989 attachment and biofilm formation after incubation with a bacterial suspension of about 10^5 cfu/mL for 1, 3, 7, 14, and 21 days, using scanning electron microscopy. Growth inhibition properties of PLGA and PLGA+C cylinders were tested on agar plates. On days 1, 3, and 21, no bacterial attachment was seen in 19.5, 9.2, and 41.4% of the titanium specimens; in 18.4, 28.7, and 34.5% of the PLGA specimens; and in 57.5, 62.1, and 57.5% of the PLGA+C specimens, respectively. During the whole study period, no biofilm was observed on 74–93% of the titanium specimens, 58–78% of the PLGA specimens, and 93–100% of the PLGA+C specimens. PLGA+C showed clear bacterial growth inhibition on agar plates, while PLGA and titanium did not show any inhibition. PLGA+C bioabsorbable material was superior to titanium in preventing bacterial attachment and biofilm formation and may have clinical applicability, for example, in prevention of infection in trauma surgery or in the treatment of chronic osteomyelitis. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 76B: 8–14, 2006

Keywords: antibiotic; bioabsorbable; biofilm; ciprofloxacin; polylactide-co-glycolide

INTRODUCTION

Tens of millions of medical devices are inserted annually in the USA.[1] The susceptibility of implanted biomaterials to bacterial attachment and biofilm formation, and thereby device-associated infections, is a serious problem in implant surgery. For example, the estimated number of fracture fixation device-associated infections was 100,000–200,000 in 2001.[1] In addition, increasing amounts of convincing data support the idea that most late-onset hip and knee prosthesis infections are consequences of peroperative contamination in primary operation.[2] This seems to occur frequently despite the use of laminar air flow and peroperative prophylactic antibiotics.[3] Most (76%) of the contaminating bacteria have been found to be coagulase-negative *staphylococci* (CoNS),[3] which belong to the normal flora of human skin and mucous membranes.[4] These bacteria are a major cause of nosocomial infections typically found in patients with indwelling or implanted foreign polymer bodies. For example, in intensive care unit, patients CoNS are the principal cause of bactere-

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mia,[5] and 87% of cases are associated with vascular access.[6] *Staphylococcus epidermidis*, which is the most frequently encountered coagulase-negative *staphylococcus* in clinical situations, has several mechanisms by which it can colonize a polymer surface and produce a multilayered biofilm.[4]

The use of different kinds of bioabsorbable materials, especially in pediatric surgery, is increasing. Unfortunately, these materials are also prone to infection. In cases of vascular access, attempts have been made to prevent the attachment of bacteria by using various types of antibiotic coating systems.[7] Similarly, new bioabsorbable osteofixation devices, which can be manufactured in the form of screws that are osteoconductive, bioabsorbable, and antibiotic-releasing, with reliable biomechanical properties,[8,9] offer the possibility of combining antibiotics with them.

This *in vitro* study with scanning electron microscopy (SEM) was performed to evaluate whether bioabsorbable polylactide-co-glycolide (PLGA) 80/20 on its own, and PLGA together with ciprofloxacin have any advantages over titanium—the standard material, for example, in craniomaxillofacial surgery—in preventing *S. epidermidis* attachment and biofilm formation in culture media.

