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Evaluation of the Effects of Curcumin/Polycaprolactone Nano Composite 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 aim of the present study was to evaluate the effect of curcumin/polycaprolactone on the healing of experimental osteomyelitis in tibia in rabbits. After induction of osteomyelitis, twenty adult male New Zealand rabbits were randomly divided into Control group in which the animals were considered as controls and no scaffolds were used, and NCMPST group in which the created bone defects were filled with the combination of polycaprolactone and curcumin. Bone samples were taken for histopathologic 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 curcumin nanocomposite significantly improved the healing process of the rabbit tibia experimental osteomyelitis model compared to the control group ($p < 0.05$). In conclusion, curcumin/polycaprolactone nanocomposite scaffold showed positive effects on the healing process.

Introduction

Bone infection is called osteomyelitis. Osteomyelitis is an inflammatory process that is associated with bone destruction and is caused by an infectious microorganism (pyogenous bacteria, tuberculosis, syphilis, some fungi, viruses, and also in the presence of foreign bodies).[1] The infection can be limited to one part of the bone or it can involve several areas such as marrow, cortex, periosteum and surrounding soft tissue in the bone.[2] Osteomyelitis is caused by the local spread of an infected source adjacent to the infection following trauma, bone surgery or joint replacement. This means that a primary infection reaches the bone. Osteomyelitis can occur at any age and can involve any bone.[2] Based on the pathogenesis of infection, osteomyelitis has different types: including hematogenous osteomyelitis, vertebral osteomyelitis, post-traumatic osteomyelitis and bone injuries and infection caused by diabetic foot: Osteomyelitis is classified based on the mechanism of entry of the microbe into the infection site or the type of inflammatory agent.[1] New diagnostic methods and better treatment for those with ready access to modern health care have led to a reduction in treatment failure rates in acute osteomyelitis. Treatment outcomes have decreased. Infection control strategies and preventive antibiotics have reduced the rate of postoperative infection. However, the large increase in reconstructive orthopedic procedures with prosthetic materials increases the overall number of infections, as no preventive measures are likely to reduce the rate of infections below 0.5%. New materials for regeneration are needed for excellent mechanical composition with

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anti-bacterial properties and greater approximation of the extracellular matrix. In chronic osteomyelitis, new surgical approaches combining orthopedic, plastic and vascular techniques are essential. Osteomyelitis imposes a high financial burden and significantly affects quality of life.[2]

Tissue engineering is the science of designing and producing new tissues to repair damaged organs for various reasons. Bone tissue has a high potential for regeneration, therefore, it is considered a suitable tissue for tissue engineering. In bone tissue engineering, like tissue engineering in other tissues, various types of cells, growth factors, cytokines and bioactive carriers such as scaffolds and hydrogels are used to maintain and support cells.[3] In general, tissue engineering depends on three basic components, engineered scaffold, cells and growth factors.[4] Considering the different types of cells and extracellular substances in different tissues of the body, a special scaffold with biological, biochemical and biomechanical properties appropriate to that tissue is necessary for tissue regeneration.[5] Materials that are used for the complete reconstruction of bone tissue must have characteristics such as biocompatibility, biodegradability, porosity to transfer cells and growth factors to the damaged tissue, mechanical properties appropriate to the desired tissue, including mechanical strength, chemistry surface, bone induction, the ability to simulate the natural bone tissue, the ability to stimulate and accelerate the differentiation towards bone-forming cells and to provide physical support for the forming tissues.[6] In order to choose the right scaffolds, attention is paid to the desired tissue structure. Choosing the method of making a scaffold is very important because by choosing the right method, a better contact surface is created for cell attachment, proliferation and differentiation. There are several methods for making and designing tissue scaffolds. Common methods such as phase separation, foam gas, dry ice emulsion, solvent casting, and electrospinning are among the methods of fabricating tissue engineering scaffolds.[7] According to the desired characteristics for a polymer to be used as a scaffold, polycaprolactone polyester (PCL) in addition to being biocompatible and biodegradable, due to its easy availability, cost-effectiveness and suitability for modification, It has become significantly important. Its adjustable physicochemical state, biological properties and mechanical strength make it resistant to physical, chemical and mechanical shocks without significant loss of its properties.[8] PCL is a biodegradable polyester. PCL is a semi-crystalline aliphatic polymer, with a melting temperature between 64-59 °C (that is, higher than body temperature), and a glass transition temperature of -60 °C. Therefore, at physiologicl temperature, semi-crystalline PCL reaches a rubbery state, which leads to high hardness and superior mechanical properties (high strength, elasticity depending on its molecular weight). PCL is non-toxic and compatible with tissue, hence it is widely used as absorbable suture, as scaffold in regenerative therapy and in drug delivery applications. PCL has a long degradation time (2-3 years) and is degraded by microorganisms or by hydrolysis of its aliphatic ester bond under physiological conditions. Due to the presence of five hydrophobic parts -CH2 in the repeating units, PCL decomposes more slowly among all polyesters.[8]

