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Surface immobilized zoledronate improves screw fixation in rat bone: A new method for the coating of metal implants

Therese Andersson · Fredrik Agholme · Per Aspenberg · Pentti Tengvall

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Abstract Previous studies show that surface immobilized bisphosphonates improve the fixation of stainless steel screws in rat tibia after 2–8 weeks of implantation. We report here about the immobilization of a potent bisphosphonate, zoledronate, to crosslinked fibrinogen by the use of another technique, i.e. ethyl-dimethyl-aminopropylcarbodiimide (EDC)/imidazole immobilization. Bone fixation of zoledronate-coated screws was compared to screws coated with crosslinked fibrinogen only and ditto with EDC/*N*-hydroxy-succinimide immobilized pamidronate. Fixation in rat tibia was evaluated by a pull-out test at either 2 or 6 weeks after implantation. Both bisphosphonate coatings increased the pull-out force at both time points, and zoledronate showed a significantly higher pull-out force than pamidronate. To further evaluate the new coating technique we also performed a morphometric study, focusing on the area surrounding the implant. The zoledronate coating resulted in an increased bone density around the screws compared to controls. No pronounced increase was seen around the pamidronate coated screws. Together, the results demonstrate the possibility of obtaining a significant local therapeutic effect with minute amounts of surface immobilized zoledronate.

Per Aspenberg and Pentti Tengvall have shares in a company with research related to the subject.

T. Andersson (✉) · F. Agholme · P. Aspenberg
Experimental Orthopaedics, Department of Clinical and Experimental Medicine, Faculty of Medicine, Linköping University, 58183 Linköping, Sweden
e-mail: therese.andersson@liu.se

P. Tengvall
Department of Biomaterials, Institute for Clinical Sciences, Sahlgrenska Academy, Gothenburg University, 40530 Gothenburg, Sweden

1 Introduction

An early mechanical fixation to bone may be important for reducing the risk of aseptic loosening of bone anchored implants [1–3]. During host response to implantation trauma, and rather similar to normal fracture healing, the balance between bone formation and resorption during the early implantation period can be shifted due to implant micromovement [4], and other reasons such as infection and bone disease. Hence, it is possible for osteoclasts to excessively resorb bone around implants or prostheses without a balancing osteoblastic activity, and vice versa. Bisphosphonates are known to suppress osteoclast activity, and can therefore yield a net anabolic effect towards enhanced bone formation. Such drugs are mainly used for the treatment of osteoporosis but have proven useful also in other applications, such as suppression of bone metastases [5] and treatment of Paget's disease [6]. They act by suppression of osteoclast metabolism [7, 8], and impair their bone resorption capacity [9]. Bisphosphonates also affect osteoblasts, at least in vitro where a positive net effect has been observed at low doses [10, 11] and a negative effect at higher doses [12]. The high affinity to hydroxyapatite [13] makes them attractive in bone tissue applications as they remain in bone for an extended period of time and can likely be reused by osteoclasts more than once, thereby amplifying the drug effect. The concept of applying bisphosphonates in implant drug-delivery systems for the improvement of implant fixation can by now be considered as an established idea.

Thus, by immobilization of bisphosphonates to implants a local bone treatment can be obtained without the need of systemic delivery. Local bisphosphonate release from screws has been studied in vivo with hydroxyapatite coated implants [14–16], protein films [17], porous metal surfaces [18] and biodegradable coatings [19]. In all the tested

bisphosphonate delivery systems, a net positive effect with increased bone formation was observed. Furthermore, a positive clinical effect of local treatment has also been observed in humans [20].

In our previous studies using a rat tibia implantation model and stainless steel screws, the bisphosphonates pamidronate and alendronate were attached to a 40–50 nm thick ethyl-dimethyl-aminopropylcarbodiimide (EDC)/*N*-hydroxy-succinimide (NHS) crosslinked fibrinogen layer via a bioconjugate technique [17, 21]. By this procedure tail amino groups of bisphosphonates were coupled to EDC/NHS activated carboxyls on fibrinogen. This chemistry hence excluded bisphosphonates without accessible amino tail groups and drugs such as ibandronate, risedronate and zoledronate could not be used. Zoledronate is a potent anti-resorptive drug with high capability [22] and high bone mineral affinity [13]. It has previously been used to accelerate fracture healing with positive results, in systemic [23, 24] as well as local [15, 19, 25] delivery studies. In order to combine the advantage of using this potent bisphosphonate with the benefit of surface immobilization via thin protein films, a new coating technique had to be developed. This technique should be cheap and fast, and provide an alternative to zoledronate-releasing hydroxyapatite-coated implants.

