Original Report

Pain Management After Bone Reconstruction Surgery Using an Analgesic Bone Cement: A Functional Noninvasive In Vivo Study Using Gait Analysis

Manon Dupleichs,[*,†] Martial Masson,[†,§,‖] Olivier Gauthier,[†,‡] Maeva Dutilleul,[†,§,‖] Jean-Michel Bouler,[*] Elise Verron,[*,†,1] and Pascal Janvier[*,1]

[*]CEISAM, CNRS UMR 6230, University of Nantes, Nantes, France, [†]RMeS-lab, INSERM UMR 1229, University of Nantes, Nantes, France, [‡]ONIRIS, Nantes Atlantic College of Veterinary Medicine, Food Science and Engineering, Nantes, France, [§]INSERM, UMS 016, CNRS 3556, Structure Fédérale de Recherche François Bonamy, SC3M facility, CHU Nantes, Université de Nantes, Nantes, France, [‖]Université de Nantes, UFR Odontologie, Nantes, F-44042, France.

Abstract: Postoperative pain after bone reconstruction is a serious complication that could jeopardize the global success of a surgery. This pain must be controlled and minimized during the first 3 to 4 postoperative days to prevent it from becoming chronic. In this study, a critical-size bone defect was created at the femoral distal end of rats and filled by an injectable calcium phosphate cement (CPC) loaded or not with local anesthetics (bupivacaine or ropivacaine). A functional evaluation of the gait was performed using the CatWalk system to compare the postoperative pain relief enhanced by the different CPCs after such a bone filling surgery. The results demonstrated significant pain relief during the short-term postoperative period, as shown by the print area and intensity parameters of the operated paw. At 24 hours, the print area decreased by 65%, 42%, and 24%, and the intensity decreased by 25%, 9%, and 1% for unloaded, ropivacaine-loaded, and bupivacaine-loaded CPCs, respectively, compared with the preoperative values. Bupivacaine-loaded CPC provided an earlier return to full functional recovery than ropivacaine-loaded CPC. Moreover, the CPCs retained their biologic and mechanical properties. For all these reasons, anesthetic-loaded CPCs could be part of the global pain management protocol after bone reconstruction surgery such as iliac crest bone grafting procedures.

Perspective: Bupivacaine-loaded CPC provided an earlier return to full gait function than ropivacaine-loaded CPC, with preserved bone filling properties. Such analgesic CPCs deserve further in vivo investigation and may be part of the global pain management protocol after bone reconstruction or bone augmentation surgery such as iliac crest bone grafting.

© 2018 The American Pain Society. Published by Elsevier Inc. All rights reserved.
Key words: Bone pain, calcium phosphate cement, drug-device combination, local anesthetic drug, CatWalk gait analysis

Postoperative pain after bone reconstruction is one of the most frequent undesirable complications, especially in bone iliac crest graft procedures.[15,24,28,36,42,54] This pain significantly disrupts patient recovery by reducing their mobility, delaying their functional recovery, increasing their hospital stay, and decreasing their quality of life and autonomy.[37,53] Furthermore, recent data suggest that chronic pain could induce changes at the immune system level by modifying immune cell gene expression.[29] This could point to a potential connection between the pain and discomfort felt and the inflammatory/healing process after bone surgery.

Pain management during the first 4 postoperative days[5,41,44] should be as efficient as possible to minimize the risk of developing chronic pain. The conventional treatment is to administer conventional systemic analgesics such as acetaminophen, nonsteroidal antiinflammatory drugs, and morphine-derived products. Paracetamol

is definitely not strong enough to relieve postoperative pain, and nonsteroidal antiinflammatory drugs can cause gastric complications[40] and delay bone healing.[21] Finally, opioids can cause multiple complications (gastric, pulmonary) that could hinder recovery and increase hospitalization length. Furthermore, analgesic prescriptions increase overall costs, thus adding an economic burden on the health care system. In addition to the systemic approach, patients are also administered local anesthetics as a bolus dose or via catheter. Human medicine surgeons often use continuous controlled administration of local anesthetics at surgical sites or around nerves that innervate the site. Local administration provides analgesia at the target site with limited systemic effects, and some studies have demonstrated the value of using local analgesia to prevent chronic pain.[3,8,43] For example, infusion of bupivacaine, either into the wound or as a local nerve block proved to be an effective analgesic technique for pain control during the recovery phase after iliac crest bone grafting. This reduced the use of opioids by 50% without decreasing the analgesic effect,[43] a finding that is of major interest in elderly patients.

Calcium phosphate (CaP) bone substitutes and CaP cements (CPC) are now considered the gold standard[28] treatment for many bone filling orthopedic surgery indications. They can be resorbed by cells, present evidenced osteoconductive properties,[2,10,28,38,39] and can be associated with bioactive molecules and therapeutic agents to act as local drug delivery systems.[47,50] We previously validated the concept of analgesic bioactive bone substitute combining CaP granules and bupivacaine, one of the most commonly used local anesthetics in bone surgery. In vivo analgesic properties of our combined device demonstrated a significant dose-dependent analgesic effect on a postoperative pain rat model using pressure algometry evaluation.[48] Because CPCs have now become the treatment of choice for many orthopedic and trauma indications,[9,55] we designed an innovative injectable biomaterial combining CPC and different local anesthetic drugs for orthopedic or bone trauma indications.

In the last decade, the CatWalk[26,52] gait analysis system has been demonstrated as a valuable, noninvasive method to assess chronic pain.[16] It has proved to be a reliable method to determine the onset or ongoing nature of a disease, set up a treatment protocol, and evidence rehabilitation. The CatWalk XT (Noldus, Wageningen, The Netherlands) has been validated for experimental rodent procedures to investigate many conditions such as neurodegenerative diseases,[46] myofascial inflammations,[31] peripheral nerve injuries,[14,18] osteoarthritis,[19,32] and trauma.[20] This method analyzes gait through video tracking and delivers a complete analysis of the spatiotemporal parameters of each paw print and dynamic interlimb coordination patterns.

The aims of this study were 1) to investigate postoperative pain after bone filling surgery through functional evaluation using gait analysis with the Catwalk system, 2) to evaluate the benefit in pain relief obtained after bone defect filling with our innovative CPCs, and 3) to assess their mechanical and biologic properties.

Materials and Methods

Preparation of anesthetic-loaded cement

The apatitic calcium phosphate cement[30] used in this study was obtained from Graftys SA (Aix-en-Provence, France). It is composed primarily of alpha-tricalcium phosphate (78%) and was loaded with either bupivacaine or ropivacaine (Sigma-Aldrich, St. Louis, MO). Local anesthetics were directly introduced into the solid phase at 8% by weight (w/w). The cement paste was prepared by mixing the powder obtained with an aqueous solution of 0.5% Na_2HPO_4. The liquid/powder (l/p) ratio was adjusted to obtain an injectable cement compatible with use in bone surgery, presenting a typical setting time of around 6 to 12 minutes. A 2-cm^3 syringe was used to inject the cement paste into a rat femoral condyle defect. After the setting process, the final product was a porous solid mainly composed of calcium deficient apatite (CDA) loaded with a local anesthetic. Before implantation, all the components were sterilized by gamma irradiation at 25 to 33 kGy. The integrity of the drugs was ensured by nuclear magnetic resonance (NMR) and mass spectrometry (MS) analyses. The ^1H NMR spectra were recorded on an Advance Bruker 300 MHz (Billerica, MA) and MS spectra on an LTQ-Orbitrap Thermo Fisher Scientific (Charlotte, NC).

Cement handling properties

The initial setting time was determined by using Gillmore's method,[1] Samples were placed in an in-house−made cell (cylindrical form, inner diameter 20 mm, depth 4.7 mm) thermostated at 37C with a water bath. The initial setting time was determined when the small Gillmore needle (diameter 2.12 mm, weight 113.4 g) failed to indent the surface of the sample.

