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Control of Bone Metastasis Caused by Breast Cancer Using Nanocomposite Scaffolds Containing Strontium Ranelate

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Introduction: Bone metastases are one of the most important consequences of breast cancer, and the possibility of developing them is greatly increased due to the late diagnosis of the cancer. In general, systemic treatments for metastases can have many side effects. To reduce this concern, tissue engineering scaffolds containing osteogenesis drugs can be used to minimize these problems as much as possible.

Methods: In this study, polycaprolactone/polyvinyl alcohol (PCL/PVA) composite scaffolds were prepared for use in bone tissue engineering with a weight percentage of strontium ranelate (SrR) drug (0, 5, and 20) by shell-core emulsion electrospinning method. Scaffolds prepared in this study were subjected to scanning electron microscopy (SEM), contact angle, and biodegradation analyses for morphological and structural investigation. Then, to check their biocompatibility, an MTT test was performed.

Results: The results of SEM images showed the nanostructure of the fibers. Also, the presence of interconnected pores with a suitable size was confirmed by the images. MTT results proved the non-toxicity of all prepared scaffolds by high cell viability (>80%). According to the obtained results, the composite scaffolds containing the drug SrR have been successfully synthesized and have the desired properties for use in bone regeneration applications.

Conclusion: Controlling bone metastases due to breast cancer is an important concern. In this study, the composite scaffold containing strontium ranlate showed suitable physicochemical, biocompatibility, and osteogenesis characteristics.

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INTRODUCTION

A large number of women are diagnosed with breast cancer every year, and due to the lack of awareness in many societies, in some cases, these cancers metastasize to other tissues [1]. One of the tissues prone to metastasis is bone, and several cases of bone metastasis due to breast cancer have been observed. In these cases, measures should be taken to prevent further bone resorption and replace the lost bone tissue [2]. During the last few decades, many efforts have been made by researchers to find suitable bone substitutes. The initial efforts were mostly focused on the use of metal substitutes, but the corrosion of these implants in the patient's body, in addition to the loss of mechanical properties and their gradual loosening, led to the release of highly toxic metal ions and subsequent inflammatory reactions. The irritant of these products is surrounded by tissues [3]. Another problem with metals is their very high modulus [4]. The elastic modulus of metals is higher than 100 GPa, which is much higher than the stiffness of dense bone (6-20 GPa) [5]. As a result of this high stiffness, the phenomenon of stress protection occurs on the growing bone, which will lead to the thinning of the new bone tissue and increase the probability of its failure again [6]. Such problems have turned the attention of many researchers to newer materials that repair bone defects with higher speed and quality. In this way, a new chapter called "bone tissue engineering" was opened, and new biomaterials were introduced to the medical community for this purpose [7]. In general, tissue engineering can be defined as the application of scientific principles for the design, construction, modification, growth, and maintenance of living tissues of the body. Whereby the properties of a biomaterial change in such a way that able to form tissue or release a mass of cells into the host's body and ultimately lead to the formation of new tissue [8-10]. In the science of bone tissue engineering, it is possible to use osteogenesis drugs whose new bone formation properties have been proven, among which the oral drug strontium ranelate can be mentioned [11].

Strontium ranelate (SrR) is a strontium (II) salt of ranelic acid that has been of interest in the treatment of severe osteoporosis [12, 13]. SrR can effectively stimulate the proliferation and differentiation of osteoblasts and reduce the differentiation of osteoclasts in laboratory conditions [14]. In addition, several in vivo studies showed that oral administration of SrR in mouse models increased bone density and reduced bone loss [15]. Despite such improvement, oral administration of SrR has several adverse effects, including the risk of cardiovascular events [16]. It has been reported that designing a scaffold as a carrier for SrR delivery could be a beneficial approach to prevent the systemic side effects of SrR while improving its therapeutic efficacy [17, 18].

In this study, the production of nanocomposite scaffolds containing the strontium ranelate was carried out by the Core-Shell emulsion electrospinning method, and several characterization studies, including biocompatibility and biodegradability, were carried out, showing the high ossification capacity of the produced nanocomposite scaffolds.

METHODS

Preparation of Emulsions

Polycaprolactone (PCL, Mn=80000 Da), PVA (Mw=89000–98000 Da), gelatin (from porcine skin, type A), acetic acid, hydrochloric acid (HCl), and formic acid were purchased from Sigma-Aldrich Co. (St. Louis, USA). In addition, SrR was brought from Servier Co. (UK). All the used reagents were of analytic grade. In this present study, composite scaffolds based on PVA–PCL were fabricated. At first, two separate solutions containing PVA and PCL were made. 8% PVA solution in formic acid was made by dissolving 0.08 grams of PVA in a mixture of half a milliliter of deionized distilled water and half a milliliter of formic acid using a magnetic stirrer for 2 hours. Finally, strontium ranelate powder (10 and 40 mg) was added to the PVA solution and stirred for 2 hours. Moreover, a 22% PCL solution containing 0.22 mg PCL in formic acid and acetic acid solution (weight ratio: 1:3) was prepared. The gradient was made in 2 hours, and then 50 μL of SPAN80 was added to the solution as a surfactant. Finally, one milliliter of the two prepared solutions was mixed in a ratio (1:1) and stirred for 2 hours. Different samples, their compositions, and the amount of PVA and PCL per milliliter of the final emulsion of the scaffold are shown in Table 1.