The aim of the present study was to evaluate a new immobilization technique by which any bisphosphonate, also without accessible tail amino groups, can be immobilized onto amino groups in a matrix. This was performed by EDC/imidazole chemistry, and zoledronate was immobilized via one of its phosphate groups to fibrinogen on stainless steel.

We investigated, by a local treatment with differently immobilized zoledronate and pamidronate, respectively, how these drugs affected the mechanical fixation after 2–6 weeks in rat tibia. We also performed a morphometric study on the area near the implant. The bisphosphonate treated screws were compared to controls with crosslinked fibrinogen coating without bisphosphonates.

2 Materials and methods

This study was performed in three parts (Fig. 1) with insertions of modified stainless steel (SS) screws and measurements of pull-out forces at 2 or 6 weeks and a morphometric study at 6 weeks. In the first experimental part, 60 rats were divided into six groups ($n = 10$ per group). The surface coatings were pamidronate-crosslinked fibrinogen, zoledronate-crosslinked fibrinogen and a control group with crosslinked fibrinogen only. Each animal had one screw inserted. In the second part, 40 animals were divided into four groups ($n = 10$ per group) receiving crosslinked fibrinogen coated SS screws or completely uncoated SS screws. Each animal had one screw inserted. The screw fixation was evaluated by pull-out tests in the first and second part of the study. In the third part, nine rats were divided into three groups. The surface coatings were the same as in part one but instead a morphometric study on the area surrounding the implant was performed 6 weeks after implantation. All screws, except the uncoated controls, were initially coated with a multilayer of crosslinked fibrinogen (thickness 40–50 nm). Bisphosphonates, pamidronate or zoledronate were then immobilized into and on this protein film.

2.1 Screw preparation

SS screws (medical quality, 316L), 1.7 mm in diameter (type M 1.7) and 3 mm in length were used in this model. The screw design has been used in several previous implant fixation studies [21, 26, 27]. Solution concentrations and rinsing protocol was adapted from earlier work by Tengvall et al. [17]. All screws were initially etched for 20 min in 40% HF, in order to create a micro-porous surface and then rinsed extensively in distilled water. Water was exchanged for an organic solution through stepwise rinsing in ethanol, acetone and xylene. Screw silanization was performed in xylene with 1% aminopropyltriethoxysilane (APTES, ABCR, Karlsruhe, Germany) for 5 min. The screws were rinsed in xylene and dried and then incubated for 5 min in PBS solution, pH 9, with 6% glutardialdehyde (GA, Sigma-Aldrich, USA). The screws were after rinsing in distilled water incubated in a PBS solution, pH 5.5, containing 10 mg/ml human plasminogen free fibrinogen (Haemochrom Diagnostica, Sweden) for 1 min, extensively rinsed in PBS, pH 5.5, and incubated during 1 min in PBS, pH 5.5, containing 37.5 mg/ml EDC and 5.75 mg/ml NHS (Sigma-Aldrich, USA). The screws were then again

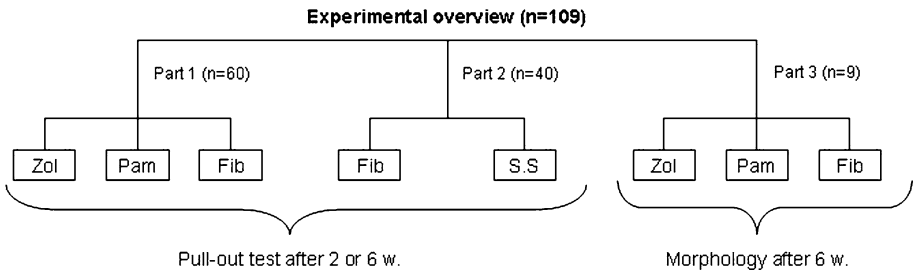

Fig. 1 Experimental setup. The three experiments that makes up the current study with number of animals in each experiment shown

rinsed in PBS, pH 5.5, incubated in the fibrinogen solution, rinsed in PBS and incubated in the EDC/NHS solution. This procedure was repeated five times until the ellipsometric crosslinked fibrinogen layer thickness was approximately 40–50 nm. The coating procedure is illustrated in Fig. 2.