The injectability assay was measured with an Advanced Material Testing System (AmetekLS55kN/1124 lbf; Ametek, Inc, Berwyn, PA) equipped with a specific syringe system, including a 2.5-mL syringe (Terumo Corp, Shibuya, Tokyo, Japan) without a needle. This test consists of measuring the force needed to extrude the volume of paste using a compression rate of 1 mm/sec. The injectability measurement was performed 3 minutes after paste preparation.

The compressive strength was measured with an Advanced Material Testing System (AmetekLS55kN/1124 lbf) at a loading rate of 1 mm/sec. The different paste samples were prepared as described above. They were then introduced into cylindrical polytetrafluoroethylene molds (6 mm in diameter and 12 mm in height) and were immersed in sodium chloride aqueous solution for 72 hours. All measurements were performed in triplicate, and results were mean $\pm$ SD.

Anesthetic drug release profiles

Two hundred milligrams of loaded cement were deposited into a six-well culture plate. After waiting 20 minutes at room temperature, cements were immersed

in sodium chloride aqueous solution (0.9% w/w) and were subsequently placed into an incubator at 37C equipped with an orbital shaker (120 rpm). Aqueous solutions were removed, filtered (0.45 μm), and replaced with fresh 0.9% sodium chloride at different times. After filtering, the amount of bupivacaine or ropivacaine released into the aqueous solution was determined by UV-visible spectrophotometer assays (UV-2501PC; Shimadzu Corp, Kyoto, Japan) at 262 nm. All experiments were performed in triplicate. Results were expressed as percentage of bupivacaine or ropivacaine released $\pm$ SD over time.

Animal experiments and surgical model

Animals

All animal procedures were approved by the institution's animal welfare committee (Approval No. 5270) and were conducted in accordance with the European guidelines for the protection of animals used for scientific purposes (2010/63/EU). Rats were housed in dedicated rodent facilities (Approval No. C44015). Eighteen female Wistar rats (Charles River Laboratories), weighing 225 to 250 g, were used. A 1-week quarantine was observed. During the experiment period, animals were housed under standard conditions with a 12-h light/12-h dark cycle and checked every day. Water and food were available as desired.

Surgical procedure

Three different CPCs were tested:

- One unloaded CPC with no anesthetic agent (CPC)
- One CPC loaded with 8% bupivacaine (CPC-Bupi)
- One CPC loaded with 8% ropivacaine (CPC-Ropi)

The animals (n = 18) were then randomly assigned to one of these groups (n = 6 per group).

Surgical procedures were conducted with the animals under general anesthesia induced with an isoflurane/oxygen mixture delivered into an induction chamber and maintained through an individual nose mask. Then a subcutaneous injection of 0.05 mg/kg of buprenorphine hydrochloride (Buprecare; The Cooper Companies, Inc, Pleasanton, CA) was performed as preemptive analgesia. A critical-size cylindrical bone defect (3.1 mm

$\emptyset$, 5 mm depth) was created in the lateral femoral condyle on the right hind limb of each animal. After hemostasis and drying of the osseous cavity, the defect was filled with one of the tested cements that was gently injected into the cavity (Fig 1). The contralateral leg was left intact. All surgical procedures were performed blindly (ie, the surgeon was unaware of which cement was being injected). Rescue analgesia with additional buprenorphine injection was available throughout the implantation period.

After surgery, a physical examination was performed twice daily with visual control of the surgical site and measurements of cranial-caudal and lateral-medial diameters to evaluate whether hematoma, edema, swelling or biomaterial intolerance occurred. If necessary, an additional injection of buprenorphine hydrochloride (0.05 mg/kg) was given.

Animals were humanely killed 3 weeks after implantation by intracardiac injection of an overdose of sodium pentobarbital (Dolethal; Vetoquinol, Fort Worth, TX) after general anesthesia induction.

Postoperative gait analysis with CatWalk system

Principle of the CatWalk analysis

Postoperative gait analysis was performed using the CatWalk XT system as previously described in rats.[25,27] Briefly, the rat had a nonforced run across an illuminated glass plate through an 8-cm wide corridor. Upon contact with the glass plate, the paw reflected the green light to a video camera below (100 Hz), which recorded the entire run (Fig 2A). Gait-related parameters were reported for each animal and each run. No food inducement was provided.

Test procedures

The gait analysis was conducted according to the following protocol:

Two weeks prior to the implantations, the rats were habituated and trained to run freely through the corridor daily. The last preoperative run, the day before surgery, was considered as the preoperative reference value. To limit interanimal variability, each operated rat was compared with its own preoperative data. CatWalk analysis was repeated after surgery at t = 2, 4, 6,

Figure 1. Implantation of cement. (A) Creation of a critical-size cylindrical defect. (B) Injection of the cement. (C) Filled defect before wound closure.
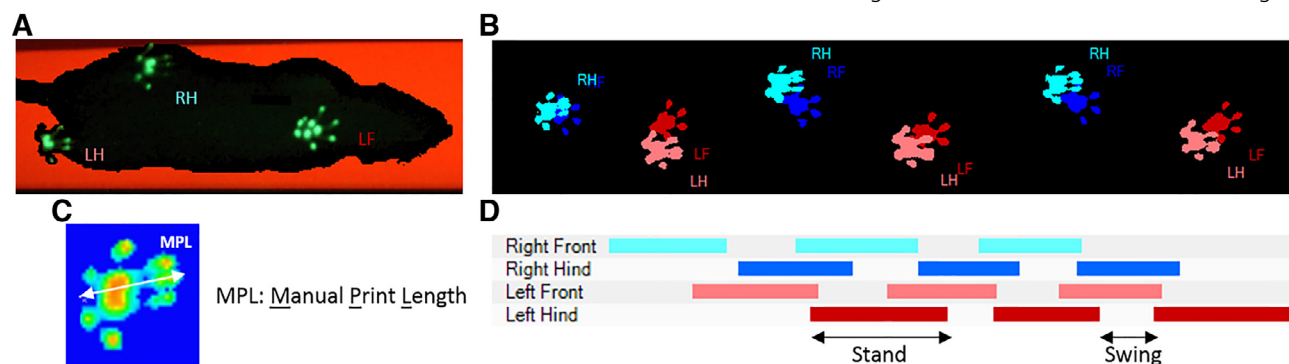
Figure 2. Gait analysis of a healthy rat by the CatWalk system. (A) Picture of the healthy rat in the Catwalk system. (B) CatWalk footprints (print area) before surgery. The footprints on the left limbs are in red while the right limbs are in blue. The hind limbs are represented in light color, and the front limbs are darker. (C) A normal right hind paw with manual print length measurement and intensity scale. (D) CatWalk gait diagram before surgery.

24, 48, and 72 hours and t = 7 and 14 days. Gait analysis experiments were conducted blindly by the same investigator who was unaware of which cement had been injected. The time of biomaterial injection was referred as t = day 0.

Parameters

Gait parameters were acquired with the CatWalk 42-cm-long corridor. The compliant run criteria were 1) a run duration between 1 and 5 seconds and 2) a maximum allowed speed variation of 80%. Runs longer than 5.5 seconds were automatically aborted. Runs with backing, sniffing, cleaning, or long stops were also discarded during the acquisition phase by the investigator. Analysis of recorded runs provides 280 raw measurements. A minimum of 5 compliant runs was necessary for statistical analysis. In most cases, the first 5 or 6 runs produced the 5 compliant runs. The number of runs was limited to avoid exhausting the rats.

