PREPARATION AND CHARACTERIZATION OF DRUG DELIVERY CARRIERS FOR LOCAL ADMINISTRATION OF SODIUM ALENDRONATE

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Abstract

Osteoporosis is often treated with the use of sodium alendronate – a drug that inhibits osteoclast-mediated bone resorption and regulates rate of bone turnover. However the disadvantage of oral administration of sodium alendronate is poor drug absorption from the gastrointestinal track and severe adverse effects. Therefore we propose local sustained drug delivery systems based on poly(lactide-co-glycolide) (PLGA) micro- and nanocarriers, which can be administered directly by simple injections to the required place in the body. In this study we encapsulated sodium alendronate into PLGA micro- and nanospheres via a double-emulsification technique. Emulsion formation in different shear rate conditions was used to optimize the size of the carriers. The prepared microspheres were observed under an inverted optical microscope which confirmed their micrometric size. The nanospheres were analyzed by atomic force microscopy, which allowed visualization of their shape and measurement of their size. Moreover the hydrodynamic diameter of the nanospheres, polydispersity index as well as zeta potential were examined by dynamic light scattering. The experiments show that drug release does not depend on the size of the carriers. Analyzed carriers do not cause cytotoxicity upon contact with osteoblast like-cells.

Keywords: *nanocarriers, microcarriers, poly(lactide-co-glycolide) (PLGA), osteoporosis, controlled drug delivery, sodium alendronate*

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Introduction

Sodium alendronate (4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid, sodium trihydrate, SA) (FIG. 1), is a potent amino-bisphosphonate that has undergone extensive clinical development for the treatment of osteoporosis and other skeletal disorders [1]. The four-carbon amino side chain of alendronate permits effective inhibition of osteoclast-mediated bone resorption [2]. Therefore, treatment with alendronate specifically inhibits increased bone resorption and thereby normalizes the rate of bone turnover.

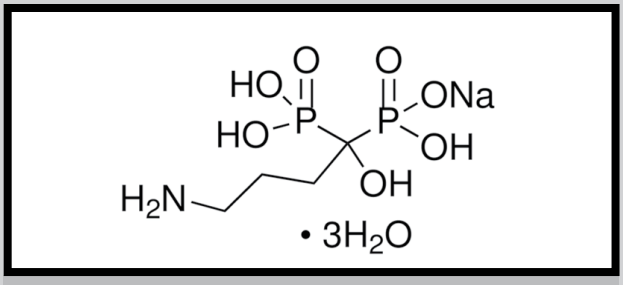

FIG. 1. Chemical formula of sodium alendronate.

Since oral administration is the most preferred for chronic drug therapy, the major disadvantage of the clinically utilized sodium alendronate is its poor absorption (less than 1% of the oral dose) from the gastrointestinal tract. In addition, the administration of sodium alendronate has been associated with severe adverse effects such as fever or stomach ulcers in humans [3]. Hence, it would be advantageous to replace systemic treatment by a local delivery system.

Drug-delivery systems are one of the most promising application fields in human health care and represent a continuously evolving field of biomedical materials science [4]. Generally, there have been two major issues in the fabrication of drug delivery systems: site specificity for the desired tissue and the duration time of the circulation of the drug carrier [5]. The challenge for novel drug delivery systems is to overcome these limitations in order to achieve optimal local absorption and bioavailability [6]. Local drug delivery systems can reduce side-effects, improve the efficacy of existing drugs and open the possibility of entire classes of new treatments. Such combined systems are able to precisely control the timing of drug release by adjusting the properties of the carriers [7].

Synthetic polymers are often used to produce drug delivery systems. For example, biodegradable poly(lactide-co-glycolide) (PLGA) is regarded as one of the most biocompatible materials used to prepare drug carriers. Different drug release profiles can be achieved by varying molecular weight, copolymer ratio, drug-loading, particle size and porosity and manufacturing conditions [8]. PLGA as a potent matrix for alendronate local administration has already been tested e. g. in the form of PLGA/PDLA/methoxyPEG films [9] or PLGA/hydroxyapatite blends [10].

