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Evaluation of effects of vancomycin/polycaprolactone nanocomposite in comparison with curcumin/polycaprolactone on the healing of experimental osteomyelitis in rabbit tibia

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Abstract

Osteomyelitis is caused by the local spread of an infected source adjacent to the infection following trauma, bone surgery or joint replacement. The present study aimed to evaluate the effect of vancomycin (Van)/polycaprolactone (PCL) nanocomposite in comparison with curcumin (Cur)/PCL on the healing of experimental osteomyelitis in tibia in rabbits. After induction of osteomyelitis forty adult male New Zealand white rabbits were randomly divided into four groups. Control group: The animals were considered as controls and no scaffolds were used. In PCL/Van group, the created bone defects were filled with the combination of PCL and Van. In PCL/Cur, the created bone defects were filled with the combination of PCL and Cur. Polycaprolactone/Cur/Van group: The created bone defects were filled with the combination of PCL, Cur and Van. Bone samples were taken for histopathological evaluation on the 30th and 60th days. For radiological evaluations of the osteomyelitis, radiographs were prepared at time intervals zero (day of surgery), 15, 30, 45, and 60 days after surgery. In order to evaluate hematology, blood was taken on days 0 (day of surgery), 15, 30, 45, and 60. The results of the present study showed that Cur nanocomposite significantly improved the healing process of the rabbit tibia experimental osteomyelitis model compared to the control group. Also, the PCL/Cur/Van group showed the best healing results. In conclusion, PCL/Cur/Van nanocomposite scaffold showed positive effects on the healing process.

Introduction

The advancement of pharmaceutical sciences and tissue engineering has made it possible to use tissue scaffolds combined with antibiotics in bone. The use of recombinant tissue scaffolds with antibiotics has prevented and treated infection as well as improved ossification in bone defects.[1,2] The use of degradable scaffolds is one of the main pillars of tissue engineering. An ideal scaffold should always have excellent biocompatibility, sufficient pore size, controllable and appropriate degradability, and ideal mechanical properties.[3] Polycaprolactone (PCL) is considered one of the suitable implants for bone tissue repair due to its significant advantages, including biocompatibility and absorbability.[4]

Nowadays, due to the antibiotic resistance of bacteria, the manufacturing and optimization of nanoparticles for drug delivery to the target tissues has been given attention. Drug-carrying nanoparticles have significant exclusive capabilities; which did not have those characteristics individually before becoming a nano drug compound.[5,6] The use of antibiotics directly at the site of infection can cause the presence of the drug with a high concentration in the focus of the infection. On the other hand, the systemic toxicity of the drug is also reduced.[7]

Glycopeptide antibiotics such as vancomycin (Van) and teicoplanin are widely used for the prevention and treatment of Gram-positive bacterial infections. Vancomycin was approved for clinical use in 1958 by the United States Food and Drug Administration.[8] Vancomycin is a special standard antibiotic for the treatment of acute infections caused by methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *S. aureus*, and also widely used for the treatment of other bacterial infections, such as pseudo colitis caused by *Clostridium difficile* and infections caused by coagulase-negative Staphylococci bacteria are

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used in hospitals.[9] The use of Van directly at the site of infection causes more effectiveness and less toxicity of this antibiotic in consumers.[10]

Curcumin (Cur) is a plant compound whose anti-bacterial and anti-inflammatory properties have been proven. Curcumin is a natural yellow phenolic substance obtained from the root of the plant (*Curcuma longa*). Its chemical name is Diferuloylmethane and its chemical formula is $C_{21}H_{20}O_6$.[11] Curcumin has attracted the attention of researchers due to its low intrinsic toxicity, and a wide range of pharmacological activities including antioxidant, anti-inflammatory, antimicrobial and anti-cancer properties.[11]

This study was aimed to evaluate the effects of PCL/Cur, PCL/Van and PCL/Cur/Van nanocomposite scaffolds in defects created in osteomyelitis in rabbit tibia bone.