Pamidronate (1 mg/ml, Toronto Research Chemicals Inc, Canada) was dissolved in PBS, pH 5.5, containing EDC and NHS (37.5, 5.75 mg/ml, respectively) [17]. The fibrinogen precoated screws were incubated in this solution for 2 h, then extensively rinsed in distilled water and dried in flowing nitrogen. Pamidronate was immobilized via its amino group to EDC/NHS activated carboxyl groups on fibrinogen, as was described earlier [17]. Zoledronate (15 mg/ml, Toronto Research Chemicals Inc, Canada) was dissolved in 0.1 M imidazole (MERCK KGaA, Germany), pH 6, containing 15 mg/ml EDC. Zoledronate was immobilized via one of its phosphate groups to a fibrinogen amine group. By forming a reactive phosphorimidazole intermediate it rapidly couples to amine containing molecules such as the fibrinogen layer on the screws (Fig. 3) [28]. The fibrinogen precoated screws were incubated for 2 h in this solution, followed by extensive rinsing in distilled water and drying in flowing nitrogen.

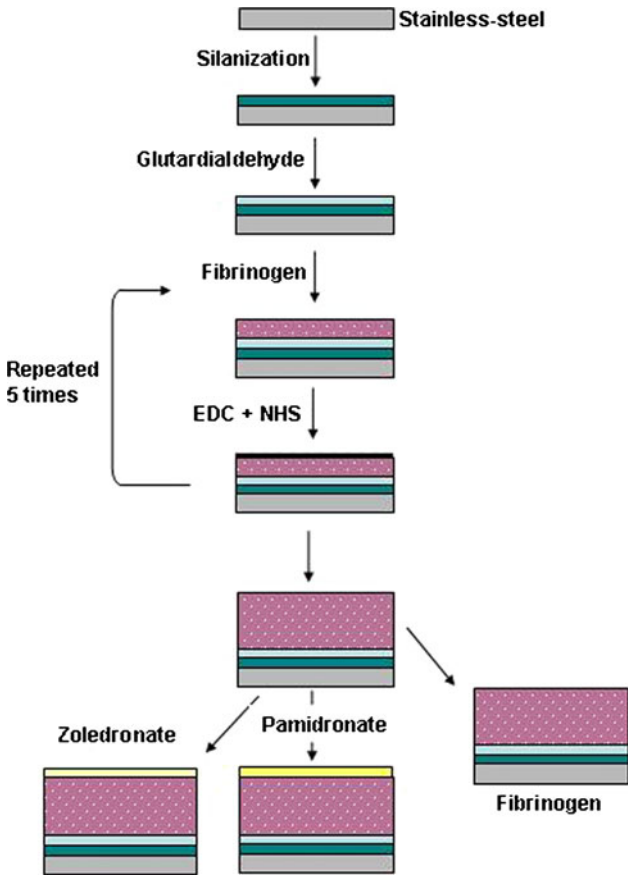

Fig. 2 Schematic description of the screw coating procedure. Layer thicknesses not to scale

2.2 Measurements of fibrinogen film and bisphosphonate thicknesses

The organic film thicknesses were measured with a null ellipsometer (Auto-El-III; Rudolph Research, Hackettstown, NJ, USA).

Ellipsometry is an optical method often used to measure the thickness of thin films adsorbed to flat surfaces. It is not possible to conduct such measurements on the uneven curved surface of the screw. Therefore, we used measurements on flat surfaces as an estimate of the amounts immobilized on the screws. This provides an approximation of the amount of bisphosphonate on the screw surface. However, because the surface area of the screw is much greater than a corresponding flat silicon surface, the film thickness on the screw is probably underestimated.