The aim of this study was to design the method of sodium alendronate encapsulation in PLGA micro- and nanocarriers for their local delivery and sustained release. To this end a double-emulsification technique was applied and the resulting carriers were characterized from the point of view of their size, morphology, surface properties, drug loading efficiency and release. Finally, biological tests were conducted using MG63 osteoblast-like cells to evaluate cell viability, distribution and morphology in the presence of the produced particles and the solution into which drugs were released.

Materials and Methods

Materials

Poly(L-lactide-co-glycolide) (PLGA, 85:15, Mn = 80 kDa, d = 1.9), synthesized by a ring-opening polymerization in bulk at 100°C with $Zr(acac)_4$ initiator [11] was kindly provided by Prof. P. Dobrzynski from the Centre of Polymer and Carbon Materials of the Polish Academy of Sciences in Zabrze, Poland. Sodium alendronate (batch no.: 504041227) was a gift from Polpharma (Poland).

Preparation of PLGA micro- and nanoparticles

The PLGA particles were fabricated using a double emulsification water/oil/water technique. An aqueous solution of sodium alendronate (0.88 mg/mL) was emulsified in a 10% (w/v) PLGA polymer solution in dichloromethane, forming the primary (water/oil) emulsion. The primary emulsion was then introduced into an aqueous solution with a stabilizer, 4% polyvinyl alcohol (PVA, Mowiol 4-88, Mn = 31 kDa) in UHQ-water and shear stress was applied, forming the secondary (water/oil/water) emulsion.

The shearing rate involved during the second emulsification stage controlled the size of the formed droplets and thereby the final size of the polymeric particles. Therefore, in order to produce nanoparticles, an ultrasonication via ultrasonic probe (Sonics VibraCellTM, USA, condition: 40% of the cycle) was used, whereas in the case of microparticles production a mechanical stirring on electromagnetic agitator at 1000 rpm was used. Afterwards, the emulsions were kept on an electromagnetic stirrer, in order to evaporate the dichloromethane. Then sodium alendronate-loaded nano- or microparticles (depending on the shear rate applied) were submitted to centrifugation, multistep washing process in UHQ-water and vacuum-drying.

Surface properties characterization

The dry particles were resuspended in 2 mL of UHQ-water using the ultrasonic bath (Polsonic® Sonic-3) for 15 min without heating.

The microparticles' size and topography were evaluated using an inverted optical microscope (Axiovert Zeiss) in the interference mode and with the AxioVision Software the size of the particles was measured.

Nanoparticles' shape was observed with atomic force microscopy (AFM, Thermomicroscopes, Explorer, Veeco). To achieve a better distribution of the nanoparticles during the measurements, the samples were 10 times diluted. The images were recorded in air using Si_3N_4 tips with a nominal radius of curvature of 30 nm (NanoProbeTM tips) in contact mode with a spring constant of 0.1 N/m. The proportional, integral and derivative parameters of the feedback mechanism applied to adjust the tip-to-sample distance, in order to keep a constant force between the tip and the sample, were 1, 0.3 and 0, respectively. Scan areas of 2 μm × 2 μm and of three random places with a 300 resolution (300 × 300 pixels) were recorded at a scan rate of 6 μm/s. All the images were flattened and treated to remove any artifacts using the software SPMLab6.0.2 Explorer.

Size distribution, zeta potential and polydispersity of nanoparticles were analyzed using dynamic light scattering (Zetasizer Nano ZS - Malvern Instrument).

Drug loading and in vitro release

In order to measure the amount of sodium alendronate in micro- and nanoparticles, a UV-spectrophotometry technique (CECIL CE2502, 2000 Series at 332 nm) with the o-phthaldialaldehyde (OPA) method was used.

