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DOSE-DEPENDENT CHONDROTOXICITY OF INTRA-ARTICULAR VANCOMYCIN IN NEW ZEALAND WHITE RABBITS: A PROSPECTIVE TRUE EXPERIMENTAL STUDY

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ABSTRACT
Local vancomycin is increasingly used in orthopaedic procedures to increase antimicrobial exposure at the surgical site, but the concentration at which direct intra-articular exposure damages intact cartilage remains uncertain. This study aimed to determine whether intra-articular vancomycin produces concentration-dependent histopathological injury in New Zealand White rabbit knee cartilage. Twenty-eight male rabbits were randomised to saline control or vancomycin at 2.5, 5, or 7.5 mg/mL and observed for 14 days. Safranin-O/Fast Green-stained cartilage was assessed by blinded observers using Modified Mankin scoring and Osteoarthritis Research Society International grading. All animals completed follow-up without clinical infection or systemic adverse events. Mean Modified Mankin scores were 2.14 ± 0.69, 4.29 ± 1.25, 4.29 ± 1.98, and 7.71 ± 2.36 in the control, 2.5, 5, and 7.5 mg/mL groups, respectively; severe lesions occurred only at 7.5 mg/mL, affecting four of seven specimens. These findings demonstrate a concentration-related in vivo cartilage safety signal and provide tissue-level evidence that complements recent chondrocyte toxicity and local vancomycin pharmacokinetic studies.

Keywords: articular cartilage; chondrotoxicity; intra-articular injection; new zealand white rabbit; vancomycin

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INTRODUCTION

Postoperative infection after arthroscopic and other intra-articular orthopaedic procedures is uncommon but potentially destructive when it involves a native joint. Infection can alter the synovial environment, trigger inflammatory cartilage degradation, require repeated debridement, and compromise long-term joint function. Recent clinical evidence in cartilage-preserving ligament surgery illustrates both the clinical relevance of infection prevention and the growing use of local vancomycin. Rougereau et al. (2024), in a two-centre cohort of arthroscopic anatomic lateral ankle ligament reconstructions, reported a reduction in postoperative infection after adoption of vancomycin-soaked grafts. The importance of prevention therefore extends beyond the absolute infection rate: a low-frequency complication can still be consequential when the affected tissue has limited capacity for biological repair.

Articular cartilage is a specialised load-bearing tissue maintained by a relatively sparse population of chondrocytes embedded within an extracellular matrix rich in type II collagen and proteoglycans. Because the tissue is avascular and has limited intrinsic repair capacity, preservation of chondrocyte viability and matrix integrity is central to cartilage-preserving surgery. Experimental rabbit studies demonstrate that structural surface injury, altered cellularity, loss of glycosaminoglycan-rich matrix, and progressive histological degeneration can be detected reproducibly with established cartilage scoring approaches (Pimenta et al., 2023; Yan et al., 2023; Yuan et al., 2023). These biological characteristics create a specific safety problem whenever a locally administered antimicrobial comes into direct contact with intact articular cartilage.

Local antibiotic delivery is attractive in orthopaedic surgery because it can generate high antimicrobial concentrations at the operative site while limiting systemic exposure. Vancomycin is frequently selected for local use because of its activity against clinically important Gram-positive organisms. Contemporary clinical studies show that intra-articular vancomycin can produce substantial local exposure with comparatively low serum concentrations. Burns et al. (2023) measured high postoperative joint-fluid levels after intra-articular vancomycin in primary arthroplasty without an observed renal or ototoxicity signal. Li et al. (2024) prospectively documented high intra-articular vancomycin concentrations after direct injection in total knee arthroplasty while serum concentrations remained below conventional systemic toxicity thresholds. Stauss et al. (2025) likewise found low systemic levels after intra-articular vancomycin powder in primary total joint arthroplasty. These data support the feasibility of local delivery, but they principally address systemic safety rather than the biological response of intact cartilage.

That distinction is critical when evidence from arthroplasty is extrapolated to procedures that preserve native articular surfaces. In total hip or knee arthroplasty, most diseased articular cartilage is resected before implantation. In arthroscopy, ligament reconstruction, intra-articular fracture treatment, cartilage repair, and selected trauma procedures, native cartilage remains present and may be exposed directly to the administered solution or to drug released from a soaked graft or local depot. A concentration that is acceptable from the perspective of serum toxicity may therefore still be harmful to chondrocytes. The clinically relevant exposure is constrained by two competing objectives: maintaining sufficient local antimicrobial activity while avoiding iatrogenic injury to host joint tissues.

Recent primary laboratory studies have raised concern that these objectives may overlap. Röhner et al. (2021) demonstrated concentration-dependent vancomycin toxicity in human chondrocytes. Hall et al. (2024) subsequently showed that 5 and 10 mg/mL vancomycin reduced viability of human primary knee chondrocytes, whereas 1.25 and 2.5 mg/mL were better tolerated; their experimental work also supported an apoptotic component of cell death. However, the toxicological literature is not uniform. Mayr et al. (2023), using a different in vitro model and comparative antibiotic conditions, found relatively favourable cartilage-cell viability with vancomycin compared with gentamicin and clindamycin. Taken together, these studies indicate that nominal concentration is important but not sufficient to predict tissue response; exposure duration, cell source, assay method, extracellular matrix, and clearance conditions can materially alter observed toxicity.

