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RESEARCH PAPER

Local controlled release of corticosteroids extends surgically induced joint instability by inhibiting tissue healing

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Background and Purpose: Corticosteroids are intra-articularly injected to relieve pain in joints with osteoarthritis (OA) or acute tissue damage such as ligament or tendon tears, despite its unverified contraindication in unstable joints. Biomaterial-based sustained delivery may prolong reduction of inflammatory pain, while avoiding harmful peak drug concentrations.

Experimental Approach: The applicability of prolonged corticosteroid exposure was examined in a rat model of anterior cruciate ligament and medial meniscus transection (ACLT + pMMx) with ensuing degenerative changes.

Key Results: Intra-articular injection of a bolus of the corticosteroid triamcinolone acetonide (TAA) resulted in enhanced joint instability in 50% of the joints, but neither instability-induced OA cartilage degeneration, synovitis, nor the OA-related bone phenotype was affected. However, biomaterial microsphere-based extended TAA release enhanced instability in 94% of the animals and induced dystrophic calcification and exacerbation of cartilage degeneration. In healthy joints, injection with TAA releasing microspheres had no effect at all. In vitro, TAA inhibited cell migration out of joint tissue explants, suggesting inhibited tissue healing in vivo as mechanisms for enhanced instability and subsequent cartilage degeneration.

Conclusions and Implications: We conclude that short-term TAA exposure has minor effects on surgically induced unstable joints, but its extended presence is detrimental by extending instability and associated joint degeneration through compromised healing. This supports a contraindication of prolonged corticosteroid exposure in tissue damage-associated joint instability, but not of brief exposure.

Abbreviations: ACLT, anterior cruciate ligament transection; IA, intra-articular; MSs, microspheres; OA, osteoarthritis; pMMx, partial medial meniscectomy; PLGA, poly(lactic-co glycolic acid); PEA, polyesteramide; TAA, triamcinolone acetonide

1 | INTRODUCTION

The musculoskeletal joint disease **osteoarthritis (OA)** is considered to be a leading cause of musculoskeletal disability in the elderly population worldwide (March et al., 2014; Vos et al., 2012). Its prevalence is rising as risk factors for OA such as high age and obesity are also increasing. Other risk factors for OA include female gender, joint injury, and abnormal loading of the joint (Palazzo, Nguyen, Lefevre-Colau, Rannou, & Poiraudeau, 2016). Clinical symptoms such as joint pain, aching, and stiffness result in physical disability and functional impairment of OA patients, leading to a large socio-economic burden (Bartley, Palit, & Staud, 2017; Litwic, Edwards, Dennison, & Cooper, 2013). Because OA is a whole joint disease affecting many different tissues of the joint, characterized by damaged articular cartilage, osteophytes, subchondral bone changes, synovitis, and thickening of the joint capsule, treatment should focus on all of these factors (Dieppe & Lohmander, 2005).

A clear predisposing factor for OA is joint instability (Onur et al., 2014), usually caused by ligament injury (Akhtar, Bhattacharya, & Keating, 2016; Christian, Anderson, Workman, Conway, & Pope, 2006) and subsequent cartilage degeneration. Intra-articular (IA) injection of corticosteroids is performed to inhibit acute inflammation associated with ligament trauma, such as rotator cuff tears (Grant, Arthur, & Pichora, 2004). Also, for treatment of OA-associated inflammation and pain, IA corticosteroid injection is frequently applied, in most cases **triamcinolone acetonide** (TAA; Kenalog®/Kenacort®). Even though corticosteroids provide excellent pain relief, their use can lead to muscle weakness and hormone imbalance, particularly when given at higher doses (Goldzweig, Carrasco, & Hashkes, 2013; Kumar, Singh, Reed, & Lteif, 2004). Also, bone loss due to increased osteoclastogenesis or induced apoptosis of osteoblasts and osteocytes is a well-known effect of corticosteroids (Brennan-Speranza et al., 2012; O'Brien et al., 2004). To decrease systemic and IA peak levels of corticosteroids, drug delivery systems for local application, in particular microspheres (MSs), are promising alternatives. These provide prolonged low-level drug exposure with a lower risk of IA and systemic side effects (Janssen, Mihov, Welting, Thies, & Emans, 2014). The MSs are injected into the joint and, given their size, cannot leave this location. Where exactly the MSs reside is unclear, but at early time points, spheroid shapes have been detected in the synovial lining (Janssen et al., 2016).

Various biodegradable polymers are being investigated for this purpose, of which in particular the FDA-approved poly(lactic-co glycolic acid) (PLGA) is used most frequently (Bodick et al., 2015; Zhang & Huang, 2012). However, degradation of PLGA is relatively fast, limiting thereby the sustained drug release period. In a recent clinical trial comparing TAA-loaded PLGA microparticles with bolus TAA injection, the primary end point of improvement in pain intensity over 12 weeks was not met (Bodick et al., 2015).

Despite the frequent use of IA application in clinical practice, IA use of TAA is contraindicated in the treatment of unstable joints. However, no scientific background or clinical evidence for this contraindication is provided in literature (McAlindon et al., 2017; McCormack, Lamontagne, Vannabouathong, Deakon, & Belzile, 2017), with even some opposing results (de la Harpe & Brighton, 1989; Geborek, Mansson, Wollheim, & Moritz, 1990; Guma et al., 2002; Kraus et al., 2018). This is in contrast to the acute tissue injury occurring in ligament tears leading to instability-induced OA in human patients (Buckwalter & Brown, 2004), which may be affected differently by prolonged exposure to corticosteroids. In the current study, the effects of extended release of TAA by polyesteramide (PEA) MSs were studied in joints destabilized by full anterior cruciate ligament transection (ACLT) with partial medial meniscectomy (pMMx), leading to OA changes. In addition, given findings in the diseased joints treated with extended release of TAA, the role of TAA in tissue healing was studied in in vitro wound healing assays with cultured ligament and joint capsule tissue explants. We demonstrated that local extended release of TAA aggravated the surgically induced instability with concurrent incongruity and subsequent cartilage degeneration. We also showed that TAA inhibits wound healing processes as fibroblasts did not grow out of joint tissue explants in its presence. Hence, extended IA corticosteroid exposure in joints with instability due to acute IA tissue damage should be avoided at all times, as it might aggravate the ensuing joint degeneration. However, only minor effects are likely to occur after single bolus injection.

What is already known

- Corticosteroids are administered intra-articularly in osteoarthritic joints and joints with acute inflammation-associated tissue trauma.
- Extended drug delivery formulations are promising approaches to prolong anti-inflammatory and analgesic effects of corticosteroids.

What this study adds

- Extended corticosteroid presence in tissue-trauma-induced instability may aggravate joint disease, while brief exposure has minor effects.
- The mechanism behind these effects is enhanced instability through corticosteroid-induced compromised tissue healing.

What is the clinical significance

- Extended corticosteroid release formulations may not be used in joints instable through acute tissue damage.

2 | METHODS

2.1 | Microspheres

PEA MSs were prepared as described before (Rudnik-Jansen et al., 2017); 20-, 30-, and 45-wt% TAA was dispersed in polymer/solvent solution, and particles were formed at 8,000 rpm with an Ultra-Turrax homogenizer and poured into a hardening bath afterwards. Particles were stirred over night at ambient temperature, cooled prior to

washing, and snap frozen with liquid nitrogen prior to freeze-drying. Particles were sterilized using γ sterilization. Dry particles were resuspended with saline to obtain three different drug concentrations per 25-μl injection volume, namely, 0.7-, 1.0-, and 1.6-mg TAA; 0.7 mg in 25-μl TAA bolus was obtained by diluting Kenacort® (40 mg·ml^{-1}, Bristol-Myers Squibb, Woerden, the Netherlands) in sterile saline. TAA (T6501-250MG, Sigma-Aldrich) used in culture experiments was dissolved in 100% ethanol and further diluted in culture medium to obtain working concentrations.

2.2 | Animal procedures

Animal care and experimental procedures were performed in agreement with the Dutch Law of Animal Experimentation and approved by the Animal Ethics Committee in Utrecht, the Netherlands (project # AVD108002015282). Animal studies are reported in compliance with the ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson, and Altman, 2010) and with the recommendations made by the *British Journal of Pharmacology*. Primary outcomes were synovial inflammation, cartilage degeneration, and bone changes, with power analysis based on previous data on the effects of TAA on synovial inflammation. Secondary outcomes were body weight, plasma TAA levels, and calcifications. To reduce animal use, first studies in which animals with OA joints receiving unloaded or loaded TAA MSs were conducted to determine the best TAA bolus dosage to use as control in the healthy animals. The effects of TAA bolus and of extended TAA release of the same TAA dosage in OA animals, in healthy joints, were evaluated in follow-up studies. Also, the six rats injected with empty MS served as control (OA) for this and a similar study on another low MW drug. Thirty-four female, 10 weeks old, Sprague–Dawley rats (RRID:RGD_70508; Charles River Laboratories International, Inc.; RRID:SCR_003792) were housed in groups under standard laboratory conditions (open cage, ad libitum food and water). Preoperatively and post-operatively, rats received 0.05 mg·kg^{-1} of buprenofine (buprecare®) and 4 mg·kg^{-1} of carporal® (AST farma B. V., Oudewater) subcutaneous as prophylactic analgesia. OA was induced by transecting the anterior cruciate ligament (ACL) and partial removal of the medial meniscus, referred to as the ACLT + pMMx model, in the experimental joint of all rats under isoflurane anaesthesia (4.5% induction, 1–2% for maintenance). After 4 weeks, animals were randomly divided into groups (each n = 6): control OA (unloaded MS), TAA bolus, MS-TAA (MSs loaded with TAA) 0.7 mg, MS-TAA 1.0 mg, MS-TAA 1.6 mg, and healthy joint MS-TAA 0.7 mg. Injections and follow-up measurements were done blinded. Two rats receiving MS-TAA in OA joints were killed before the end of the study, one due to renal infection (MS-TAA 1.0 mg) and the other due to severe weight loss after being exposed to high dose TAA release (MS-TAA 1.6 mg), leading to a reduction of n = 5 in the experimental group MS-TAA 1.0 mg and MS-TAA 1.6 mg.

2.3 | Plasma TAA retrieval and measurement

At 0, 2, 4, 6, and 8 hr after IA-MS injection, venous blood samples were taken and collected in K2-EDTA blood capillary tubes (T-MQK Capiject, Terumo Medical Corporation), daily on the following week and after that once a week. TAA concentrations were determined from plasma by LC–MS/MS, using a Thermo Fisher Scientific triple quadrupole Quantum Acces LC–MS/MS system (Waltham, MA) as previously described (Rudnik-Jansen et al., 2017).