The experiments on drug release from the carriers were carried out by diffusion through semipermeable membrane and spectrophotometric quantitative analysis of sodium alendronate with OPA. In brief, dried particles were introduced in a membrane with MWCO (molecular weight cut off) of 12 kDa (ZelluTrans/Roth T3 made of regenerated cellulose) previously washed with UHQ-water. The membranes with the samples were transferred to the flasks containing 10 mL of UHQ-water and 1 mM of nitric acid on the electromagnetic stirrer at 50 rpm. The release samples were collected for 7 days (every 3 h in the first three days, twice on the fourth day and once on days 5, 6 and 7).

Biological tests

Cell culture studies were performed using MG63 osteoblast-like cells (European Collection of Cell Cultures, Salisbury, UK). These cells were seeded on 24-well plates (Tissue Culture Plate, Sarstedt, USA) at a density of $1.5 \cdot 10^4$ cells/cm^2 and cultured in MEM with Earle's Salts (supplemented with 10% FBS, 1% penicilin/streptomicin, 2 mM L-glutamine) at 37°C under 5.0% CO_2 atmosphere. One day later, the medium was substituted by 1 mL of new medium and solution from the sodium alendronate release assay at a 8:2 v/v ratio. For cell culture studies, the release solutions were collected after 1, 3 and 7 days. After the following day of cell culture, the supernatants were collected to assess the nitric oxide (NO) level by the Griess method, the cells were stained using Hematoxylin and Eosin (H&E) and their viability was analyzed by the MTT test. Tissue culture polystyrene (TCPS, i.e. bottom of the well plates) was used as a control.

Statistics

The results are shown as mean ± SEM (standard error of the mean). Statistical analysis was performed using a distribution t-test with SPSS (Statistical Package for the Social Sciences) software. Significant differences were assumed at $p^* < 0.05$.

Results and Discussion

The shape and size of the microparticles were evaluated using an inverted optical microscope with AxioVision software. FIG. 2 A and 2 B display the morphology of unloaded and sodium alendronate loaded microparticles. It can be noticed that all unloaded particles (FIG. 2 A) display a black color, whereas in FIG. 3 B almost all microparticles show greater transparency. These results can lead us to distinguish loaded from unloaded microparticles from their transparency. Based on the provided software it was possible to measure the size of the microparticels. Thus, the sodium alendronate unloaded micropaticles' average diameter was 8.7 μm ± 1.8 μm, while the loaded particles showed a significantly higher diameter of 15.3 μm ± 6.1 μm.

Sodium alendronate loading efficiency in the microparticles (which reflects the mass of drug incorporated into the particles in relation to the mass of drug initially added to the system), was 50% ± 5%. This means that it is desirable to improve this parameter, by changing, in the future experiments for example, concentration of PVA in the external aqueous phase or providing higher ionic strength by addition of NaCl [12].

The morphology and size distribution, zeta potential and polydispersity of the nanoparticles were evaluated with the use of atomic force microscopy and dynamic light scattering, respectively. FIG. 3 A and B show the 2 μm × 2 μm three-dimensional AFM pictures of unloaded and loaded nanoparticles, which confirm their nanometric size and spherical shape. In FIG. 3 C and D the size distribution of unloaded and loaded nanoparticles as measured by DLS is also presented. The results show that particles' diameter is in the range of 100 nm to 600 nm, in both cases. TABLE 1 summarizes the results obtained by the DLS method. The average diameter of unloaded and loaded nanoparticles is very similar, 252 ± 5 nm and 241 ± 8 nm, respectively. PLGA nanoparticles produced have a polydispersity in the standard range, 0.219 ± 0.011 for unloaded and 0.194 ± 0.016 for sodium alendronate loaded nanoparticles. The results of zeta potential distribution show negative values in both cases that are due to the negative charges of the polymer [13].

