Enhancing Therapeutic Efficacy in Osteoarthritis Through the Formulation of Resveratrol and Phloretin Loaded Poly (Lactic-Co-Glycolic Acid) (PLGA) Nanoparticles

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Osteoarthritis (OA) is a chronic joint disease characterized by cartilage degradation, alterations in bone formation or subchondral bone remodeling and progressive synovial inflammation [1]. Natural products are among the frequently utilized pharmaceutical components intended to target and treat a number of emerging and developing disorders. These compounds exhibit diverse pharmacological properties, including anticancer, anti-inflammatory, antibacterial, anti-oxidative, immunosuppressive activity, among others. Nevertheless, their use is limited by their poor water solubility, low bioavailability, low stability, and rapid metabolism [2]. Nanotechnological approaches and specifically nanomaterials as drug delivery vectors can solve some of major drawbacks of existing pharmacological strategies [1].Different nanocarriers such as polylactic co-glycolic acid (PLGA) nanoparticles (NPs) have been developed as effective methods for sustained drug release in OA treatment due to their stability and in vivo cell uptake [3,4,5,6]. For this reason, the goal of this study was to encapsulate the therapeutic compounds resveratrol (Resv) and phloretin (Phl) within PLGA nanoparticles and to study their cytotoxic and anti-inflammatory effects related to OA on a chondrogenic cell line (ATDC-5). PLGA NPs were synthesized using the simple emulsification-solvent evaporation method, with resveratrol or phloretin loaded into the inner core of the emulsion. Scanning electron microscopy (SEM) was used to assess the morphology and size of the particles. NP size, size distribution and electrokinetic potential were determined by Dynamic Light Scattering (DLS). The drug content of Resv in NPs was determined directly by measuring the encapsulated resveratrol amount in PLGA NPs after dissolution using UV-Visible. The drug content of Phloretin was quantitatively determined by UPLC. The release of Resv and Phl from prepared NPs was assessed in PBS media (pH 7.4) containing SDS 0.3% (w/v) and quantified by UV-Visible and UPLC method, respectively. The cytotoxicity of PLGA nanosystems in ATDC-5 cell line was evaluated by the Blue Cell Viability Assay, after 24h of incubation with different concentrations (0.1 -2 mg/ml) of PLGA NPs or loaded with Resv (Resv@PLGA-NPs) or Phl (Phl@PLGA-NPs) and with the equivalent doses of free drug. To test the anti-inflammatory activity, ATDC-5 cells were treated with 250 ng/mL of lipopolysaccharide (LPS) for 24h after being pre-incubated with the subcytotoxic concentration of Resv@PLGA- NPs, Ph@PLGA-NPs and the equivalent doses of free drugs for 4h. The Griess reaction was used to assess the nitrite accumulation in culture media as a hallmark of inflammation. Both Resv@PLGA-NPs and Phl@PLGA-NPs exhibited spherical morphology with an average size of 117 ± 26 nm and 132 ± 33 nm, respectively, and a negative charge of -27.65 mV and -25.02 mV, respectively (Figure 1 A,B). Both Resv@PLGA-NPs and Phl@PLGA-NPs exhibited remarkable encapsulation efficiency (EE%) (36.48 and 7.57 %) and drug loading (DL%) (17.37 and 3.41 %), respectively. The obtained Resv@PLGA-NPs and Phl@PLGA-NPs showed an initial burst release followed by a sustained release. On the other hand, the viability results demonstrated the cytocompatibility of both Resv@PLGA-NPs and Phl@PLGA-NPs within the tested concentration range, with percentages exceeding 70% in most cases, complying with ISO 10993-5 recommendations. The experiments suggested that resveratrol and phloretin could be successfully incorporated into polymeric nanoparticles, as demonstrated by their absence of negative effects on ATDC-5 cells and effective encapsulation in PLGA. Moreover, these nanoformulations exhibited significant anti-inflammatory effects while maintaining the activity of the loaded therapeutic molecules, and therefore, are a potential candidates for OA treatment.

References

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Figures

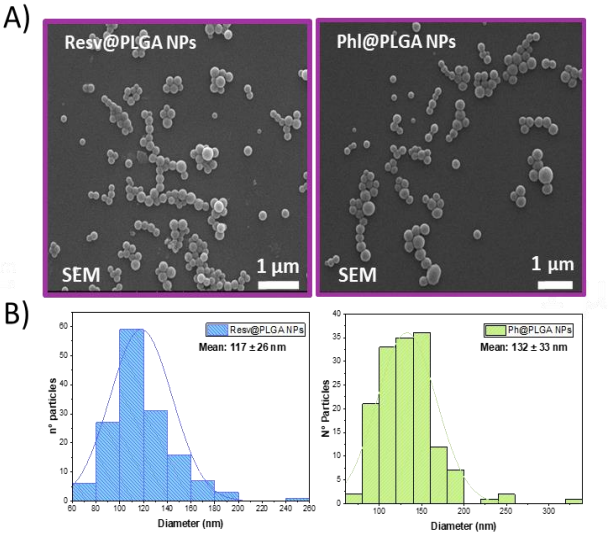

Figure 1.SEM images of Resv@PLGA and Phl@PLGA nanoparticles display a monodisperse population of NPs (A). Particle size distribution histogram derived from the analysis of 150 NPs from SEM images (B).