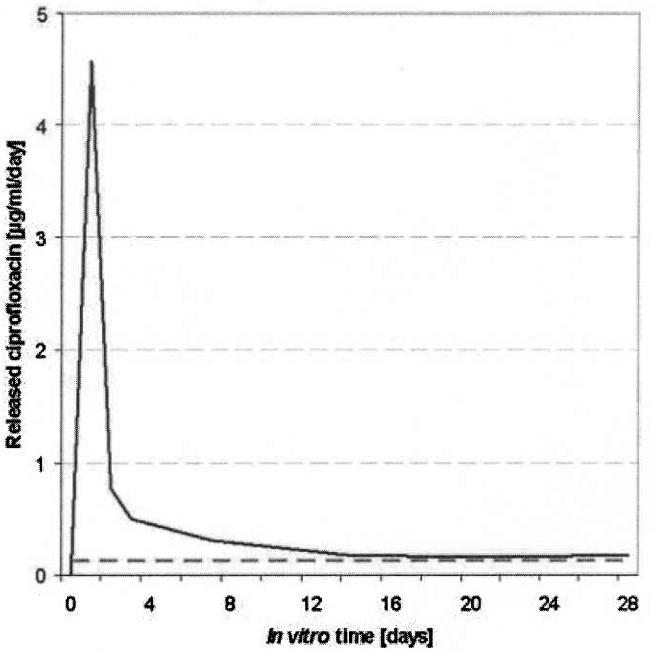

Figure 1. The concentration of released ciprofloxacin as a function of immersion time. The antibiotic was released from bioabsorbable γ-sterilized PLGA cylinders during the first 28 days of the *in vitro* drug release test. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

MATERIALS AND METHODS

Biomaterials

Cylindrical specimens (7 mm long and 6.4 mm diameter) of bioabsorbable polymers were tested. They were made of either (1) polylactide-co-glycolide (PLGA) 80/20, or (2) PLGA 80/20 containing ciprofloxacin. The cylinders were prepared at the Institute of Biomaterials, Tampere University of Technology, Tampere, Finland, and they were sterilized using γ-irradiation. Titanium control cylinders of the same dimensions were prepared at KI-Technology Oy, Oulu, Finland, and gas-sterilized.

The bioabsorbable matrix was composed of a copolymer of L-lactide and glycolide with a starting monomer ratio of 80% L-lactide and 20% glycolide. Commercial PuraSorb®PLG (Purac Biochem bv., Gorinchem, Netherlands), which is a semicrystalline form of bioabsorbable synthetic PLGA 80/20, was used. The inherent viscosity of PLGA 80/20 is 6.28 dL/g (0.1%, chloroform, 25°C). The antibiotic was ciprofloxacin ($C_{17}H_{18}FN_3O_3$), which is a synthetic fluoroquinolone. It has solubility in water of <50 μg/mL, a half-life of 3–5 h, and a molecular weight of 331.4. Ciprofloxacin comprised 8% by weight of resulting implants. PLGA and ciprofloxacin starting materials were in powder form. PLGA and ciprofloxacin were melt extruded into billets (6.4 mm ϕ) with a small laboratory scale mixer so that the antibiotic was distributed throughout the polymer matrix. Resulting billets were then cut mechanically into cylinders (7 mm long and 6.4 mm ϕ).

Bacterial Strains

Staphylococcus epidermidis ATCC 35989 was selected in this study because it was shown in a tube adherence test[10] to attach strongly onto the surfaces of both polystyrene and polypropylene tubes when compared with common laboratory strains (*S. epidermidis* ATCC 29887 showing slight attachment; and *S. aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 showing no attachment). The selected strain was confirmed by E-test (Biodisc AB, Solna, Sweden) to be sensitive to ciprofloxacin; the minimum inhibitory concentration (MIC) was found to be 0.125 μg/mL, which is clearly lower than the drug concentration released from the cylinder (Figure 1). The bacterial strain was stored in equal portions in skimmed milk at −80°C until use.

S. epidermidis ATCC 35989 was attached to the surface of the cylinders by incubation in glass tubes (one cylinder per tube) containing 4 mL of freshly prepared bacterial suspension (~10^5 cfu/mL, corresponding to McFarland turbidity standard 0.5) in Trypticase soy broth (TSB) medium at 35°C. As controls, similar cylinders were incubated in TSB medium without bacteria. After 1, 3, 7, 14, and 21 days, one specimen of each type of material was retrieved under aseptic conditions and prepared for scanning electron microscopy (SEM). The concentrations of the test bacteria in each tube were determined after each incubation period by quantitative plating on blood agar plates. The sensitivity of the test strain to ciprofloxacin was checked at the end of each incubation period. Each test was performed in triplicate.