Based on the expected properties of the CPCs tested, the following parameters were studied:

- Print area (in cm^2) is the surface of the complete paw print during the stance phase. The stand phase (in seconds) is the duration of the contact between the paw and the glass plate (Fig 2B).
- Intensity (AU) is the mean intensity of the complete paw during the stand phase in the whole step cycle. The print intensity depends on the degree of contact between a paw and the glass plate. The intensity ranges from 0 to 255 and is converted to a color scale from green to red (Fig 2C).
- Manual print length (in cm) is the distance between the middle of the third toe and the heel of a paw during stance (Fig 2C).
- Duty cycle (in %) expresses the stance phase as a percentage of the step cycle. It is calculated according to the formula: stance/[stance + swing] × 100, where the stance is the duration of the contact between the paw and the glass plate and the swing is the duration in seconds of the no-contact phase of a paw with the glass plate (Fig 2D).

Variations in these parameters provided functional data related to the analgesic effects of the implanted cements and the expected benefits in pain relief.

Standard error of the mean (SEM) and histologic studies

Sample preparation

Once explanted, the distal ends of the implanted femurs were fixed in formalin. After this, the bone specimens (n = 18) were dehydrated with graded ethanol. They were then infiltrated and embedded in glycol methyl-methacrylate (GMMA, VWR International SAS, Fontenay sous Bois, France) obtained by mixing 90% purified methyl-methacrylate (VWR, Radnor, PA), 9% polyethylene glycol (VWR) and 1% benzoyl peroxide (Merck, Kenilworth, NJ). Polymerization was started with a mixture of N,N-dimethylaniline, 99% (Sigma-Aldrich) and propan-2-ol (Prolabo, France) at 4̄C for 1 week. The resulting resin blocks were then polished and cut into 2 pieces: one half of each sample was used for either SEM or histologic analysis.

SEM analysis

After polishing, the remaining bone-implant surfaces were coated with gold-palladium and femoral samples were observed by SEM (Leo 1450VP, Zeiss, magnification x50). Images were acquired with the back-scattered electron mode.[51] A 2-dimensional micro-architectural analysis was performed using Leica QWin Pro software. A bioactive zone (region of interest) was drawn manually in a ring-shape on the interface between the resorbed surface of the CPC and the concomitant new bone (Fig 3). The ring included the reactive part of the cement, which was seen as lighter compared to the inactive part, and the newly formed bone, which was seen as slightly darker gray compared with the old bone around it. The percentages of the newly formed bone surface in the total ring surface were then calculated after a manual grayscale thresholding for the elimination of pores from the surface calculus. The values were the then recalculated from square pixels to square micrometers.

Histologic studies

Undecalcified serial 7-μm sections of each sample were cut perpendicularly to the drilling axis of the
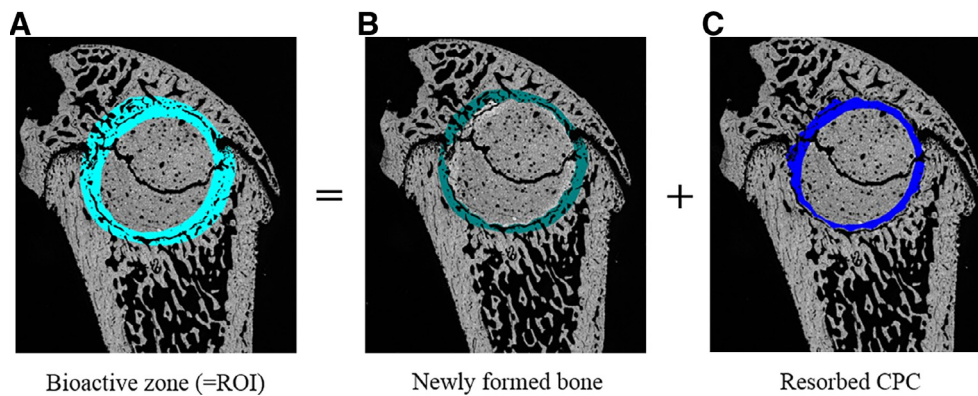
Figure 3. SEM images after femoral implantation. The ROI analyzed using Leica QWin Pro software corresponded to (A) a bioactive zone (lighter-blue area), which included (B) the newly formed bone (green area) and (C) the resorbed surface of the cement (blue area).

implantation area using a hard tissue microtome (Leica SM2500; Leica Biosystems Inc, Buffalo Grove, IL) equipped with a D-profile tungsten-carbide knife (Leïca). Movat's pentachrome and hematoxylin-eosin stains were performed on bone sections, which were then observed under a light microscope (Axioplan 2; ZEISS, Oberkochen, Germany). The Movat's pentachrome stain is bone-tissue specific and was used to distinguish mineral bone (yellow-green), osteoid tissue (red lines), soft tissue (light pink), and cement (blue).[49,51] The hematoxylin-eosin stain was used as a reference to analyze more specific tissue components. Each slice was scanned and observed using NDP.view2 software (Hamamatsu, Shizuoka Prefecture, Japan) for histological analysis.

Statistical analysis

For each animal, the mean ($\pm$ SD) of the 5 compliant runs was calculated for the 4 selected parameters: print area, intensity, duty cycle, and manual print length. Mean values were then calculated for unloaded and loaded cements. Unloaded CPC and anesthetic-loaded CPC gait parameters were compared using analysis of variance (ANOVA) tests. When significant effects were found, the Fisher test was used for inter-groups comparisons. Each time we observed a significant difference between 2 groups, the associated F-test was checked to be positive. The threshold for significance was set at 99% ($P < .01$). SEM images were analyzed using the Kruskal-Wallis Test.

Results

Influence of anesthetics on cement properties

The addition of anesthetics did not significantly modify the setting reaction of the different cements. For the CPC-Bupi, the setting time was identical to the CPC (6.5−7.0 min range) without any change in the l/p ratio. Conversely, the setting time was shorter for the CPC-Ropi (4.0−4.5 min). The l/p ratio was then adjusted from 0.45 to 0.48 in order to have a similar setting time to the other cements. The new setting time was in the 6.0- to

6.5-minute range. Injectability was not significantly different among the 3 different cements (4.5 $\pm$ 1.5 N for the CPC and 5.2 $\pm$ 0.2 N and 4.3 $\pm$ 1.1 N for the CPC-Bupi and the CPC-Ropi, respectively). The association of both bupivacaine and ropivacaine led to a significant decrease in compressive strength from 25.5$\pm$1.2 MPa for CPC to 18.4 $\pm$ 0.1 MPa for the CPC-Bupi and 16.3 $\pm$ 1.6 MPa for the CPC-Ropi. For the CPC-Ropi, this was the direct consequence of a greater l/p ratio.

Anesthetic release kinetics

The linear correlation coefficient for UV measurement of both anesthetics was .999 at 262 nm. The release of drugs (Fig 4) was studied over several days (up to 11 days). First, an initial burst of approximately 44% and 30% of bupivacaine and ropivacaine, respectively, was released within the first hours (8 hours = .33 days). During the first 4 days, the CPC-Bupi released 72% of the drug, whereas the CPC-Ropi released 64% of the drug over the same period. At the end of the test (11 days), the cumulative drug release was about 74% and 70% for bupivacaine and ropivacaine, respectively.

Integrity of the analgesic drugs

The NMR (Supplementary Fig 1) and MS (Supplementary Fig 2) spectra indicated that the integrity of the released bupivacaine and ropivacaine was preserved.

Catwalk results

Before surgery, the animals presented a classic gait pattern with no significant difference between the print area and the print length of the right hind paw compared to the contralateral one. Mean preoperative values were 1.88 $\pm$ 0.03 cm^2 for print area, 115.28 $\pm$ 0.55 AU for intensity, 1.86 $\pm$ 0.02 cm for manual print length, and 66.34% $\pm$ 0.56% for duty cycle, and were considered as the reference baselines. After surgery, the animals were able to place their operated paw (right hind limb) in step cycles at any time of the experiment, and weight bearing on the operated limb was constant throughout the postoperative period.