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.[9] 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, and on the other hand, the systemic toxicity of the drug is also reduced.[10]

Curcumin is an active polyphenolic substance derived from plants, with broad-spectrum antibacterial properties. Curcumin has antioxidant effects and due to its hydrophobicity and other characteristics, the bioavailability of curcumin is limited.[11] However, researchers have increased its bioavailability in recent studies through several methods such as nanoemulsion and electrospinning. In addition, curcumin 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, curcumin exhibits bactericidal activity and can be used in combination with other substances to further increase its antibacterial properties.[12] Curcumin is a plant compound whose antibacterial 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 (C21H20O6).[13] Curcumin has attracted the attention of researchers due to its low intrinsic toxicity, but a wide range of pharmacological activities including antioxidant, anti-inflammatory, antimicrobial and anti-cancer properties.

In 1949, Schraufstatter and colleagues were the first to report the antibacterial properties of curcumin.[14] In the past seventy years, several studies on the broad-spectrum inhibitory effect of curcumin against various gram-negative and gram-positive bacteria have been performed.[15] The important point is that curcumin also shows specific antibacterial activities against multidrug-resistant gram-negative microbes such as methicillin-

resistant *Staphylococcus aureus*.[16] A recent study by Batista de Andrade Neto *et al*. reported that the minimum inhibitory concentration (MIC) for curcumin against clinical isolates of methicillin-resistant *Staphylococcus aureus* was in the range of 125-500 microgram per milliliter.[17] The aim of the present study was to evaluate the effects of curcumin/polycaprolactone scaffold in defects created in osteomyelitis in rabbit tibia bone.

Materials and Methods

Preparation and Synthesis of Curcumin/ Polycaprolactone Nanocomposite Nanofiber Using Electrospinning Method

The preparation of nanofibers was carried out at Shaya Nanomeyar Technologists Company (National ID: 14009867750, registration number: 52983) located in Science and Technology Park of East Azarbaijan Province, Tabriz. For this purpose, polycaprolactone with a molecular weight of 80,000 was dissolved in a two-solvent system of acetic acid-formic acid (AA:FA) at a ratio of 70:30 and a concentration of 20% by weight/volume, and then curcumin was added gradually. It was added to the prepared polymer solution at a ratio of 5% w/w. In the next step, the polymer solution was drawn into a 5 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 and after the electrospinning process was completed, the foil containing the electrospun nanofibers was placed in an oven at a temperature of 37 °C for 48 to 72 hours to evaporate the solvent.[18] The scaffold with a size of 1 cm × 1 cm were used inside the defects and were sterilized by UV device for 20 minutes before being placed inside the bone.

Investigating the Morphology of Nanofibrous Scaffold

Scanning electron microscope (SEM) was used to observe the surface morphology of the scaffold. A small piece of the scaffold was covered with a thin layer of gold and then it was imaged on the stage of the SEM microscope under vacuum and a potential difference of 15 kV. Nanofiber diameter analysis was done with Digimizer image analysis software. For this purpose, the diameter of 50 fibers was randomly measured in the image of the nanofiber network. Structural features, arrangement and orientation of nanofibers are shown in Figure 1.