Scanning Electron Microscopy

For SEM, the cylinders were washed in phosphate buffer solution at pH 7.4 and fixed with 3% glutaraldehyde/phos-

phate buffer at pH 7.3 for 1.5 h. After fixation, each specimen was washed three times in phosphate buffer and dehydrated with ethanol at 30% (for 10 min), 50% (10 min), 70% (2 × 10 min), 80% (5 min), 90% (5 min), and 100% (2 × 5 min). In absolute ethanol, the samples were subjected to critical-point drying with carbon dioxide in a BAL-TEC CPD 030 critical point dryer (BAL-TEC Ltd., Balzers, Liechtenstein). The cylinders were then mounted on metal stubs with conductive carbon cement, coated with platinum-palladium in an Agar high resolution sputter coater (Agar Scientific Ltd., Stansted, UK) and examined by SEM (FESEM, JEOL JSM-6300F scanning electron microscope; JEOL, Tokyo, Japan) at an accelerating voltage of 6 kV.

Pilot studies showed that bacteria did attach to the lateral surface. Since this surface is heat-treated, the antibiotic is thought not to be close enough to the surface to affect bacterial attachment in the early stages. On the other hand, the mechanically cut surface was thought to have closer-to-the-surface antibiotic exerting an effect on bacterial attachment. Hence, it was decided to analyze this surface, as screws planned to be made from this composite material would be mechanically prepared by peeling off the heat-treated lateral surface. Thus, the mechanically cut surface would be that seen in the finished product. For each time point (1, 3, 7, 14, and 21 days) three new cylinders were analyzed, resulting in a total of 45 specimens; 15 titanium, 15 PLGA, and 15 PLGA with ciprofloxacin.

Standard examination fields were determined by SEM from the mechanically cut circular surface of cylindrical biomaterials and titanium specimens, so that the examination procedure would be reproducible and provide a basis for drawing comparative results from all the examined specimens. At a magnification of 3000×, we proceeded by moving ½ mm at a time from the central point of the circular surface to the edge of the biomaterial along x and y axes in both directions. The presence or absence of bacteria and biofilm were documented in the examined areas, which were 1.2×10^{-3} mm^2 in size. The number of bacterial cells present in each area was determined, and the amount of extracellular polymer material was estimated by using a scale of –(no biofilm formation), + (weak or moderate biofilm formation), and ++ (strong biofilm formation). The standardized method of SEM analysis and evaluation of the degree of biofilm formation are shown in Figure 2.

Statistical Evaluation

For each material, daily means of bacterial cell count were calculated for the 29 examined areas, taking into account only the non-zero values. The frequency of zeros (no bacterial cells) was recorded for each specimen. Since the data contained excess zeros, the analysis was carried out in two parts (two-part model): (1) the proportion of zeros was analyzed by the χ^2 test or Fisher's exact test. (2) The nonzero part was analyzed by analysis of variance (ANOVA) or the nonparametric Kruskal-Wallis test. Two-tailed p values are reported. Biofilm formation was analyzed by Fisher's exact test. The