The measurements were made on 20 Si sample pieces (1 cm × 1 cm), which were all treated according to the above protocol, except that the Si surfaces were not etched in HF. A crosslinked fibrinogen layer was prepared on the surfaces. Ten of the surfaces were incubated in a pamidronate solution and ten in a zoledronate solution (see Fig. 2). The organic layer thicknesses were calculated according to the McCrackin evaluation algorithm [29], and converted into an approximate adsorbed amount per unit area using de Feijter's formula [30]. The assumed refractive index of the protein and bisphosphonate films were $n_f = 1.465$ [31]. During the measurements, 1 nm ellipsometric thickness approximated a surface mass concentration of 0.12 μg/cm^2.

2.3 Animal experiments and surgical procedure

A total of 109 male Sprague–Dawley rats with a mean weight of 366 ± 28 g (8–10 weeks old) were used. The rats were housed three per cage at 21°C in a 12 h light and 12 h dark cycle and were given free access to food and water. The study was approved by the Regional Ethics Committee for Animal Experiments. Institutional guidelines for the care and treatment of laboratory animals were followed.

The rats were anaesthetized with isoflurane gas, antibiotics and analgesics were given subcutaneously, pre- and postoperatively. The surgery was performed under aseptic conditions. A 5–6 mm longitudinal incision was made along the right tibia. The periosteum was reflected proximally to the growth plate. A 1.2 mm hole was drilled through one of the cortices, approximately 3 mm from the growth plate, using a hand held drill. Finally, one screw per animal was inserted and the skin sutured. The investigator was blinded for screw coatings during the surgery.

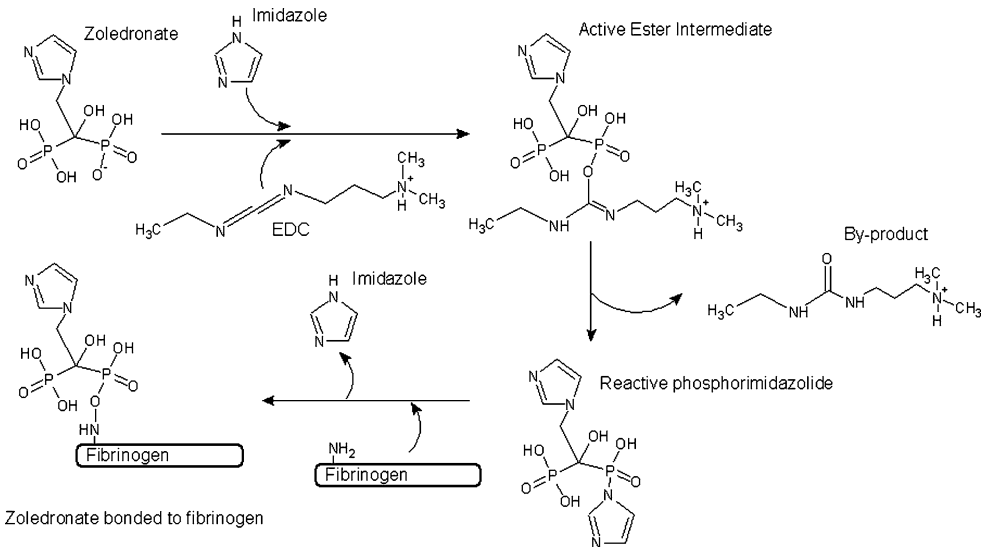

Fig. 3 Theoretical reaction pathway of Zoledronate bonding to fibrinogen, adapted from reference [28]

2.4 Biomechanical analysis

The pull-out analyses were performed while blinded. The rats from the first and second part of the study were killed either 2 or 6 weeks after surgery, and the tibia harvested. The screws were tested for pull-out strength in a materials testing machine (100R, Admet, Norwood, MA, USA). The tibias were mounted in the machine with the head of the screw pointing out of a hole (3.5 mm in diameter) in a metal plate. The head of the screw was fixed by a metal pin, passing through a hole in the screw head and a horseshoe shaped connector (Fig. 4). When mounted, the screw were pulled out from the bone until failure at a constant speed of 0.1 mm/s. Peak force and energy uptake were calculated by the software of the testing machine (MtestW 5.0.1. Admet, Norwood, MA, USA). The stiffness was calculated as the slope of the linear part in the elastic phase of the force/distance curve, as marked by the investigator. We consider the peak force to be the value of most importance to determine screw fixation.