Material and Methods

Preparation and synthesis of nanocomposite nanofibers. The preparation of nanofibers was carried out at Shaya Nanomeyar Technologists Company located in the Science and Technology Park of East Azarbaijan Province, Tabriz, Iran. Polycaprolactone (Sigma-Aldrich, St. Louis, USA) with a molecular weight of 80,000 was dissolved in a two-solvent system of acetic acid-formic acid (Merck, Darmstadt, Germany) at a ratio of 70:30 and a concentration of 20.00% (w/v), and then Cur (Sigma-Aldrich) and Van (Afa chemi, Tehran, Iran) were added gradually. They were added alone and together to the prepared polymer solution at a ratio of 5.00% (w/w). In the next step, the polymer solution was drawn into a 5.00 mL plastic syringe without a valve and placed in the feeding pump of the electrospinning machine. The electrospinning process was carried out at room temperature. Then, the foil containing the electrospun nanofibers was placed in an oven at a temperature of 37.00 ˚C for 48 to 72 hr to evaporate the solvent.[12] The scaffolds with a size of 1.00 × 1.00 cm were used inside the defects and sterilized by a ultraviolet device for 20 minbefore being placed inside the bone.A MIRA 3 scanning electron microscope (SEM; Tescan, Brno, Czech Republic) was used to observe the surface morphology of the scaffolds. A small piece of the scaffold was covered with a thin layer of gold and then imaged on the stage of the SEM microscope under vacuum, with a potential difference of 15.00 kV. Nanofiber diameter analysis was done using Digimizer image analysis software (version 6.3; MedCalc bvba, Ostend, Belgium). For this purpose, the diameter of 50fibers was randomly measured in the image of the nanofiber network. Structural features, arrangement, orientation, average and diameter distribution of nanofibers are shown in Figure 1.[13]

Preparation of bacteria. Standard *S. aureus* was obtained from the Persian Type Culture Collection (Tehran, Iran). After 18 - 24 hr, a young culture of bacteria was prepared in a tryptic soy broth (TSB) medium (Merck). The TSB medium containing bacteria was centrifuged three times with saline buffer at 3,000 rpm for 10 min, bacterial sediment was prepared and different amounts of bacteria were prepared. To use a specific volume of bacteria, a standard solution of half McFarland was used. In each mL of microbial suspension, equivalent to half McFarland, (CFU mL^{-1}) there are 1.50×10^8 bacteria. For injection, the amount of CFU mL^{-1} 1.00×10^7 of bacteria was required, and 100 µL of *S. aureus* suspension was prepared and injected.

Induction of osteomyelitis. To induce osteomyelitis, the method described in a previous study was used.[14] Rabbits were anesthetized by intramuscular injection of 50.00 mg kg^{-1} ketamine (Alfasan, Woerden, Netherlands) and 5.00 mg kg^{-1} xylazine (Alfasan) and regional hair was completely shaved at the right tibial bone site. The intended

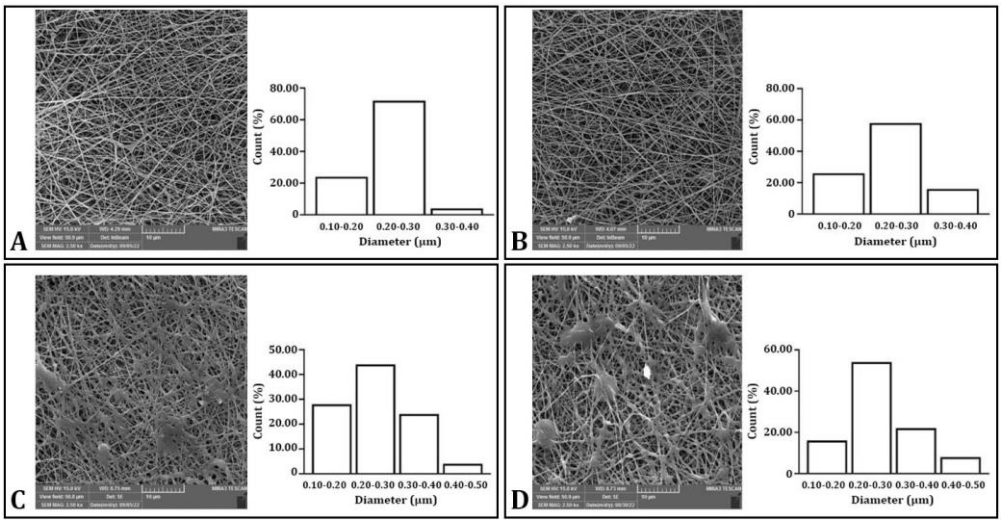

Fig. 1. Field emission scanning electron microscopy images of the synthesized **A)** Polycaprolactone (PCL)/Curcumin (Cur), **B)** PCL, **C)** PCL/Vancomycin (Van), and **D)** PCL/Cur/Van nanocomposites.

position was prepped. The injection site was selected on the proximal inner surface of the tibia, one centimeter below the knee joint and posterior to the tibial tuberosity. The solution containing *S. aureus* bacteria was administered percutaneously using a needle size 18. Normal saline (0.10 mL) was injected to ensure that the needle was placed in the right place. Then, 0.10 mL tetradecyl sulfate (Std Pharmaceutical Products Ltd., Hereford, UK), 0.10 mL tryptic broth containing 1.00×10^6 colony units of *S. aureus* administered. At the end, 0.10 mL normal saline was injected again to wash the tip of the needle to ensure complete entry of the bacteria. Four weeks after the injection of bacteria, in order to confirm the process of osteomyelitis, radiographs were taken from the bones and after confirming the results, the rabbits were divided into four experimental groups and prepared for the surgical procedure. The procedures of this work were approved by the University Ethical Committee and filed under IR.IAU.SRB.REC.1401.161 code. We followed instructions of the National Academy of Sciences Publication with numbers 85-23 that was revised in 1985.