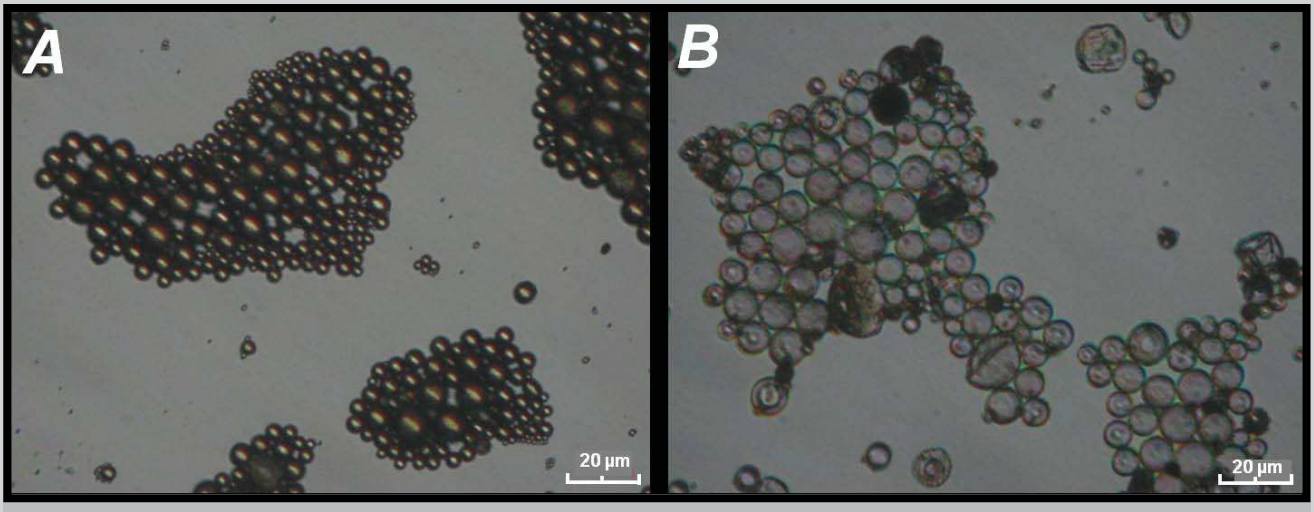

FIG. 2. Inverted optical microscopy pictures of A – unloaded microparticles and B – loaded microparticles. Magnification 40x. Scale bar 20 µm.