2.5 Morphometric analysis

The nine rats from the third part of the study were killed 6 weeks after surgery. The proximal section of the tibia with the screw still in place were fixed in formaldehyde, sequentially dehydrated in increasing concentrations of ethanol and then embedded in polymethylmethacrylate (Technovit 9100 embedding system; Heraeus Kulzer, Wehrheim, Germany). The embedded bones were sawed parallel to the long axis of the screw and the tibial shaft, generating two sections showing the cross section of the screw and the area surrounding it. Images of the sections were obtained by using a scanning electron microscope (SEM Leo, Carl Zeiss, Oberkuchen, Germany) operating in backscatter mode with an accelerating voltage of 20 kV. The sawed sections were coated with a thin layer of gold (~20 nm) to prevent charging. One image was taken per specimen. Three blinded investigators independently tried to sort the images into three groups based on the amount of bone surrounding the screw.

Fig. 4 Experimental set-up for pull-out analyses. Tibia mounted in the materials testing machine. *Black arrow* points at the inserted screw

For bone density measurements, the images were converted to grey scale and analyzed using Image J imaging software (Rasband, W.S., Image J, U. S. National Institutes of Health, Bethesda, MD, USA). Thresholds were set and areas of mineralized bone were detected using prespecified grey scale levels. A region of interest was defined as 1400 μm along the long axis of the screw, excluding the intracortical portion and the tip. Perpendicular to this axis, the region extended 500 μm away from the outermost part of the screw threads, on both sides of the screw. The screw area was subtracted and then the imaging software calculated the area occupied by mineralized bone compared to the total area of the region of interest.

2.6 Statistics

Biomechanical data were analyzed by two-way Anova, using time and surface treatments as independent variables and with maximum force, stiffness and energy as the observed parameters. Peak force was regarded as the primary outcome variable. Multiple comparisons between treatment groups were done using Scheffe's post hoc test. Differences in variance between groups were checked with Levene's test. Because the variance in the groups increased with the mean value in the first part of the study, data were ln-transformed before analysis. The results were considered significant at the 5% level. Bone density data and data from blinded rating were analyzed by Kruskal–Wallis nonparametric test, followed by Mann–Whitney test for intergroup comparisons. Data were evaluated using SPSS for Windows, Rel. 15.0.0. 2006 (SPSS Inc.,Chicago).

3 Results

3.1 Ellipsometry

The ellipsometric thickness of the crosslinked fibrinogen layer was approximately 45 ± 5 nm. The pamidronate layer thickness was approximately 2.3 ± 0.4 nm and the zoledronate layer thickness 0.9 ± 1.4 nm, corresponding to approximately 279 ng/cm^2 of pamidronate and 108 ng/cm^2 of zoledronate.

3.2 Biomechanical analysis

Five animals were excluded from the first part of the study. Three died due to surgical complications. Two were excluded due to technical problems during testing. In the second part, two rats were excluded due to technical problems during testing. All exclusions were made while blinded.

In the first experimental series, both implantation time and surface treatments affected the three evaluated biomechanical parameters (Table 1, $P < 0.01$). A tendency was observed towards increasing screw stability by bisphosphonates with time although no significant increase was observed when ln-transformed values were used.

Importantly, both types of bisphosphonate coated screws showed a stronger implant fixation than controls without bisphosphonate. The zoledronate peak force and energy were also significantly higher than those observed for pamidronate (Table 2, Fig. 5). Bisphosphonate treatments increased the standard deviations within the 2 and 6 week experiments, respectively. In the second experimental series we evaluated whether fibrinogen-only coated screws differed from non-coated SS screws, but no such difference was found (Tables 3, 4; Fig. 6).

3.3 Morphometric analysis

The three images classified as surrounded by the most bone were all zoledronate-coated screws. The three images classified as surrounded by the least bone were all fibrinogen coated screws. The images of the pamidronate coated screws were all classified as in between ($P = 0.03$ for all groups). This rating was repeated independently by two other investigators, who all came to the same result.