Experimental animal model. Forty adult male rabbits of New Zealand white breed with an average weight of 2.50 to 3.00 kg were obtained from the Laboratory Animal Breeding and Maintenance Center of Guilan University. Animals were placed separately in propylene cages. To adapt to the environment, all the rabbits were kept for one week in the animal house under the standard conditions of natural day light cycle at 25.00 ˚C. All animals were kept under the same nutritional conditions with food and free access to water. After the injection of bacteria and the induction of osteomyelitis the animals were randomized into four groups of 10 animals each. Control group: The animals were considered as controls and no scaffolds were used. In PCL/Van group, the bone defects were filled with the combination of PCL and Van; in PCL/Cur, the bone defects were filled with the combination of PCL and Cur; and in PCL/Cur/Van group, the bone defects were filled with the combination of PCL, Cur and Van.

Surgical procedures. Induction of general anesthesia in rabbits with tibial osteomyelitis was performed by intramuscular injection of 50.00 mg kg^{-1} ketamine and 5.00 mg kg^{-1} xylazine. Then the medial surface of tibia was prepped surgically. The skin and muscles were dissected by a surgical blade. Abscesses and infectious soft tissue sinuses were debrided. A bone defect was created by a 3.50 mm drill head on the proximal medial cortex of the tibia. After creating a defect, the affected area was washed with normal saline, then 1.00 × 1.00 cm scaffolds were placed inside the defects, and muscles and skin was closed routinely. In the first 5 days after the surgery, every 24 hr, the wound and suture site were checked for bleeding and infection or suture opening and re-dressing was performed with sterile gauze. After 14 days, the skin sutures were removed and no tears were seen. The surgical site was treated according to grouping of each rabbit in each group. The surgical site was inspected daily to prevent infection or postoperative bleeding.[15]

Histopathologic studies. Five rabbits from each group were euthanized using an over dose of phenobarbital sodium (ChemiDaru, Tehran, Iran) on days 30 and 60 post operation. After general anesthesia with intravascular injection of phenobarbital sodium, the area of the scaffolds implantation were harvested in different groups. The samples were fixed in 10.00% buffered formalin fixing solution and paraffin embedding were prepared for histological study. Also, the selected bone tissues were placed in formic acid for decalcification. Hematoxylin and Eosin staining was done to study histopathology using light microscope (DM500; Leica, Wetzlar, Germany). Bone samples were graded in terms of filling of the defect site, inflammatory reaction, and bone recovery.[16]

Radiological evaluations. To carry out radiological evaluations, radiographs were prepared from the desired area at time intervals of before induction of osteomyelitis, on 0 (day of surgery), 15, 30, 45, and 60 days post-operation in lateral and ventrodorsally positions using a mobile CR X-ray (EPX-f2800; Konica Minolta, Tokyo, Japan) adjusted on 60.00 kV and 2.50 mAs. Sequestrum formation, periosteal new bone formation, destruction of bone, the extent of disease, soft tissue swelling and new bone formation were noted and scored based on the works of Nelson *et al.,* and Reizner *et al.* (Table 1).[17,18]

Table 1. Scoring system of radiologic evaluations.[16,17]

Variables	Definition	Score
Sequestrum formation	+: Present	2
	-: Absent	1
Periosteal new bone formation	+: Present	1
	±: Equivocal	½
	-: Absent	0
Bone destruction	++: Severe. Multiple areas involved	2
	+: Moderate. Only one area involved	1
	±: Mild. Only one area involved	½
	-: No Destruction	0
Disease extent: Involvement of each of three areas (proximal, middle, and distal of tibia)	+: Present	1
	±: Equivocal	½
	-: Absent	0

Hematological studies. Blood samples were collected from the jugular vein of all rabbits on days 0, 15, 30, 45 and 60. The 1.00 mL of blood, in tubes containing ethylenediaminetetraacetic acid was used for hematological evaluations. A clinical pathologist performed hematology evaluations using a Nihon Kohden Celltac alpha MEK-6500 device (Tokyo, Japan).