2.4 | Static weight-bearing asymmetry assessment

During follow-up, body weights and difference in weight borne by experimental compared to contralateral hind limb were obtained weekly using a Linton Incapacitance Tester (Linton Instrumentation). Rats were placed in a plastic chamber with each hind paw resting on transducer pads, to record weight distribution over 3 s for five trials. Percent ipsilateral weight bearing was calculated as weight on the experimental hind limb divided by weight on both hind limbs.

2.5 | Bone changes and calcifications determined using μCT images

All rats were imaged directly after CO_2/O_2 asphyxiation, using a Quantum FX μCT scanner (PerkinElmer, Waltham, MA) with parameters time = 3 min, isotropic voxel size = 30 μm^3, tube voltage of 90 kV, and tube current = 180 μA. 3D reconstructed images were obtained, and serial 2D images were reconstructed using software Analyze 11.0 (PerkinElmer; RRID:SCR_009120). ImageJ software (ImageJ; RRID:SCR_003070) was used for all analyses. Serial 2D scans were evaluated for subchondral sclerosis, osteophytes, bone cysts, and loose bodies according to a multi-modality scoring system evaluated previously (Panahifar et al., 2014). Joint alignment was evaluated using sagittal serial 2D scans. Periarticular dystrophic calcifications were quantified by the mean average of each pixel value above soft tissue pixel value of each μCT scan, based on histograms representing air, soft tissue, and calcified tissue values. To obtain comparable region of interests, the same distance from growth plate to the lower end of tibia on the scan was measured.

2.6 | Macroscopic and histopathological analysis

All hind limbs were collected for histological analysis and were fixed in 4% formaldehyde solution (Klinipath BV, the Netherlands) for 1 week. Thereafter, joints were decalcified in 0.5-M EDTA (VWR international BV) solution for a total of 8 weeks, refixating for 3 days in 4% formaldehyde solution every 2 weeks, and embedded in paraffin. Five μm thick transversal knee joint sections were cut and stained either with Safranin-O/Fast green or haematoxylin/eosin to evaluate cartilage degeneration using the Mankin score (0 complete healthy–14 total joint destruction) or synovitis using the Krenn score (0 healthy–9 severe synovitis) respectively. Scoring was done blinded by two observers (A.T./I.R.). All rat cadavers were blinded macroscopically and microscopically assessed for systemic side effects of TAA, by veterinary pathologists (M.J.L.K./R.E.T.).

2.7 | Primary ligament explants and fibroblast outgrowth

From 10 healthy surplus rats, patella, collateral, and cruciate ligament explants (n = 5 per ligament type) were isolated, washed with PBS + 1% penicillin/streptomycin (Gibco), weighted, and cultured in flasks (25 cm^2, cellstar®) in DMEM (high glucose, GlutaMAX™, pyruvate, Gibco, Life Technologies, Carlsbad, CA) supplemented with 10% heat inactivated FBS (HyClone, Life Technologies), 1% ascorbic acid 2-phosphate (Sigma-Aldrich), and 1% penicillin/streptomycin (Gibco) for 10 days in the presence of 10^{-6}-M TAA or ethanol control or 4 days in the presence of 10^{-6}-M TAA, then washed with PBS and subsequently exposed to medium containing ethanol as vehicle control for the remaining days, mimicking bolus TAA injection. Then, cells were trypsinized and counted in Bürker-Türk counting chambers. Cell number was corrected for mg tissue. At Day 1, medium was collected and LDH content as indicator for cytotoxicity was measured, using a Cytotoxicity Detection Kit (Roche) according to the manufacturer's protocol. The colorimetric signals were measured in duplicates on a Versamax microplate reader (Molecular Devices LLC, Sunnyvale, CA) at 490 nm subtracted by a background reference.

2.8 | Wound closure assay

Patella ligament cells of four healthy surplus rats were isolated via the fibroblast outgrowth method described above. Cells were expanded in DMEM supplemented with 10% heat inactivated FBS, 1% ascorbic acid 2-phosphate and 1% penicillin/streptomycin and passage 2 cells were used for the scratch assay. Cells were seeded in 96-well plate (1,000 per well), and 24 hr later, a scratch was made in the monolayer with a 20-µl pipette tip across the centre of each well. Subsequently, wells were washed and replenished with fresh medium containing 10^{-6}-M (=1 µM) TAA or ethanol control. The concentration was chosen based on previously shown relationships of synovial fluid–serum concentration ratios (Hunder & Gleich, 1974; Wallis, Simkin, & Nelp, 1987), with plasma level of 2 µM corresponding to synovial fluid concentrations of approximately 4 µM or higher, and on a study showing that injection with PLGA MSs loaded with half the amount of TAA used here in human OA joints, that resulted in a concentration of 0.7 µM in aspirated synovial fluid after 1 week (Kraus et al., 2018). Images were obtained at time point 0, 24, and 48 hr after scratch. Gap distance was quantified using ImageJ, and end point 48 hr was used to calculate migration rates.

2.9 | Data and statistical analysis

The data and statistical analysis comply with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2018). Data were analysed using IBM® SPSS® Statistics version 21. For each animal experiment carried out at least six different animals were included in each group, based on an a priori power analysis with 85% power, α-error level of confidence (α = .008) corrected for the number of relevant comparisons between the groups set at 0.85% and assuming SDs are as observed as in previous studies (Rudnik-Jansen et al., 2017). Power analysis was done to detect differences between TAA as bolus and TAA released by MSs. Our previous results demonstrated that an n of 6 allows for detection of differences in synovial inflammation, cartilage degeneration, and bone changes of given intervention (Tellegen et al., 2018). However, in treatment groups "MS-TAA 1.0 mg" and "MS-TAA 1.6 mg," one rat was killed before the end of the study, leading to a reduction in n value of 5. Distribution of plasma TAA was analysed by non-parametric Kruskal–Wallis test with post hoc pairwise comparisons using the Dunn–Bonferroni approach. Body weight curves and weight-bearing asymmetry over time were analysed using linear mixed model, followed by pairwise comparisons with Sidak post hoc tests. As dose was not a significant predictor for weight-bearing asymmetry, all extended TAA release groups were taken together and compared to control (OA) and TAA bolus weight bearing. Model selection was based on the lowest Akaike information criterion. Donor and time served as random effect factors, and treatment served as fixed effect factor in both models. Regression coefficients were estimated by the maximum likelihood method. Differences in dystrophic calcium amounts were evaluated by one-way ANOVA with Tukey's post hoc test after Ln transformation. All post hoc tests were performed only if the F value for the ANOVA achieved statistical significance. All bone changes were assessed for femur, tibia, and patella, medial and lateral sides. Subchondral sclerosis and osteophytes were analysed using one-way ANOVA with post hoc Tukey's test. Bone cysts and loose bodies data were assessed using the nonparametric Kruskal–Wallis test with post hoc pairwise Dunn–Bonferroni comparisons. No significant differences in scores of contralateral joints were observed between groups and thus included as one control group. Cartilage degeneration and synovitis scores were analysed with one-way ANOVA with Tukey's post hoc test after Ln transformation. Statistically significant differences of all post hoc tests were found at $^*P < .05$. Ligament cell number outgrowth was analysed with one-way ANOVA with Tukey's post hoc test for each ligament type separately. Non-parametric Kruskal–Wallis test with post hoc pairwise comparisons using the Dunn–Bonferroni approach was used for capsule outgrowth data. Cell migration of TAA was compared to ethanol control treated explants using independent Student t-test.

2.10 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017).

3 | RESULTS

3.1 | Local extended release of TAA resulted in body weight loss without recovery

Extended TAA release from MSs showed lower peak plasma TAA levels compared to TAA bolus release. In none of the conditions, TAA was not detected in the systemic circulation at 120 hr post-injection (Figure 1a). To determine possible adverse effects of the systemic load, body weight was monitored. Local extended release of TAA in healthy joints and joints destabilized by ACL transection and meniscectomy significantly decreased body weight, irrespective of the dose (Figure 1b). Body weight increased again 3 weeks post IA injection but was not regained to the level of untreated animals in the remaining study period. Animals receiving 0.7-mg TAA bolus, the same dose as the lowest dose of TAA released by MSs, also lost body weight after IA injection but regained body weight to similar levels as untreated animals. Macroscopical and microscopical examination of several body tissues post-mortem otherwise showed no abnormalities related to TAA administration.

3.2 | Local TAA delivery increased asymmetrical weight bearing in surgically induced OA joints

Static weight-bearing measurements were performed to monitor asymmetry in hind limb weight bearing as indication of pain. However, apart from a temporary weight-bearing asymmetry 28 days after induction, the destabilizing surgery and associated OA did not change static weight bearing on the long term. In contrast, animals with OA injected with TAA (either as bolus or by extended release) in general unloaded their experimental joint, suggesting a painful limb and/or postural instability due to the extended joint instability (Figure 2).

3.3 | Extended TAA release in surgically instability-induced OA joints caused dystrophic calcification

The 3D reconstructions showed a clear image of calcium deposition surrounding the OA joints subjected to extended TAA release. By histology, these were verified to be cell-free masses indicative of pathological processes (Riley, Harrall, Constant, Cawston, & Hazleman, 1996), rather than ectopic bone formation. Dystrophic calcifications were not encountered in unstable OA joints injected with TAA bolus (Figure 3), nor in healthy joints injected with TAA-loaded MSs. As a chance finding, joint malalignment was observed in the sagittal plane on μCT images made immediately post-mortem (Figure 4) and recorded as possible parameter of joint instability. In 94% of OA joints with extended TAA delivery, joint subluxation with concurrent incongruity and subsequent contracture of the joint was observed and in 50% of OA joints injected with bolus TAA (Table 1). In OA joints with unloaded MS, healthy joints treated with MS-TAA, and all contralateral joints, no joint subluxation was observed. However, the number of observations did not allow for statistical analysis.