The bone density measurements demonstrated that zoledronate markedly increased the bone density around the

Table 1 Comparison of bisphosphonates and controls (fibrinogen), mechanical data for the different treatment groups and implantation time points

Group	Time (weeks)	Number (*n*)	Peak force (N)		Stiffness (N/mm)		Energy (Nmm)	
			Mean	SD	Mean	SD	Mean	SD
Fibrinogen	2	9	53	12	125	34	21	5
Pamidronate	2	10	60	8	125	28	23	5
Zoledronate	2	9	72	12	135	40	29	7
Fibrinogen	6	8	72	11	123	50	30	5
Pamidronate	6	10	102	27	196	57	36	12
Zoledronate	6	9	128	34	228	62	56	21

Table 2 Pairwise comparison between treatment groups at both time points

Compared groups	95% Confidence interval			*P*-value
	Lower (%)	Mean (%)	Upper (%)	
Peak force (N)				
Pamidronate–Fibrinogen	6	27	53	0.007
Zoledronate–Fibrinogen	28	54	86	0.000
Zoledronate–Pamidronate	1	21	45	0.032
Stiffness (N/mm)				
Pamidronate–Fibrinogen	−3	25	61	0.098
Zoledronate–Fibrinogen	7	39	80	0.009
Zoledronate–Pamidronate	−13	12	43	0.543
Energy (Nmm)				
Pamidronate–Fibrinogen	−8	17	48	0.276
Zoledronate–Fibrinogen	24	59	102	0.000
Zoledronate–Pamidronate	8	36	72	0.007

The confidence interval describes the percent increase of the mean of the first group compared to the second. Scheffe's Post-hoc test used for *P* values

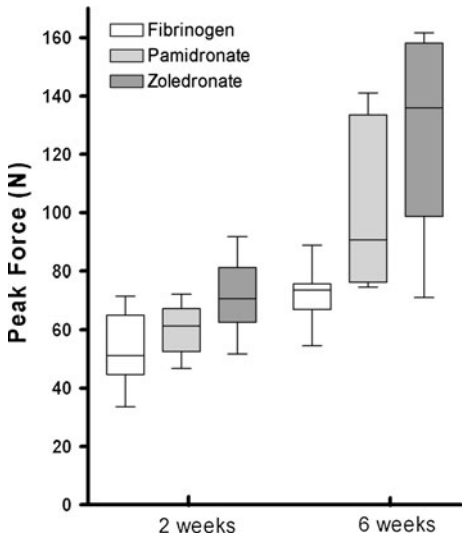

Fig. 5 Peak forces at screw removal, 2 and 6 weeks after implantation in rat tibia. Scheffe post-hoc test following the Anova demonstrated that all three treatment groups differed significantly from each other

screws in comparison to both pamidronate and fibrinogen coatings (Fig. 7). Pamidronate also had a positive effect on the bone density compared to fibrinogen, but the effect was less pronounced. All intergroup comparisons showed statistically significant differences ($P = 0.05$ for all).

Table 4 Pairwise comparison between fibrinogen and steel groups at both time points

	Compared groups	95% Confidence interval		*P*-value
		Lower (%)	Upper (%)	
Force	Fibrinogen–Stainless steel	−7	17	0.39
Stiffness	Fibrinogen–Stainless steel	−38	22	0.58
Energy	Fibrinogen–Stainless steel	−3	8	0.30

The confidence interval describes the percent increase of mean of the first group compared to the second. Both time points are analyzed together

4 Discussion

By immobilizing zoledronate to a fibrinogen coated stainless steel implant we observed a significantly increased mechanical implant fixation after two and six weeks of implantation. The zoledronate coating increased the pull-out force by 28–86% compared to controls. Zoledronate also

Table 3 Comparison of control treatment (fibrinogen) and no treatment (stainless steel). Mechanical data at two and six weeks of implantation

Group	Time (weeks)	Number (*n*)	Peak force (N)		Stiffness (N/mm)		Energy (Nmm)	
			Mean	SD	Mean	SD	Mean	SD
Fibrinogen	2	9	39	5	89	24	16	3
Stainless steel	2	10	46	10	87	35	19	4
Fibrinogen	6	9	93	14	177	39	34	6
Stainless steel	6	10	79	16	190	18	27	6