Statistical analyses. SPSS Software (version 18.0; IBM Corp., Armonk, USA) was used to perform statistical tests. First, the normality and homogeneity of data variance were checked by the Kolmogorov-Smirnov test. Then, based on those results, the differences between the test groups and the control groups were evaluated using One Way ANOVA in blood biochemistry parameters and Kruskal-Wallis non parametric ANOVA in radiology parameters. Numerical data were expressed as mean ± SD. The *p*-value less than 0.05 was considered as a significant level.

Results

Histopathology. In the histopathological examination of the experimental groups, there were no sign of secondary infection or colonization in any section evidenced by the absence of all criteria in any slides of control and experimental groups on the 30th and 60th days after surgery in any of the slides of the control and experimental groups on the 30th and 60th days after surgery. In the control group, the connection of fracture surfaces by fibrotic tissue, the beginning of spongy bone formation in a small amount (on the 30th day) to a large amount (on the 60th day) and the absence of dense bone and bone marrow formation were observed. In the PCL/Van group, the filling of the lesion spaces with high amounts of connective tissue and nanocomposite could be seen in the examination of day 30 slides, however, on the day 60 tissue slides, the filling of bone defect spaces with smaller amounts of connective tissue and bone plates was observed.

In the examination of the slides of the PCL/Cur group on the 30th day, as in the PCL/Van group, large amounts of connective tissue and nanocomposite were observed, while the examination of the slides of this group on the 60th day showed large amounts of bone tissue, the formation of bone marrow cavities and connection of the defect site with dense bone was observed. Examination of the histopathological sections from the PCL/Cur/Van implantation site on the 30th day showed the accumulation of large amounts of connective tissue and bone trabeculae. Similar to the other groups, the examination of the slides of this group also on the 60th day showed the accumulation of connective tissue, the formation of dense bone in the bone defect site, the filling of the defect site with spongy bone, the formation of bone marrow cavities and the rearrangement of bone plates (Fig. 2).

Radiological evaluations. The findings of analyses of scores of sequestrum formation, periosteal new bone formation, destruction of bone, extent of disease, soft tissue swelling and new bone formation are as shown in Table 2. The analyzed parameters showed that in the PCL/Cur/Van group the parameters were improved significantly compared to other groups ($p < 0.001$; Figs. 3 and 4).

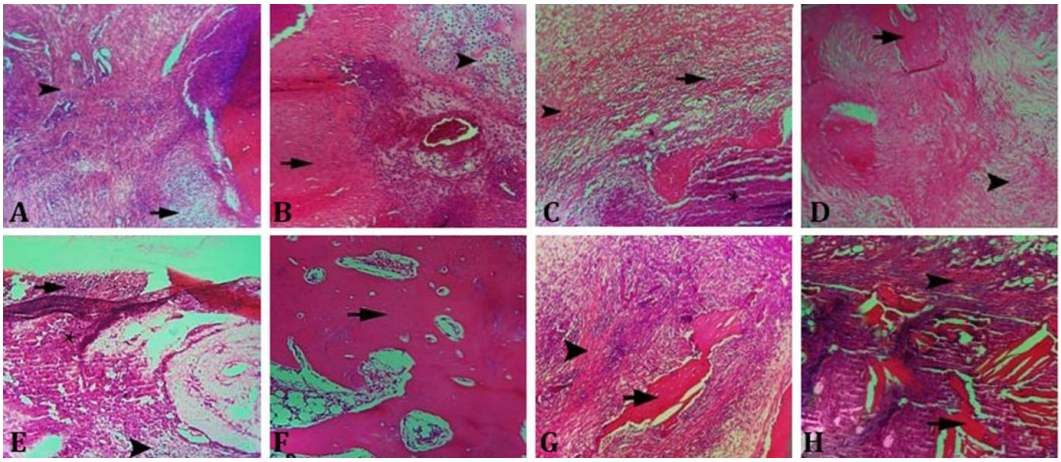

Fig. 2. A) Bone defect in the control group on day 30, large amounts of connective tissue (arrowhead) and small amounts of cartilage tissue (arrow) are observed. **B)** Bone defect in the control group on day 60, some connective tissue (arrowhead) and a large amount of cartilage tissue (arrow) are observed. **C)** Bone defect in polycaprolactone (PCL)/vancomycin (Van) group on day 30, accumulation of large amounts of connective tissue (arrowhead) and inflammation (arrow) and composite (*) can be seen. **D)** Bone defect in PCL/Van group on day 60, some connective tissue (arrowhead) and bone trabeculae (arrow) are observed. **E)** Bone defect in PCL/curcumin (Cur) group on day 30, the accumulation of large amounts of connective tissue (arrowhead) and inflammation (arrow) and composite (*) are observed. **F)** Bone defect in PCL/Cur on day 60, large amounts of bone tissue (arrow) can be observed. **G)** Bone defect in PCL/Cur/Van group on day 30, accumulation of large amounts of connective tissue (arrowhead) and bone trabeculae (arrow) can be observed. **H)** Bone defect in PCL/Cur/Van group on day 60, significant amounts of connective tissue (arrowhead) and many bone trabeculae (arrow) can be observed (Hematoxylin and Eosin staining; 10×).