The intact joint environment adds another layer of complexity. After intra-articular administration, cartilage exposure depends on the delivered concentration and volume, native synovial fluid, joint size, postoperative bleeding, irrigation, drainage, tissue binding, inflammation, and time-dependent clearance. Bue et al. (2021) demonstrated in a porcine microdialysis model that intra-articular vancomycin produces high and measurable synovial concentrations over time. Pharmacokinetic evidence therefore confirms that cartilage can be exposed to clinically meaningful local concentrations, but it does not establish whether structural tissue injury follows. Controlled in vivo histological studies are needed because intact extracellular matrix, synovium, subchondral bone, and joint biomechanics can modify effects observed in isolated cells.

New Zealand White rabbits provide a practical model for this question because their knees permit standardised injection, tissue harvesting, histological preparation, and comparison across controlled treatment groups. Contemporary rabbit studies have used Mankin-type and OARSI-based histological assessment to characterise experimentally induced cartilage degeneration and chondrotoxicity (Pimenta et al., 2023; Yan et al., 2023; Yuan et al., 2023). At the same time, spontaneous background lesions and species-specific histological features must be recognised when interpreting small-animal experiments (Eggers Carroll et al., 2024). The model is therefore suitable

for identifying a preclinical concentration-response safety signal, but not for defining a direct human dosing threshold.

A further methodological issue is that concentration, total dose, and exposure route are not interchangeable. Vancomycin powder placed in a wound, vancomycin dissolved for direct intra-articular injection, and a tendon graft soaked in vancomycin may deliver very different concentration-time profiles to cartilage even when the nominal amount of drug is similar. Direct injection provides a defined starting concentration but is subsequently modified by synovial dilution and clearance; powder may dissolve gradually; and graft soaking may localise drug around the graft before diffusion into the joint. This distinction is relevant to experimental design because a biologically safe total dose could still create a transiently high local concentration at the cartilage surface, whereas the same dose distributed in a larger volume could produce a lower peak exposure. A concentration-response experiment therefore isolates a clinically important variable that cannot be inferred reliably from dose alone.

The objective of this study was to determine whether direct intra-articular vancomycin exposure causes concentration-dependent structural cartilage injury in New Zealand White rabbit knees. Specifically, saline control was compared with vancomycin concentrations of 2.5, 5, and 7.5 mg/mL over a 14-day observation period, with cartilage evaluated using Safranin-O/Fast Green staining, Modified Mankin scoring, and Osteoarthritis Research Society International (OARSI) grading. The prespecified hypothesis was that concentrations of 5 mg/mL or higher would be associated with measurable and progressively greater histopathological cartilage damage.

METHOD

Ethics, reporting, and use of artificial intelligence

Ethical approval was obtained before the experiment from the Health Research Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara (protocol no. 2603265-KEPK-USU). All procedures were conducted in accordance with institutional animal-care procedures, the principles of replacement, reduction, and refinement (3Rs), and ARRIVE 2.0 reporting principles for in vivo animal experiments. Animals were euthanised under deep anaesthesia by trained laboratory personnel according to the approved protocol, followed by confirmation of death before tissue harvesting. No human participants or identifiable human data were involved in this experiment.

Artificial intelligence assistance was used only for grammatical revision and language refinement during manuscript preparation. It was not used for study conception, study design, data collection, statistical analysis, interpretation of results, reference generation, or development of the scientific conclusions. All data, references, intellectual content, and final manuscript decisions were independently verified and approved by the authors.

Study design, setting, and animals

This prospective true experimental animal study used a post-test-only control group design. The experiment was conducted from March to April 2026 in an institutional pharmacology laboratory. Twenty-eight male New Zealand White rabbits weighing 2-3 kg were included. Only male rabbits were used to reduce biological variability in this exploratory experiment; consequently, sex-related biological variation was not assessed. Animals underwent clinical examination during acclimatisation. Exclusion criteria were death during acclimatisation, evidence of clinical illness, anorexia, or abnormal limb function before intervention. Animals were housed at 22 ± 2 °C and 55 ± 5% humidity under a 12:12-hour light-dark cycle, with standard chow and water available ad libitum.

The ethical design sought to balance the need for a controlled concentration-response experiment with reduction of unnecessary animal use. Standardised procedures, consistent follow-up, and

blinded histological assessment were used so that each animal contributed interpretable outcome data. Humane clinical observation was maintained throughout follow-up for signs of infection, altered feeding, lameness, wound complications, or systemic adverse effects.