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Table 1: Samples and Amounts of Polymers and Strontium Ranelate in Electrospinning Solution[a]

	Amount of PCL, gr	Amount of PVA, gr	Amount of SrR, gr
PP	0.22	0.08	0
5SrPP	0.22	0.08	0.05
10SrPP	0.22	0.08	0.20

[a] Abbreviations: PCL, Poly Caprolactone; PP, Poly Caprolactone/Poly Vinyl Chloride; PVA, Poly Vinyl Chloride; SrPP, Strontium Ranelate/ Poly Vinyl Chloride/ Poly Caprolactone; SrR, Strontium Ranelate

Figure 1: Prepared Emulsion Containing the Drug Strontium Ranelate

Core-Shell Emulsion Electrospinning

The optimization of electrospinning parameters, such as the distance between the needle and rotating drum collector, applied voltage, the concentration of the electrospinning solution, and the solution flow rate was first done. Therefore, the electrospinning solution was electrospun with the optimal electrospinning parameters mentioned in Table 2.

Table 2: Electrospinning Parameters

	Amount
Voltage, kV	18
Feeding Rate, ml/h	0.15
Needle Distance to the Collector, cm	11
Needle Diameter, G	20

Field emission scanning electron microscopy (FESEM) analysis was used to examine the morphology and diameter of electrospun nanofibers. To determine the amount of water uptake and the hydrophilicity of the scaffolds, the initial weight of the scaffold (W1) was immersed in 20 ml of PBS solution for 24 hours. Then, the scaffold was taken out of the solution, and the weight of the scaffold immersed in water was recorded (W2) after excess water was removed by filter paper. The amount of water uptake by the scaffold was measured through Equation 1.

Equation 1:

$$WaterUptake(\%) = \frac{W_2 - W_1}{W_1} \times 100$$

The biodegradability of the scaffolds was evaluated by measuring the weight loss of samples after immersion in PBS. Here, the composite scaffolds with precalculated dimensions were located in a plastic container containing PBS at 37◦C and at specific times. The initial weight of the samples was measured before immersing them in PBS (W0). After each certain period, the samples were washed and dried in a vacuum oven at room temperature for 24h (Wt). The weight loss percentage was calculated by the following formula:

Equation 2:

$$WeightLoss(\%) = \frac{W_0 - W_t}{W_0} \times 100$$

Dimethylthiazoldiphenyltetrazolium bromide (MTT) test was used to evaluate the level of cytotoxicity. In this way, the first 104 Mesenchymal Stem Cells (MSCs) were poured into a 96-well cell culture plate and then placed in an incubator at 37°C for 24 hours so that the cells stick to the bottom of the plate. The extract taken from each sample was added to the culture well, and the cells were placed in the vicinity of these extracts for another 24 hours. Then, the culture medium was removed, and 100 µL of MTT with a concentration of 0.5 mg/ml was added to each well. After 4 hours, the solution was removed from the cells, and isopropanol was added to each well to dissolve the translucent crystals formed inside the cells. After half an hour, the intensity of the color created at the wavelength of 545 nm was calculated. The well with more cells shows a higher optical density (OD) than the one with fewer cells. Therefore, it can be determined from the relationship below the well with more cells and compared with the control sample (Equation 3).

Equation 3:

$$Viability(\%) = \frac{(average\ optical\ density\ of\ samples)}{(average\ optical\ density\ of\ control)} \times 100$$

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RESULTS

Scanning Electron Microscopy Analysis (SEM)