Table 2. Radiographic evaluation of the healing process of defects on the 15th, 30th, 45th, and 60th days after surgery in different experimental groups.

Parameters	Groups	Day 15		Day 30		Day 45		Day 60	
		Score	*p*-value	Score	*p*-value	Score	*p*-value	Score	*p*-value
Sequestrum formation	Control	18	<0.001	10½	1.00	15½	<0.001	18	<0.001
	PCL/Van	8		10½		15½		10½	
	PCL/Cur	8		10½		15½		10½	
	PCL/Cur/Van	8		10½		5½		3	
Periosteal new bone formation	Control	3	<0.001	8	<0.001	10½	1.00	10½	1.00
	PCL/Van	10½		8		10½		10½	
	PCL/Cur	10½		8		10½		10½	
	PCL/Cur/Van	18		18		10½		10½	
Destruction of bone	Control	18	<0.001	18	<0.001	18	<0.001	18	<0.001
	PCL/Van	8		13		13		13	
	PCL/Cur	8		5½		5½		8	
	PCL/Cur/Van	8		5½		5½		3	
Extent of disease: mid tibia	Control	10½	1.00	18	<0.001	10½	1.00	18	<0.001
	PCL/Van	10½		8		10½		8	
	PCL/Cur	10½		8		10½		8	
	PCL/Cur/Van	10½		8		10½		8	
Extent of Disease: Proximal tibia	Control	10½	1.00	10½	1.00	18	<0.001	13	<0.001
	PCL/Van	10½		10½		8		13	
	PCL/Cur	10½		10½		8		13	
	PCL/Cur/Van	10½		10½		8		3	
Extent of disease: distal tibia	Control	10½	1.00	10½	1.00	10½	1.00	10½	1.00
	PCL/Van	10½		10½		10½		10½	
	PCL/Cur	10½		10½		10½		10½	
	PCL/Cur/Van	10½		10½		10½		10½	
Soft tissue swelling	Control	10½	1.00	15½	<0.001	18	<0.001	18	<0.001
	PCL/Van	10½		5½		8		8	
	PCL/Cur	10½		15½		8		8	
	PCL/Cur/Van	10½		5½		8		8	

PCL: Polycaprolactone; Cur: Curcumin; Van: Vancomycin.

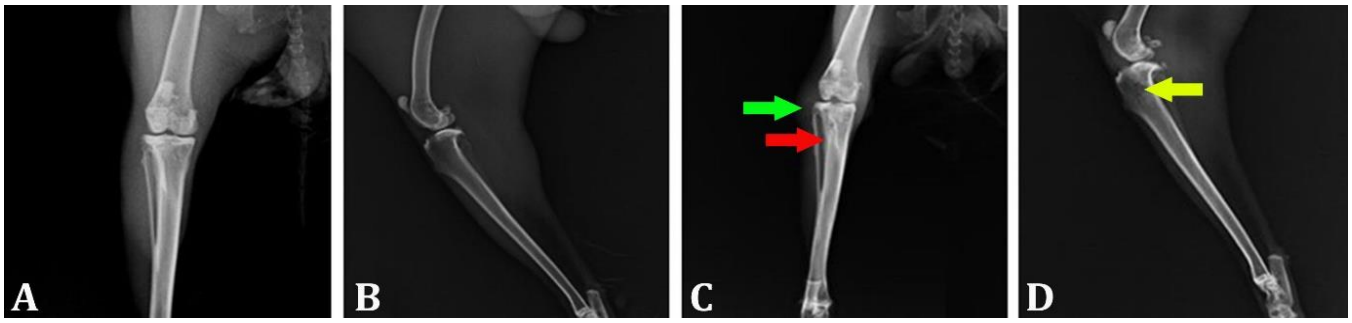

Fig. 3. Radiographic images of the defects **A** and **B)** before induction of osteomyelitis; **C** and **D)** four weeks after induction of osteomyelitis. Periosteal new bone formation (red arrow), destruction of bone (yellow arrow) and soft tissue swelling (green arrow) are shown.