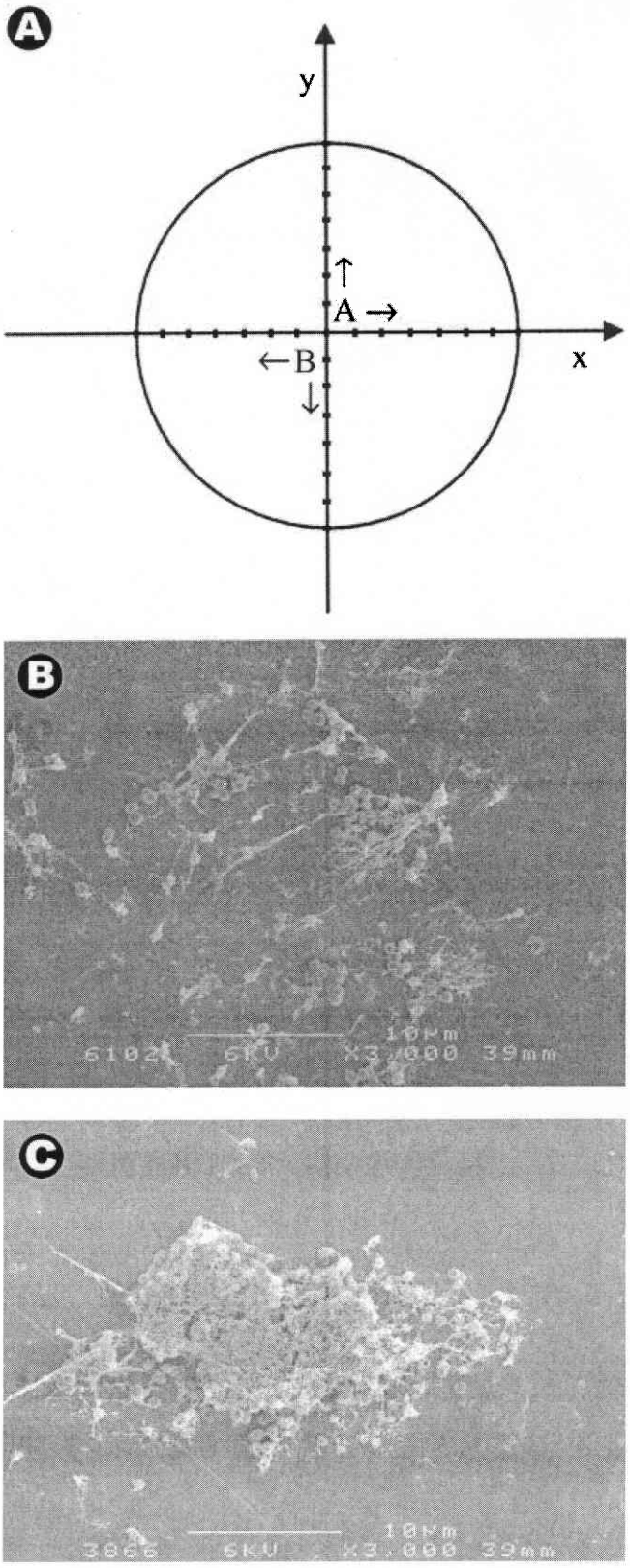

Figure 2. Schematic illustration showing the standardized method of SEM analysis (A). Examined areas (magnification ×3000, 1.2×10^{-3} mm^2) were on the mechanically cut circular surfaces of the cylindrical specimens (7 mm length, 6.4 mm diameter): x-axis, A direction (y = 0), millimeter from the central point: 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.2, and at similar points in the B direction and on the y-axis in both directions. Evaluation of the degree of biofilm formation: slight or moderate (+) (B), and strong (++) (C).

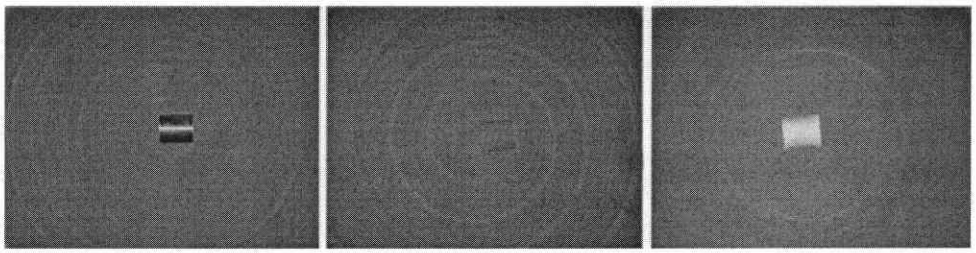

Figure 3. Growth inhibition of *S. epidermidis* ATCC 35989 on agar plates. (A) titanium, (B) PLGA 80/20, and (C) PLGA 80/20 containing ciprofloxacin. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

analyses were carried out using SPSS for Windows (version 11.5).[11]

Growth Inhibition Test on Agar Plates

Cylinders made of titanium, PLGA 80/20, and PLGA 80/20 containing ciprofloxacin were tested for their bacterial growth inhibitive properties by embedding one specimen of each in a blood agar plate containing *S. epidermidis* ATCC 35989 suspension at a concentration of about 10^7 cfu/mL in TSB medium. The plates were incubated overnight at 35°C and the diameters of the inhibition areas were measured. Five parallel samples of each material were examined.

RESULTS

PLGA containing ciprofloxacin inhibited bacterial growth on blood agar plates, showing an average diameter of growth inhibition area of 34 mm (Figure 3), while no inhibition was seen with pure PLGA and titanium specimens.