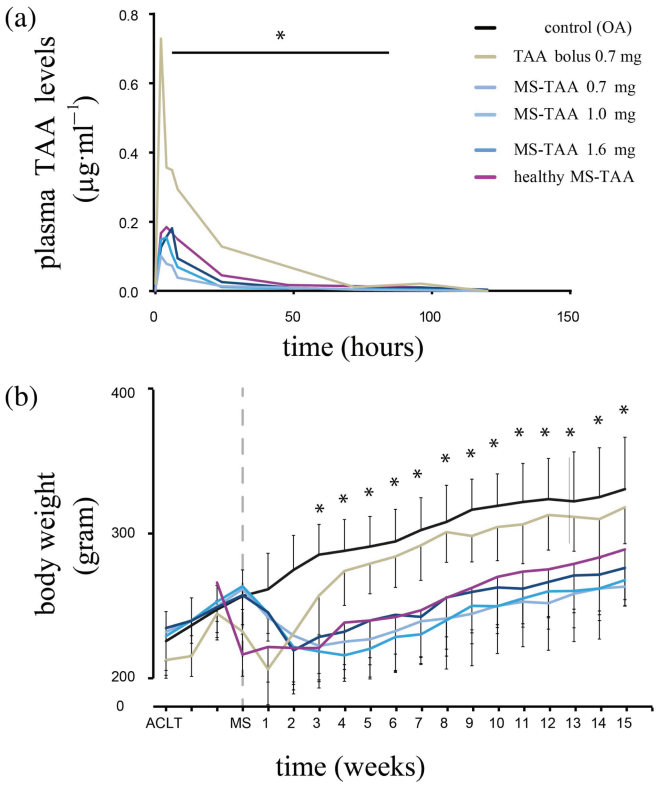

FIGURE 1 Systemic effects of local TAA delivery. (a) Bolus TAA gives a significantly different serum profile than all other burst releases in OA joints. (b) Local extended release of TAA results in body weight loss compared to control (OA). All data shown as mean ± SD with control (OA) $n = 6$, TAA bolus 0.7 mg $n = 6$, MS-TAA (TAA loaded in microsphere), 0.7 mg $n = 6$, MS-TAA 1.0 mg $n = 5$, MS-TAA 1.6 mg $n = 5$, healthy joint MS-TAA 0.7 mg $n = 6$. MS, microsphere; $^*P < .05$, significantly different as indicated

3.4 | TAA extended delivery decreased bone cyst number

To look into the effect of TAA on bone at the in vivo level, subchondral sclerosis, osteophyte formation, bone cyst number, and loose bodies were scored (Figure 5). TAA bolus did not affect any of these parameters in the instability-induced OA joints (Figure 5). The extent of joint degeneration, as characterized by histological evaluation using the Mankin score (Mankin & Lippiello, 1970), together with the calcifications, made it impossible to score bone changes in MS-TAA-treated induced OA joints, except for bone cyst number. The number of bone cysts in joints exposed to 0.7- and 1.0-mg MS-TAA was significantly lower compared to non-treated controls and were reduced to levels comparable to the healthy joints either contralateral or treated with 0.7-mg TAA in MSs (Figure 5c). TAA bolus and 1.6-mg MS-TAA were not able to reduce bone cyst numbers substantially, suggesting that there is an optimal MS-delivered TAA dose that provides a protective role in bone cyst occurrence.

3.5 | Extended TAA release leads to substantial cartilage degeneration

Cartilage integrity was evaluated using the Mankin score, representing the sum score of the structure, cell morphology, matrix content, and tidemark integrity of the cartilage (Mankin & Lippiello, 1970). The medial tibia plateau region in the joint was shown to develop more severe and reproducible OA changes than the lateral side in surgical OA models (Hayami et al., 2006) and was therefore analysed. While IA injection of bolus TAA did not affect cartilage degeneration, extended TAA release effectuated by the MSs in the surgically induced joints lead to a substantial increase of cartilage destruction with even bone-on-bone contact in most joints (Figure 6a,b). However, in healthy joints exposed to extended TAA release by the MSs, tissue structure, cell morphology, and tidemark integrity were unaffected. Still, the separate sub-categories of the Mankin score did not present a different pattern between all conditions, as is also shown for the total cartilage degeneration score (Figure 6b). No hypertrophy of the remaining cartilage in any of the joints was found, as evaluated by collagen type X IHC (Figure S1).

The extent of joint degeneration together with calcifications made it impossible to properly score synovitis in OA joints exposed to extended TAA release. Hence, the effect of extended TAA release on inflammation in OA joints could not be established and could not be graphically depicted. The OA joints treated with TAA bolus injections showed no effect on synovitis (Figure 7). However, in a few surgically induced joints, the synovial capsule appeared rich in cartilage-like cells, which was not seen in the contralateral joints (Figure S2; OA control 3/6, TAA bolus 3/6, MS-TAA [all dosages] 5/16, and healthy MS-TAA 2/6).

3.6 | TAA inhibits migration of ligament and synovial capsule cells

We investigated to what extent healing processes such as cell outgrowth and migration were affected by TAA. Fibroblasts did not migrate out of rat ligament or capsule explants when exposed to 10^{-6}-M TAA during the culture period of 10 days (Figure 8a). Temporary exposure to TAA, mimicking the bolus TAA injection, did show a reduction in numbers of fibroblasts migrating out of the explants in all tissues, which was statistically significant for the collateral ligaments compared to vehicle control. No cytotoxicity was found in any of the conditions. Since cell migration was completely inhibited in the presence of 10^{-4}-M TAA (data not shown), we further focussed on migration as crucial part of the healing process by performing scratch assays with primary patella ligament cells in the presence or absence of 10^{-6}-M TAA. Consistent with the results of the tissue outgrowth assay, we found that in vitro wound closure by primary patella ligament cells was significantly impaired by long-term TAA exposure (Figure 8b).

4 | DISCUSSION

The aim of the current study was to evaluate the effects of biomaterial-based sustained delivery of the corticosteroid TAA, compared to TAA bolus delivery, in joint degeneration associated with acute tissue damage-induced instability.

Although plasma TAA was detectable only until 120 hr post-injection, in all animals injected with TAA, considerable weight loss was induced during the 2 weeks after injection. While the weight of the animals receiving TAA bolus caught up with the weight of non-treated animals 3 weeks post-injection, extended release of TAA prevented this recovery, suggesting an extended systemic, though

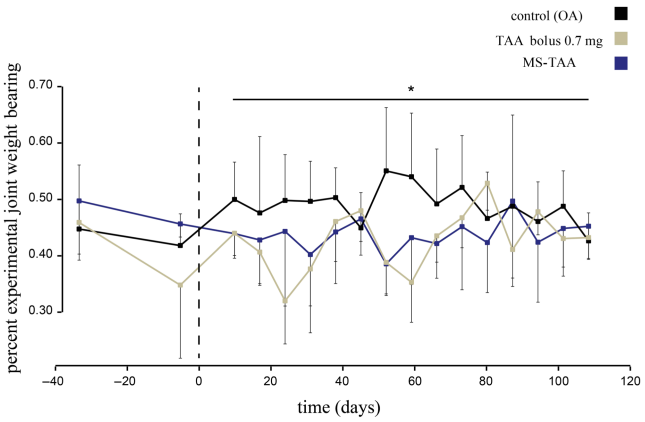

FIGURE 2 Weight-bearing asymmetry after local TAA delivery. MS-TAA loaded with a dose range of 0.7- to 1.6-mg TAA and bolus TAA (0.7 mg) in OA joints induced less weight bearing of the experimental joint, as indicator for pain. Dotted line represents time of MS/bolus injection. All data shown as mean ± SD with control (OA) $n = 6$, TAA bolus $n = 6$, MS-TAA $n = 16$. MS-TAA dose groups were taken together since dose was not a significant fixed effect in this statistical model. MS, microsphere; *$P < .05$, significantly different as indicated

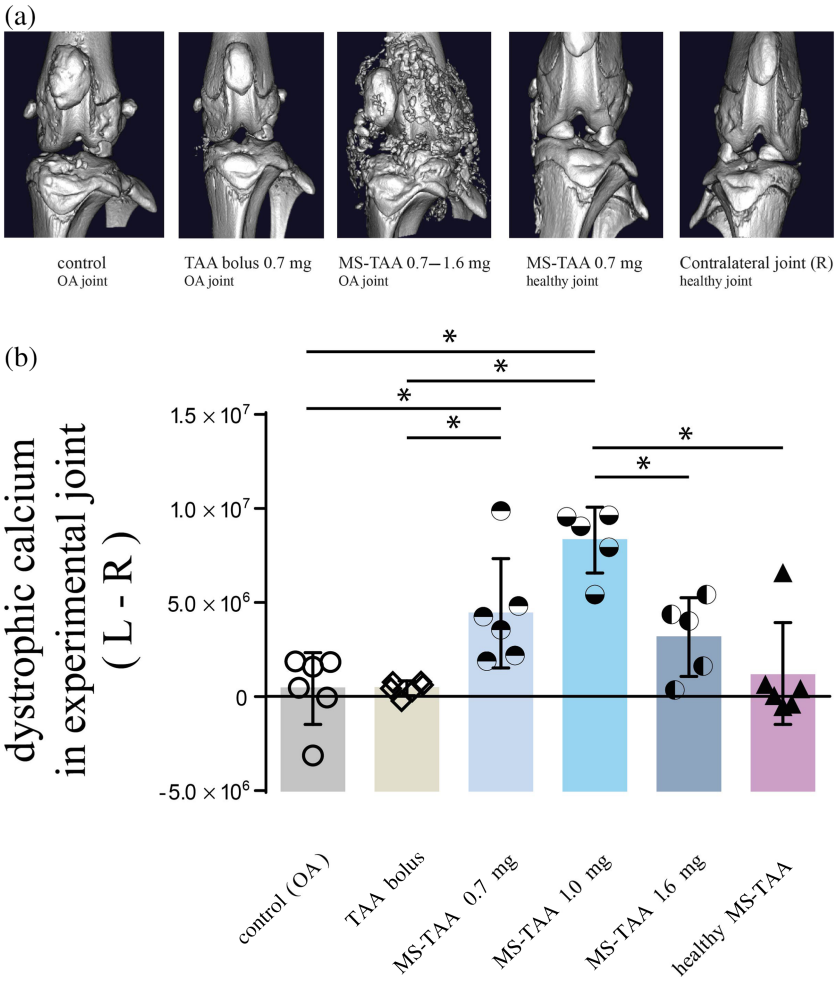

FIGURE 3 Extended TAA release in OA joints lead to large amount of dystrophic calcification. (a) 3D reconstruction of μCT scans. MS-TAA 0.7 mg is employed as a typical example of all three loading doses. (b) Quantification of dystrophic calcification in experimental joint (L), corrected for the contralateral joint (R). Individual data shown for control (OA) $n = 6$, TAA bolus $n = 6$, MS-TAA 0.7 mg n = 6, MS-TAA 1.0 mg $n = 5$, MS-TAA 1.6 mg $n = 5$, healthy joints MS-TAA 0.7 mg $n = 6$, as well as mean ± SD. MS, microsphere; *$P < .05$, significantly different as indicated

not detectable, presence of TAA. Weight loss in rats upon systemic oral exposure (Caparroz-Assef, Bersani-Amado, Kelmer-Bracht, Bracht, & Ishii-Iwamoto, 2007) and after weekly IA injections with a cumulative dose of 1.1-mg TAA (Siebelt et al., 2015) was described before. This is in contrast to the weight gain through enhanced fluid retention and increased appetite found in human patients (Tataranni et al., 1996). The mechanism behind this discrepancy is difficult to pinpoint but may be explained by species differences in receptor binding (Su et al., 2007) and/or signalling (Young, Yoxall, & Wagner, 1978) or differences in balance between mineralocorticoid and glucocorticoid activities of the drug (Nakabayashi et al., 2001; Tanigawa et al., 2002; Zhu et al., 2000). Glucocorticoids can induce muscle atrophy in rodents at relatively low concentrations (Sato et al., 2017) and hence might elicit weight loss at undetectable systemic concentrations. However, although no systemic side effects were found macroscopically and microscopically 12 weeks after treatment, we cannot exclude a possible toxic effect causing the weight loss in TAA-treated animals. In general, rodent models have their limitations in evaluation