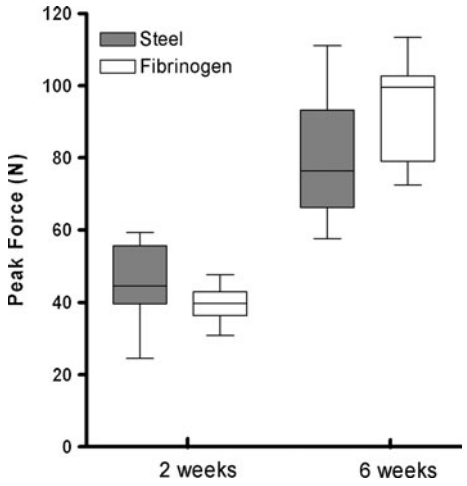

Fig. 6 Peak force at screw removal, 2 and 6 weeks after implantation in rat tibia. A comparison between fibrinogen coated stainless steel screws and uncoated stainless steel screws. No significant differences were detected

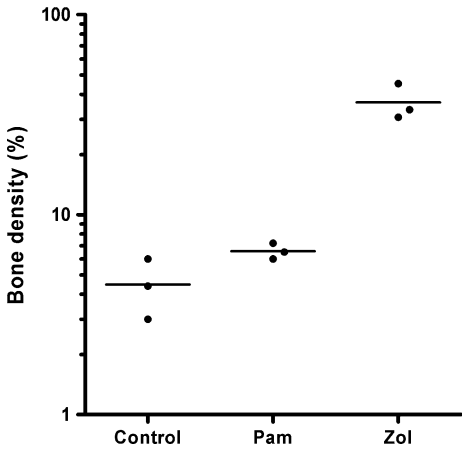

Fig. 7 Bone density around screws as determined by image analysis. Zoledronate-coated screws had a much higher bone density than screws coated with pamidronate or fibrinogen only

showed a superior fixation in comparison to pamidronate. The biomechanical data correlates well with the morphological results showing an increased bone density around zoledronate coated screws compared to both pamidronate and fibrinogen coated, 6 weeks after implantation. Importantly, the biomechanical values for fibrinogen coated screws did not differ from those of bare SS screws. This implies that surface composition and chemistry did not influence the mechanical fixation, i.e. the pull-out force in this model appeared relatively independent of the underlying immobilization matrix properties.

Several bisphosphonate studies have shown an improved implant fixation to bone [14–19]. The results in the present study agree with and confirm these findings. In earlier studies, we introduced the concept of a thin protein film as the immobilization matrix [17, 21, 27]. In these studies we used EDC/NHS techniques for the immobilization of bisphosphonates with a tail amino group. However, zoledronate lacks a primary amino group that is the requirement for the use of EDC/NHS immobilization chemistry, and therefore this chemistry can not be used. Also, previous experiments showed that a simple physical bisphosphonate adsorption to SS screws was not sufficient for increasing the mechanical fixation in vivo [21]. Hence, by use of EDC/imidazole instead for the immobilization of zoledronate we are now able to broaden the concept of bisphosphonate immobilization in bone applications. The results from the present animal experiments, in combination with ellipsometry data imply that the immobilization techniques worked well.

Previous measurements in our laboratory confirmed that the immobilized amounts of alendronate, measured as increase in the ellipsometric thicknesses, correspond well with amounts of radioactively labelled alendronate [32, 33]. Because of similarities in molecular size, the relation between ellipsometric thickness and amounts should be roughly similar for different bisphosphonates. The approximate amount of pamidronate in the present study was 280 ng/cm^2 corresponding to about 150 ng per screw [32], and yet this minute amount was sufficient to improve fixation. Assuming the ellipsometric readings are valid also for zoledronate, we managed to immobilize approximately 1/3 of the amount of pamidronate. However, zoledronate is a much more potent bisphosphonate than pamidronate [9], and this likely explains the increased positive effect of zoledronate compared to pamidronate. In a previous study using a fibrinogen immobilization matrix and ^{14}C-alendronate, 60% of the immobilized bisphosphonate was released after 8 h, but the release continued slowly for up to 8 days [33]. Unfortunately we do not have access to ^{14}C labelled zoledronate so the release kinetics for zoledronate cannot be studied in the same fashion. However, the release kinetics is likely not a critical issue here, as bisphosphonates possess high affinity to bone mineral and stay locally for long time. During osteoclastic bone resorption, bisphosphonates can be released, but they are likely to reattach to nearby bone surfaces again. This local recycling makes bisphosphonates exceptionally useful for localized applications, such as implant surfaces.