Hematological studies. No significant difference was observed on day zero between the control and other experimental groups ($p \geq 0.05$). A significant difference was observed between the experimental groups and the control group on day 15. Application of Van and Cur on this day caused a decrease on day 60 in the average number of white blood cells (WBCs) in all groups compared to the control group ($p < 0.001$). No significant difference was observed between groups receiving Cur and Van ($p \geq 0.05$; Fig. 5). Analyzing the results on the 30th day showed a significant difference in all experimental groups compared to the control group ($p < 0.001$). On day 30, no significant difference was observed between PCL/Cur and PCL/Van groups ($p \geq 0.05$) and a significant difference was observed between the experimental groups and the control group on day 45 ($p < 0.001$; Fig. 5). There was no significant difference in the mean number of WBCs between the PCL/Cur and PCL/Van groups ($p \geq 0.05$), and the evaluation of the mean number of WBCs on the 60th day showed a significant difference in experimental groups compared to the control group ($p < 0.001$). Application of Cur along with Van caused a significant increase in the number of WBCs in the PCL/Cur/Van group compared to other experimental groups ($p < 0.001$; Fig. 5).

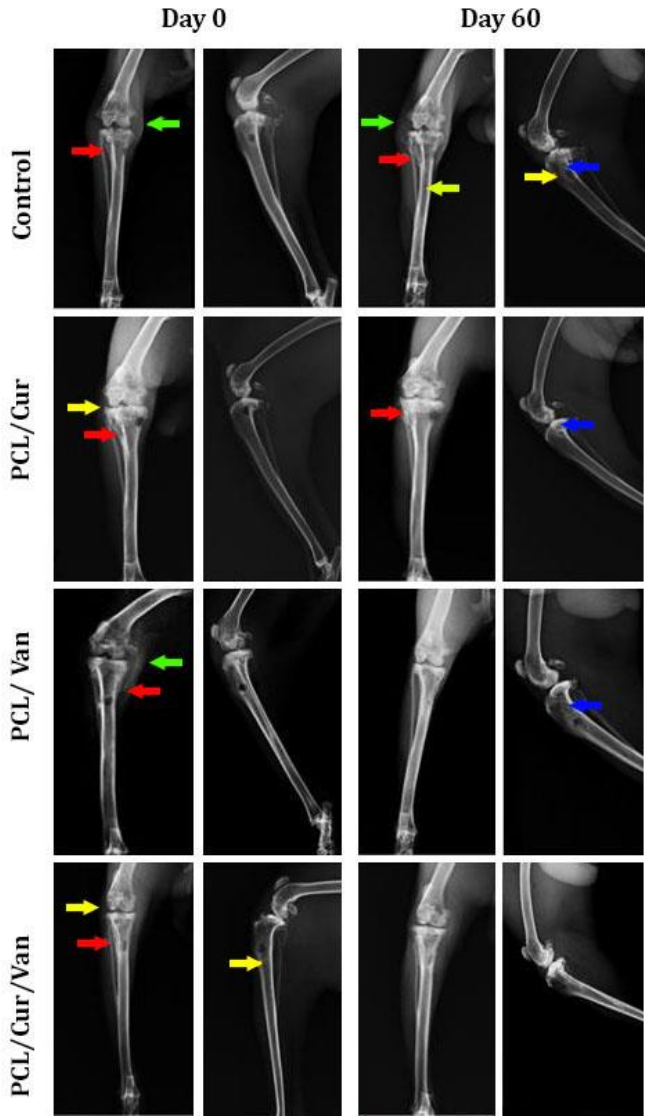

Fig. 4. Radiographic images of the defects in Control, polycaprolactone (PLC)/curcumin (Cur), PLC/vancomycin (Van), PLC/Cur/Van and groups on days 0 and 60. Periosteal new bone formation (red arrow), sequestrum formation (blue arrow), destruction of bone (yellow arrow) and soft tissue swelling (green arrow) are shown.

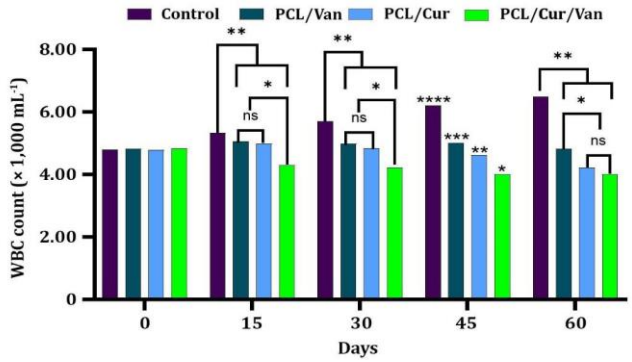

Fig. 5. The effect of polycaprolactone (PCL), curcumin (Cur) and vancomycin (Van) nanocomposites in different experimental groups on the number of rabbit white blood cells (WBCs) on different days during study period. The number of WBCs in different groups on days 0, 15, 30, 45, and 60. *, **, ***, **** = $p < 0.05$ compared to the other groups.