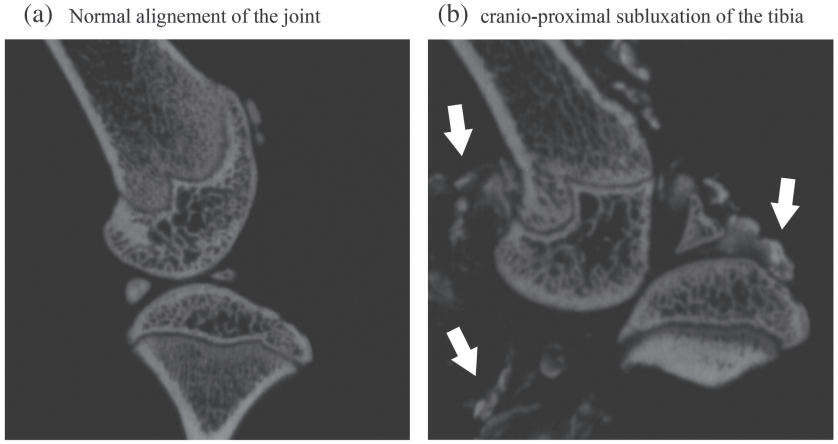

FIGURE 4 Local TAA delivery in OA joints retain instability, leading to joint subluxation. 2D sagittal μCT image example used to evaluate joint alignment throughout the whole joint. (a) Normal joint alignment of control (OA) joint. (b) Representative example of a MS-TAA 0.7 mg treated OA joint with cranio-proximal subluxation of the tibia and evident calcification of the periarticular tissues, indicated by white arrows. These chronic changes most probably represent joint contracture

TABLE 1 Local TAA in OA joints retain instability, regardless of delivery system

Condition	Joint subluxation		
	Yes	No	%
Control (OA)	0	6	0
TAA bolus	3	3	50
MS-TAA 0.7 mg	6	0	100
MS-TAA 1.0 mg	5	0	100
MS-TAA 1.6 mg	4	1	80
Healthy MS-TAA	0	6	0
Contralateral joints	0	34	0

Abbreviation: MS, microsphere.

of effectivity of drugs for clinical practice and do not completely reflect human disease. Still, novel treatments are commonly developed and tested initially in rodent models. A large animal model would be the next step in the testing of the current drug delivery system before going to the human patient.

In addition to inducing irreversible weight loss, extended exposure to TAA also enhanced cartilage degeneration and dystrophic calcification. Why this pathological accumulation of calcium salts in necrotic or degenerated tissue was found is as yet unclear. Possibly the prolonged instability caused tissue and cell death, the debris of which could subsequently not be cleared by the innate phagocytic system under the immunosuppressive action of TAA. The observed effects of extended TAA exposure on cartilage integrity and the induction of dystrophic calcification in the current model were not likely to be merely due to overdosing. Based on joint space volume, the 0.7-mg dose (the lowest dosage used here) corresponds to 56-mg TAA in a human knee joint, which is only 1.4 times higher the 40-mg TAA bolus generally administered in patients. Even the 80 mg of bolus TAA administered in arthritic knees in several clinical studies did not result in adverse effects (C. E. Chavez-Chiang et al., 2011; N. R. Chavez-Chiang et al., 2012; Kumar, Dhir, Sharma, Sharma, & Singh, 2017; Popma et al., 2015). Moreover, the IA release of a dose of 60-mg TAA from a PLGA MS platform, equivalent to the lowest dosage used in the current study, was shown to be safe in knee OA patients (Bodick et al., 2013). Also in a different, collagenase-induced, rat model of OA, the extended release of TAA by PEA MSs did not show any effects on cartilage integrity nor induced tissue calcification (Rudnik-Jansen et al., 2017). Given the absence of effects in previous studies of unloaded PEA MSs in OA animals, the MS platform per se is not likely to have contributed to the effects found either (Janssen et al., 2016; Rudnik-Jansen et al., 2017), nor would have their combination with TAA bolus, given the absence of effects of either when administered separately. Together with the lack of effect of the bolus in OA animals and of TAA-releasing PEA MSs in non-operated healthy animals, our observations rather point towards an interaction of extended release of TAA with the pathological processes occurring in the ACLT + pMMx OA model.

Extension of tissue trauma-induced instability may have incited the effects of extended exposure found here. Although the differences in

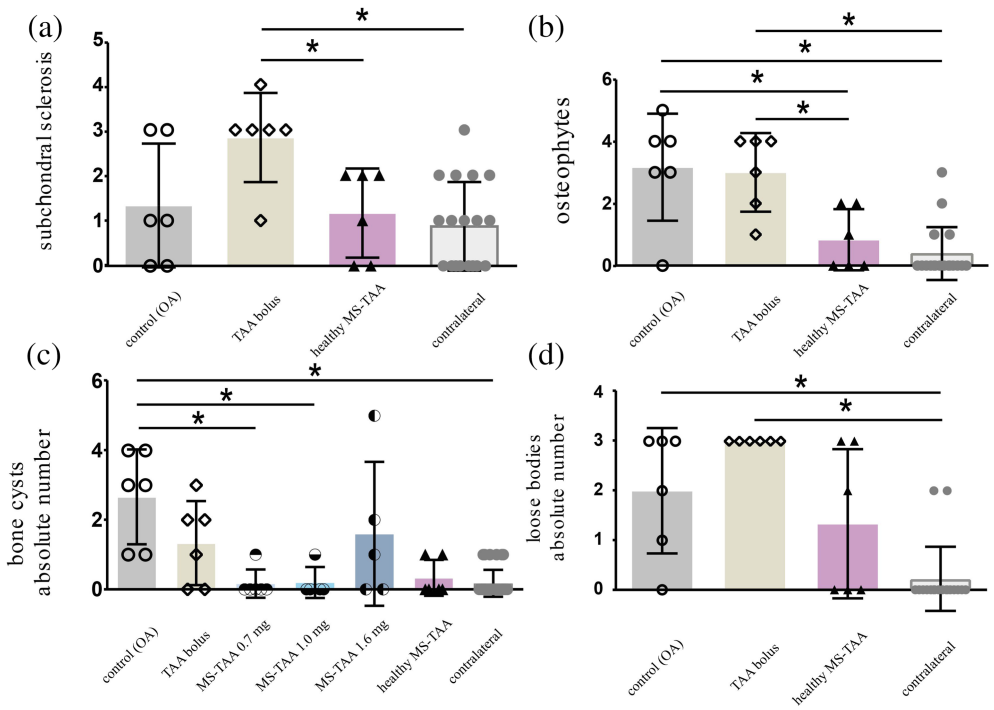

FIGURE 5 TAA extended delivery decreases bone cyst number, while bolus TAA does not alter boney changes in OA joints. μCT scans of medial tibia plateau were scored for (a) subchondral sclerosis, (b) osteophytes, (c) bone cysts, and (d) loose bodies. Individual data shown for control (OA) $n = 6$, TAA bolus $n = 6$, MS-TAA 0.7 mg $n = 6$, MS-TAA 1.0 mg $n = 5$, MS-TAA 1.6 mg $n = 5$, healthy joint MS-TAA 0.7 mg $n = 6$, and all contralateral joints $n = 18$, as well as mean ± SD. The extent of joint destruction together with calcifications made it impossible to score OA associated bone changes in MS-TAA treated OA joints. MS, microsphere; *$P < .05$, significantly different as indicated

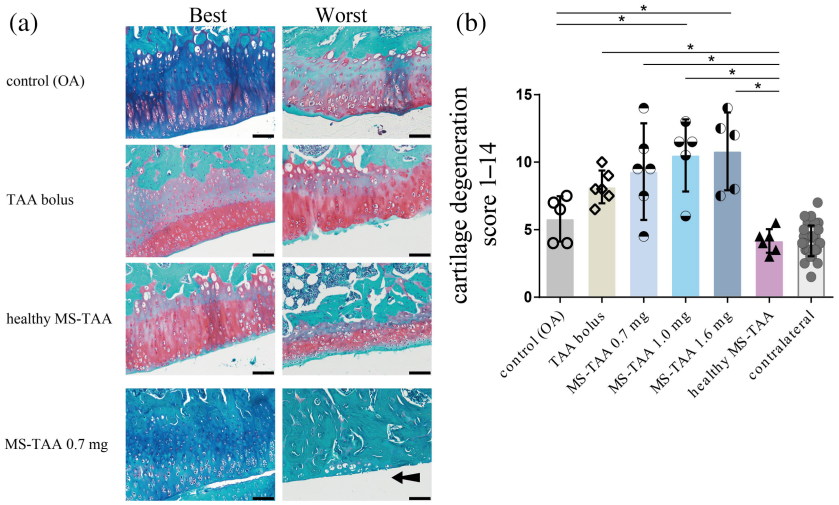

FIGURE 6 Extended TAA release in OA joints causes cartilage destruction. (a) Histological overview of best and worst outcomes (medial tibia plateau), stained with Safranin-O/Fast green. Arrow indicates the denuded bone surface. Scale bars: 100 µm. (b) Quantification of cartilage degeneration using Mankin score (1–14). Individual data shown for control (OA) n = 6, TAA bolus n = 6, MS-TAA 0.7 mg n = 6, MS-TAA 1.0 mg n = 5, MS-TAA 1.6 mg n = 5, healthy joint MS-TAA 0.7 mg n = 6, and all contralateral joint n = 32, as well as mean ± SD. MS, microsphere; *P < .05, significantly different as indicated