Sample size, randomisation, and blinding

Sample size was estimated before the experiment using a resource-equation approach for a four-group exploratory animal design. Seven animals were allocated to each group, giving a total sample of 28 rabbits. After acclimatisation, animals were randomised to control, vancomycin 2.5 mg/mL, vancomycin 5 mg/mL, or vancomycin 7.5 mg/mL. The allocation sequence was generated before intervention. Histological observers were blinded to group assignment. Before formal scoring, representative slides were reviewed to harmonise application of the scoring criteria; final scoring was then conducted without knowledge of treatment allocation.

Anaesthesia, intervention, and follow-up

Anaesthesia was induced with ketamine hydrochloride 35 mg/kg and xylazine hydrochloride 5 mg/kg intramuscularly. Ketamine hydrochloride, xylazine hydrochloride, vancomycin hydrochloride powder, and sterile 0.9% sodium chloride were obtained from licensed institutional laboratory or pharmacy stock; corresponding batch information was recorded in the institutional laboratory logbook. Vancomycin hydrochloride powder was reconstituted aseptically with sterile 0.9% sodium chloride to produce the assigned concentrations. The control group received sterile 0.9% sodium chloride alone.

Intra-articular injection was performed using a sterile 1 mL disposable syringe and 27-gauge needle through a lateral knee approach. Synovial fluid aspiration was used to confirm intra-articular placement before injection. An equal injection volume was administered to all groups so that the intended experimental difference was vancomycin concentration rather than delivered volume. Animals were observed for 14 days after intervention for clinical signs of infection, lameness, anorexia, wound complication, or systemic adverse event. The 14-day endpoint was selected to identify acute to subacute histological changes after a single controlled exposure.

Tissue harvesting and histopathology

After the observation period, bilateral knee joints underwent arthrotomy, and cartilage specimens were harvested in a standardised manner from the medial femoral condyle and medial tibial plateau using microsaw irrigation. Standardisation of harvest location was used to reduce variation attributable to regional differences in cartilage thickness and loading environment.

Samples were fixed in 10% neutral buffered formalin, decalcified with 10% ethylenediaminetetraacetic acid (EDTA), embedded in paraffin, and sectioned sagittally at 5 µm with perpendicular orientation to the articular surface. Sections were stained with Safranin-O/Fast Green according to the institutional histopathology laboratory protocol. Safranin-O was used to visualise proteoglycan-rich cartilage matrix, and reduced staining intensity was interpreted together with structural findings as evidence of matrix glycosaminoglycan depletion. Slides were examined using a standard bright-field light microscope, and digital images were captured for blinded histopathological assessment and figure preparation. Recent experimental rabbit cartilage studies have used comparable histological staining and semiquantitative scoring approaches to characterise matrix and structural injury (He et al., 2024; Pimenta et al., 2023).

Outcome measures

The primary outcome was the Modified Mankin score, which evaluates structural architecture, cellularity, proteoglycan staining, and tidemark-related changes. The secondary outcome was OARSI cartilage histopathology grading, which provides lesion-oriented assessment of the depth and extent of cartilage degeneration. Recent rabbit experiments support the use of Mankin-type

scoring for cartilage injury and repair (Pimenta et al., 2023; Yuan et al., 2023) and OARSI-based grading for experimental cartilage degeneration (Yan et al., 2023).

Two independent observers assessed all slides while blinded to group allocation. Before formal scoring, the observers reviewed representative training slides to harmonise interpretation of scoring criteria. Disagreements in histopathological interpretation were reviewed jointly and resolved by consensus before final data entry. The analysis therefore used one final consensus score for each specimen.

Statistical analysis

Data were analysed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro-Wilk test. Because histological scores were ordinal or non-normally distributed, intergroup comparisons were conducted using the Kruskal-Wallis test. Pairwise exploratory comparisons were assessed using the Mann-Whitney U test and interpreted against a Bonferroni-adjusted threshold for six planned comparisons. A two-sided $P < 0.05$ was considered statistically significant for omnibus tests, and $P < 0.0083$ was used for pairwise comparisons after Bonferroni adjustment.

Descriptive results are presented as mean ± standard deviation where this form was available in the source dataset, together with categorical severity distributions and mean ranks. For statistical consistency, the Kruskal-Wallis result $H = 13.5$ with 3 degrees of freedom is reported with its corresponding chi-square approximation of $P = 0.004$. Pairwise values reported only as $P < 0.01$ in the source table are presented as such and are not over-interpreted against the stricter Bonferroni threshold when an exact P value is unavailable.

RESULT

Animal follow-up and clinical observations

All 28 animals completed the 14-day observation period, with seven animals analysed in each group. No dropout, clinical infection, lameness, anorexia, or systemic adverse event was recorded. Macroscopic inspection before tissue harvesting did not identify gross septic changes. These observations indicate that the experimental exposure was completed without overt systemic illness or clinically apparent joint infection that could independently confound the histological findings.