When the percentages of zero-findings (i.e. without *S. epidermidis* growth) were compared between the three materials at each time point (Table I), only 19.5% of the examined areas of the titanium specimens and 18.4% of PLGA specimens were negative on the first day, while the corresponding figure for ciprofloxacin-containing PLGA was 57.5%. On days 3 and 7, PLGA specimens were more often negative than the titanium ones. However, on days 14 and 21, the situation had changed, and titanium seemed to be slightly better than PLGA in preventing *S. epidermidis* growth. On the other hand, the proportion of zero findings was always clearly higher in the ciprofloxacin-containing PLGA group. As indicated in Table I, the zero findings in the three groups differed highly significantly at each time point, with p values of less or much less than 0.025.

In the situations with *S. epidermidis* growth, the mean bacterial count in the titanium group was between those in the two PLGA groups. At each time point, the PLGA group showed the highest growth and the ciprofloxacin-containing PLGA group the lowest *S. epidermidis* growth. During the whole study period apart from day 21, the means of bacterial growth differed statistically between the groups (Table I). The platings on blood agar of media from the incubation tubes contained no viable bacteria after each incubation period in the cases with ciprofloxacin-containing PLGA, while clear growth was observed in the culture media from tubes containing pure PLGA and titanium. The reason for the low percentages of zeros and the slightly increased means of *S.*

TABLE I. Bacterial Attachment to Cut Surfaces of Titanium, PLGA, and Ciprofloxacin-Releasing PLGA (PLGA+C)

Material	1 Day ($p < 0.001$* $p = 0.025$**)	3 Days ($p < 0.001$* $p = 0.004$**)	7 Days ($p < 0.025$* $p = 0.024$**)	14 Days ($p < 0.006$* $p = 0.017$**)	21 Days ($p = 0.007$* $p = 0.13$**)	Whole Study Period ($p = 0.004$**)
Titanium						
% of zero-values	19.5	9.2	17.2	32.2	41.4	—
Mean (SD)	11.8 (5.4)	9.2 (4.7)	6.1 (1.9)	4.3 (3.4)	3.9 (2.4)	7.1 (4.8)
PLGA						
% of zero-values	18.4	28.7	19.5	23.0	34.5	—
Mean (SD)	19.9 (7.2)	29.1 (12.6)	15.9 (6.5)	10.7 (2.1)	20.0 (16.0)	19.1 (5.9)
PLGA+C						
% of zero-values	57.5	62.1	33.3	46.0	57.5	—
Mean (SD)	1.9 (0.2)	1.7 (0.4)	4.1 (1.7)	3.2 (0.9)	2.6 (0.9)	2.7 (0.6)

Negative findings (% of zero-values), and means and standard deviations (SDs) of the numbers of attached bacteria in the examined areas of each material (non-zero parts) were calculated for each time point.

* p values for differences between material groups: zero *versus* nonzero data (χ^2 test or Fisher's exact test).

** p values for differences between groups in nonzero data (ANOVA or nonparametric Kruskal-Wallis test).

TABLE II. Biofilm Formation on Cut Surfaces of Titanium, PLGA and Ciprofloxacin-Releasing PLGA (PLGA+C)

	Biofilm Grade (%)														
	Day 1 ($p < 0.001$*)			Day 3 ($p < 0.001$*)			Day 7 ($p < 0.001$*)			Day 14 ($p < 0.001$*)			Day 21 ($p < 0.001$*)		
Biomaterial	−	+	++	−	+	++	−	+	++	−	+	++	−	+	++
Titanium	82	17	1	74	24	2	90	9	1	92	7	1	93	7	0
PLGA	67	33	0	73	20	7	58	41	1	78	21	1	78	20	2
PLGA+C	100	0	0	100	0	0	93	7	0	99	1	0	98	2	0

The amount of biofilm was estimated in the examined areas by using a grading scale of − (no biofilm formation), + (weak or moderate biofilm formation), and ++ (strong biofilm formation). It is indicated in the table in terms of percentage.

* p values for differences between material groups.

epidermidis growth in ciprofloxacin-containing PLGA specimens on days 7 and 14 remains open, but it may be the result of chance, owing to the small numbers involved.