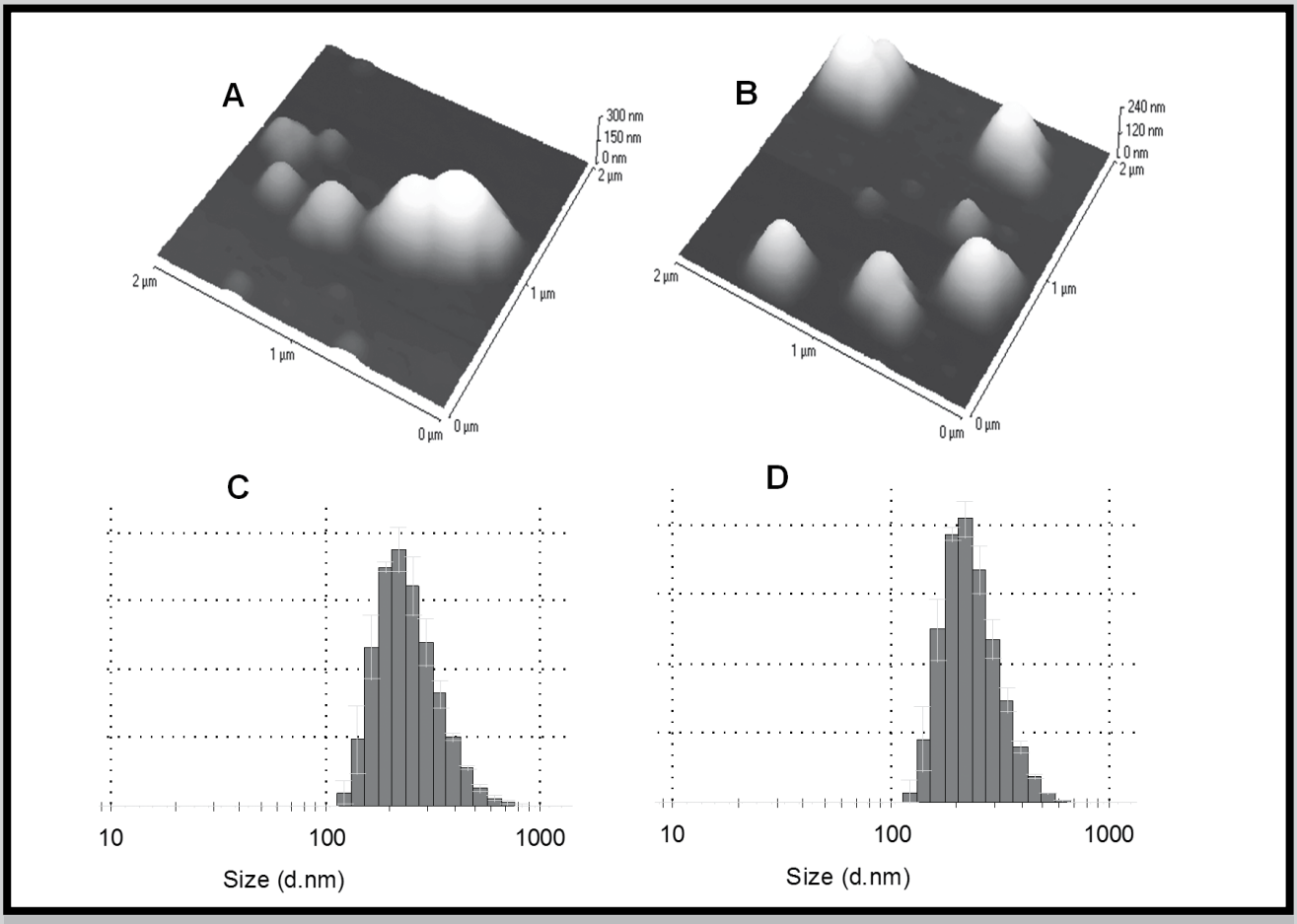

FIG. 3. Three dimensional AFM topography pictures (A, B) and size distribution (C, D) of - unloaded nanoparticles (A, C) and - loaded nanoparticles (B, D).

TABLE 1. Properties of PLGA nanoparticles unloaded and loaded with sodium alendronate.

	Diameter (nm)	Polydispersity	Zeta Potential (mV)
Unloaded	252 ± 5	0.219 ± 0.011	-14.9 ± 0.4
Loaded	241 ± 8	0.194 ± 0.016	-16.6 ± 0.1

Zeta potential plays an important role in the chemical properties of the interface between solid and liquid and is characterized by the result of an electric double-layer, an electric potential across the surface of the material. The magnitude of zeta potential reflects the magnitude of charges absorbed by the solid [14]. Therefore, zeta potential values are negative due to the negative charge of the PLGA [13] and confirm that the sodium alendronate is situated inside the carriers and not on their surface. If this drug was on the surface the zeta potential value should have been approximately -5 mV [15]. The absolute value of zeta potential obtained can also indicate that the particles produced have an acceptable short-term stability [16].

Spectroscopic evaluations of drug concentration in surrounding medium show sustained linear release of sodium alendronate during the three days of the experiment (FIG. 4). Afterwards the log phase was achieved. Interestingly, release kinetics was the same for both nano- and microspheres, what indicates that the size of particles does not influence release profile. Further it may be concluded that drug release from PLGA carriers seems to be Fickian diffusion controlled, not degradation controlled. In other words, the drug release is mainly governed by the rate of dissolution of the alendronate through complex porosities of the PLGA material [17].