Modified Mankin score and cartilage damage severity

Modified Mankin scores demonstrated a concentration-related increase in cartilage injury. The control group had a mean score of 2.14 ± 0.69. Mean scores in the vancomycin 2.5 mg/mL and 5 mg/mL groups were both 4.29, with standard deviations of 1.25 and 1.98, respectively. The highest mean score was observed in the 7.5 mg/mL group at 7.71 ± 2.36. The increasing dispersion at higher exposure levels was accompanied by a shift toward more advanced structural abnormalities.

Table 1.
Mean Modified Mankin score after intra-articular vancomycin exposure.

Treatment group	n	Mean Modified Mankin score	Standard deviation
G1, control	7	2.14	0.69
G2, vancomycin 2.5 mg/mL	7	4.29	1.25
G3, vancomycin 5 mg/mL	7	4.29	1.98
G4, vancomycin 7.5 mg/mL	7	7.71	2.36
Total	28	4.61	2.59

The categorical distribution of damage severity showed the same directional pattern. All seven control specimens were classified as minimal damage. In the 2.5 mg/mL group, five specimens showed minimal damage and two showed moderate damage. In the 5 mg/mL group, three specimens showed minimal damage and four showed moderate damage. In the 7.5 mg/mL group, one specimen showed minimal damage, two showed moderate damage, and four showed severe

damage. Severe lesions were therefore observed only in the highest-concentration group, where they affected four of seven specimens.

Table 2.
Cartilage damage severity based on Modified Mankin scoring

Treatment group	Minimal, n	Moderate, n	Severe, n	Total, n
G1, control	7	0	0	7
G2, vancomycin 2.5 mg/mL	5	2	0	7
G3, vancomycin 5 mg/mL	3	4	0	7
G4, vancomycin 7.5 mg/mL	1	2	4	7

The Kruskal-Wallis analysis identified an intergroup difference in Modified Mankin scores (H = 13.5, df = 3, P = 0.004). Mean ranks increased across the concentration gradient from 8.50 in controls to 11.93 at 2.5 mg/mL, 15.36 at 5 mg/mL, and 22.21 at 7.5 mg/mL. The monotonic increase in mean ranks supports a concentration-response pattern in the ordinal distribution of histological injury.

Table 3.
Mean ranks for intergroup differences in Modified Mankin scores.

Treatment group	n	Mean rank
G1, control	7	8.50
G2, vancomycin 2.5 mg/mL	7	11.93
G3, vancomycin 5 mg/mL	7	15.36
G4, vancomycin 7.5 mg/mL	7	22.21

Kruskal-Wallis H = 13.5, df = 3, P = 0.004.

OARSI cartilage histopathology grading

OARSI grading supported the same overall direction of effect. The control group showed the mildest lesions, whereas higher vancomycin concentrations showed progressively deeper and more extensive cartilage damage. The OARSI grade distribution differed across groups (Kruskal-Wallis H = 21.756, df = 3, P < 0.001).

Exploratory pairwise Mann-Whitney analysis was interpreted against the Bonferroni-adjusted threshold of P < 0.0083. The comparisons of 2.5 versus 5 mg/mL (P = 0.001) and 2.5 versus 7.5 mg/mL (P = 0.002) met the adjusted threshold, whereas control versus 2.5 mg/mL (P = 0.202) and 5 versus 7.5 mg/mL (P = 0.08) did not. For control versus 5 mg/mL and control versus 7.5 mg/mL, the source dataset reports P < 0.01 without an exact value; these comparisons are therefore reported descriptively without classifying them against the stricter P < 0.0083 threshold. Representative Safranin-O/Fast Green-stained sections show increasing surface irregularity, chondrocyte abnormalities, vascular invasion, and thinning in higher-score specimens.

Table 4.
Mann-Whitney post-hoc analysis of OARSI scores between treatment groups

Comparison	Mean rank	Mann-Whitney U	P value
G1 vs G2	6.29 vs 8.71	16	0.202
G1 vs G3	4.00 vs 11.00	0	<0.01
G1 vs G4	4.00 vs 11.00	0	<0.01
G2 vs G3	4.14 vs 10.86	1	0.001
G2 vs G4	4.14 vs 10.86	1	0.002
G3 vs G4	11.00 vs 10.86	12.5	0.08

Bonferroni-adjusted significance threshold: P < 0.0083. Values reported as P < 0.01 in the source dataset are retained without further classification because exact P values are unavailable. OARSI = Osteoarthritis Research Society International.