A

B
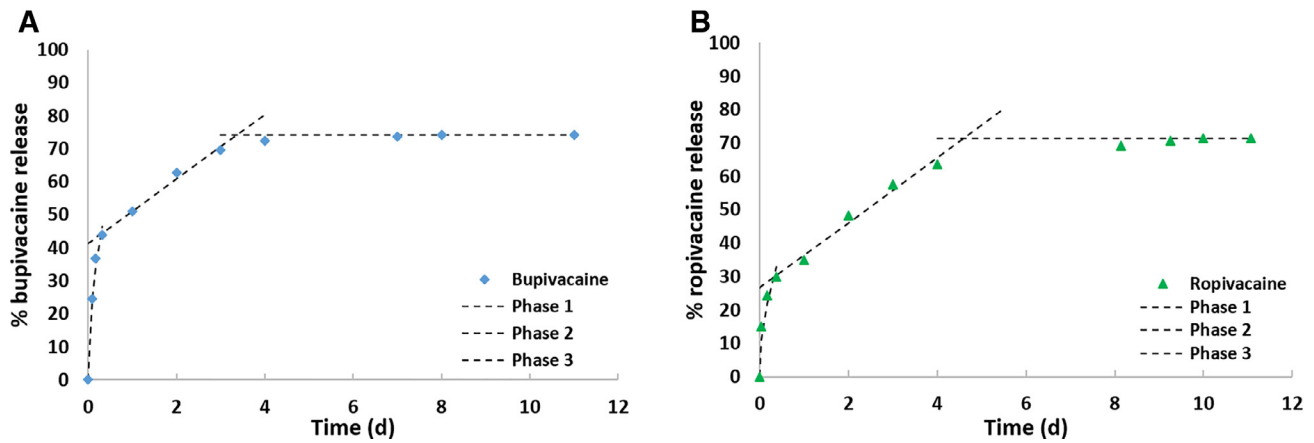
Figure 4. Release profiles from CPC loaded with (A) 8% bupivacaine and (B) 8% ropivacaine.

Figs 5A and 6A show the evolution of the print area parameter during the postoperative period. After implantation of unloaded cement, the print area decreased from 2.04 ± 0.07 cm^2 before surgery to a minimum of 0.66 ± 0.06 cm^2 at 24 hours postoperatively. The print area then increased gradually to 1.60 ± 0.06 cm^2 at 14d. With both loaded cements, after an initial postoperative decrease, a faster return to the baseline value than for unloaded CPC at 14d was observed. At 24 hours postoperatively, gait modification reached its highest level for rats treated with the unloaded cement. It can be seen that the rats treated with both loaded cements had a significantly ($p<0.01$) higher print area than rats treated with the unloaded cement: 1.44 ± 0.06 cm^2 for bupivacaine and 1.10 ± 0.06 cm^2 for ropivacaine. This can be seen in the footprint pictures of the operated rats according to the different implantation conditions (Fig 5B). Moreover, the two loaded CPCs can be differentiated at this point in time, with the CPC-Bupi showing a significantly higher print area. 48 hours

postoperatively, it was still possible to differentiate significantly between the unloaded CPC and loaded CPCs. Finally at day 7 after surgery, the print area of rats implanted with the CPC-Bupi was not significantly different from the preoperative baseline.

The intensity parameter and the print area parameter displayed similar variation patterns. 24 hours after implantation (Fig 6B), the intensity decreased by $25\% \pm 2\%$, $9 \pm 2\%$ and 1 ± 2 % for CPC, CPC-Ropi and CPC-Bupi respectively compared with the baseline. The differences between the three cements were significant ($P < .01$). 48 hours after surgery; the CPC provided significantly lower intensity ($79\% \pm 2\%$) than both loaded cements ($96\% \pm 2\%$ for CPC-Ropi and $98\% \pm 2\%$ for CPC-Bupi). Interestingly, during the entire evaluation period the intensity provided by the CPC-Bupi was not significantly different from the preoperative reference baseline. At the end of the evaluation period (14 days after surgery), the intensity provided by CPC and CPC-Ropi returned to the baseline value and was no different from the preoperative baseline.

A

B
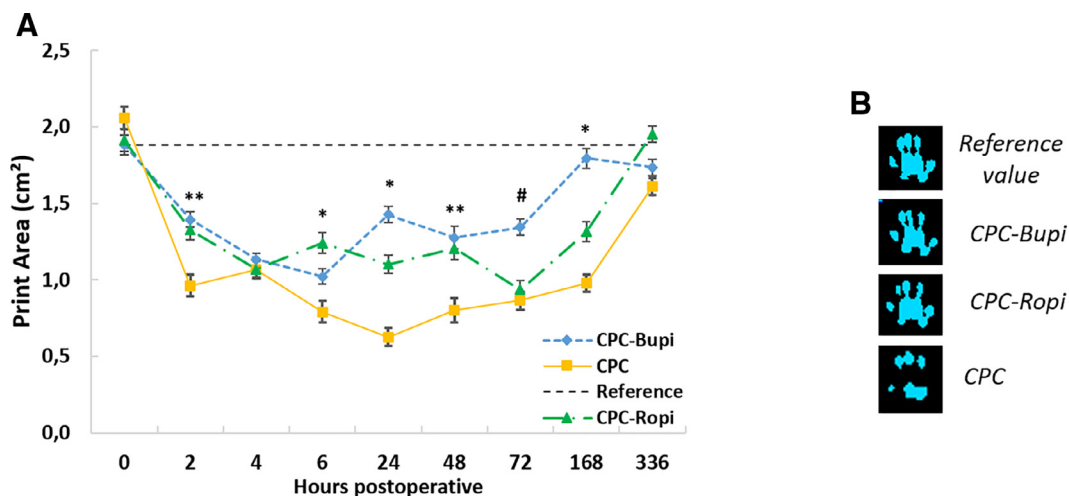
Figure 5. Print area (in cm^2) of the operated paw (right hind limb) for the 3 types of cements. CPC in yellow (plain line), CPC-Bupi in blue (dotted line), CPC-Ropi in green (dash-dotted line), and the reference preoperative baseline is the enlarged black dotted line. Statistical analysis used ANOVA with the Fisher test. The results are the means $\pm$ SD (N = 6), * $P < .01$ (CPC-Bupi vs CPC-Ropi vs CPC), ** $P < .01$ anesthetic loaded-CPCs vs CPC, # $P < .01$ CPC-Bupi vs others (A), print area pictures for each condition at 24 hours postoperative (B).
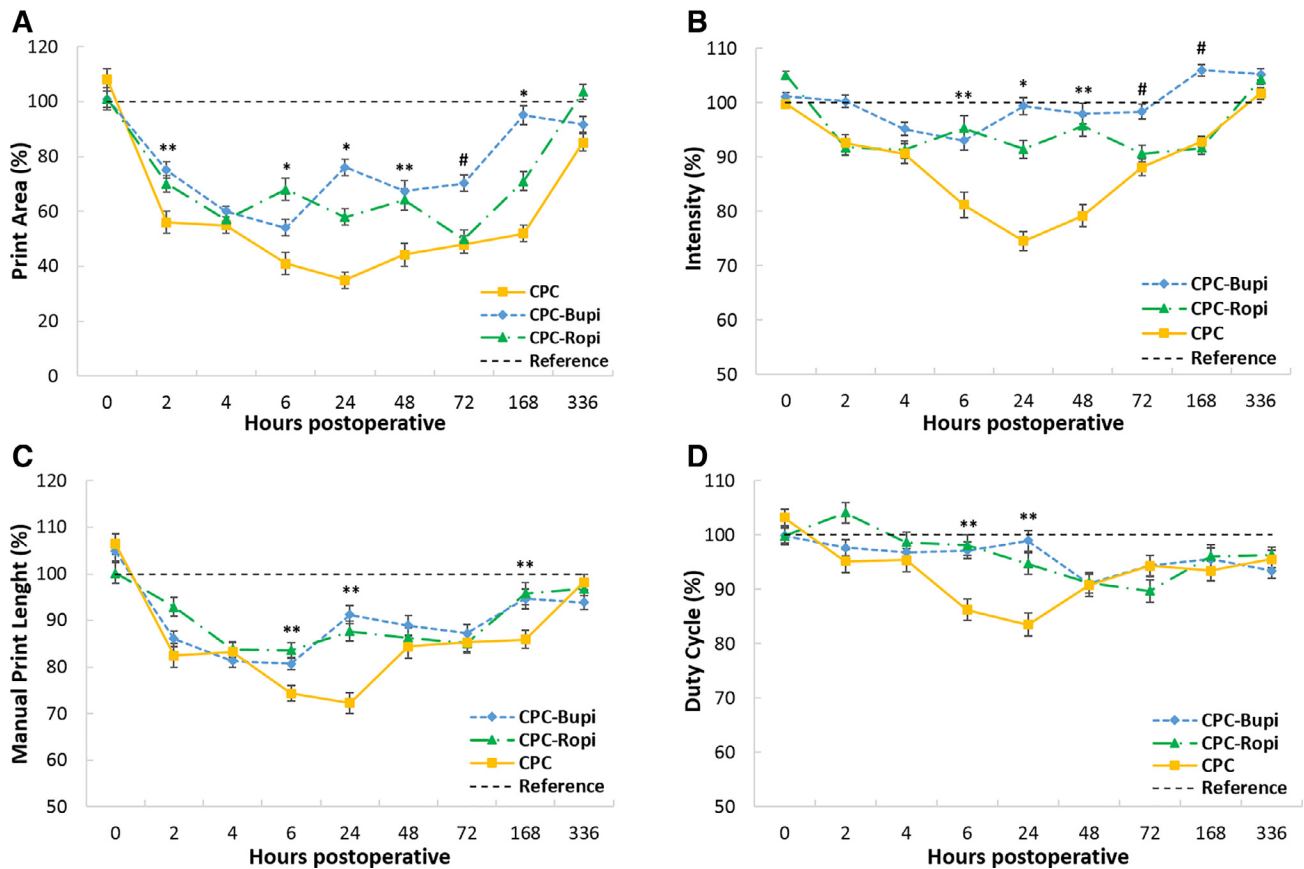
Figure 6. Four tested parameters for the 3 types of cements: print area in % of the preoperative baseline (A), intensity in % of the preoperative baseline (B), manual print length in % of the preoperative baseline (C), and duty cycle in % of the preoperative baseline (D). CPC in yellow (plain line), CPC-Bupi in blue (dotted line), CPC-Ropi in green (dash-dotted line), and the reference preoperative baseline is the enlarged black dotted line. Statistical analysis used ANOVA with the Fisher test. The results are the means $\pm$ SD (N = 6), * $P < .01$ (CPC-Bupi vs CPC-Ropi vs CPC), ** $P < .01$ anesthetic loaded-CPCs vs CPC, # $P < .01$ CPC-Bupi vs others.