FIG. 5 shows the absorbance values of MTT test (FIG. 5 A) and nitric oxide concentration (FIG. 5 B) for MG63 osteoblast-like cells cultured for 1, 3 and 7 days in release solution (R) of micro- (M) and nanoparticles (N), loaded (+) and unloaded (-) with sodium alendronate and also cultured for one day with loaded and unloaded nanoparticles (N+ and N-). On day 1, the viability of cells in all conditions of culture was similar or higher than on TCPS. After 3 days of culture, the cell viability increased in almost all conditions (RM+, RM-, RN+) and decreased in RN- sample but to a similar value as the control. On day 7, in conditions RM- and RN- the cell viability increased and in conditions RM+ and RM- decreased. It is worth noting that changes in cells viability measured in all conditions and for all days were always similar or higher than the viability on TCPS. Since this material is probably the most appropriate for cell culture, it is possible to verify that both release solutions from micro- and nanoparticles, loaded or unloaded, do not influence negatively cell viability. The presence of nanoparticles also does not influence viability of cells. For all materials and culture times studied the concentration of nitric oxide was similar to that of control TCPS, suggesting that the nano-and microparticles or release solution do not have cytotoxic effects on MG63 cells (FIG. 5 B).

FIG. 6 presents the pictures obtained under an inverted optical microscope which show that the MG63 cells effectively adhered in the presence of the release solution or PLGA nanoparticles, which were loaded with sodium alendronate or unloaded. Cells' morphology was the same as that on reference TCPS.

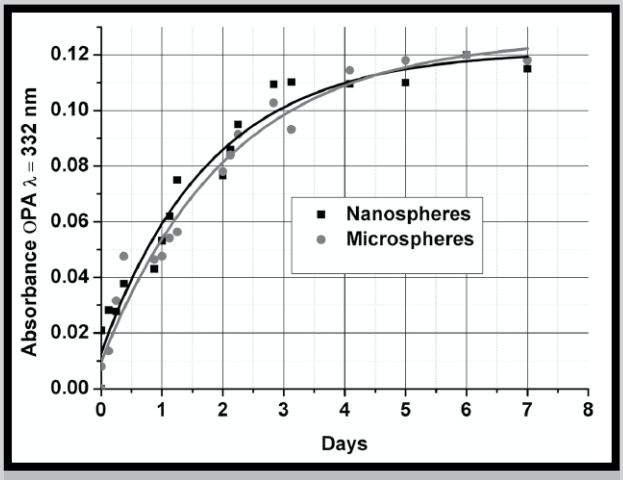

FIG. 4. Release of sodium alendronate from PLGA nano- and microspheres measured by OPA method.

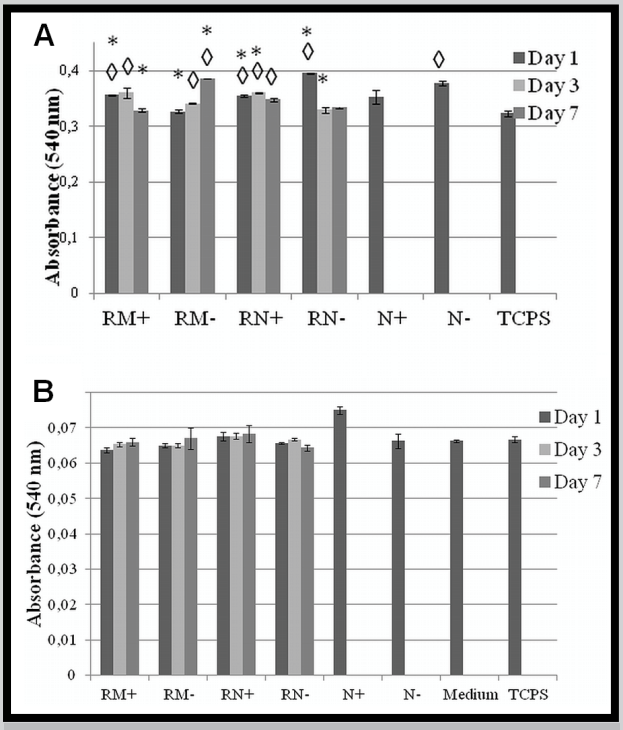

FIG. 5. Absorbance values of the MTT assay (A) and nitric oxide concentration (NO) (B) performed on MG63 osteoblast-like cells cultured for 1, 3 and 7 days in release solution (R) of micro- (M) and nanoparticles (N), loaded (+) and unloaded with sodium alendronate as well as with nanoparticles loaded (N+) and unloaded (N-). TCPS was used as control. ◊ Significant differences as compared with TCPS control, * Significant differences between release solution of nano- and microparticles loaded and unloaded ($p<0.05$).