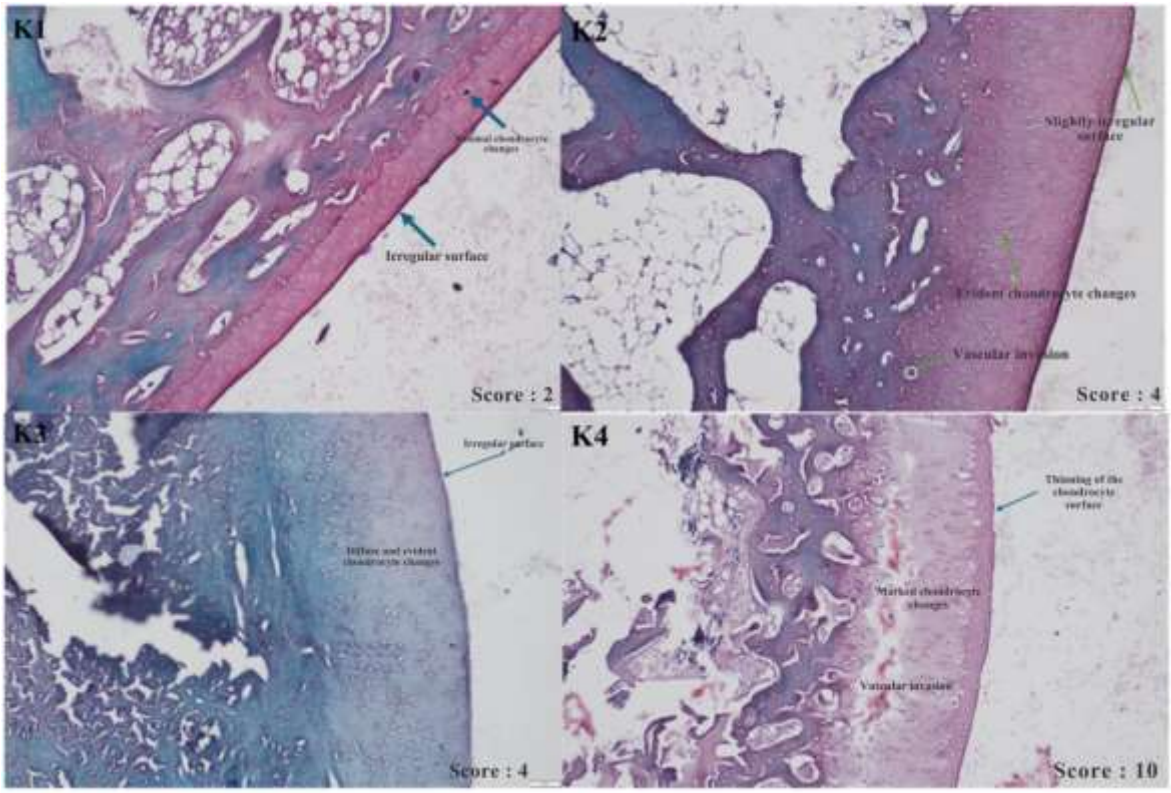

Figure 1. Representative Safranin-O/Fast Green-stained cartilage sections demonstrating Modified Mankin scoring patterns across treatment groups.

DISCUSSION

The principal finding of this in vivo experiment was a concentration-related pattern of cartilage injury after direct intra-articular vancomycin exposure. Controls had the lowest Modified Mankin and OARSI injury burden, whereas the 7.5 mg/mL group had the highest Modified Mankin scores and contained all specimens classified as severe damage. The convergence of a composite histological score, lesion-oriented grading, categorical severity distribution, and representative Safranin-O/Fast Green morphology supports the interpretation that the observed changes reflect a coherent tissue response rather than an isolated scoring artefact.

The present findings are consistent with recent primary chondrocyte studies. Röhner et al. (2021) demonstrated concentration-dependent toxicity of vancomycin in human chondrocytes. Hall et al. (2024) extended this evidence using human primary knee chondrocytes and found reduced viability at 5 and 10 mg/mL, while 1.25 and 2.5 mg/mL were better tolerated; their mechanistic experiments also supported apoptotic cell death. The rabbit data in the current study do not replicate an isolated-cell assay, but the direction of effect is concordant: tissue injury became more prominent as vancomycin concentration increased, with the clearest structural damage at 7.5 mg/mL.

The apparently more favourable results reported by Mayr et al. (2023) are also important because they demonstrate that vancomycin chondrotoxicity should not be reduced to a single universal numerical threshold. Their human cartilage-cell experiments compared vancomycin with gentamicin and clindamycin and found relatively favourable viability with vancomycin under the tested conditions. Differences in exposure duration, cell preparation, antibiotic formulation, culture environment, outcome assay, and extracellular matrix protection can change the magnitude of toxicity. The present experiment addresses a complementary question by examining tissue-level structural injury in a living joint, where synovial dilution, matrix diffusion, local clearance, and biomechanics modify exposure.

A recent in vivo rabbit study by Pimenta et al. (2023) provides a useful methodological parallel. Those investigators evaluated the chondrotoxic effects of intra-articular tranexamic acid and povidone-iodine using a New Zealand White rabbit model and Mankin histological parameters, demonstrating that local agents considered useful in orthopaedic practice can produce measurable cartilage changes in vivo. Although the tested compounds and exposure conditions differed from the present experiment, both studies support the broader principle that local intra-articular interventions require host-tissue safety evaluation rather than relying solely on systemic safety data. The distribution of injury across the present concentration groups is clinically informative. At 2.5 mg/mL, the mean Modified Mankin score was higher than in controls, but five of seven specimens remained in the minimal-damage category and no severe lesions occurred. At 5 mg/mL, the mean remained 4.29 but the distribution shifted, with four of seven specimens classified as moderate damage. At 7.5 mg/mL, the mean score increased to 7.71 and four of seven specimens were severe. This pattern illustrates why group means alone can conceal a clinically relevant redistribution of ordinal histological severity. For preclinical safety assessment, central tendency and the proportion of specimens crossing into higher injury categories should be interpreted together.