Like the print area parameter, the manual print length was expected to decrease after surgery. Manual print length was 72% $\pm$ 2% at 24 hours (Fig 6C) for the CPC, whereas for CPC-Bupi and CPC-Ropi the manual print length was 91% $\pm$ 2% and 88% $\pm$ 2%, respectively. This difference was significant ($P < .01$) compared with the CPC but without any notable difference between the two loaded CPCs. At day 7 after surgery, the print length parameter was not found to be significantly different from the preoperative baseline for both loaded CPCs.

The duty cycle parameter (Fig 6D) did not exhibit significant variation throughout the entire implantation period, with no significant difference between the two loaded CPCs. Conversely, for CPC, the duty cycle decreased postoperatively and a significant difference ($P < .01$) between the unloaded cement (84% $\pm$ 2%) and the loaded CPCs (95% $\pm$ 2% for ropivacaine and 99% $\pm$ 2% for bupivacaine, respectively) was noted at 24 hours. At 48 hours after surgery, the duty cycle was not significantly different for all cements and progressively increased back to the reference value at 7 days after surgery.

SEM and histologic studies

Three weeks after implantation, the injected cements were still present in all implantation sites and new bone apposition was observed on the surface of each one of them (Fig 7A). As depicted in Fig 7B, the percentage of the newly formed bone was not significantly different between the 3 conditions. In accordance with this, osteoid tissue bordered by an osteoblast cell line was observed for all cements. The local release of the drugs did not modify the bone-cement interfaces and the osteointegration of both loaded CPCs as compared to the unloaded CPC. In addition, histological analysis did not reveal any adverse inflammatory reaction (Fig 8).

Discussion

Pain caused by bone harvesting for reconstruction is regarded as one of the major complications after autologous bone grafting procedures.[15,24,28,36,37,42,53,54] Multimodal analgesia appears to be the best method to limit postoperative pain and its evolution to chronicity. It includes peripheral nerve blocks through locoregional analgesia and systemic administration of analgesic drugs.[3,8,43]

CPCs are considered to be gold standard materials for new therapeutic approaches such as percutaneous or minimally-invasive techniques. These biomaterials are better suited to the shape of a bone defect than granules[22,23] and thanks to their moldability, they can fit
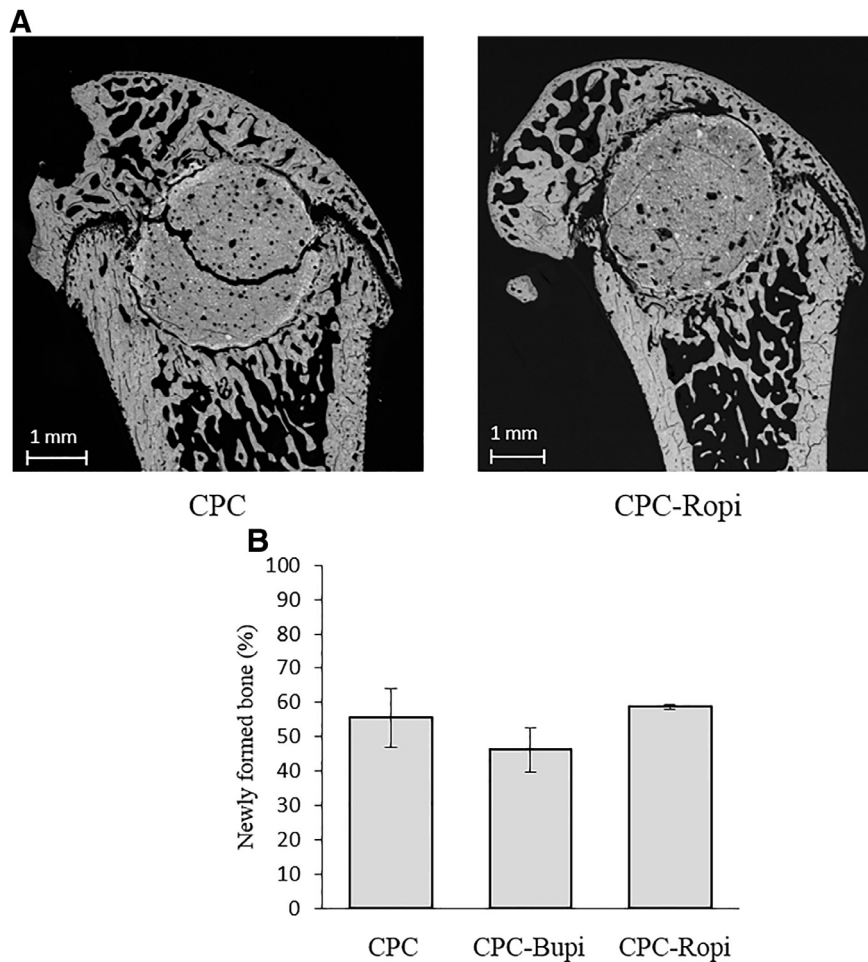
Figure 7. A. SEM images after femoral implantation. B. Results were expressed as the percentage of the newly formed bone relative to the ROI.

perfectly into implantation sites, thus optimizing bone-biomaterial contact in geometrically complex bone defects. Moreover, CPCs are based on a mixture of CaP mineral compounds that react together to provide a material that hardens in situ after injection, through a nonexothermic reaction in contrast to PMMA cements. The association of CaP bone cements with bioactive molecules has been investigated for 3 major clinical purposes in the bone environment: the local delivery of antibiotics, the promotion of osteointegration or bone augmentation by the release of growth factors or cytokines, and the prevention of further bone resorption by the incorporation and subsequent in vivo release of antiresorptive agents such as bisphosphonates.[49] In this study, it was possible to incorporate local anesthetics into CPCs to propose them as suitable candidates for pain management after bone surgery. Such bone substitutes, which can both fill the harvest site and deliver an analgesic substance in situ, could constitute a novel therapeutic approach.