As Table II shows, in the titanium group, no biofilm production was observed in most cases over a three-week period (74–93% of the cases). However, weak or moderate biofilm formation was seen in up to one-fourth of the cases (7–24%) and even strong biofilm formation was occasionally observed (Table II, Figure 2(C)). In harmony with the aforementioned bacterial results, biofilm production was strongest in the PLGA group, in which bacteria were attached to the substratum by fibrous strands and were partially or entirely covered by extracellular polymer matrix (Figure 4). Weak or moderate biofilm formation was commonly seen in the PLGA group (20–41% of the examined areas of the specimens at different times). No biofilm was observed in the ciprofloxacin-releasing PLGA group on days 1 and 3. Biofilm production in this group was very slight over the entire study period, and even when present, extracellular glycocalyx matrix occurred only slightly as amorphous material surrounding bacterial cells in some areas. As indicated in Table II, biofilm production differed significantly between the three groups at each time point ($p < 0.001$).

DISCUSSION

Our results show that ciprofloxacin-releasing bioabsorbable material was superior to titanium in preventing *S. epidermidis* attachment and biofilm formation *in vitro*, whereas pure PLGA was poorer than titanium in this respect.

The results support the idea that ciprofloxacin-releasing bioabsorbable materials are promising in preventing *S. epidermidis* attachment and biofilm formation: PLGA+C resulted in a clear inhibition zone of *S. epidermidis* growth on agar plates and PLGA+C cylinders incubated in liquid culture medium did not result in any growth of viable bacteria after various incubation periods, in contrast to pure PLGA and titanium. Earlier, SEM analysis has been used in morphological studies and direct counting of bacteria attached to surfaces.[12,13] Our SEM analysis showed no bacterial growth in 57.5% of the examined PLGA+C areas on the first day, whereas less than a fifth of the findings in the other two groups were negative. Moreover, during the whole study period, the mean bacterial count was lowest in PLGA+C specimens. Similarly, in the PLGA+C group, exopolysaccharide matrix was not seen at all after various incubation periods in 93–100% of the specimens or it surrounded some cells only slightly. The situation was totally different in the other two groups (Table II, Figure 4).

Our approach does have some limitations. Quantitative assessment of attached bacteria using SEM is probably neither ideal nor optimally practical because of the small fields and time consuming work. Thus, only a few experiments

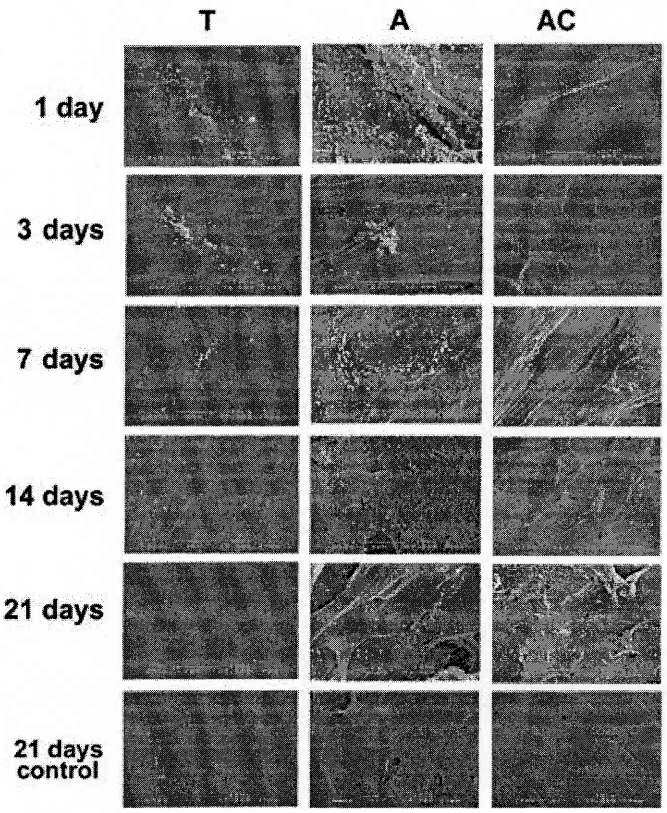

Figure 4. Scanning electron micrographs of materials incubated with *S. epidermidis* ATCC 35989 for 1, 3, 7, 14, and 21 days, and control cylinders after 21 days of incubation in TSB medium without bacteria. Tested materials were titanium (T), PLGA 80/20 (A), and PLGA 80/20 containing ciprofloxacin (AC).