Ethical Considerations

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 National Academy of Sciences Publication with number of 85-23 that was revised in 1985. In the present study, following injecting bacteria into the tibia bone, the rabbits were transferred into their boxes and were evaluated daily for signs of septicemia including lethargy, loss of appetite, failure to perform grooming habits, and increased temperature. In order to reduce the possibility of septicemia and eliminate the effects of bacterial injection, aggressive fluid therapy using Ringer's lactate serum was performed for 5 days. Rabbits were kept for 4 weeks and their daily tissue changes were investigated.

Preparation of Bacteria

Standard *Staphylococcus aureus* was obtained from Persian Type Culture Collection (PTCC, Tehran, Iran). After 18-24 hours, a young culture of bacteria was prepared in Tryptic Soy Broth (TSB) medium. The TSB medium containing bacteria was centrifuged three times with saline buffer (PBS) at 3000 rpm for ten minutes, and 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 cc of microbial suspension, equivalent to half McFarland, (CFU/ml) there are 1.5 × 108 bacteria. For injection, the amount of CFU/ml 1 × 107 of bacteria was required, and 100 microliters of *Staphylococcus aureus* suspension was prepared and injected.[19]

Induction of Osteomyelitis

To induce osteomyelitis, the surgical method of Norden and colleagues was used.[19] Rabbits were anesthetized by intramuscular injection of 10% ketamine (50 mg/kg, Alfasan, The Netherlands) and 2% xylazine (5 mg/kg, Alfasan, The Netherlands) and regional hair Surgery was completely shaved at the site of the right tibial bone. The desired position was disinfected by using betadine scrub solution and alcohol, following the principles of asepsis. 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. To inject the solution containing *Staphylococcus aureus* bacteria prepared in advance with a needle size 18 percutaneously (from inside the skin) entered the soft tissue and then the inner cortex of the bone. The needle went towards the proximal and metaphysis to cause regional infection. This condition prevents it from entering the diaphysis of the bone and causing infection in the entire bone (Figure 2). When the needle was placed in the right place, first 0.1 cc of normal saline was injected to ensure that the injection path was open. Then, 0.1 cc of tetradecyl sulfate drug, 0.1 cc of tryptic broth containing *Staphylococcus aureus* bacteria, which had approximately

1 million colony units, were injected. At the end, in order to wash the tip of the needle to ensure the complete entry of bacteria, 0.1 cc of normal saline was injected again. Four weeks after the injection of bacteria, in order to confirm the process of osteomyelitis, radiographs were taken from the tibia bones of the rabbits, and after confirming the results, the rabbits were divided into four experimental groups and prepared for the surgical procedure.

Experimental Animal Model

To carry out this study, 40 adult male rabbits of New Zealand breed with an average weight of 2.5 to 3 kg were obtained from the Laboratory Animal Breeding and Maintenance Center of Gilan University. At first, in order to ensure the health of the animals used in this research, all the rabbits were clinically evaluated, and oral and skin antiparasitic treatment was prescribed for all of them as well as vitamin C and vitamins A + D for 7 days. Animals were placed separately in propylene cages. The bottom of the cages was covered with hay. In order 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 degrees Celsius. All animals were kept under the same nutritional conditions with food and free access to water. In order to prevent any contamination, their bedding and drinking water were also changed daily. After the injection of bacteria and the induction of osteomyelitis the animals were randomized into two groups of 10 animals each: Control group: The animals were considered as controls and no scaffolds were used. NCMPST group: The created bone defects were filled with the combination of polycaprolactone and curcumin.