The use of both Modified Mankin and OARSI assessment strengthened the histological interpretation. Mankin-type scoring captures structural architecture, cellularity, matrix staining, and tidemark-related changes, whereas OARSI grading emphasises lesion depth and extent. Recent rabbit studies have used these frameworks to characterise experimental cartilage injury and degeneration. Yuan et al. (2023) applied Mankin scoring in a rabbit cartilage-defect experiment, while Yan et al. (2023) used OARSI histological scoring to demonstrate progressive post-meniscal-tear degeneration. Agreement between two complementary systems in the current study reduces the likelihood that the concentration-response pattern was driven by a single scoring construct.

Safranin-O/Fast Green morphology adds a biological anchor to the numerical scores. Reduced Safranin-O staining is compatible with loss or redistribution of proteoglycan-rich matrix when interpreted with structural changes, while surface irregularity, hypocellularity or abnormal cellular organisation, vascular invasion, and thinning indicate progressive tissue disturbance. He et al. (2024), in a rabbit cartilage-repair model, similarly used histological matrix assessment to evaluate structural changes after intervention. In the present study, the higher-score representative sections displayed progressively abnormal surface and cellular morphology, supporting the conclusion that increasing vancomycin concentration affected both matrix and structural compartments.

Clinical pharmacokinetic studies explain why this tissue-level question matters. Burns et al. (2023) found that intra-articular vancomycin in primary arthroplasty produced high joint-fluid concentrations with low systemic levels and no observed short-term renal or ototoxicity signal. Li et al. (2024) prospectively demonstrated high intra-articular concentrations after direct vancomycin injection in total knee arthroplasty while avoiding systemic toxicity. Stauss et al. (2025) likewise reported low serum vancomycin levels after intra-articular powder application in primary total joint arthroplasty. These studies support systemic tolerability of local delivery but do not establish preservation of intact native cartilage, because arthroplasty removes most articular surfaces before definitive reconstruction.

The distinction is especially relevant in cartilage-preserving procedures. Rougereau et al. (2024) reported reduced postoperative infection after introduction of vancomycin-soaked grafts in arthroscopic ankle ligament reconstruction, illustrating why local vancomycin is attractive outside arthroplasty. Burns et al. (2024) also reported reduced prosthetic infection after intra-articular vancomycin in a primary hip and knee arthroplasty series. These clinical studies address antimicrobial effectiveness, whereas the present experiment addresses a different endpoint: structural cartilage safety during direct intra-articular exposure. The two domains should be considered together rather than treating antimicrobial efficacy as evidence of host-tissue safety.

Pharmacokinetics provides the mechanistic bridge between dose and tissue injury. In a porcine knee microdialysis study, Bue et al. (2021) demonstrated high synovial vancomycin concentrations after intra-articular administration. The concentration actually experienced by cartilage after a clinical application will depend on dose, dilution volume, joint volume, irrigation, bleeding, drainage, tissue binding, inflammation, and clearance. A nominal dose in milligrams therefore cannot be interpreted independently from the volume and residence time that determine local concentration. The present experiment isolated one component of this relationship by maintaining an equal injection volume while varying vancomycin concentration.

The biological pattern is also compatible with a mechanism in which increasing extracellular drug concentration produces progressively greater cellular stress and matrix disturbance. Hall et al. (2024) identified loss of chondrocyte viability and apoptosis-related changes at higher vancomycin concentrations, while the present histology showed increasing abnormalities in cellular organisation and Safranin-O staining across the exposure gradient. These outcomes are not identical endpoints, but they are mechanistically complementary: chondrocyte injury can reduce matrix maintenance, and proteoglycan depletion can alter cartilage hydration and mechanical behaviour. The 14-day interval in the current study allowed these cellular and matrix effects to be expressed as structural histopathological changes. Because apoptosis, inflammatory signalling, and matrix-catabolic enzymes were not measured directly, the study cannot assign a single molecular pathway; however, the concordance between recent cellular experiments and the present tissue-level response supports biological plausibility for a concentration-dependent toxic effect.

Delivery method may further modify the relationship between antimicrobial efficacy and cartilage exposure. A vancomycin-soaked graft, as evaluated clinically by Rougereau et al. (2024), creates a local depot associated with soft tissue and graft material, whereas direct solution injection immediately exposes the whole synovial compartment. In arthroplasty studies by Burns et al. (2023, 2024), Li et al. (2024), and Stauss et al. (2025), cartilage preservation was not the principal safety endpoint because native surfaces were largely resected. These differences explain why apparently reassuring systemic safety results cannot be transferred automatically to arthroscopy or cartilage-preserving trauma surgery. For those procedures, the relevant safety question is not simply whether serum vancomycin remains low, but whether the concentration-time profile at the surviving articular surface remains compatible with chondrocyte and matrix integrity.

