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Stem cell-extracellular vesicles as drug delivery systems: New frontiers for silk/curcumin nanoparticles

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ABSTRACT

The aim of this work was to develop a novel carrier-in-carrier system based on stem cell-extracellular vesicles loaded of silk/curcumin nanoparticles by endogenous technique. Silk nanoparticles were produced by desolvation method and curcumin has been selected as drug model because of its limited water solubility and poor bioavailability. Nanoparticles were stable, with spherical geometry, 100 nm in average diameter and the drug content reached about 30%. Cellular uptake studies, performed on mesenchymal stem cells (MSCs), showed the accumulation of nanoparticles in the cytosol around the nuclear membrane, without cytotoxic effects. Finally, MSCs were able to release extracellular vesicles entrapping silk/curcumin nanoparticles. This combined biological-technological approach represents a novel class of nanosystems, combining beneficial effects of both regenerative cell therapies and pharmaceutical nanomedicine, avoiding the use of viable replicating stem cells.

1. Introduction

In recent years, novel drug delivery systems have been developed to optimize the efficacy of therapeutics enhancing their bioavailability, reducing their degradation rate, allowing targeting and thus their control release, cellular uptake and reducing side effects (Mottaghitalab et al., 2015). Nanotechnology is giving a great impact in drug delivery field and nanoparticles are at the leading edge, since they can improve solubility, stability and efficacy of drugs, achieving a reduction in administration frequency and lower risks of toxicity in patients (Mishra et al., 2013; Parveen et al., 2012). Thanks to their tunable properties (particle size, surface charge, chemical modifications) nanoparticles can be envisioned as the future of drug delivery technology as their "nanosize" is crucial to their cellular internalization, which is the key to achieve a "real" therapeutic efficacy (Kumari et al., 2010). Bio-inspired nanoparticles, based on natural polymers or bio-macromolecules, mirror natural compounds. Among them, silk fibroin is a natural polymer widely studied for tissue engineering and drug delivery (Altman et al., 2002; Chlapanidas et al., 2011, 2016, 2013; Farago et al., 2016; Vepari and Kaplan, 2007; Vigani et al., 2016). Silk fibroin is an effective polymer for the delivery of therapeutic agents because it has shown biocompatibility, controllable biodegradability, low toxicity/immunogenicity, chemical modification potential, appropriate mechanical properties and therapeutic retention at target sites (Kundu et al., 2013; Wang and Zhang, 2015). Moreover, silk nanoparticles can be prepared by different techniques, mainly desolvation and salting out, selected on drug physicochemical properties (Zhao et al., 2015).

Extracellular vesicles (EVs) are small membrane bound-vesicles, ranging in size from 40 to 1000 nm, produced by most of mammalian cell lineages both under physiological and pathological conditions. EVs can be found in body fluids such

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as in saliva, urine, bile, cerebrospinal fluid (Kim et al., 2016; Tompkins et al., 2015), and can act as physiological delivery nanosystems. The main advantages of EV-based drug delivery than synthetic delivery nanosystems (e.g. liposomes) are lower immunogenicity and toxicity and higher stability in circulation and tissues (van der Meel et al., 2014). Moreover, EVs mirror the genetic and proteomic content of their secreting cells, thus those derived from mesenchymal stem/stromal cells (MSCs) retain several biological activities that are able to reproduce the beneficial effects of stem cells (Camussi and Quesenberry, 2013) including migration to injured tissues (i.e. homing), inflammatory and immune response modulation through the secretion of cytokines and trophic factors (Caplan, 2010; de Girolamo et al., 2013; Torre et al., 2015).

Recently, our research group has developed an innovative carrier-in-carrier system for hydrophobic drugs mediated by micelle-loaded MSCs: micelles were considered as the first drug carrier, while the second carrier were constituted by MSCs that could also assure an adequate drug targeting due to their innate homing. We demonstrated that the uptake of micelles by MSCs has resulted effective and quick without any relevant cytotoxic effects and cells, loaded of micelles, were able to release the entrapped drug (Tripodo et al., 2015a).

The aim of this study was to evolve a novel carrier-in-carrier delivery system based on EVs loaded of silk/curcumin nanoparticles. Silk nanoparticles were produced by desolvation method and curcumin has been selected as drug model because of its limited water solubility and poor bioavailability. This natural polyphenolic compound, isolated from the rhizome of *Curcuma longa*, could be used in the treatment of many chronic diseases, such as multiple sclerosis, rheumatoid arthritis, colitis, Alzheimer's disease and potentially in cancer prevention and therapy (Aggarwal and Harikumar, 2009) due to its proved antioxidant, anti-inflammatory, apoptosis-inducing and anti-angiogenic activities. Thus, nanoparticles were uptaken by adipose-derived MSCs and subsequently MSC-EVs, loaded of silk/curcumin nanoparticles, were secreted and characterized. Our idea represents a novel approach, coupling stem cell therapy and nanomedicine.