Surgical Procedures

Induction of general anesthesia in rabbits with tibial osteomyelitis was performed by intramuscular injection of 10% ketamine (50 mg/kg, Alfasan, The Netherlands) and 2% xylazine (5 mg/kg, Alfasan, The Netherlands), then the inner surface of the region was shaven and prepped surgically. The skin and muscles were dissected by a surgical blade. Abscesses and infectious soft tissue sinuses were debrided. After reaching the bone, necrotic and infected pieces were debrided and a bone defect was created by a 3.5 mm drill head on the proximal inner cortex of the tibia. The bone was washed with 10 cc of normal saline solution and the muscles were sutured with 3-0 polyglycolic acid suture and the skin with 3-0 nylon suture. In the first 5 days after the surgery, every 24 hours, the wound and suture site were checked for bleeding and infection or suture opening and re-dressing was performed with sterile gauze. 14 days later, the skin sutures were removed and no tears were seen. The surgical site was treated according to the treatment protocol for each rabbit. The surgical site was inspected daily to prevent infection or postoperative bleeding (Figure 3).

Histopathologic Studies

Five rabbits from each group were euthanized on days 30 and 60 post operation. After general anesthesia with intravascular injection of sodium phenobarbital, the tissues of the scaffold implantation area of NCMPST group was sampled. The samples were fixed in 10% buffered formalin fixing solution and after fixing, passage and paraffin embedding were prepared for histologic study. Also, the selected bone tissues were placed in acid for decalcification. Hematoxylin-eosin staining was done to study histopathology. Bone samples were graded in terms of filling of the defect site, inflammatory reaction, and bone recovery.

Radiological Evaluations

In order to carry out radiological evaluations, radiographs were prepared from the desired area at time intervals of 0 (day of surgery), 15, 30, 45 and 60 days post operation. Sequestrum formation, periosteal new bone formation, destruction of bone, extent of disease, soft tissue swelling and new bone formation they were scored based on the works of others.[20]

Statistical Analyses

SPSS version 18 software was used to perform statistical tests. First, the normality and homogeneity of data variance were checked by Kolmogorov-Smirnov test. Then, based on those results, the differences between the

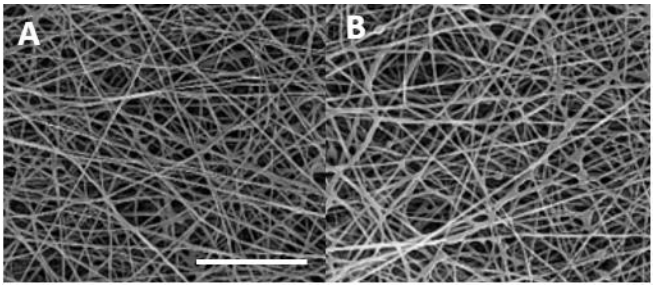

Figure 1. FESEM images of the synthesized (A) polycaprolactone and (B) curcumin/polycaprolactone nanocomposite. Scale bar: 10 µm.

Figure 2. Stages of induction of osteomyelitis and injection of *Staphylococcus aureus* bacteria in the tibia bone.