Generally, in core-shell emulsion electrospinning, many crucial parameters are involved in the flawless nanofibrous structure formation. Among others, we can mention the polymer emulsion flow rate, voltage, the distance between the needle tip and the collector, and the viscosity of the shell and core target solution. Therefore, in this research, to investigate the effect of the drug strontium ranelate, different solutions containing different doses of the drug were used. These different doses change the viscosity of the core solution, which is one of the crucial parameters of shell-core emulsion electrospinning. Figure 2 shows the results obtained from the scanning electron microscopy of the electrospun scaffolds. As expected, in the scaffolds prepared by the emulsion electrospinning method, the diameter of the nanofibers increased with the increase in the amount of drug in the scaffold and the subsequent increase in the viscosity of the core. Furthermore, the absence of drug particles on the surfaces of nanofibers indicates the proper compatibility of the drug with the hydrophilic polymer polyvinyl alcohol (PVA) in the core. According to Figure 1, all samples produced by the core-shell electrospinning method are continuous, smooth, beadles, and in the nano-size range. When the drug is added to the core solution and subsequently to the final emulsion, there are changes in the properties and viscosity of the solution and the final emulsion. Similar to the conventional electrospinning method, the emulsion electrospinning method is a one-step production process during which electrical energy leads to the solidification of the microfluidic jet. The process leads to nanofibers production in a short period of time (~10-2 sec) [19].

Water Uptake Analysis

The results of water uptake of nanofibers are shown as the equilibrium amount of water absorption in Figure 3. As expected, due to the hydrophilic nature of strontium ranelate and polyvinyl alcohol, the amount of water absorption for the G-20SrPP sample was the highest (5.12±18.01 g/g). However, this value has decreased to 4.97±46.06 g/g and 1.23±71.71 g/g in G-5SrPP and G-PP samples, respectively.

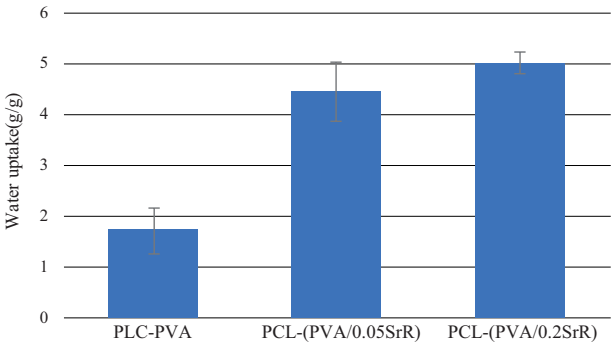

Figure 3: Equilibrium Water Uptake Values of Scaffolds

Degradation Behavior

Figure 4 shows the weight loss values of different samples during 28 days of immersion in PBS. As expected, the G-20SrPP scaffold has the highest degradation rate, where 66.73±2.43% of the studied sample is degraded after 28 days. This value has decreased with the increase in the shell thickness compared to the core, so 5SrPP and G-PP showed 57.83±1.49% and 53.13±1.68% degradation after

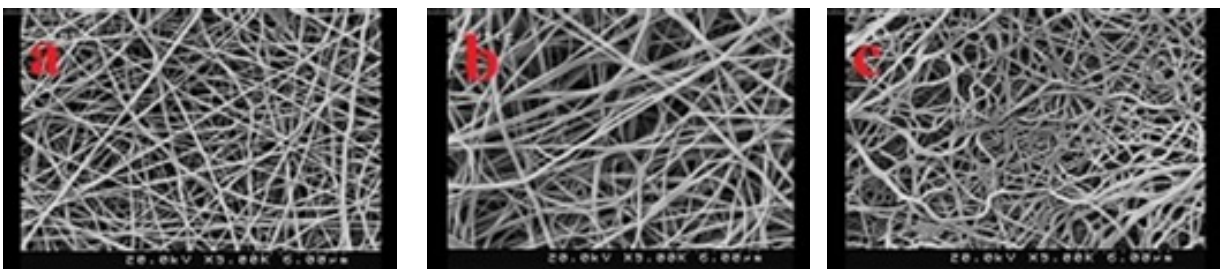

Figure 2: Scanning Electron Microscopy Images
A) PP scaffold; B) 5SrPP scaffold; C) 20SrPP scaffold

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28 days. This finding can be due to the decrease in the water absorption capacity of the scaffold with the increase in the ratio of the thickness of the shell to the core. Increasing the thickness of the core and subsequently increasing water absorption facilitates the penetration of water into the nanofibers and thus accelerates the hydrolysis process.