joint subluxation as change finding could not be statistically substantiated and would require additional studies with larger sample sizes to be proven, a role of extension of instability was supported by inhibition of cell outgrowth from tissue explants in culture: To address the possible mechanisms responsible for the effects of extended exposure to TAA, we studied its effect on wound healing in vitro, using a TAA concentration based on the range expected to be present in the synovial fluid (Hunder & Gleich, 1974; Kraus et al., 2018; Wallis et al., 1987). The continuous presence of TAA resulted in a complete block of migration of ligament or synovial capsule cells out of tissue explants in culture. Temporary exposure to TAA resulted in an intermediate effect, with a mere reduction of outgrowth of cells, that was significant for medial collateral, cruciate ligament, and synovial capsule tissue. This indicated that temporal TAA exposure diminished migration, but cells were able to resume the wound healing processes. This notion is in line with the suggested lower frequency of joint subluxation as determined post-mortem we found in the OA joints treated with bolus injections, compared to the apparent increase of instability in the joints exposed to MS-delivered TAA. The effect of TAA on tissue outgrowth was at least partly due to the inhibition of cell migration, as shown in the cell migration assay, in accordance with previous data on the effects of **dexamethasone** and TAA on tenocytes (Tempfer et al., 2009; Tsai, Tang, Wong, & Pang, 2003). TAA was also shown to inhibit human tenocyte collagen synthesis (Wong, Tang, Fu, Lee, & Chan, 2004), suggesting that tissue healing is impaired in many aspects. Hence, the extended exposure to TAA in the current study may have inhibited the general and naturally occurring arthrofibrosis in OA, a normal process leading to stabilization and

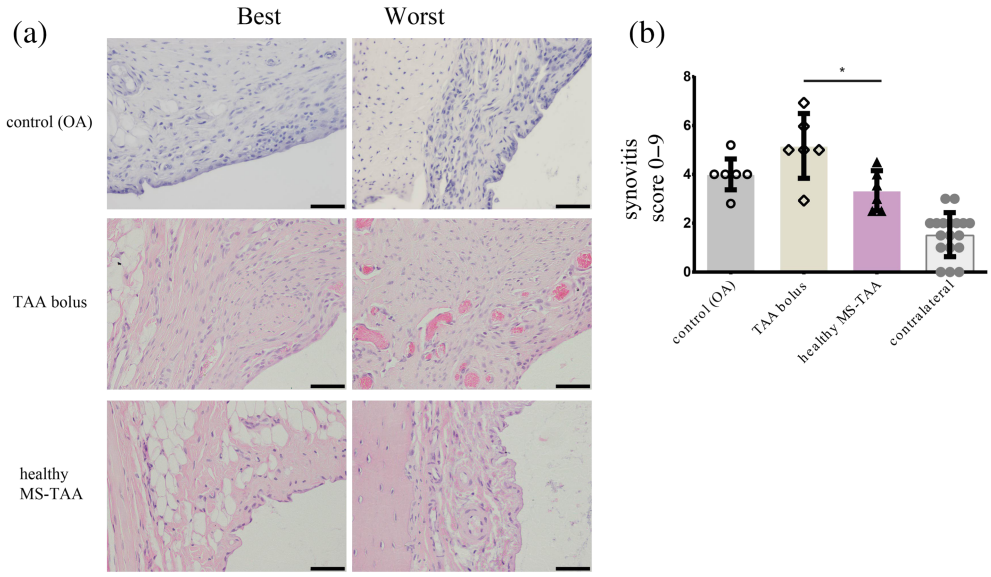

FIGURE 7 TAA bolus release in OA joints does not reduce synovitis. (a) Histological overview of best and worst outcomes. Scale bars: 50 µm. (b) Quantification of synovitis, using Krenn score (0–9). Individual data shown for control (OA) n = 6, TAA bolus n = 6, healthy joint MS-TAA 0.7 mg n = 6, and all contralateral joints n = 18, as well as mean ± SD. MS-TAA conditions are not scored or displayed, since the calcifications made it impossible to distinguish representable synovial tissue. MS, microsphere; *P < .05, significantly different as indicated

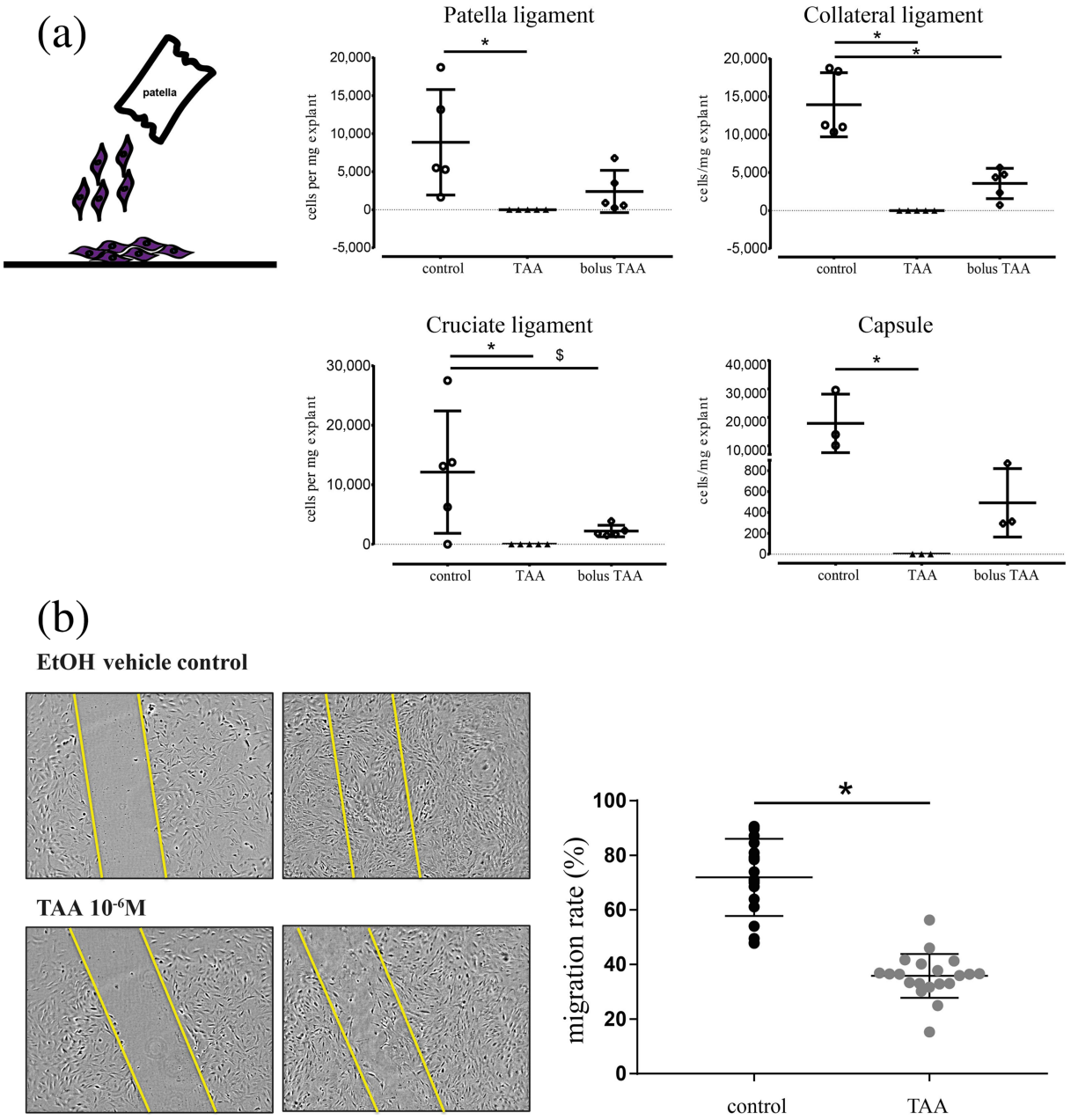

FIGURE 8 Continuous TAA exposure abolished outgrowth; temporary TAA exposure inhibited cell outgrowth. (a) Patella n = 5, collateral n = 5 and cruciate ligament n = 5 and capsule n = 3 rat explants exposed to vehicle control (EtOH), TAA (10-day exposure), or bolus TAA (4-day exposure). Individual data shown with mean ± SD, \$ P = .054 and *P < .05. (b) Quantified migration rates of primary rat patella ligament cells of four rats when exposed to vehicle control (EtOH) and TAA over 48 hr. n = 5 per rat, per condition. Individual data shown with mean ± SD, *P < .05, significantly different as indicated

stiffness of the joint (Shelbourne, Patel, & Martini, 1996). It has to be noted that the in vitro models used may have been suboptimal in reflecting the dynamic changes in synovial fluid concentrations over time, as only one dosage was used at a concentration well over the anticipated receptor saturation concentration (Mayer, Kaiser, Milholland, & Rosen, 1974), and exposure was limited to 10 days, whereas TAA released from MSs in vivo was detected up until 12 weeks after IA injection (Kraus et al., 2018). However, this is more likely to have caused an underestimation rather than overestimation of the effects found in vivo.

Although this was not a major aim and this study was underpowered to demonstrate the absence of differences, effects of TAA bolus injections in OA joints compared to non-treated joints, in contrast to the effects of the extended presence of TAA, are supported by various in vivo and clinical studies. Post-surgical injection of the corticosteroid dexamethasone for inhibition of acute inflammation in a rabbit model of surgically induced OA showed no side effects (Heard et al., 2015; Huebner, Shrive, & Frank, 2014). In patients, corticosteroids have been intravenously administered perioperatively and post-operatively during ACL repair surgery and orally afterwards to reduce

inflammation and pain, without complications (Dahl, Spreng, Waage, & Raeder, 2012; Vargas & Ross, 1989). A recent clinical trial showed that bolus TAA injections in patients with ACL injury, with the aim to delay or prevent post-traumatic OA, had no adverse events after 5 weeks of follow-up and even transiently exposure reduced some biomarkers indicative of joint degeneration and inflammation after injury (Lattermann et al., 2017). Hence, it is most likely that acute tissue trauma may be treated safely by a short acting dose of corticosteroids. However, it should be noted that small calcified depots have been described upon IA injections of patients with corticosteroids (Boulman, Slobodin, Rozenbaum, & Rosner, 2005; Harigane et al., 2011), possibly similar to the dystrophic calcifications found in the current study. In addition, corticosteroids can increase the elasticity of the ligament, thereby increasing knee joint laxity and the susceptibility for ligament ruptures (Khowailed, Petrofsky, Lohman, Daher, & Mohamed, 2015; Stijak et al., 2015), so even short-term IA exposure is not entirely without risks.