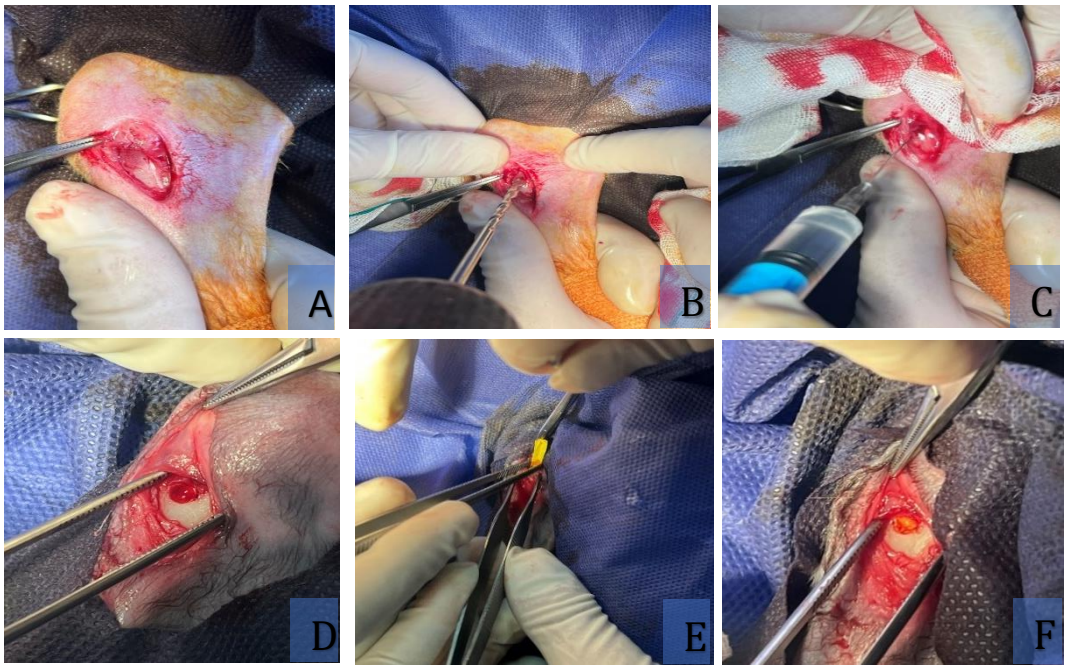

Figure 3. Intraoperative photos of stages (A-F) of the creation of defects and the replacement of scaffolds in NCMPST group in the rabbit tibia bone.

test groups and the control groups were evaluated using one-way parametric ANOVA in blood biochemistry parameter and Kruskal-Wallis non parametric ANOVA in histopathology and radiology parameters. Numerical data were expressed as mean ± SD. p-value < 0.05 was considered as a significant level.

Results

Histopathology

In the histopathologic examinations of the experimental groups, no signs of infection were observed in any of the slides of the Control and NCMPST 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 examination of the slides of the NCMPST group on the 30th day 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 (Figure 4).

Radiologic Evaluations

Regarding sequestrum formation, a significant difference was evident between Control and NCMPST group on days 30, 15 and 60 ($p < 0.001$). Regarding, the new bone periosteum formation on days 15 and 30 a significant difference was observed between two groups ($p < 0.001$). The highest average amount of bone periosteum formation was observed on both the 15th and 30th days in the NCMPST group. Examining the parameter of bone tissue destruction on days 15, 30, 45 and 60 days showed a significant difference between Control and NCMPST groups ($p < 0.001$). Bone destruction was observed with an average of 18 in the Control group on all days. The lowest amount of bone destruction was observed in the NCMPST group. The spread of the disease was measured in different areas of the tibia. In the examination of the spread of the disease in the middle part of the tibia on the 15th and 45th days, no significant difference was observed between two groups ($p < 0.05$). However, on the 30th and 60th days, the range in the Control group was more than twice that of NCMPST groups. In the proximal part of the tibia bone on days 40 and 60, there was a significant difference between control group and NCMPST group ($p < 0.05$). The lowest spread of the disease was observed in the proximal part of the bone on the 60th day in NCMPST group. Regarding the spread of the disease, no significant difference was observed between NCMPST and control groups in the distal part of the tibia ($p < 0.05$) Soft tissue swelling was measured on days 15, 30, 45 and 60 after surgery using radiology. The statistical results showed no significant difference on the 15th day between the Control and NCMPST group ($p < 0.05$). The lowest amount of soft tissue swelling was observed in the NCMPST group on day 30 (Figure 5, Table 1).

