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# Mesenchymal stem cells as delivery vehicle of porphyrin loaded nanoparticles: Effective photoinduced *in vitro* killing of osteosarcoma

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ABSTRACT

Mesenchymal stem cells (MSC) have the unique ability to home and engraft in tumor stroma. These features render them potentially a very useful tool as targeted delivery vehicles which can deliver therapeutic drugs to the tumor stroma. In the present study, we investigate whether fluorescent core-shell PMMA nanoparticles (FNPs) post-loaded with a photosensitizer, namely meso-tetrakis (4-sulfonatophenyl) porphyrin (TPPS) and uploaded by MSC could trigger osteosarcoma (OS) cell death *in vitro* upon specific photoactivation. In co-culture studies we demonstrate using laser confocal microscopy and time lapse imaging, that only after laser irradiation MSC loaded with photosensitizer-coated fluorescent NPs (TPPS@FNPs) undergo cell death and release reactive oxygen species (ROS) which are sufficient to trigger cell death of all OS cells in the culture. These results encourage further studies aimed at proving the efficacy of this novel tri-component system for PDT applications.



## 1. Introduction

Osteosarcoma (OS) is the most common primary tumor of human bone [1]. Current treatments consist of multiple modalities, traditionally including amputation or limb sparing surgery as well as chemotherapy. Despite great strides in the diagnosis and treatment of OS to date, substantial improvement in overall survival rate has been elusive and has remained constant for over two decades [2–4]. Various chemotherapeutics have been investigated for the treatment of OS in the adjuvant setting. Efficacy has been observed with platinum analogues, e.g. cisplatin and carboplatin, the anthracycline antibiotic doxorubicin (DOX), and combinations of these drugs [2,5]. However, patients receiving these agents frequently experience severe side effects, such as hypersensitivities, extravasation injuries, arrhythmias, gastrointestinal toxicity, myelosuppression, and cumulative cardiotoxicity. Such a scenario is worsened by lack of OS-associated/specific markers, which has hampered the development of targeted therapeutics. Although alternatives to conventional chemotherapeutic treatments have been proposed and studied [6–8], there is still a large need to identify new, safer and more effective therapeutic approaches for OS.

Despite progress in basic research which has given a better understanding of tumor biology and has led to the design of new generations of targeted drugs, recent large clinical trials for cancer have only been able to detect small differences in treatment outcomes [9]. These facts indicate that to promote further progress it is necessary to emphasize on other therapeutic approaches. To this regard, photodynamic therapy (PDT) has the potential to meet many currently unmet medical needs. Although still emerging, PDT is already a successful and clinically approved therapeutic modality used for the management of neoplastic and other malignant diseases [10]. PDT is a minimally invasive procedure in which target cells are destroyed by reactive oxygen species (ROS) and especially by singlet oxygen, generated by excitation of nontoxic photosensitizer with light at an appropriate wavelength.

There are very few reports describing the use of PDT to treat structural lesions within bone [11,12]. However, a promising pilot study in spontaneous canine OS has recently demonstrated that PDT induces tumor necrosis in treated animals [13]. In order for PDT to be both effective and safe, it is crucial that the photosensitizers are delivered in therapeutic concentrations preferentially to targeted cells, while being scarcely absorbed by non-target tissues, thus minimizing undesirable side effects [14,15]. To this regard, nanotechnology has been

*Abbreviations:* NPs, Nanoparticles; OS, Osteosarcoma; MSC, mesenchymal stem cells; PDT, photodynamic therapy; TPPS, meso-tetrakis(4-sulfonatophenyl) porphyrin; FNPs, Fluorescent nanoparticles; TPPS@FNPs, Fluorescent nanoparticles loaded with TPPS.

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shown to play a crucial role. Nanomedicine has recently drawn strong interest from the scientific community because of its potential to radically change cancer therapies [16–18]. Within the last decade many studies have described nanoparticles (NPs) as effective drugs carriers which allow to considerably decrease drug dosages and therefore toxicity effects associated with free chemotherapeutic agents administration [19]. However, their ability to deliver chemotherapeutic agents to subcellular targets and to control their behavior within the cell requires further studies. In addition, quantitative descriptions on the kinetics, amount, mechanisms, and trajectories of NPs uptake and trafficking are lacking. These barriers continue to hamper the clinical utility and benefit of nanoparticle-based technologies [20].

From this starting point, cells and in particular non-hematopoietic mesenchymal stem cells (MSC) could represent an ideal vehicle for targeted drug delivery, since they can be loaded with therapeutic agents, while maintaining their ability to migrate to sites of disease [21,22]. MSC are primary cells that can be expanded *ex-vivo* to reach a clinically relevant number. They are found in different tissues, such as bone marrow, fat and muscle [23,24], and these cell types are intimately involved in tissue regeneration and repair [25,26]. The therapeutic potential of MSC is linked to a broad spectrum of biological activities such as anti-inflammatory, immunomodulative and tissue reparative activities combined with low immunogeniticy. In addition MSC express genes encoding a large variety of arteriogenic cytokines and promote *in vitro* and *in vivo* arteriogenesis through paracrine mechanisms [27]. Besides these activities, MSC have the unique ability to home to sites of inflammation/injury and to tumors stroma, which makes them useful for the targeted delivery of therapeutics to these sites [28,29]. Furthermore, in the last decade researchers have taken advantage of MSC tropism to inflamed tissues to target and kill cancer cells [30–32].