In general, controversy on the effect of corticosteroids on joint cartilage is still considerable. Negative effects of corticosteroids on cartilage integrity have been mainly described in vitro, in monocultures of chondrocytes or cartilage tissue explants (Euppayo et al., 2016; Suntiparpluacha, Tammachote, & Tammachote, 2016), although loss of safranin-O positivity was found upon IA injection of TAA-delivering MSs in healthy joints (Bodick et al., 2018). However, we showed that while TAA indeed inhibited proteoglycan content of cartilage explants in monoculture, in co-cultures of cartilage explants with synovial tissue, the same concentration of TAA enhanced proteoglycan synthesis (Beekhuizen et al., 2011). Also, in several clinical studies, no deleterious effects of IA corticosteroid use on tissue integrity were found (Bellamy et al., 2006; Raynauld et al., 2003; van Middelkoop et al., 2016), altogether suggesting that in the more complex context of the pathological joint, corticosteroids per se are not harmful.

Despite the increase of cartilage degeneration and dystrophic calcification, MS-delivered TAA did result in a decrease in number of bone cysts in the tibia. Other aspects of the boney phenotype of OA, such as osteophyte formation, could not be evaluated due to the disorganization of the joints and the dystrophic calcification found. Notwithstanding, the reduction in bone cysts by extended TAA release may point towards its potency for application in stable OA joints, as bone cysts have been suggested to be associated with pain, although not unequivocally demonstrated (Barr et al., 2015). Unfortunately, we discovered that pain could not be measured reliably in the current model using static weight-bearing analysis, in contrast to the applicability of this assay in detection of OA pain in mice models (Khatab et al., 2018; van Buul et al., 2014). Possibly, the weight changes between the hind paws were too subtle to detect with a static weight-bearing measurement device in rats. Other types of analyses previously used for detecting pain in rat studies, such as the von Frey or dynamic weight-bearing analysis, may be more suitable for measuring pain in future studies (Rudnik-Jansen, Schrijver, et al., 2019).

A factor complicating optimal development of IA drug delivery systems is the limited tools for analysis of local drug concentrations over time. Serum concentrations are inherently low as, after diffusion of the drug from the synovial space to the blood, the drug is diluted and cleared by the liver, easily falling below the limit of detection by HPLC (Derendorf, Mollmann, Gruner, Haack, & Gyselby, 1986). In rodent models commonly used in the first stage of preclinical development of novel therapies, synovial fluid sampling over time is impossible and very small quantities of plasma are available for analysis. In the current study, plasma TAA levels in the first 2 days reflected the initial burst release of TAA-loaded PEA occurring in vitro (Rudnik-Jansen et al., 2017). The area under the curve (AUC) of the plasma levels, indirectly showing loss from the synovial cavity and hence delivery by the MSs, was approximately 10 times smaller for the MS-based versus the bolus formulation of 0.7-mg TAA. However, whether the drug was released for the full period of 16 weeks after injection, as was demonstrated before in vitro (Rudnik-Jansen, Woike, et al., 2019), cannot be stated with certainty. PEA MSs loaded with a hydrophobic near IR dye were shown to still contain dye up until 70 days after injection in a rat OA model (Rudnik-Jansen et al., 2017), suggesting that release continued until at least the first 10 weeks. Moreover, in knee OA patients treated with a PLGA-based platform releasing TAA (Kraus et al., 2018), TAA was detected in the synovial fluid of knee OA patients up to 12 weeks after IA injection, but not in bolus injected joints. As we have shown in an acute arthritis model that PEA MS-based TAA release inhibited inflammation for a longer time period than PLGA-based TAA release (Rudnik-Jansen, Woike, et al., 2019), it is very likely that in the current study, release spanned even more than these 12 weeks. Nevertheless, more insight of the IA delivery kinetics, possibly using drug labelling, will be required for optimal development and prevention of the side effects found here.

In conclusion, extended TAA release in the joint has the potential to reduce systemic exposure to TAA and treat OA-associated pain and inflammation. However, we show that in a joint destabilized through ligament and meniscus transection, increased instability, dystrophic calcification, enhanced cartilage degeneration, and pain are induced. Most likely, extended TAA release, but not temporal exposure, in traumatic unstable joints leads to impaired wound healing and thereby extended instability leading to more tissue damage. Hence, in clinical practice, OA patients with (traumatic) unstable joints should not be treated with IA extended release formulations of TAA. To evaluate the efficacy of the used MS platform releasing TAA in the inhibition of pain, another animal model focused on inflammation and pain, without acute joint tissue damage is recommended.

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AUTHOR CONTRIBUTIONS

I.R. and A.R.T. conceived, designed, and performed most of the experiments and analysed the data, and I.R. wrote the manuscript. A.R.T. also critically reviewed the manuscript. N.W., G.M., and J.T. conceived and designed the experiments, manufactured and characterized provided products, and reviewed the manuscript. K.S. contributed to animal handling, in vitro studies and review of the manuscript. B.P. contributed to all μCT analysis, helpful discussions, and review of the manuscript. B.P.M. and P.J.E. contributed to animal surgery and review of the manuscript. R.E.T. and MJ.L.K. contributed to (histo) pathological examination of animals and review of the manuscript. E.G. contributed to histological processing of samples and review of the manuscript. H.V. contributed to animal handling, assistance of μCT, and review of the manuscript. A.E. and E.M. contributed to the detection of TAA in plasma samples and review of the manuscript. H.W. contributed to μCT analysis and review of the manuscript. M.A.T and L.B.C conceived and designed experiments, critically reviewed the manuscript, and oversaw the project.

CONFLICT OF INTEREST

P.E. received research grants to his department from Stryker, Active Implants, DePuy-Synthes, DSM Biomedical, Regentis, and Zimmer-Biomet for unrelated projects. J.T. and G.M. are co-inventors on the patent for the PEA biomaterial platform.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design & Analysis and Animal Experimentation, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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REFERENCES

Akhtar, M. A., Bhattacharya, R., & Keating, J. F. (2016). Generalised ligamentous laxity and revision ACL surgery: Is there a relation? *The Knee*, 23(6), 1148–1153. doi:10.1016/j.knee.2015.11.006

Alexander, S. P. H., Kelly, E., Marrion, N. V., Peters, J. A., Faccenda, E., Harding, S. D., … CGTP Collaborators (2017). THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Overview. *British Journal of Pharmacology*, 174, S1–S16. https://doi.org/10.1111/bph.13882

Barr, A. J., Campbell, T. M., Hopkinson, D., Kingsbury, S. R., Bowes, M. A., & Conaghan, P. G. (2015). A systematic review of the relationship between subchondral bone features, pain and structural pathology in peripheral joint osteoarthritis. *Arthritis Research & Therapy*, 17, 228. https://doi.org/10.1186/s13075-015-0735-x

Bartley, E. J., Palit, S., & Staud, R. (2017). Predictors of osteoarthritis pain: The importance of resilience. *Current Rheumatology Reports*, 19(9), 57. https://doi.org/10.1007/s11926-017-0683-3

Beekhuizen, M., Bastiaansen-Jenniskens, Y. M., Koevoet, W., Saris, D. B., Dhert, W. J., Creemers, L. B., & van Osch, G. J. (2011). Osteoarthritic synovial tissue inhibition of proteoglycan production in human osteoarthritic knee cartilage: Establishment and characterization of a long-term cartilage-synovium coculture. *Arthritis and Rheumatism*, 63(7), 1918–1927. https://doi.org/10.1002/art.30364

Bellamy, N., Campbell, J., Robinson, V., Gee, T., Bourne, R., & Wells, G. (2006). Intraarticular corticosteroid for treatment of osteoarthritis of the knee. *Cochrane Database of Systematic Reviews*, 2, CD005328. https://doi.org/10.1002/14651858.CD005328.pub2

Bodick, N., Lufkin, J., Willwerth, C., Blanks, R. C., Inderjeeth, C. A., Kumar, A., & Clayman, M. D. (2013). FX006 prolongs the residency of triamcinolone acetonide in the synovial tissues of patients with knee osteoarthritis. *Osteoarthritis and Cartilage*, 21, S144–S145. https://doi.org/10.1016/j.joca.2013.02.307

Bodick, N., Lufkin, J., Willwerth, C., Kumar, A., Bolognese, J., Schoonmaker, C., … Clayman, M. (2015). An intra-articular, extended-release formulation of triamcinolone acetonide prolongs and amplifies analgesic effect in patients with osteoarthritis of the knee: A randomized clinical trial. *The Journal of Bone and Joint Surgery. American Volume*, 97(11), 877–888. https://doi.org/10.2106/JBJS.N.00918

Bodick, N., Williamson, T., Strand, V., Senter, B., Kelley, S., Boyce, R., & Lightfoot-Dunn, R. (2018). Local effects following single and repeat intra-articular injections of triamcinolone acetonide extended-release: Results from three nonclinical toxicity studies in dogs. *Rheumatology and Therapy*, 5(2), 475–498. https://doi.org/10.1007/s40744-018-0125-3

Boulman, N., Slobodin, G., Rozenbaum, M., & Rosner, I. (2005). Calcinosis in rheumatic diseases. *Seminars in Arthritis and Rheumatism*, 34(6), 805–812. https://doi.org/10.1016/j.semarthrit.2005.01.016

Brennan-Speranza, T. C., Henneicke, H., Gasparini, S. J., Blankenstein, K. I., Heinevetter, U., Cogger, V. C., … Seibel, M. J. (2012). Osteoblasts mediate the adverse effects of glucocorticoids on fuel metabolism. *The Journal of Clinical Investigation*, 122(11), 4172–4189. https://doi.org/10.1172/JCI63377

Buckwalter, J. A., & Brown, T. D. (2004). Joint injury, repair, and remodeling: Roles in post-traumatic osteoarthritis. *Clinical Orthopaedics and Related Research*, (423), 7–16.