Discussion

Several surgical methods and antibiotic bone scaffolds have been developed to manage osteomyelitis.[21] However, bone infection is still considered a medical and

veterinary challenge. Curcumin is one of the plant compounds whose antibacterial and anti-inflammatory properties have been proven. Curcumin has little inherent toxicity, however, it shows a wide range of pharmacological activities including antioxidant, anti-inflammatory and antimicrobial.[21] Curcumin loading in nanoparticles has increased its antimicrobial power against bacteria. In this study, the effects of curcumin/polycaprolactone nanocomposite scaffolds was evaluated and compared in defects involved in osteomyelitis in rabbit tibia. The bone composition of rabbits is similar to humans and they have a similar Haversian system. The use of natural antioxidants in the treatment of osteomyelitis has been done in previous studies.[22] Among the natural organic antibacterial agents extracted from plants, polyphenols and essential oils are primary antibacterial agents. Hydrophilic polyphenols increase the permeability of the membrane and affect the fluidity of the membrane and thus the normal function of the bacterial cell membrane.[22] It was reported that tea polyphenols affected the bacterial cell membrane by changing the permeability of the cell membrane and disrupting the membrane function.[23] To further investigate the antibacterial effect of tea polyphenols, researchers conducted antibacterial experiments using porous silica nanoparticles containing green tea polyphenols in a mildly acidic microenvironment simulated from osteomyelitis. The results showed that green tea polyphenol nanoparticles effectively destroyed *Staphylococcus aureus* and its bacterial inhibition rate.[24] Other herbal medicines also have antibacterial properties and can be used in the treatment of osteomyelitis. Others

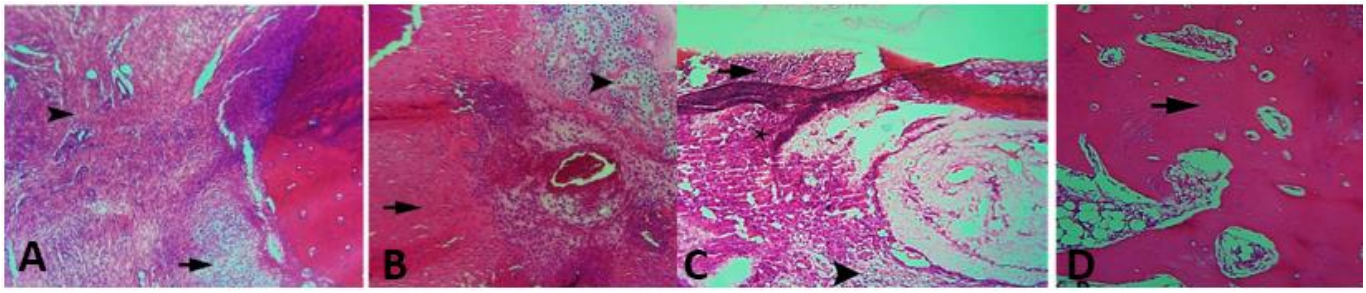

Figure 4. Representative histologic micrographs: (A) Bone defect in the Control group on day 30, large amounts of connective tissue (arrowhead) and small amounts of cartilage tissue (arrow) are observed. (B) Bone defect in the Control group on day 60, some connective tissue (arrow) and a large amount of cartilage tissue (arrowhead) are observed. (C) Bone defect in NCMPST group on day 30, the accumulation of large amounts of connective tissue (arrowhead) and inflammation (arrow) and composite (*) are observed. (D) Bone defect in NCMPST on day 60, large amounts of bone tissue (arrow) can be observed (H&E staining, ×10).