Considering these preliminary remarks, we hypothesized that combining photodynamic therapy, nanoparticles, and MSC would represent a very promising tool for targeted drug delivery. In fact, a suitably designed photosensitizer@NPs system loaded into MSC would at the same time overcome the still unascertained issues associated with NPs administration alone and be safe for cells activity, being active only once MSC reach the target site and when they are exposed to an appropriate stimulus. Moreover, we considered that a system with these characteristics could potentially overcome many of the limits associated with current therapeutic approaches, both in terms of inefficient cells accumulation into the tumor stroma and of ineffective/uncontrolled drug release.

In the present study we describe a novel tri-component biomaterial system, composed of core-shell PMMA nanoparticles (FNPs) post-loaded with a photosensitizer, namely meso-tetrakis (4-sulfonatophenyl) porphyrin (TPPS) and incorporated into MSC for targeted osteosarcoma photo-dynamic treatment. Our NPs are fluorescently labeled through incorporation of a fluorescein derivative in the inner hydrophobic core, while the external shell is decorated with primary and quaternary ammonium salts which are able to electrostatically bind anionic porphyrins [33].

The aim of our study is therefore to demonstrate that MSC can be loaded with TPPS@FNPs and that, upon light irradiation, an energy transfer occurs from the TPPS@FNPs–MSC system to the free oxygen, resulting in the generation of ROS that cause cell death. In principle this system can overcome all the issues still associated with selective NPs biodistribution and uptake by exploiting the tumor homing ability of MSC and their low immunogenicity [34]. Moreover, the high photosensitizer payload on the NPs ensures the transport of a massive pro-drug quantity to the tumor site. The internalization and cytotoxicity of TPPS@FNPs was determined by FACS analysis and Methylene blue/WST-1 assays respectively, whereas the amount of released ROS was established by monitoring H2DCFDA activation [35]. Then, through laser confocal microscopy and time lapse imaging of TPPS@FNPs–MSC co-cultured with OS cells *in vitro*, we tested the ability of this system to induce controlled cell death when stimulated with laser light. In order to roughly mimic *in vivo* conditions, we carried out the co-culture experiment with a 1:5 (MSC *vs* OS cells) ratio, demonstrating the high effectiveness of our system even in this environment.

## 2. Materials and methods

### 2.1. Materials

2-(Dimethylamino)ethyl methacrylate (DMAEMA), 1-bromooctane, 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AIBA), methyl methacrylate (MMA, 99.0%) (distilled before use), TPPS, 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA), human osteosarcoma cell line U2OS-RFP-TUBA1B (we will refer to these cells as U2OSTubRFP cells), mouse anti-βTubulin [T4026], anti-mouse IgG Cy3 [C2181], and TRITC-Phalloidin [P1951] were purchased from Sigma-Aldrich. α-Modified minimum Essential Medium (α-MEM), and fetal bovine serum (FBS) were purchased from Lonza (Verviers, Belgium). McCoy's 5A, GlutaMAX™ 1%, TripLe™ Select, Hoechst 33342 [H1399], and LIVE/DEAD viability kit (L3224) were purchased from Invitrogen-Life Technologies (Paisley, UK). Mouse anti-Lamp1/CD107a [H4A3] was purchased from BD Pharmingen™ (Frankiln Lakes, NJ, USA). U2OS (HTB-96) cells were purchased from ATCC (Teddington, UK). Fluoromount-G was purchased from Southern Biotech (Birmingham, AL, USA). WST-1 was purchased from Roche (Mannheim, Germany).

### 2.2. Fluo nanoparticles (FNPs) synthesis and characterization

#### 2.2.1. Synthesis

FNPs were obtained using the following procedure. Briefly, an aqueous solution (50 ml) of 2-(dimethyloctyl)-ammonium ethylmethacrylate bromide (0.52 g, 1.5 mmol) was introduced, at room temperature, into a 250 ml three-neck reactor equipped with a condenser, a mechanical stirrer, a thermometer and nitrogen inlet. The mixture was purged with nitrogen, at a stirring rate of 300 rpm, and heated to 80 °C. Subsequently, 2-aminoethyl methacrylate hydrochloride (AEMA, 0.25 g, 1.48 mmol) was added to the previously obtained solution until complete solubilization was obtained.

The fluorescent co-monomer allyl 2-(3-allyloxy-6-oxo-6H-xanthen-9-yl) benzoate [36] (0.003 g, 0.007 mmol) was then added to methylmethacrylate (0.93 ml, 9.35 mmol) and the obtained mixture was added to the water solution [37,38]. After additional 10 min equilibration time, 15 mg (0.05 mmol) of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AIBA), dissolved in 0.5 ml of mQ water was added to the mixture, which was then allowed to react for 4 h. The reaction product was purified by dialysis (against water) to remove residual monomer and stabilizer.

#### 2.2.2. Characterization

The FNPs UV spectrum and the TPPS release amount were determined by spectrophotometric measurement with a Lamda 20 Perkin Elmer spectrophotometer (Waltham, MA, USA). Supernatants were filtered with an Amicon Ultra 0.5 ml 100 K Millipore Filter (Billerica, MA, USA) by using a EBA 12 HETTIC centrifuge equipped with a F-205 FALC tubes rotator. The hydrodynamic diameter of the nanospheres was determined by photon correlation spectroscopy (PCS) at 25 °C using a Zetasizer 3000 HS system (Malvern, UK) equipped with a 10 mV He – Ne laser. Data was analyzed based on the viscosity and refractive index of pure water at 25 °C. The instrument was calibrated with standard polystyrene latex particles of 200 nm in diameter. To achieve a constant ionic background, the sample was diluted in 10 mM NaCl to a concentration of 20 mg/ml. As far as the electrophoretic mobility is concerned, ζ-potential was measured at 25 °C by means of the same Zetasizer 3000 HS system. The instrument calibration was checked using standard polystyrene latexes,



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