The effectiveness of the analgesia and the resulting functional recovery needed to be evaluated as objectively as possible. In this study, the CatWalk automated quantitative gait analysis system was deemed to be the best method. It provided a functional assessment of the pain relief provided by injecting CPC loaded with 2 different analgesic drugs, bupivacaine and ropivacaine, and compared with the pain relief obtained with the cement alone. Reproducible and objective gait analysis data were obtained in an animal model that mimics the clinical situation. In vivo studies in rats have been widely used to evaluate both bone reconstruction ability of CaP bone substitutes[4,7,12] and neurologic and orthopedic pain,[34,35] with the Von Frey monofilament test being the most commonly used method to evaluate mechanical allodynia in rats. Several articles[6,52] have discussed the difference between the Von Frey monofilament method and the CatWalk system. It appears that the major drawback of the Von Frey method is the difficulty in establishing a correct threshold. Moreover, the force of the filament is influenced by ambient humidity and temperature. In addition, animals are tested under restrictive conditions that are very different from physiological gait conditions. Conversely, the Catwalk system allows the animals to walk freely, which minimizes handling and reduces confounding stress factors. Moreover, it provides an objective, non-invasive analysis for investigating a large number of gait parameters. In this study, changes in the gait and gait expression parameters in the operated rats were induced by surgical pain and shown by either a reluctance or an inability to use the operated limb.
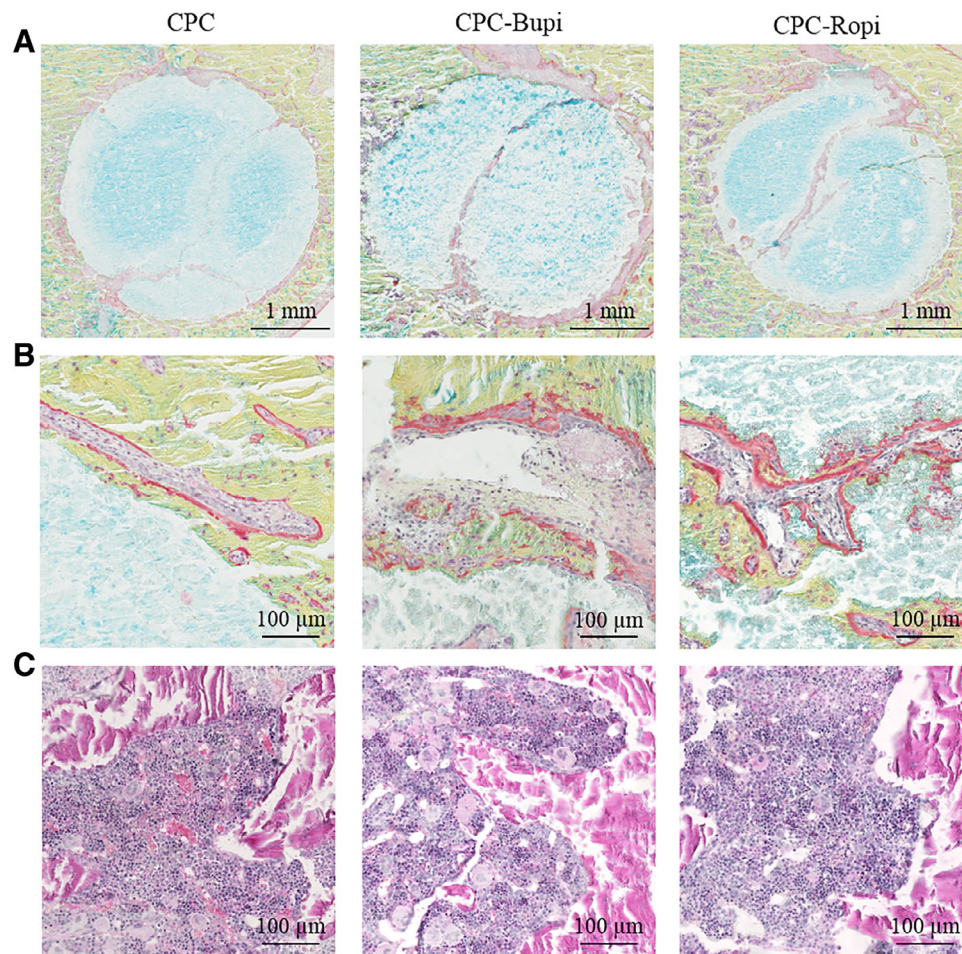
Figure 8. Histological analysis of femoral samples 3 weeks after implantation. A. Observation of the whole cement after implantation with Movat's stain (blue = cement; yellow-green = bone, light pink = soft tissue). B. Detailed images of the new bone formation from Movat's stain (red line = osteoid tissue, purple line: osteoblast cell line). C. Bone marrow observation from hematoxylin-eosin stain.

Gait analysis and functional recovery

The chosen parameters were of great interest because they concerned both spatial (print area, manual print length) intensity, and temporal (duty cycle) data. Catwalk gait analysis showed that all these parameters decreased after surgery. Rats treated with the unloaded cement exhibited the highest level of pain at 24 hours after surgery, as shown by the significant decrease observed on all tested parameters compared with pre-operative baselines. However, implantation of analgesic drug-loaded cements provided a clear and continuous improvement of the postoperative gait, especially during the short-term postoperative period. Due to the significant difference among CPC-Bupi, CPC-Ropi, and the CPC observed at 24 hours, the print area and intensity parameters revealed simple and reliable criteria to evaluate the postoperative weight-bearing ability and functional recovery of the operated rats. These 2 parameters reflected the induced postoperative pain by the fact that the animals became reluctant or unable to support weight-bearing pressure on their operated paw, and the postoperative functional benefits provided by the implantation of analgesic CPCs.

During the short-term postoperative period, the study of the duty cycle of the implanted hind limb revealed a decrease in the paw-floor contact (stance duration) associated with an increase in the swing duration. Reduced manual print length was directly related to the decrease in the print area. Moreover, the manual print length and duty cycle parameters were not significantly different between the 2 loaded CPCs, but still significantly different compared to the unloaded cement, emphasizing the ability to contrast the analgesic effect compared to the unloaded cement. Although both CPC-Bupi and CPC-Ropi displayed similar pain relief benefits at 24 and 48 hours postoperative, CPC-Bupi seemed to provide an earlier and more complete return to preoperative baselines than CPC-Ropi, in accordance with the compared pharmacokinetic properties and the respective efficiency of these 2 drugs.[13]

During surgery, for ethical and medical reasons, buprenorphine hydrochloride was administered subcutaneously as preemptive analgesia. This drug has a longer duration of action than morphine (up to 6 hours [17]). The long-acting effect of the perioperative analgesia could have influenced the results obtained during the early stages of Catwalk assessment. However, all the surgical procedures and gait analyses were performed blindly to increase the reliability of the results obtained.

Release of the drug during the critical period

Modifications to the setting reaction kinetics along with the mechanical and rheological properties of loaded-CPCs could impact their future clinical performance. The objective of this study was to provide and validate a new approach to decrease the risk of developing chronic pain after iliac crest autologous bone grafting involving the use of an original drug-biomaterial combination device that can provide significant pain relief during the first 3 to 4 postoperative days.