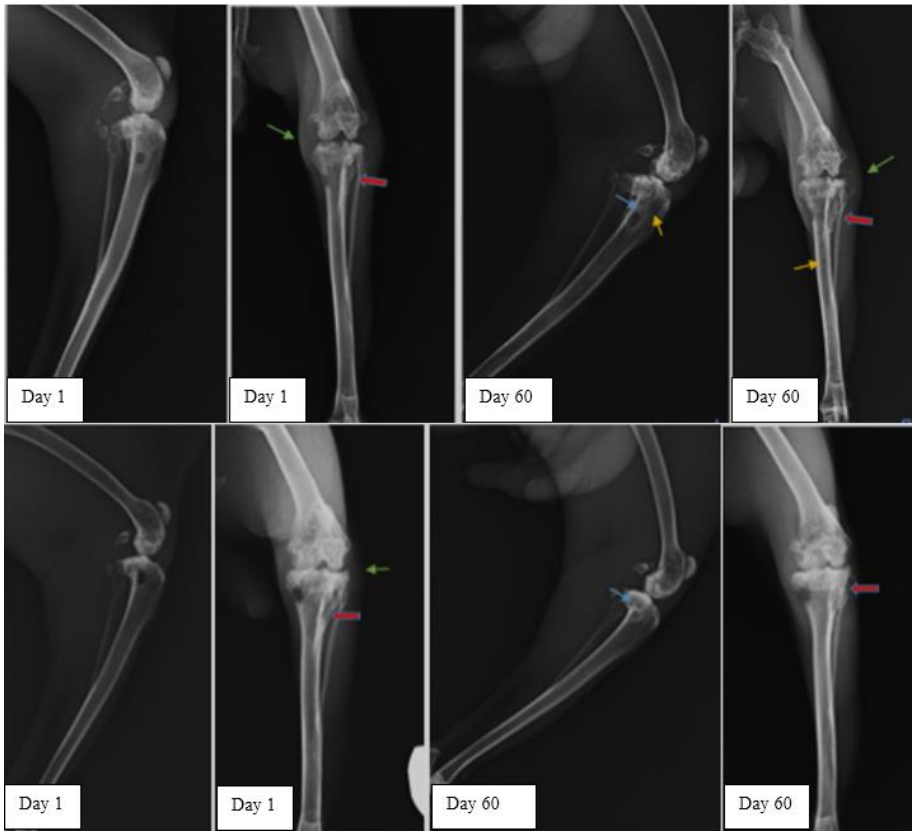

Figure 5. Radiographic images of the defects in Control (upper row) and NCMPST (lower row) groups on days 1 and 60. Periosteal new bone formation (red arrow), sequestrum formation (blue arrow), destruction of bone (orange arrow) and soft tissue swelling (green arrow) are shown.

Table 1. Radiographic evaluation of the healing process of defects on the 15th, 30th, 45th and 60th days after surgery in Control and NCMPST groups.

Parameters	Group	Day	Score	Group	Day	Score	Group	Day	Score	Group	Day	Score
Sequestrum formation	Control	60	18	Control	45	15.5	Control	30	10.5	Control	15	18
	NCMPST	60	10.5	NCMPST	45	15.5	NCMPST	30	10.5	NCMPST	15	8
Periosteal new bone formation	Control	60	10.5	Control	45	10.5	Control	30	8	Control	15	3
	NCMPST	60	10.5	NCMPST	45	10.5	NCMPST	30	8	NCMPST	15	10.5
Destruction of bone	Control	60	18	Control	45	18	Control	30	18	Control	15	18
	NCMPST	60	8	NCMPST	45	5.5	NCMPST	30	5.5	NCMPST	15	8
Extent of disease: Mid tibia	Control	60	18	Control	45	10.5	Control	30	18	Control	15	10.5
	NCMPST	60	8	NCMPST	45	10.5	NCMPST	30	8	NCMPST	15	10.5
Extent of disease: Proximal tibia	Control	60	13	Control	45	18	Control	30	10.5	Control	15	10.5
	NCMPST	60	13	NCMPST	45	8	NCMPST	30	10.5	NCMPST	15	10.5
Extent of disease: Distal tibia	Control	60	10.5	Control	45	10.5	Control	30	10.5	Control	15	10.5
	NCMPST	60	10.5	NCMPST	45	10.5	NCMPST	30	10.5	NCMPST	15	10.5
Soft tissue swelling	Control	60	18	Control	45	18	Control	30	15.5	Control	15	10.5
	NCMPST	60	8	NCMPST	45	8	NCMPST	30	15.5	NCMPST	15	10.5

extracted essential oils from traditional Chinese herbal medicines. Cell membrane conductance measurements showed that essential oils increased the permeability of *S. aureus* cell membranes and had significant antibacterial effects.[25]