Caparroz-Assef, S. M., Bersani-Amado, C. A., Kelmer-Bracht, A. M., Bracht, A., & Ishii-Iwamoto, E. L. (2007). The metabolic changes caused by dexamethasone in the adjuvant-induced arthritic rat. *Molecular and Cellular Biochemistry*, 302(1–2), 87–98. https://doi.org/10.1007/s11010-007-9430-9

Chavez-Chiang, C. E., Sibbitt, W. L. Jr., Band, P. A., Chavez-Chiang, N. R., DeLea, S. L., & Bankhurst, A. D. (2011). The highly accurate anteriolateral portal for injecting the knee. *Sports Medicine, Arthroscopy, Rehabilitation, Therapy & Technology: SMARTT*, 3(1), 6. https://doi.org/10.1186/1758-2555-3-6

Chavez-Chiang, N. R., Sibbitt, W. L., Band, P. A., DeLea, S. L., Park, K. S., & Bankhurst, A. D. (2012). The outcomes and cost-effectiveness of intraarticular injection of the rheumatoid knee. *Rheumatology International*, 32(2), 513–518. https://doi.org/10.1007/s00296-010-1718-5

Christian, S. R., Anderson, M. B., Workman, R., Conway, W. F., & Pope, T. L. (2006). Imaging of anterior knee pain. *Clinics in Sports Medicine*, 25(4), 681–702. https://doi.org/10.1016/j.csm.2006.06.010

Curtis, M. J., Alexander, S., Cirino, G., Docherty, J. R., George, C. H., Giembycz, M. A., … Ahluwalia, A. (2018). Experimental design and analysis and their reporting II: updated and simplified guidance for authors

and peer reviewers. *British Journal of Pharmacology*, 175(7), 987–993. https://doi.org/10.1111/bph.14153

Dahl, V., Spreng, U. J., Waage, M., & Raeder, J. C. (2012). Short stay and less pain after ambulatory anterior cruciate ligament (ACL) repair: COX-2 inhibitor versus glucocorticoid versus both combined. *Acta Anaesthesiologica Scandinavica*, 56(1), 95–101. https://doi.org/10.1111/j.1399-6576.2011.02584.x

de la Harpe, A. L., & Brighton, S. W. (1989). The effect of long-term corticosteroid therapy on joint mobility. *Clinical and Experimental Rheumatology*, 7(1), 79–80.

Derendorf, H., Mollmann, H., Gruner, A., Haack, D., & Gyselby, G. (1986). Pharmacokinetics and pharmacodynamics of glucocorticoid suspensions after intra-articular administration. *Clinical Pharmacology and Therapeutics*, 39(3), 313–317. https://doi.org/10.1038/clpt.1986.45

Dieppe, P. A., & Lohmander, L. S. (2005). Pathogenesis and management of pain in osteoarthritis. *Lancet*, 365(9463), 965–973. https://doi.org/10.1016/S0140-6736(05)71086-2

Euppayo, T., Siengdee, P., Buddhachat, K., Pradit, W., Chomdej, S., Ongchai, S., & Nganvongpanit, K. (2016). In vitro effects of triamcinolone acetonide and in combination with hyaluronan on canine normal and spontaneous osteoarthritis articular cartilage. *In Vitro Cellular & Developmental Biology. Animal*, 52(7), 723–735. https://doi.org/10.1007/s11626-016-0022-4

Geborek, P., Mansson, B., Wollheim, F. A., & Moritz, U. (1990). Intraarticular corticosteroid injection into rheumatoid arthritis knees improves extensor muscles strength. *Rheumatology International*, 9(6), 265–270. https://doi.org/10.1007/BF00541322

Goldzweig, O., Carrasco, R., & Hashkes, P. J. (2013). Systemic adverse events following intraarticular corticosteroid injections for the treatment of juvenile idiopathic arthritis: Two patients with dermatologic adverse events and review of the literature. *Seminars in Arthritis and Rheumatism*, 43(1), 71–76. https://doi.org/10.1016/j.semarthrit.2012.12.006

Grant, H. J., Arthur, A., & Pichora, D. R. (2004). Evaluation of interventions for rotator cuff pathology: A systematic review. *Journal of Hand Therapy*, 17(2), 274–299. https://doi.org/10.1197/j.jht.2004.02.013

Guma, M., Olive, A., Roca, J., Forcada, J., Duro, J. C., Holgado, S., … Tena, X. (2002). Association of systemic lupus erythematosus and hypermobility. *Annals of the Rheumatic Diseases*, 61(11), 1024–1026. https://doi.org/10.1136/ard.61.11.1024

Harding, S. D., Sharman, J. L., Faccenda, E., Southan, C., Pawson, A. J., Ireland, S., … NC-IUPHAR (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Res.*, 46, D1091–D1106. https://doi.org/10.1093/nar/gkx1121

Harigane, K., Mochida, Y., Ishii, K., Ono, S., Mitsugi, N., & Saito, T. (2011). Dystrophic calcinosis in a patient with rheumatoid arthritis. *Modern Rheumatology*, 21(1), 85–88. https://doi.org/10.1007/s10165-010-0344-0

Hayami, T., Pickarski, M., Zhuo, Y., Wesolowski, G. A., Rodan, G. A., & Duong, L. T. (2006). Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. *Bone*, 38 (2), 234–243. https://doi.org/10.1016/j.bone.2005.08.007

Heard, B. J., Barton, K. I., Chung, M., Achari, Y., Shrive, N. G., Frank, C. B., & Hart, D. A. (2015). Single intra-articular dexamethasone injection immediately post-surgery in a rabbit model mitigates early inflammatory responses and post-traumatic osteoarthritis-like alterations. *Journal of Orthopaedic Research*, 33(12), 1826–1834. https://doi.org/10.1002/jor.22972

Huebner, K. D., Shrive, N. G., & Frank, C. B. (2014). Dexamethasone inhibits inflammation and cartilage damage in a new model of post-traumatic osteoarthritis. *Journal of Orthopaedic Research*, 32(4), 566–572. https://doi.org/10.1002/jor.22568

Hunder, G. G., & Gleich, G. J. (1974). Immunoglobulin E (IgE) levels in serum and synovial fluid in rheumatoid arthritis. *Arthritis and Rheumatism*, 17 (6), 955–963. https://doi.org/10.1002/art.1780170606

Janssen, M., Mihov, G., Welting, T., Thies, J., & Emans, P. (2014). Drugs and polymers for delivery systems in OA joints: Clinical needs and opportunities. *Polymers*, 6(3), 799–819. https://doi.org/10.3390/polym6030799

Janssen, M., Timur, U. T., Woike, N., Welting, T. J., Draaisma, G., Gijbels, M., … Emans, P. J. (2016). Celecoxib-loaded PEA microspheres as an auto regulatory drug-delivery system after intra-articular injection. *Journal of Controlled Release*, 244(Pt A), 30–40. https://doi.org/10.1016/j.jconrel.2016.11.003

Khatab, S., van Buul, G. M., Kops, N., Bastiaansen-Jenniskens, Y. M., Bos, P. K., Verhaar, J. A., & van Osch, G. J. (2018). Intra-articular injections of platelet-rich plasma releasate reduce pain and synovial inflammation in a mouse model of osteoarthritis. *The American Journal of Sports Medicine*, 46(4), 977–986. https://doi.org/10.1177/0363546517750635

Khowailed, I. A., Petrofsky, J., Lohman, E., Daher, N., & Mohamed, O. (2015). 17β-estradiol induced effects on anterior cruciate ligament laxness and neuromuscular activation patterns in female runners. *Journal of Women's Health (2002)*, 24(8), 670–680. https://doi.org/10.1089/jwh.2014.5184

Kilkenny, C., Browne, W., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Animal research: Reporting in vivo experiments: The ARRIVE guidelines. *British Journal of Pharmacology*, 160, 1577–1579.

Kraus, V. B., Conaghan, P. G., Aazami, H. A., Mehra, P., Kivitz, A. J., Lufkin, J., … Bodick, N. (2018). Synovial and systemic pharmacokinetics (PK) of triamcinolone acetonide (TA) following intra-articular (IA) injection of an extended-release microsphere-based formulation (FX006) or standard crystalline suspension in patients with knee osteoarthritis (OA). *Osteoarthritis and Cartilage*, 26(1), 34–42. https://doi.org/10.1016/j.joca.2017.10.003

Kumar, A., Dhir, V., Sharma, S., Sharma, A., & Singh, S. (2017). Efficacy of methylprednisolone acetate versus triamcinolone acetonide intra-articular knee injection in patients with chronic inflammatory arthritis: A 24-week randomized controlled trial. *Clinical Therapeutics*, 39(1), 150–158. https://doi.org/10.1016/j.clinthera.2016.11.023

Kumar, S., Singh, R. J., Reed, A. M., & Lteif, A. N. (2004). Cushing's syndrome after intra-articular and intradermal administration of triamcinolone acetonide in three pediatric patients. *Pediatrics*, 113(6), 1820–1824. https://doi.org/10.1542/peds.113.6.1820

Lattermann, C., Jacobs, C. A., Proffitt Bunnell, M., Huston, L. J., Gammon, L. G., Johnson, D. L., … Spindler, K. P. (2017). A multicenter study of early anti-inflammatory treatment in patients with acute anterior cruciate ligament tear. *The American Journal of Sports Medicine*, 45(2), 325–333. https://doi.org/10.1177/0363546516666818

Litwic, A., Edwards, M. H., Dennison, E. M., & Cooper, C. (2013). Epidemiology and burden of osteoarthritis. *British Medical Bulletin*, 105, 185–199. https://doi.org/10.1093/bmb/lds038

Mankin, H. J., & Lippiello, L. (1970). Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. *The Journal of Bone and Joint Surgery. American Volume*, 52(3), 424–434. https://doi.org/10.2106/00004623-197052030-00002

March, L., Smith, E. U., Hoy, D. G., Cross, M. J., Sanchez-Riera, L., Blyth, F., … Woolf, A. D. (2014). Burden of disability due to musculoskeletal (MSK) disorders. *Best Practice & Research. Clinical Rheumatology*, 28(3), 353–366. https://doi.org/10.1016/j.berh.2014.08.002

Mayer, M., Kaiser, N., Milholland, R. J., & Rosen, F. (1974). The binding of dexamethasone and triamcinolone acetonide to glucocorticoid receptors in rat skeletal muscle. *The Journal of Biological Chemistry*, 249(16), 5236–5240.