To our knowledge, only two other techniques have been described for the immobilization of zoledronate to implant surfaces with subsequent testings in vivo. In one study, zoledronate was incorporated in a biodegradable coating of poly(D,L-lactide; PDLLA) [19]. The implants, containing about 20 μg zoledronate in each coating were subsequently used as intramedullary nails for the stabilisation of a rat tibial midshaft fracture. After 6–12 weeks, the fractured bone was removed and the fracture callus evaluated radiographically and biomechanically. By using zoledronate,

there was a significant improvement of both mechanical parameters and radiographic appearance [19]. In other studies, varying amounts of zoledronate was adsorbed directly onto hydroxyapatite precoated titanium implants, and implanted into the distal femur of rats [15]. The femurs were harvested after 3 weeks for histomorphometric and pull-out analyses. The incorporation of 2 μg of zoledronate increased the pull-out force by 42%, and increased the peri-implant bone density [15]. Positive results were also found in a canine model, where bone ingrowth into hydroxyapatite coated porous tantalum implants increased upon the adsorption of zoledronate [18]. In a recent publication, the effects on bone formation after adsorption of different bisphosphonates to hydroxyapatite coated titanium were compared [25]. Zoledronate, pamidronate or ibandronate coated implants were inserted into the tibial medullary canal of ovariectomized rats, and compared to untreated controls. The results after three months of implantation showed increased implant fixation with zoledronate coatings compared to controls, but also compared to ibandronate and pamidronate. Histology, biomechanical testing and μ-CT images revealed that all bisphosphonates improved the bone-implant integration and early bone formation. Zoledronate showed the most striking effect [25], and supports our observation that tiny amounts of immobilized zoledronate improve implant fixation.

Why not always utilize apatite coated bone implants and prior to insertion adsorb bisphosphonate onto them? In some orthopaedic settings, a hydroxyapatite coating may not be beneficial. For instance, when high friction with a hydroxyapatite coating may preclude screw removal and bisphosphonate mediated enhancement of fixation is desirable, the present fibrinogen coating offers an alternative. For example, due to the formation of a mechanically stabilizing bone "sleeve" [27], bisphosphonate immobilization onto a non-CaP containing matrix may decrease the risk of screw loosening (cut out) in fracture fixation of osteoporotic cancellous bone. Hydroxyapatite coatings lead to structural integration between bone and the implant surface. However, this does not necessarily imply a better fixation, because the fixation of a screw depends on the strength of the surrounding bone at a certain distance from the surface, rather than surface friction. Together with the previously used EDC/NHS techniques, we are now able to immobilize several types of bisphosphonates to crosslinked fibrinogen and other protein coatings. One limitation in the present model is, however, the small size of the screws, meaning that improved bone quality less than a mm away from the implant could dramatically improve its fixation. In order to improve an arbitrary screw fixation it is likely that the surrounding bone density should be enhanced at distances corresponding to the depth of the thread or more. With a larger screw, therefore, this should be obtained outside bisphosphonate coated threads at a larger distance than observed in the present study. This may be more difficult to achieve, but remains to elucidate.

5 Conclusions

Zoledronate and pamidronate were immobilized to crosslinked-fibrinogen on SS screws by the use of EDC/imidazole and EDC/NHS chemistries, respectively. Biomechanical pull-out tests showed that bisphosphonates improved implant fixation at 2 and 6 weeks in rat tibia. Furthermore, zoledronate showed a significantly better fixation than pamidronate, and no significant differences in pullout force were found between bare SS and SS with a crosslinked fibrinogen coating. Bone density around the screws was increased by both types of bisphosphonate coating compared to the fibrinogen coating. In correlation with the biomechanical results, zoledronate generated the greatest increase in density. It appears that tiny amounts of immobilized bisphosphonates, at the order of a few hundreds of nanograms, are sufficient to improve implant fixation to bone.

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