In this study, the release profile of the 2 tested drugs consisted of 3 successive phases, as shown in Fig 4:

1. An initial burst in which approximately 44% and 30% of the drugs (bupivacaine and ropivacaine, respectively) were released within the first 8 hours. According to the literature,[23,33,45] this release corresponds to the penetration of sodium chloride aqueous solution into the cement and follows the Higuchi model.
2. After this initial massive release, a linear drug release was observed from 8 hours to 4 days, with a release rate of 9.7% per day for the 2 different cements.
3. Finally, during the third stage, the release rate slowed considerably, and the drug release reached a plateau (4 and 5 days for bupivacaine and ropivacaine, respectively).

These in vitro results showed an efficient release from the 2 loaded cements during the first 3 to 4 postoperative days, in contrast to a previous study[48] in which bupivacaine-loaded CDA granules were characterized by a burst release of the drug (80% within 5 h). The choice of a CPC as drug delivery system appears to be of considerable interest to control the progressive release of the drugs and thus the duration of the analgesic effect during the most critical period. It could therefore be possible to include these kinds of loaded CPCs into the multimodal analgesia protocol to fill the donor site in autologous iliac bone grafting procedures.

Biologic response to loaded-CPC implantation

Considering the cardiotoxicity and vasodilatory effects of bupivacaine,[11] both the local tolerance and safety of loaded CPCs had to be documented in situ. A preliminary in vivo safety study (ie, femoral implantation in rabbits) was conducted over 8 weeks (unpublished work). Briefly, plasma assays were performed at successive times following in vivo implantation. Bupivacaine was under the detectability threshold in all samples (<100 ng/mL). To summarize, these unpublished preliminary data demonstrated the absence of systemic passage, strongly suggesting that this loaded cement displayed a good safety profile. In addition, monitoring of cardiovascular function revealed no adverse effects from the time of femoral implantation until the animals were sacrificed.

In this study, histologic analysis showed that local release of bupivacaine or ropivacaine from the 2 tested cements did not generate any adverse bone tissue reaction. Furthermore, the loaded CPCs retained their biological properties and their bone healing and bone filling ability due to their bioactive, osteoconductive, and injectability properties.[2,38,39] Moreover, by extrapolating the drug release profile and the volume of cement implanted, no dysfunctions were expected. No neurological problems such as skin damage or self-trauma, and no dysfunction such as recurrent non weight-bearing lameness or muscle atrophy that could have been attributed to local side-effects were observed in the rats, regardless the time of implantation. These data are of major interest as part of multimodal analgesia in orthopedic and fracture management surgery.

Conclusion

To our knowledge, this is the first time that the Cat-Walk system was used to evaluate the pain relief provided by the implantation of bone substitutes loaded with analgesic drugs. This appeared to be an appropriate method to study functional recovery after a bone harvesting procedure followed by filling the bone defect created with an analgesic-loaded biomaterial. The method demonstrated and differentiated the potential analgesic effects produced by the carrier alone and by the eluted drug. In addition, this preliminary in vivo study showed that the injectable cements were well tolerated and supported controlled release of local anesthetic over the course of several days (up to 2 weeks). Moreover, these new cements demonstrated the properties required for use in preclinical studies and in a global pain management protocol after bone surgery.

Acknowledgements

Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jpain.2018.04.014.

References

1. ASTM: Standard Test Method for Time of Setting of Hydraulic-Cement Paste by Gillmore Needles. ASTM C266-08, 2008.

2. Bajammal SS, Zlowodzki M, Lelwica A, Tornetta P, Einhorn TA, Buckley R, Leighton R, Russell TA, Larsson S, Bhandari M: The Use of Calcium Phosphate Bone Cement in Fracture Treatment: A Meta-Analysis of Randomized Trials. J Bone Jt Surg-Am Vol 90:1186-1196, 2008

3. Banks A: Innovations in postoperative pain management: continuous infusion of local anesthetics. AORN J 85:904-914; quiz 915-18, 2007

4. Basso DM, Beattie MS, Bresnahan JC: A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 12:1-21, 1995

5. Beaussier M: Frequency, intensity, development and repercussions of postoperative pain as a function of the type of surgery. Ann Fr Anesth Reanim 17:471-493, 1998

6. Berryman ER, Harris RL, Moalli M, Bagi CM: Digigait quantitation of gait dynamics in rat rheumatoid arthritis model. J Musculoskelet Neuronal Interact 9:89-98, 2009

7. Beyreuther B, Callizot N, Stöhr T: Antinociceptive efficacy of lacosamide in the monosodium iodoacetate rat model for osteoarthritis pain. Arthritis Res Ther 9:R14, 2007

8. Bigby J, Reader A, Nusstein J, Beck M, Weaver J: Articaine for supplemental intraosseous anesthesia in patients with irreversible pulpitis. J Endod 32:1044-1047, 2006

9. Bohner M, Gbureck U, Barralet JE: Technological issues for the development of more efficient calcium phosphate bone cements: A critical assessment. Biomaterials 26:6423-6429, 2005

10. Bouler JM, Pilet P, Gauthier O, Verron E: Biphasic calcium phosphate ceramics for bone reconstruction: A review of biological response. Acta Biomater 53:1-12, 2017

11. Bourne E, Wright C, Royse C: A review of local anesthetic cardiotoxicity and treatment with lipid emulsion. Local and Regional Anesthesia 3:11-19, 2010

12. Buerkle H, Schäpsmeier M, Bantel C, Marcus MA, Wüsten R, Van Aken H: Thermal and mechanical antinociceptive action of spinal vs peripherally administered clonidine in the rat inflamed knee joint model. Br J Anaesth 83:436-441, 1999

13. Casati A, Putzu M: Bupivacaine, levobupivacaine and ropivacaine: are they clinically different? Best Pract Res Clin Anaesthesiol 19:247-268, 2005

14. Chen Y-J, Cheng F-C, Sheu M-L, Su H-L, Chen C-J, Sheehan J: Pan H-C: Detection of subtle neurological alterations by the Catwalk XT gait analysis system. J Neuroengineering Rehabil 11:1, 2014

15. Chung F, Ritchie E, Su J: Postoperative pain in ambulatory surgery. Anesth Analg 85:808-816, 1997

16. Coulthard P, Pleuvry BJ, Brewster M, Wilson KL, Macfarlane TV: Gait analysis as an objective measure in a chronic pain model. J Neurosci Methods 116:197-213, 2002

17. Curtin LI, Grakowsky JA, Suarez M, Thompson AC, DiPirro JM, Martin LBE, Kristal MB: Evaluation of buprenorphine in a postoperative pain model in rats. Comp Med 59:60-71, 2009

18. Deumens R, Jaken RJP, Marcus MAE, Joosten EAJ: The CatWalk gait analysis in assessment of both dynamic and static gait changes after adult rat sciatic nerve resection. J Neurosci Methods 164:120-130, 2007

19. Ferland CE, Laverty S, Beaudry F, Vachon P: Gait analysis and pain response of two rodent models of osteoarthritis. Pharmacol Biochem Behav 97:603-610, 2011

20. Gabriel AF, Marcus MAE, Honig WMM, Walenkamp GHIM, Joosten EAJ: The CatWalk method: A detailed analysis of behavioral changes after acute inflammatory pain in the rat. J Neurosci Methods 163:9-16, 2007

21. Geusens P, Emans PJ, de Jong JJA, van den Bergh J: NSAIDs and fracture healing: Curr Opin Rheumatol 25:524-31, 2013.

22. Ginebra MP, Espanol M, Montufar EB, Perez RA, Mestres G: New processing approaches in calcium phosphate cements and their applications in regenerative medicine. Acta Biomater 6:2863-2873, 2010

23. Ginebra MP, Traykova T, Planell JA: Calcium phosphate cements as bone drug delivery systems: A review. J Controlled Release 113:102-110, 2006