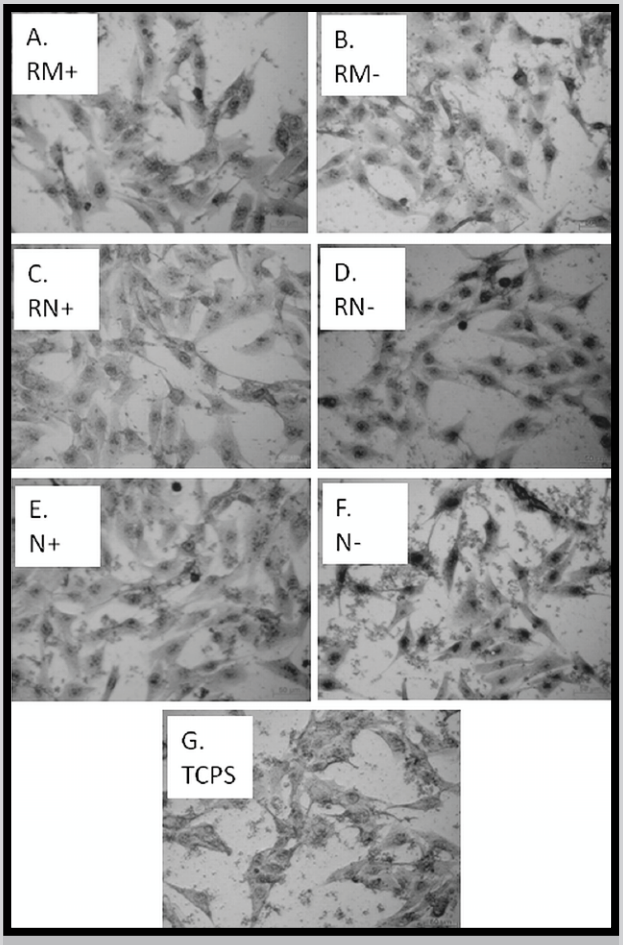

FIG. 6. Morphology of MG63 osteoblast-like cells cultured for 1 day in release solution (R) of micro- (M) and nanoparticles (N), loaded (+) and unloaded with sodium alendronate as well as with loaded (N+) and unloaded (N-) Nanoparticles. TCPS was used as control.

Conclusions

In this study the encapsulation of sodium alendronate into PLGA micro- and nanoparticles via a double-emulsification technique and analysis of the particles' physicochemical and biological properties were performed. Optical microscopy and atomic force microscopy evaluations confirmed that by changing the shear stress applied to the second emulsion of the particles' fabrication process it is possible to change the size of the produced carriers. The results of zeta potential show that sodium alendronate is incorporated inside the particles, since loaded and unloaded samples have similar values of surface charge. Moreover it was found that the micro- and nanospheres obtained show sustainable release of the drug during short-term studies. Finally, the biological tests lead us to conclude that incubation of osteoblast-like cells in nanoparticles or release solutions from both nano- and microcarriers has no negative effect on cell morphology, adhesion and proliferation.