Discussion

In recent years, the pathogenesis of osteomyelitis has been almost completely determined and many factors that contribute to the persistence of this infection have been identified. In its treatment, several antimicrobial agents with different spectrums of action have been used.[19]

Curcumin loading in nanoparticles has increased its antimicrobial power against bacteria.[20] The bone composition of rabbits is similar to humans and they have a similar Haversian system. Hydrophilic polyphenols increase the permeability of the membrane and affect the fluidity of the membrane, thus, affecting the normal function of the bacterial cell membrane.[21] Herbal medicines bear antibacterial properties and can be used in the treatment of osteomyelitis. Others extracted essential oils from traditional Chinese herbal medicines.[22] Cell membrane conductance measurements showed that essential oils increased the permeability of *S. aureus* cell membranes and had significant antibacterial effects.[23] Curcumin disrupts the metabolism of bacterial cells. It has been reported that treatment of *S. aureus* with Cur affected kynurenine levels, nitric oxide and thiol levels and caused bacterial death by disrupting metabolic pathways.[24] Also, Cur, with its ability to increase the host's defense against intracellular bacterial infections, is considered as a strong immune regulator.[23]

Bone mineral crystal nanoparticles create a large surface area and the binding of these materials is very weak causing better absorption of the materials by osteoclasts. In other words, the small size of these materials with high levels in unit volume creates a suitable structure for use in bone restorations.[25] By producing particles in nanoscales the compatible bio-properties such as bone conductivity, deposition solubility and mechanical capability are improved.[25] Curcumin is an active polyphenolic substance derived from plants, with broad-spectrum antibacterial properties. Due to its hydrophobicity, the bioavailability of Cur is limited. However, researchers have increased its bioavailability in recent studies through several methods such as nano-emulsion and electrospinning. In addition, Cur has many beneficial properties such as low intestinal absorption, fast metabolism and low toxicity in high doses in humans, which has led to its potential applications as an antibacterial agent. In fact, Cur exhibits bactericidal activity and can be used in combination with other substances to increase its antibacterial properties further.[26] Curcumin has a synergistic effect with beta-lactam antibiotics, including penams (penicillins), cephems

(cephalosporins), monobactams and beta-lactam carbapenems. Curcumin causes synergism with polypeptide antibiotics including Van that is commonly used as antibacterial drugs against gram-negative and gram-positive bacteria.[27] In the present study, Cur improved the histopathological damage caused by osteomyelitis in the experimental groups especially where was combined with Van. On different days, the formation of primary growing bone was observed in all the treatment groups from the edges of the defect. However, the bone defect on the 30th day in the Control group was filled with only fibrotic tissue, and in the PCL/Van group with a lot of fibrotic tissue and a little cartilage. In the PCL/Cur/Van and the PCL/Cur group the bone defects were filled with bony trabeculae. The best filling speed of the defect site, on day 60, with a significant difference was observed in the PCL/Cur/Van group. On the 60th day, similar findings with no significant difference were observed between PC/Van and PCL/Cur groups. In the present study, the use of nanocomposite PCL/Cur and PCL/Cur/Van induced the formation of bone layers. In the PCL/Van group, the filling of the lesion spaces with high amounts of connective tissue and nanocomposite was visible on day 30, however, on day 60, the filling of the bone defect spaces was visible with smaller amounts of connective tissue and bone plates. Polycaprolactone/Cur group on day 30 showed large amounts of connective tissue. Unlike the PCL/Van group, the PCL/Cur group on day 60, showed large amounts of bone tissue, formation of bone marrow cavities, and the connection of the defect site with dense bone. The PCL/Cur/Van nanocomposite implantation site on day 30 showed the accumulation of large amounts of connective tissue and bone trabeculae. Similar to the PCL/Cur group, the PCL/Cur/Van group on day 60 showed the accumulation of connective tissue, the formation of dense bone in the bone defect site, the filling of the defect site with spongy bone, the formation of bone marrow cavities and the rearrangement of bone plates.