could be performed, allowing fluctuations caused by chance (decreased numbers of zero findings on days 7 (33.3%) and 14 (46.0%) with slightly increased bacterial counts in ciprofloxacin-releasing PLGA specimens). Of course, the possibility of acquired resistance of *S. epidermidis* during exposure to ciprofloxacin is also a realistic explanation. This is a common problem in clinical situations concerning almost all antimicrobial agents used for the treatment of *S. epidermidis* infections, except for vancomycin[14] and probably linezolid.[15] However, our negative culture results after different incubation periods argue against acquired resistance. An *in vitro* model also has other limitations. Insufficient nutrient supply may hinder the growth of bacteria, which could be one explanation for the increasing number of negative bacterial findings towards the end of the experiment. Moreover, in the pathogenesis of device-associated infections, there is multifaceted interaction of microbes, device, and the host.[1]

In a classic human study as little as 100 cfu of *S. aureus* were infective in the presence of silk sutures.[16] When foreign material was not present, even 10^7 organisms did not cause infection in experimental studies.[17,18] Although our use of *S. epidermidis* at a concentration of 10^5 cfu is in harmony with that used in a recent *in vitro* study,[19] from a practical point of view, this concentration should be regarded cautiously, because a host response was lacking.

The multifaceted aspects of the pathogenesis of device-associated infections are currently being viewed from a new perspective, since in an experimental study *S. epidermidis* bacteria have proven to be viable in pericatheter macrophages, suggesting the possibility of chronic intracellular infection in typical extracellular bacteria.[20] The complexity of host-foreign material interaction was further stressed in one experiment where titanium-stimulated human peripheral blood monocytes induced significant increases in TNFα and interleukin 6 synthesis, which was inhibited by ciprofloxacin.[21] However, we do not yet know what may happen to cytokine production from monocytes in association with pure PLGA or ciprofloxacin-containing bioabsorbable material.[22]

All in all, it is too early to say what will happen in clinical settings, but our *in vitro* study clearly showed that the first step in the pathogenesis of device-associated infection—the attachment of bacteria—can be significantly decreased through the use of PLGA+C, a ciprofloxacin delivery system that seems ideal for clinical purposes. Fluoroquinolones have a concentration-dependent killing capacity for microbes.[23] Our results show that in the first few days locally released ciprofloxacin concentrations exceed by more than 30 times the observed MIC for the *S. epidermidis* strain used in our experiment. It has recently been mentioned that "devices constructed of biomaterials that fully prevent infections remain a tantalizing prospect."[24] Our results support the idea that ciprofloxacin-releasing bioabsorbable material may allow us to achieve this goal.

In conclusion, PLGA ciprofloxacin-releasing material appeared to reduce staphylococcal attachment and biofilm formation significantly *in vitro* when compared with pure PLGA or titanium. This novel controlled drug delivery implant material may have a promising future with clinical applicability, for example, for prevention of infection in trauma surgery or in the treatment of chronic osteomyelitis.