The experimental 14-day endpoint should therefore be viewed as a defined toxicological window rather than a model of all clinical exposure scenarios. A single intra-articular administration may be cleared substantially earlier than 14 days, but structural consequences of an early injury can remain detectable after the drug itself has disappeared. Conversely, the model does not reproduce repeated dosing, postoperative irrigation, drains, haemarthrosis, active infection, or inflamed synovium, each of which could alter exposure and tissue response. The strength of the design is internal control of concentration under otherwise standardised conditions; its limitation is that this control is achieved by simplifying the clinical environment. The observed concentration gradient is consequently most useful for establishing biological hazard and relative dose response, not for predicting the exact histological effect of a particular clinical protocol.

The statistical pattern should be interpreted with attention to the ordinal nature and small sample size of the experiment. The omnibus Modified Mankin analysis showed an intergroup difference, and mean ranks increased monotonically with concentration. OARSI grading showed an even stronger omnibus difference. The source dataset reported two pairwise comparisons only as $P < 0.01$; because the Bonferroni-adjusted threshold was $P < 0.0083$, the revised analysis avoids declaring those comparisons significant without exact P values. This conservative presentation does not alter the central descriptive finding that the 7.5 mg/mL group had the greatest histological injury burden, but it prevents overstatement of pairwise inferential evidence.

Several limitations constrain interpretation. First, seven animals per group provide an exploratory safety signal but limited precision for smaller effects. Second, the 14-day endpoint captures acute to subacute histological injury and cannot establish reversibility, chronic degeneration, or functional consequences. Third, synovial vancomycin concentrations were not measured, so histological severity cannot be linked directly to peak concentration, area-under-the-curve exposure, or time above a putative toxicity threshold. Fourth, the study did not include apoptosis markers, inflammatory cytokines, synovial histology, matrix immunohistochemistry, or cartilage biomechanics. Hall et al. (2024) identified apoptosis-related effects in human chondrocytes, making mechanistic validation in intact cartilage an important unresolved question. Fifth, only male rabbits were studied, so sex-related variation was not assessed.

The rabbit model also has inherent translational limitations. Species differences in cartilage thickness, cellularity, joint volume, baseline histopathology, and drug clearance mean that the observed concentrations should not be converted directly into a human dosing threshold. Eggers Carroll et al. (2024) documented the range of spontaneous histopathological findings in New Zealand White rabbits, highlighting the importance of controlled comparison with contemporaneous animals. Yan et al. (2023) likewise emphasised caution when extrapolating rabbit cartilage degeneration to humans. The current results are therefore best interpreted as comparative preclinical evidence of concentration-related injury within this model.

Another interpretive strength is the use of a saline control rather than comparison only among antibiotic concentrations. Background cartilage variation can occur even in healthy rabbits, and spontaneous lesions may influence histological scores (Eggers Carroll et al., 2024). The control group establishes the baseline distribution under the same housing, anaesthesia, injection, observation, tissue-harvesting, and processing conditions. The progressive increase in mean ranks from control through 7.5 mg/mL therefore occurs against a contemporaneous procedural reference rather than against historical normal values. In addition, all animals completed follow-up and there was no clinically apparent infection, making infection-related cartilage destruction an unlikely explanation for the between-group pattern. These design features do not eliminate all confounding, but they strengthen attribution of the observed concentration gradient to the experimental exposure within the limits of the model. The absence of severe lesions in control, 2.5 mg/mL, and 5 mg/mL groups, followed by four severe specimens at 7.5 mg/mL, is particularly relevant because it shows that the response involved not only incremental score differences but also a qualitative shift in the upper end of lesion severity.

The study nevertheless has several strengths: a controlled in vivo design, a prespecified concentration gradient, equal injection volumes, complete 14-day follow-up, standardised tissue harvesting, blinded assessment, and two complementary cartilage scoring systems. Recent primary evidence from cell culture, animal experiments, arthroscopic reconstruction, arthroplasty pharmacokinetics, and local infection prevention provides a coherent contemporary framework for interpretation. Within that framework, the present study adds tissue-level evidence that local vancomycin concentration is a relevant determinant of native cartilage safety and helps define the toxicological side of the risk-benefit balance for cartilage-preserving orthopaedic procedures.

CONCLUSION

Intra-articular vancomycin produced concentration-related histopathological cartilage injury in New Zealand White rabbit knees. The greatest Modified Mankin injury burden and all severe lesions occurred in the 7.5 mg/mL group, while OARSI grading showed the same overall concentration-related direction. The agreement between complementary histological assessments provides preclinical tissue-level evidence that native cartilage response depends on local vancomycin concentration. This study contributes in vivo safety data to the evaluation of local vancomycin in orthopaedic settings in which intact articular cartilage remains exposed.