Vancomycin is a glycopeptide antibiotic that prevents the synthesis of peptidoglycan layer in gram positive bacteria.[28] Curcumin can reduce Van-induced nephrotoxicity by inhibiting oxidative stress and inflammatory response in a mouse model.[27] The evaluation of the mean number of WBCs in the rabbit serum on different days of the experiment in the present study also showed a significant difference in all experimental groups compared to the control group, and the consumption of PCL/Cur/Van caused a significant increase in the number of WBCs compared to other experimental groups. Also, in the histopathological examination of the experimental groups of the current study, the highest presence of inflammatory cells (50.00 - 75.00%) was observed on the 30th day after surgery in the control group. The animals receiving nanocomposite PCL/Cur/Van showed the lowest presence of inflammatory cells with a significant difference from that of the control group. Also, on the 60th day after surgery, the results were similar and the highest presence of inflammatory cells (50.00 - 75.00%) was observed in the control group. In 2009, Kumar Nandi and his colleagues examined various types of antibiotic delivery systems regionally and locally in the treatment of osteomyelitis. They pointed out that compounds that were structurally biodegradable and had compounds closer to bone compounds, ended up with better healing.[29]

In a study, gelatin/β-tricalcium phosphate composite porous scaffolds was used as a local controlled release system for Van and the efficiency of the scaffolds in eliminating infections and repairing osteomyelitis defects in rabbits was confirmed.[30] It has been reported that treatment with Van loaded calcium sulphate and autogenous bone improved rabbit model of bone infection.[31] Improved repair of bone defect by degradable bioactive borate glass releasing Van has also been reported.[32] In a clinical study on chronic osteomyelitis patients, negative pressure closed drainage combined with Van loaded calcium sulfate and autogenous bone enhanced treatment of chronic osteomyelitis.[33]

The combination therapy with Van-loaded calcium sulfate and Van-loaded polymethyl methacrylate was reported to achieved more effective control of infection in the treatment of osteomyelitis through synergistic effect.[34] Others indicated that Van -loaded Bicera™ was a potential bone substitute that could prevent implant-associated osteomyelitis and postoperative infection.[35] Vancomycin-loaded bone-like hydroxyapatite/poly(amino acid) scaffold was demonstrated to have good potential for the repair of infectious bone defects because of its ability to deliver antibiotics and promote bone regeneration.[36]

In radiological studies of infectious osteomyelitis, the positive effects of Van in reducing the number of bacteria have been reported. Vancomycin is often combined with other antibiotics to treat serious infections caused by *S. aureus*. In the present study, the positive therapeutic effects of Van in radiological examinations were observed compared to the control group. In the present study, in order to investigate the process of bone defect healing in experimental groups, radiographic evaluations of the process of healing defects were performed on the 15th, 30th, 45th and 60th days after surgery. The highest mean amount of bone periosteum formation was observed on both the 15th and 30th days in the PCL/Cur/Van group. The parameter of bone tissue destruction on days 15, 30, 45, and 60 days showed a significant difference between control and other groups ($p < 0.001$). The lowest amount of bone destruction was observed in the PCL/Cur/Van and PCL/Cur groups. The spread of osteomyelitis in different areas of the tibia was also measured by radiographic evaluation. The lowest spread of osteomyelitis was observed on the 60th day in the proximal part of the bone in the PCL/Cur/Van group. Soft tissue swelling was also

measured on days 15, 30, 45, and 60 after surgery using radiology. The lowest amount of soft tissue swelling was observed in the PCL/Cur/Van group on the 30th day.

Hematology investigations and the average number of WBCs in the blood samples taken from different experimental groups of the present study showed the effect of PCL/Cur nanocomposite on days 15, 30, 45, and 60 on the number of blood cells. The WBC count of the rabbits were higher compared to the control group, and the consumption of PCL/Van and Cur on this day caused an increase in the average number of WBCs in all groups compared to the control group ($p < 0.001$). Also, no significant difference was observed between PCL/Cur and PCL/Van groups. The results of the present study completely showed the synergistic effect of Van and Cur in findings of histopathology, radiology and hematology.

In conclusion, the results of the present study showed that PCL/Cur, PCL/Van and PCL/Cur/Van nanocomposites met all the criteria of tissue engineering scaffolds for bone regeneration. The PCL/Cur significantly improved the healing process of the experimental osteomyelitis model of rabbit tibia compared to Van nanocomposite in an equal or better way. In addition, the PCL/Cur/Van nanocomposite group showed the best healing results. It seems that polycaprolactone/Cur /Van nanocomposite had a better effect on the reconstruction of bone defects and could be used as a scaffold in bone fractures. Further biochemical and genetic studies of the experimental groups to detect cellular events remain to be investigated.

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Conflict of interests

There are no conflicts of interests to be declared.

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