24. Goulet JA, Senunas LE, DeSilva GL, Greenfield ML: Autogenous iliac crest bone graft. Complications and functional assessment. Clin Orthop 339:76-81, 1997

25. Hamers FPT, Lankhorst AJ, van Laar TJ, Veldhuis WB, Gispen WH: Automated Quantitative Gait Analysis During Overground Locomotion in the Rat: Its Application to Spinal Cord Contusion and Transection Injuries. J Neurotrauma 18:187-201, 2001

26. Koopmans GC, Deumens R, Brook G, Gerver J, Honig WMM, Hamers FPT, Joosten EAJ: Strain and locomotor speed affect over-ground locomotion in intact rats. Physiol Behav 92:993-1001, 2007

27. Lankhorst AJ, ter Laak MP, van Laar TJ, van Meeteren NLU, de Groot JCMJ, Schrama LH, Hamers FPT, Gispen W-H: Effects of Enriched Housing on Functional Recovery After Spinal Cord Contusive Injury in the Adult Rat. J Neurotrauma 18:203-215, 2001

28. Maharaj MM, Phan K, Mobbs RJ: Anterior cervical discectomy and fusion (ACDF) autograft versus graft substitutes: what do patients prefer?—A clinical study. J Spine Surg 2:105-110, 2016

29. Massart R, Dymov S, Millecamps M, Suderman M, Gregoire S, Koenigs K, Alvarado S, Tajerian M, Stone LS, Szyf M: Overlapping signatures of chronic pain in the DNA methylation landscape of prefrontal cortex and peripheral T cells. Sci Rep 6:19615, 2016

30. Mellier C, Fayon F, Boukhechba F, Verron E, LeFerrec M, Montavon G, Lesoeur J, Schnitzler V, Massiot D, Janvier P, Gauthier O, Bouler J-M, Bujoli B: Design and properties of novel gallium-doped injectable apatitic cements. Acta Biomater 24:322-332, 2015

31. Miyagi M, Ishikawa T, Kamoda H, Orita S, Kuniyoshi K, Ochiai N, Kishida S, Nakamura J, Eguchi Y, Arai G, Suzuki M, Aoki Y, Toyone T, Takahashi K, Inoue G, Ohtori S: Assessment of Gait in a Rat Model of Myofascial Inflammation Using the CatWalk System: Spine 36:1760-4, 2011.

32. KÄ Möller, O-G Berge, Hamers FPT: Using the CatWalk method to assess weight-bearing and pain behaviour in walking rats with ankle joint monoarthritis induced by carrageenan: Effects of morphine and rofecoxib. J Neurosci Methods 174:1-9, 2008

33. Noukrati H, Cazalbou S, Demnati I, Rey C, Barroug A, Combes C: Injectability, microstructure and release properties of sodium fusidate-loaded apatitic cement as a local drug-delivery system. Mater Sci Eng C 59:177-184, 2016

34. Peter B, Gauthier O, Laïb S, Bujoli B, Guicheux J, Janvier P, van Lenthe GH, Müller R, Zambelli P-Y, Bouler J-M, Pioletti DP: Local delivery of bisphosphonate from coated orthopedic implants increases implants mechanical stability in osteoporotic rats. J Biomed Mater Res A 76:133-143, 2006

35. Peter B, Pioletti DP, Laïb S, Bujoli B, Pilet P, Janvier P, Guicheux J, Zambelli P-Y, Bouler J-M, Gauthier O: Calcium phosphate drug delivery system: influence of local zoledronate release on bone implant osteointegration. Bone 36:52-60, 2005

36. Pollock R, Alcelik I, Bhatia C, Chuter G, Lingutla K, Budithi C, Krishna M: Donor site morbidity following iliac crest bone harvesting for cervical fusion: a comparison between minimally invasive and open techniques. Eur Spine J 17:845-852, 2008

37. Rasche S, Koch T: [Regional anaesthesia versus general anaesthesia-pathophysiology and clinical implications]. Anaesthesiol Reanim 29:30-38, 2004

38. Rey C: Calcium phosphate biomaterials and bone mineral. Differences in composition, structures and properties. Biomaterials 11:13-15, 1990

39. Russell TA, Leighton RK: Comparison of Autogenous Bone Graft and Endothermic Calcium Phosphate Cement for Defect Augmentation in Tibial Plateau Fractures: A Multicenter, Prospective, Randomized Study. J Bone Jt Surg-Am Vol 90:2057-2061, 2008

40. Scheiman JM: NSAID-induced Gastrointestinal Injury: A Focused Update for Clinicians. J Clin Gastroenterol 50:5-10, 2016

41. Schobelock MJ: Pain management: engineering successful outcomes. J Healthc Mater Manage 10:61-66, 1992

42. Silber JS, Anderson DG, Daffner SD, Brislin BT, Leland JM, Hilibrand AS, Vaccaro AR, Albert TJ: Donor site morbidity after anterior iliac crest bone harvest for single-level anterior cervical discectomy and fusion. Spine 28:134-139, 2003

43. Singh K, An HS, Samartzis D, Nassr A, Provus J, Hickey M, Andersson GBJ: A prospective cohort analysis of adjacent vertebral body bone mineral density in lumbar surgery patients with or without instrumented posterolateral fusion: a 9- to 12-year follow-up. Spine 30:1750-1755, 2005

44. Smith G: Pain after surgery. Br J Anaesth 67:233-234, 1991

45. Tung I-C: In vitro drug release of antibiotic-loaded porous hydroxyapatite cement. Artif Cells Blood Substit Biotechnol 23:81-88, 1995

46. Vandeputte C, Taymans J-M, Casteels C, Coun F, Ni Y, Van Laere K, Baekelandt V: Automated quantitative gait analysis in animal models of movement disorders. BMC Neurosci 11:1, 2010

47. Verron E, Bouler JM, Guicheux J: Controlling the biological function of calcium phosphate bone substitutes with drugs. Acta Biomater 8:3541-3551, 2012

48. Verron E, Gauthier O, Janvier P, Le Guen H Holopherne D, Cavagna R, Bouler J-M: Analgesic properties of calcium phosphate apatite loaded with bupivacaine on postoperative pain. J Biomed Mater Res B Appl Biomater 94:89-96, 2010

49. Verron E, Gauthier O, Janvier P, Pilet P, Lesoeur J, Bujoli B, Guicheux J, Bouler J-M: In vivo bone augmentation in an osteoporotic environment using bisphosphonate-loaded calcium deficient apatite. Biomaterials 31:7776-7784, 2010

50. Verron E, Khairoun I, Guicheux J, Bouler J-M: Calcium phosphate biomaterials as bone drug delivery systems: a review. Drug Discov Today 15:547-552, 2010

51. Verron E, Pissonnier M-L, Lesoeur J, Schnitzler V, Fellah BH, Pascal-Moussellard H, Pilet P, Gauthier O, Bouler J-M: Vertebroplasty using bisphosphonate-loaded calcium phosphate cement in a standardized vertebral body bone defect in an osteoporotic sheep model. Acta Biomater 10:4887-4895, 2014

52. Vrinten DH, Hamers FF: 'CatWalk'automated quantitative gait analysis as a novel method to assess mechanical allodynia in the rat; a comparison with von Frey testing. Pain 102:203-209, 2003

53. Wilder-Smith OH, Arendt-Nielsen L: Postoperative Hyperalgesialts Clinical Importance and Relevance. J Am Soc Anesthesiol 104:601-607, 2006

54. Zaslansky R, Eisenberg E, Peskin B, Sprecher E, Reis DN, Zinman C, Brill S: Early administration of oral morphine to orthopedic patients after surgery. J Opioid Manag 2:88-92, 2006

55. Zhang J, Liu W, Schnitzler V, Tancret F, Bouler J-M: Calcium phosphate cements for bone substitution: Chemistry, handling and mechanical properties. Acta Biomater 10:1035-1049, 2014