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References

[1] Karageorgiou V., Kaplan D.: Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 26(27) (2005) 5474-5491.
[2] Liberman U. A., Weiss S. R., Bröll J., Minne H. W., Quan H., Bell N. H., Rodriguez-Portales J., Downs R. W. Jr., Dequeker J., Favus M.: Effect of Oral Alendronate on Bone Mineral Density and the Incidence of Fractures in Postmenopausal Osteoporosis. N. Engl. J. Med. 333(22) (1995) 1437-1444.
[3] Ezra A., Golomb G.: Administration routes and delivery systems of bisphosphonates for the treatment of bone resorption. Adv. Drug Delivery Rev. 42(3) (2000) 175-195.
[4] Vallet-Regí M., Balas F., Arcos D.: Mesoporous Materials for Drug Delivery. Angew. Chem. Int. Ed. 46(40) (2007) 7548-7558.
[5] Choi S. W., Kim J. H.: Design of surface-modified poly(d,l-lactide-co-glycolide) nanoparticles for targeted drug delivery to bone. J. Controlled Release 122(1) (2007) 24-30.
[6] Peter B., Pioletti D. P., Laďb S., Bujoli B., Pilet P., Janvier P., Guicheux J., Zambelli P. Y., Bouler J., Gauthier O.: Calcium phosphate drug delivery system: influence of local zoledronate release on bone implant osteointegration. Bone 36(1) (2005) 52-60.
[7] Verron E., Khairoun I., Guicheux J., Bouler J. M.: Calcium phosphate biomaterials as bone drug delivery systems: a review. Drug Discovery Today 15(13-14) (2010) 547-552.
[8] Cenni E., Granchi D., Avnet S., Fotia C., Salerno M., Micieli D., Sarpietro M. G., Pignatello R., Castelli F., Baldini N.: Biocompatibility of poly(d,l-lactide-co-glycolide) nanoparticles conjugated with alendronate. Biomaterials 29(10) (2008) 1400-1411.
[9] Long K. A., Jackson J. K., Yang C., Chehroudi B., Brunette D. M., Burt H. M.: Controlled release of alendronate from polymeric films. J Biomater Sci Polym Ed. 20(5-6) (2009) 653-72.
[10] Veena H. R., Prasad D.: Evaluation of an aminobisphosphonate (alendronate) in the management of periodontal osseous defects. J. Indian Soc Periodontol 14 (2010) 40-45.
[11] Dobrzynski P.: Synthesis of biodegradable copolymers with low-toxicity zirconium compounds. III. Synthesis and chain-microstructure analysis of terpolymer obtained from L-lactide, glycolide, and ε-caprolactone initiated by zirconium(IV) acetylacetonate. J. Polym. Sci., Part A: Polym. Chemi. 40(18) (2002) 3129-3143.
[12] Miladi K., Sfar S., Fessi H., Elaissari A.: Drug carriers in osteoporosis: Preparation, drug encapsulation and applications. Int. J. Pharm. 445(1-2) (2013) 181-195.
[13] Taetz S., Nafee N., Beisner J., Piotrowska K., Baldes C., Mürdter T. E., Huwer H., Schneider M., Schaefer U. F., Klotz U., Lehr C. M.: The influence of chitosan content in cationic chitosan/PLGA nanoparticles on the delivery efficiency of antisense 2′-O-methyl-RNA directed against telomerase in lung cancer cells. Eur. J. Pharm. Biopharm. 72(2) (2009) 358-369.
[14] Lin Y., Mao S.: ζ Potential and its effect on compaction of biomass fuel logs. Biomass Bioenergy 20(3) (2001) 217-222.
[15] Mondal T., Sunny M. C., Khastgir D., Varma H. K., Ramesh P.: Poly (l-lactide-co-ε-caprolactone) microspheres laden with bioactive glass-ceramic and alendronate sodium as bone regenerative scaffolds. Mater. Sci. Eng., C 32(4) (2012) 697-706.
[16] Wu L., Zhang J., Watanabe W.: Physical and chemical stability of drug nanoparticles. Adv. Drug Delivery Rev. 63(6) (2011) 456-469.
[17] Cruz L., Assumpção E., Staniscuaski-Guterres S., Raffin-Pohlmann A.: High encapsulation efficiency of sodium alendronate in eudragit S100/HPMC blend microparticles. Quím. Nova 32(5) (2009) 1170-1174.