Curcumin disrupts the metabolism of bacterial cells and it has been reported that treatment of *Staphylococcus aureus* with curcumin affected kynurenine levels, nitric oxide and thiol levels, and caused bacterial death by disrupting metabolic pathways. Activation of the kynurenine pathway probably reduces the cellular reserve of L-tryptophan available to support bacterial growth, thereby limiting bacterial cells access to an essential nutrient.[26] Also, curcumin, with its ability to increase the hosts defense against intracellular bacterial infections, is considered as a strong immune regulator.[26]

Bone mineral crystals nanoparticles create a large surface area and 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. [27] By producing particles in nanoscales the compatible bio-properties such as bone conductivity, deposition and solubility and mechanical capability are improved.[27] Curcumin is an active polyphenolic substance derived from plants, with broad-spectrum antibacterial properties. Curcumin 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, curcumin exhibits bactericidal activity and can be used in combination with other substances to further increase its antibacterial properties.[12] In the present study, curcumin improved the histopathologic damage caused by osteomyelitis in NCMPST group. In the histopathologic examinations of the samples on different days, the formation of primary growing bone was observed in curcumin/polycaprolactone nano composite treated group 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 NCMPST group the bone defects were filled with bony trabeculae. In the present study, the use of curcumin/polycaprolactone nanocomposite induced the formation of bone layers. In the histopathologic examinations of the experimental groups performed in the NCMPST 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. NCMPST group on day 30 showed large amounts of connective tissue. The histopathologic sections from the vancomycin/curcumin nanocomposite implantation site on day 30 showed the accumulation of large amounts of connective tissue and bone trabeculae.

Researchers in 2017 compared hydroxyapatite tissue scaffold and polycaprolactone nanoparticle in the experimental defect created in rabbit bone, which was reported by histopathologic examination on the 45th day after polycaprolactone nanoparticle surgery. Lamellar bone was better than hydroxyapatite and the Control group.[28] Various types of antibiotic delivery systems regionally and locally in the treatment of osteomyelitis have been developed and in consistent with the present study, it was demonstrated that compounds that were

structurally biodegradable and had compounds closer to bone compounds, ended up better healing.[29]

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 was performed on the 15th, 30th, 45th and 60th days after surgery. In the statistical analysis of new bone periosteum formation, the highest mean amount of bone periosteum formation was observed on both the 15th and 30th days in the NCMPST group. The parameter of bone tissue destruction on days 15, 30, 45 and 60 days showed a significant difference between Control and NCMPST group ($P < 0.001$). The lowest amount of bone destruction was observed in the curcumin/polycaprolactone nano composite treated group. The spread of osteomyelitis in different areas of the tibia was also measured by radiographic evaluation. In the examination of the spread of osteomyelitis in the proximal part of the tibia, the lowest spread of osteomyelitis was observed on the 60th day in the proximal part of the bone in the curcumin/polycaprolactone nano composite treated group. Also, soft tissue swelling was measured on days 15, 30, 45 and 60 after surgery using radiology. The statistical results showed that the lowest amount of soft tissue swelling was observed in curcumin/polycaprolactone nano composite treated group on the 30th day.

Curcumin induces its antibacterial effect by different mechanisms. In fact, increasing the permeability of the bacterial membrane caused by curcumin can increase the absorption of other drugs. Increasing bacterial membrane permeability is a critical mechanism to explain the synergistic effect of curcumin combined treatment with other antibiotic drugs or natural products.[15] The results of the present study completely showed the positive effect curcumin in findings of histopathology and radiology.

In conclusion, the results of the present study showed that curcumin/polycaprolactone nanocomposite, met all the criteria of tissue engineering scaffolds for bone regeneration. Curcumin/polycaprolactone significantly improved the healing process of experimental osteomyelitis model of rabbit tibia. It seems that curcumin/polycaprolactone nanocomposite had a better effect on the reconstruction of bone defects and could be used as a scaffold in bone fractures. Therefore, the findings of the present study showed that the curcumin/polycaprolactone nanocomposite scaffold had a positive effect on the healing process.

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Conflict of Interest

There are no conflicts of interests to be declared.

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