McAlindon, T. E., LaValley, M. P., Harvey, W. F., Price, L. L., Driban, J. B., Zhang, M., & Ward, R. J. (2017). Effect of intra-articular triamcinolone vs saline on knee cartilage volume and pain in patients with knee osteoarthritis: A randomized clinical trial. *JAMA*, 317(19), 1967–1975. https://doi.org/10.1001/jama.2017.5283

McCormack, R., Lamontagne, M., Vannabouathong, C., Deakon, R. T., & Belzile, E. L. (2017). Comparison of the 3 different injection techniques used in a randomized controlled study evaluating a cross-linked sodium hyaluronate combined with triamcinolone hexacetonide (cingal) for osteoarthritis of the knee: A subgroup analysis. *Clinical Medicine Insights. Arthritis and Musculoskeletal Disorders*, 10, 1179544117725026. https://doi.org/10.1177/1179544117725026

Nakabayashi, H., Koyama, Y., Sakai, M., Li, H. M., Wong, N. C., & Nishi, S. (2001). Glucocorticoid stimulates primate but inhibits rodent α-fetoprotein gene promoter. *Biochemical and Biophysical Research Communications*, 287(1), 160–172. https://doi.org/10.1006/bbrc.2001.5564

O'Brien, C. A., Jia, D., Plotkin, L. I., Bellido, T., Powers, C. C., Stewart, S. A., … Weinstein, R. S. (2004). Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength. *Endocrinology*, 145(4), 1835–1841. https://doi.org/10.1210/en.2003-0990

Onur, T. S., Wu, R., Chu, S., Chang, W., Kim, H. T., & Dang, A. B. (2014). Joint instability and cartilage compression in a mouse model of post-traumatic osteoarthritis. *Journal of Orthopaedic Research*, 32(2), 318–323. https://doi.org/10.1002/jor.22509

Palazzo, C., Nguyen, C., Lefevre-Colau, M. M., Rannou, F., & Poiraudeau, S. (2016). Risk factors and burden of osteoarthritis. *Annals of Physical and Rehabilitation Medicine*, 59(3), 134–138. https://doi.org/10.1016/j.rehab.2016.01.006

Panahifar, A., Jaremko, J. L., Tessier, A. G., Lambert, R. G., Maksymowych, W. P., Fallone, B. G., & Doschak, M. R. (2014). Development and reliability of a multi-modality scoring system for evaluation of disease progression in pre-clinical models of osteoarthritis: Celecoxib may possess disease-modifying properties. *Osteoarthritis and Cartilage*, 22(10), 1639–1650. https://doi.org/10.1016/j.joca.2014.06.013

Popma, J. W., Snel, F. W., Haagsma, C. J., Brummelhuis-Visser, P., Oldenhof, H. G., van der Palen, J., & van de Laar, M. A. (2015). Comparison of 2 dosages of intraarticular triamcinolone for the treatment of knee arthritis: Results of a 12-week randomized controlled clinical trial. *The Journal of Rheumatology*, 42(10), 1865–1868. https://doi.org/10.3899/jrheum.141630

Raynauld, J. P., Buckland-Wright, C., Ward, R., Choquette, D., Haraoui, B., Martel-Pelletier, J., … Pelletier, J. P. (2003). Safety and efficacy of long-term intraarticular steroid injections in osteoarthritis of the knee: A randomized, double-blind, placebo-controlled trial. *Arthritis and Rheumatism*, 48(2), 370–377. https://doi.org/10.1002/art.10777

Riley, G. P., Harrall, R. L., Constant, C. R., Cawston, T. E., & Hazleman, B. L. (1996). Prevalence and possible pathological significance of calcium phosphate salt accumulation in tendon matrix degeneration. *Annals of the Rheumatic Diseases*, 55(2), 109–115. https://doi.org/10.1136/ard.55.2.109

Rudnik-Jansen, I., Colen, S., Berard, J., Plomp, S., Que, I., van Rijen, M., … Creemers, L. (2017). Prolonged inhibition of inflammation in osteoarthritis by triamcinolone acetonide released from a polyester amide microsphere platform. *Journal of Controlled Release*, 253, 64–72. https://doi.org/10.1016/j.jconrel.2017.03.014

Rudnik-Jansen, I., Schrijver, K., Woike, N., Tellegen, A., Versteeg, S., Emans, P., … Creemers, L. (2019). Intra-articular injection of triamcinolone acetonide releasing biomaterial microspheres inhibits pain and inflammation in an acute arthritis model. *Drug Delivery*, 26(1), 226–236. https://doi.org/10.1080/10717544.2019.1568625

Rudnik-Jansen, I., Woike, N., de Jong, S., Versteeg, S., Kik, M., Emans, P., … Creemers, L. (2019). Applicability of a modified rat model of acute arthritis for long-term testing of drug delivery systems. *Pharmaceutics*, 11(2). https://doi.org/10.3390/pharmaceutics11020070

Sato, A. Y., Richardson, D., Cregor, M., Davis, H. M., Au, E. D., McAndrews, K., … Bellido, T. (2017). Glucocorticoids induce bone and muscle atrophy by tissue-specific mechanisms upstream of E3 ubiquitin ligases. *Endocrinology*, 158(3), 664–677. https://doi.org/10.1210/en.2016-1779

Shelbourne, K. D., Patel, D. V., & Martini, D. J. (1996). Classification and management of arthrofibrosis of the knee after anterior cruciate ligament reconstruction. *The American Journal of Sports Medicine*, 24(6), 857–862. https://doi.org/10.1177/036354659602400625

Siebelt, M., Korthagen, N., Wei, W., Groen, H., Bastiaansen-Jenniskens, Y., Muller, C., … Weinans, H. (2015). Triamcinolone acetonide activates an anti-inflammatory and folate receptor-positive macrophage that prevents osteophytosis in vivo. *Arthritis Research & Therapy*, 17, 352. https://doi.org/10.1186/s13075-015-0865-1

Stijak, L., Kadija, M., Djulejic, V., Aksic, M., Petronijevic, N., Aleksic, D., … Filipovic, B. (2015). The influence of sex hormones on anterior cruciate ligament ruptures in males. *Knee Surgery, Sports Traumatology, Arthroscopy*, 23(12), 3578–3584. https://doi.org/10.1007/s00167-014-3247-3

Su, X., Vicker, N., Lawrence, H., Smith, A., Purohit, A., Reed, M. J., & Potter, B. V. (2007). Inhibition of human and rat 11β-hydroxysteroid dehydrogenase type 1 by 18β-glycyrrhetinic acid derivatives. *The Journal of Steroid Biochemistry and Molecular Biology*, 104(3–5), 312–320. https://doi.org/10.1016/j.jsbmb.2007.03.019

Suntiparpluacha, M., Tammachote, N., & Tammachote, R. (2016). Triamcinolone acetonide reduces viability, induces oxidative stress, and alters gene expressions of human chondrocytes. *European Review for Medical and Pharmacological Sciences*, 20(23), 4985–4992.

Tanigawa, K., Nagase, H., Ohmori, K., Tanaka, K., Miyake, H., Kiniwa, M., & Ikizawa, K. (2002). Species-specific differences in the glucocorticoid receptor transactivation function upon binding with betamethasone-esters. *International Immunopharmacology*, 2(7), 941–950. https://doi.org/10.1016/S1567-5769(02)00036-X

Tataranni, P. A., Larson, D. E., Snitker, S., Young, J. B., Flatt, J. P., & Ravussin, E. (1996). Effects of glucocorticoids on energy metabolism and food intake in humans. *The American Journal of Physiology*, 271(2 Pt 1), E317–E325. https://doi.org/10.1152/ajpendo.1996.271.2.E317

Tellegen, A. R., Rudnik-Jansen, I., Beukers, M., Miranda-Bedate, A., Bach, F. C., de Jong, W., … Tryfonidou, M. A. (2018). Intradiscal delivery of celecoxib-loaded microspheres restores intervertebral disc integrity in a preclinical canine model. *Journal of Controlled Release*, 286, 439–450. https://doi.org/10.1016/j.jconrel.2018.08.019

Tempfer, H., Gehwolf, R., Lehner, C., Wagner, A., Mtsariashvili, M., Bauer, H. C., … Tauber, M. (2009). Effects of crystalline glucocorticoid triamcinolone acetonide on cultered human supraspinatus tendon cells. *Acta Orthopaedica*, 80(3), 357–362. https://doi.org/10.3109/17453670902988360

Tsai, W. C., Tang, F. T., Wong, M. K., & Pang, J. H. (2003). Inhibition of tendon cell migration by dexamethasone is correlated with reduced α-smooth muscle actin gene expression: A potential mechanism of delayed tendon healing. *Journal of Orthopaedic Research*, 21(2), 265–271. https://doi.org/10.1016/S0736-0266(02)00151-1

van Buul, G. M., Siebelt, M., Leijs, M. J., Bos, P. K., Waarsing, J. H., Kops, N., … van Osch, G. J. (2014). Mesenchymal stem cells reduce pain but not degenerative changes in a mono-iodoacetate rat model of osteoarthritis. *Journal of Orthopaedic Research*, 32(9), 1167–1174. https://doi.org/10.1002/jor.22650

van Middelkoop, M., Arden, N. K., Atchia, I., Birrell, F., Chao, J., Rezende, M. U., … Bierma-Zeinstra, S. M. (2016). The OA Trial Bank: Meta-analysis of individual patient data from knee and hip osteoarthritis trials show that patients with severe pain exhibit greater benefit from intra-articular glucocorticoids. *Osteoarthritis and Cartilage*, 24(7), 1143–1152. https://doi.org/10.1016/j.joca.2016.01.983

Vargas, J. H. 3rd, & Ross, D. G. (1989). Corticosteroids and anterior cruciate ligament repair. *The American Journal of Sports Medicine*, 17(4), 532–534. https://doi.org/10.1177/036354658901700414

Vos, T., Flaxman, A. D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., … Memish, Z. A. (2012). Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, 380(9859), 2163–2196. https://doi.org/10.1016/S0140-6736(12)61729-2

Wallis, W. J., Simkin, P. A., & Nelp, W. B. (1987). Protein traffic in human synovial effusions. *Arthritis and Rheumatism*, 30(1), 57–63. https://doi.org/10.1002/art.1780300108

Wong, M. W., Tang, Y. N., Fu, S. C., Lee, K. M., & Chan, K. M. (2004). Triamcinolone suppresses human tenocyte cellular activity and collagen synthesis. *Clinical Orthopaedics and Related Research*, (421), 277–281.

Young, J. M., Yoxall, B. E., & Wagner, B. M. (1978). Topical betamethasone 17-valerate is an anticorticosteroid in the rat. 1. Dermal atrophy. *The British Journal of Dermatology*, 99(6), 655–663. https://doi.org/10.1111/j.1365-2133.1978.tb07060.x

Zhang, Z., & Huang, G. (2012). Intra-articular lornoxicam loaded PLGA microspheres: Enhanced therapeutic efficiency and decreased systemic toxicity in the treatment of osteoarthritis. *Drug Delivery*, 19(5), 255–263. https://doi.org/10.3109/10717544.2012.700962

Zhu, W., Song, L., Zhang, H., Matoney, L., LeCluyse, E., & Yan, B. (2000). Dexamethasone differentially regulates expression of carboxylesterase genes in humans and rats. *Drug Metabolism and Disposition*, 28(2), 186–191.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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