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REFERENCES

1. Darouiche RO. Device-associated infections: A macroproblem that starts with microadherence. Clin Infect Dis 2001;33:1567–1572.
2. Widmer AF. New developments in diagnosis and treatment of infection in orthopedic implants. Clin Infect Dis 2001;33 (Suppl 2):S94–S106.
3. Davis N, Curry A, Gambhir A, Panigrahi H, Walker C, Wilkins E, Worsley M, Kay P. Intraoperative bacterial contamination in operations for joint replacement. J Bone Joint Surg Br 1999;81: 886–889.
4. von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative *staphylococci.* Lancet Infect Dis 2002;2:677–685.
5. National Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1990–May 1999, issued June 1999. Am J Infect Control 1999;27:520–532.
6. Richards M, Edwards J, Culver D, Gaynes R. Nosocomial infections in medical intensive care units in the United States. National Nosocomial Infections Surveillance System. Crit Care Med 1999;27:887–892.
7. Raad II, Hanna HA. Intravascular catheter-related infections: New horizons and recent advances. Arch Intern Med 2002;162: 871–878.
8. Leinonen S, Suokas E, Veiranto M, Törmälä P, Waris T, Ashammakhi N. Holding power of bioabsorbable ciprofloxacin-containing self-reinforced poly-L/DL-lactide 70/30 bioactive glass 13 miniscrews in human cadaver bone. J Craniofac Surg 2002;13:212–218.
9. Tiainen J, Veiranto M, Suokas E, Törmälä P, Waris T, Ninkovic M, Ashammakhi N. Bioabsorbable ciprofloxacin-containing and plain self-reinforced polylactide-polyglycolide 80/20 screws: Pullout strength properties in human cadaver parietal bones. J Craniofac Surg 2002;13:427–433.
10. Christensen G, Simpson W, Bisno A, Beachey E. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect Immun 1982;37:318–326.
11. Lachenbruch P. Analysis of data with excess zeros. Stat Methods Med Res 2002;11:297–302.
12. An Y, Friedman R, Draughn R, Smith E, John J. Bacterial adhesion to biomaterial surfaces. In: Wise D, Trantolo D, Altobelli D, Yaszemski M, Gresser J, editors. Human Biomaterials Applications. Totowa, New Jersey: Humana Press; 1996. p 19–57.
13. Franson T, Sheth N, Rose H, Sohnle P. Scanning electron microscopy of bacteria adherent to intravascular catheters. J Clin Microbiol 1984;20:500–505.
14. Biavasco F, Vignaroli C, Varaldo PE. Glycopeptide resistance in coagulase-negative *staphylococci.* Eur J Clin Microbiol Infect Dis 2000;19:403–417.
15. Curtin J, Cormican M, Fleming G, Keelehan J, Colleran E. Linezolid compared with eperezolid, vancomycin, and gentamicin in an *in vitro* model of antimicrobial lock therapy for *Staphylococcus epidermidis* central venous catheter-related bio-

film infections. Antimicrob Agents Chemother 2003;47:3145–3148.
16. Elek SD, Conen PE. The virulence of *Staphylococcus pyogenes* for man: A study of the problems of wound infection. Br J Exp Pathol 1957;38:573–586.
17. James RC, MacLeod CJ. Induction of staphylococcal infections in mice with small inocula introduced on sutures. Br J Exp Pathol 1961;42:266–277.
18. Noble WC. The production of subcutaneous staphylococcal skin lesions in mice. Br J Exp Pathol 1965;46:254–262.
19. Lyte M, Freestone PP, Neal CP, Olson BA, Haigh RD, Bayston R, Williams PH. Stimulation of *Staphylococcus epidermidis* growth and biofilm formation by catecholamine inotropes. Lancet 2003;361:130–135.
20. Boelens JJ, Dankert J, Murk JL, Weening JJ, van der Poll T, Dingemans KP, Koole L, Laman JD, Zaat SA. Biomaterial-associated persistence of *Staphylococcus epidermidis* in pericatheter macrophages. J Infect Dis 2000;181:1337–1349.
21. Blaine TA, Pollice PF, Rosier RN, Reynolds PR, Puzas JE, O'Keefe R. Modulation of the production of cytokines in titanium-stimulated human peripheral blood monocytes by pharmacological agents. The role of cAMP-mediated signaling mechanisms. J Bone Joint Surg Am 1997;79:1519–1528.
22. Dalhoff A, Shalit I. Immunomodulatory effects of quinolones. Lancet Infect Dis 2003;3:359–371.
23. Craig WA. Pharmacodynamics of antimicrobials: General concepts and applications. In: Nightingale CH, Murakawa T, Ambrose PJ, editors. Antimicrobial Pharmacodynamics in Theory and Clinical Practice. New York: Marcel Dekker; 2002. p 1–22.
24. Burke JP. Patient safety: Infection control—A problem for patient safety. N Engl J Med 2003;348:651–656.