2. Material and methods

2.1. Materials

Sodium carbonate, lithium bromide, calcium chloride, acetone, curcumin, ethanol, collagenase, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), nile red, sodium alginate and dimethyl sulfoxide were obtained from Sigma-Aldrich (Milan, Italy). Dialysis tubes were purchased from Visking (London, United Kingdom). All reagents used for cell cultures were purchased from Euroclone (Milan, Italy).

2.2. Silk fibroin extraction and nanoparticle preparation

Bombyx mori cocoons were cut into pieces, added to a boiling 0.02 M Na_2CO_3 solution for 30 min and then rinsed four times in distilled water. Degummed fibers were dried at room temperature and dissolved in 9.3 M LiBr solution at 60 °C for 4 h (Aggarwal and Harikumar, 2009). The raw silk solution was dialyzed against distilled water using cellulose tubes (MWCO 3000–5000 Da) at room temperature for 72 h to remove the residual LiBr chaotropic salts. The final concentration of aqueous silk solution was about 8% w/v, determined by freeze-drying (Modulyo® Edwards Freeze dryer, Kingston, NY) of known silk volumes.

Silk nanoparticles were prepared by acetone desolvation method (Seib et al., 2013). Briefly, aqueous silk solution was diluted to achieve the concentration 1.5% w/v and it was added drop wise in acetone under gentle stirring for 1 min at room temperature (volume ratio silk:acetone 1:5). Silk/curcumin nanoparticles were obtained adding silk solution into acetone, in which previous curcumin powder was solubilized in different concentrations (Table 1). Nanoparticles were then dialyzed against distilled water using cellulose tubes (MWCO 3000–5000 Da) until complete solvent removal. Nanoparticles were stored at 4 °C or subjected to freeze dried process at $8*10^{-1}$ mbar and −50 °C for 72 h for further investigations. Five different formulations, consisting of silk nanoparticles or silk/curcumin nanoparticles, were prepared (Table 1).

2.3. Characterization of silk and silk/curcumin nanoparticles

2.3.1. Drug loading determination

The quantification of curcumin content in silk nanoparticles was evaluated using a direct spectrophotometer method (Uvikon 860, Kontron Instruments, Switzerland) at 425 nm. Briefly, 1 mg of freeze-dried nanoparticles was dissolved in 10 ml of 96% v/v ethanol, maintaining mild magnetic stirring. Curcumin concentration was measured from a calibration curve of nine curcumin solutions in ethanol at the concentration range of $8{\cdot}10^{-3}$–$6.25{\cdot}10^{-4}$ mg/ml, with a correlation coefficient $r^2 > 0.9895$. Ethanol was considered as control solution. Each measurement was performed in triplicate.

2.3.2. Nanoparticle-tracking analysis (NTA)

Nanoparticle mean diameter was determined by nanoparticle tracking using NanoSight (NS300, Malvern Instruments, Malvern, Italy), fitted with a NS300 flow-cell top plate and a 405 nm laser. The NanoSight sample pump was used in conjunction with 1 ml syringes. Fresh nanoparticles were diluted 1:100 in phosphate buffer saline (PBS) immediately prior to analysis. All measurements were carried out at 26.4 °C with detection angle of 90° and done in triplicate. A single analysis represented a fresh dilution of the stock particle solution and consisted of three 30 s video captures. Results were analyzed with the NTA software 3.0 (Malvern Instruments, Malvern, Italy).

2.3.3. Morphological evaluation

A Zeiss EVO MA10 (Carl Zeiss, Oberkochen, Germany) was used to analyze the morphology of nanoparticles. The samples were gold-sputter coated under argon to render them electrically conductive prior to microscopy.

Table 1
Composition of silk and silk/curcumin nanoparticles (Np): five formulations were obtained after solubilization of different curcumin amount in acetone. The percentage of curcumin loading is reported as mean values and standard error (SE).

Nanoparticles	Curcumin concentration in acetone (mg/ml)	Curcumin loading (%) (mean ± SE)
Np0	–	–
Np1	0.03	0.95 ± 0.127
Np1.5	0.1	1.50 ± 0.111
Np14	0.5	13.66 ± 1.518
Np32	1.5	31.97 ± 1.522