REFERENCES

Bue, M., Thomassen, M. B., Larsen, O. H., Jørgensen, A. R., Stilling, M., Søballe, K., & Hanberg, P. (2021). Local vancomycin concentrations after intra-articular injection into the knee joint: An experimental porcine study. The Journal of Knee Surgery, 34(9), 936-940. https://doi.org/10.1055/s-0039-3402078

Burns, A. W. R., Chao, T., Tsai, N., Lynch, J. T., & Smith, P. N. (2023). The use of intra-articular vancomycin is safe in primary hip and knee arthroplasty. Journal of Orthopaedics, 46, 161-163. https://doi.org/10.1016/j.jor.2023.10.017

Burns, A. W. R., Smith, P., & Lynch, J. (2024). Intra-articular vancomycin reduces prosthetic infection in primary hip and knee arthroplasty. Arthroplasty Today, 26, 101333. https://doi.org/10.1016/j.artd.2024.101333

Eggers Carroll, E., Kumar, A., Romao, P., Ross, C. L., Henderson, W., & Sharma, A. K. (2024). Spontaneous histopathology in New Zealand White rabbits: Ten years of control data. Journal of Toxicologic Pathology, 37(3), 109-126. https://doi.org/10.1293/tox.2023-0132

Hall, S., Grayson, J., Grant, G., Vertullo, C., & Anoopkumar-Dukie, S. (2024). In vitro evaluation of vancomycin-induced toxicity in human primary knee chondrocytes. International Journal of Toxicology, 43(2), 177-183. https://doi.org/10.1177/10915818231216413

He, X., Han, Q., Zhang, Y., Zhang, H., Liu, J., & Zhou, X. (2024). Effect of collagen-based scaffolds with hydroxyapatite on the repair of cartilage defects in the rabbit knee joint. Journal of Orthopaedic Surgery and Research, 19, 818. https://doi.org/10.1186/s13018-024-05323-5

Li, X., Lai, J., Yang, X., Xu, H., & Xiang, S. (2024). Intra-articular injection of vancomycin after arthrotomy closure following gentamicin-impregnated bone cementation in primary total knee arthroplasty provides a high intra-articular concentration while avoiding systemic toxicity: A prospective study. Journal of Orthopaedic Surgery and Research, 19, 856. https://doi.org/10.1186/s13018-024-05357-9

Mayr, H. O., Regenbrecht, N., Mayr, M. F., Riedel, B., Hart, M. L., Schmal, H., & Seidenstuecker, M. (2023). Effect of vancomycin, gentamicin and clindamycin on cartilage cells in vitro. Biomedicines, 11(12), 3143. https://doi.org/10.3390/biomedicines11123143

Pimenta, F. S., de Oliveira Campos, T. V., de Abreu e Silva, G. M., Buzelin, M. A., Nunes, C. B., & de Andrade, M. A. P. (2023). Chondrotoxic effects of tranexamic acid and povidone-iodine on the articular cartilage of rabbits. International Orthopaedics, 47(10), 2429-2437. https://doi.org/10.1007/s00264-023-05820-y

Rougereau, G., Kassab Hassan, S., Valentin, E., Jehan, S., Bauer, T., Baudrier, N., & Hardy, A. (2024). Incidence of septic arthritis after vancomycin soaking of the graft during arthroscopic anatomic anterior talofibular ligament and calcaneofibular ligament reconstruction. Orthopaedic Journal of Sports Medicine, 12(3), 23259671241228276. https://doi.org/10.1177/23259671241228276

Röhner, E., Zippelius, T., Böhle, S., Rohe, S., Matziolis, G., & Jacob, B. (2021). Vancomycin is toxic to human chondrocytes in vitro. Archives of Orthopaedic and Trauma Surgery, 141(3), 375-381. https://doi.org/10.1007/s00402-020-03431-5

Stauss, R., Savov, P., Seeber, G. H., Brand, S., Ettinger, M., & Beheshty, J. A. (2025). Analysis of systemic serum vancomycin levels following intraarticular application in primary total joint arthroplasty. Archives of Orthopaedic and Trauma Surgery, 145, 60. https://doi.org/10.1007/s00402-024-05688-6

Yan, W., Fan, Y., Dai, W., Cheng, J., Zhao, F., Yang, S., Li, Y., Maimaitimin, M., Cao, C., Shao, Z., Li, Z., Wang, H., Hu, X., & Ao, Y. (2023). Earlier and more severe cartilage degeneration occurs after meniscal tears in juvenile rabbits compared with adults. Cartilage, 14(1), 106-118. https://doi.org/10.1177/19476035221138959

Yuan, L. B., Jin, T., Yao, L., Yin, D. H., & Xu, Y. Q. (2023). The role and mechanism of biological collagen membranes in repairing cartilage injury through the p38MAPK signaling pathway. Journal of Orthopaedic Surgery and Research, 18, 837. https://doi.org/10.1186/s13018-023-04261-y.