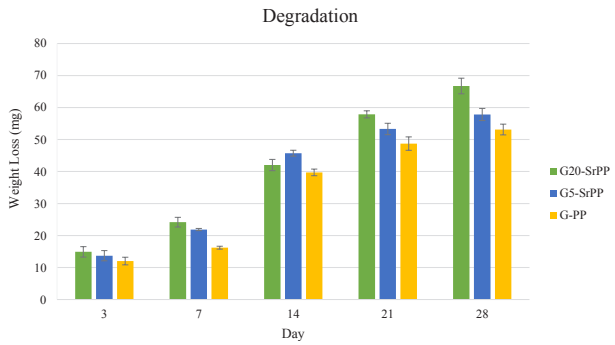

Figure 4: Degradation Behavior of the Prepared Scaffolds

MTT Assay

Figure 5 shows the results of the MTT test during 1, 3, and 5 days of cell culture. The results indicate that the cell viability in scaffold samples containing strontium ranelate was higher than the strontium ranelate-free samples, representing the non-toxic effect of the SrR in the nanofibrous scaffolds of this research. Moreover, in the G-20SrPP sample, the cell viability is significantly higher than the G-5SrPP electrospun scaffold sample ($P<0.01$) (Figure 4). The results of the MTT test showed that the release of SrR in the culture medium during the first three days had no cytotoxic activity and had a suitable range for stimulating cell growth and proliferation. The vital role of SrR in bone regeneration has been completely examined in previous studies, and a wide variety of mechanisms have thus far been adopted (e.g., stimulating prostaglandin production, sensing by activation of several cellular signaling pathways, such as fibroblast growth factor receptors, ERK1/2-MAPK, and NFATc/Wnt) [18, 20]. Furthermore, the exclusive act of the SrR on the calcium-sensing receptor (CaSr) results in the upregulation of osteoblast proliferation regulators, such as ETS-related gene 1 and c-fos [21, 22].

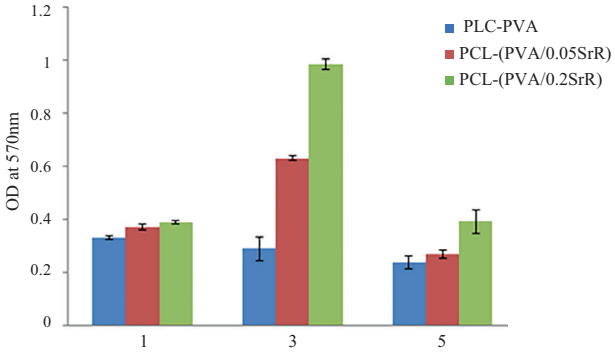

Figure 5: The Results of the MTT Test of the Synthesized Scaffolds on Days 1, 3, and 5

DISCUSSION

Generally, in core-shell emulsion electrospinning, many crucial parameters are involved in flawless nanofibrous structure formation. Among others, we can mention the polymer emulsion flow rate, voltage, the distance between the needle tip and the collector, and the viscosity of the shell and core target solution. Therefore, in this research, to investigate the effect of the drug strontium ranelate, different solutions containing different doses of the drug were used. These different doses change the viscosity of the core solution, which is one of the crucial parameters of shell-core emulsion electrospinning.

According to SEM results, all samples produced by the core-shell electrospinning method are continuous, smooth, beadles, and in the nano-size range. When the drug is added to the core solution and subsequently to the final emulsion, there are changes in the properties and viscosity of the solution and the final emulsion. Furthermore, the absence of drug particles on the surfaces of nanofibers indicates the proper compatibility of the drug with the hydrophilic polymer polyvinyl alcohol (PVA) in the core. Since PVA and SrR are hydrophilic, by increasing their amount in the structure of scaffolds, as expected, the water uptake of the scaffolds increased. These results were confirmed by the water uptake analysis. According to the degradation results, it can be seen that by reducing the amount of SrR, the total degradation is reduced. This finding can be due to the decrease in the water absorption capacity of the scaffold with the increase in the ratio of the thickness of the shell to the core. Increasing the thickness

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of the core and subsequently increasing water absorption facilitates the penetration of water into the nanofibers and thus accelerates the hydrolysis process. The results of the MTT test showed that the release of SrR in the culture medium during the first three days had no cytotoxic activity and had a suitable range for stimulating cell growth and proliferation. The vital role of SrR in bone regeneration has been completely examined in previous studies, and a wide variety of mechanisms have thus far been adopted (e.g., stimulating prostaglandin production, sensing by activation of several cellular signaling pathways, such as fibroblast growth factor receptors, ERK1/2-MAPK, and NFATc/Wnt) [18, 20]. Furthermore, the exclusive act of the SrR on the calcium-sensing receptor (CaSr) results in the upregulation of osteoblast proliferation regulators, such as ETS-related gene 1 and c-fos [21, 22].

In the present study, strontium ranelate was obtained from scaffolds produced by the emulsion electrospinning method. In this method, nanofibrous scaffolds with a core-shell structure made of polyvinyl alcohol/polycaprolactone were prepared, and the drug strontium ranelate was loaded in the core part of the nanofibers. Generally, numerous and vital factors in choosing an ideal scaffold for bone tissue engineering applications should be investigated and analyzed. In the research study, the use of strontium ranelate in nanofiber scaffolds and the effect of adding strontium ranelate to the scaffold using the emulsion electrospinning method were investigated. The results demonstrated the appropriate physicochemical and biocompatibility properties of the synthesized scaffolds.

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None declared.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS APPROVAL

This study doesn't need an ethical approval number.

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