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Selectivity and sensitivity of molybdenum oxide-polycaprolactone nanofiber composites on skin cancer: Preliminary *in-vitro* and *in-vivo* implications

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ABSTRACT

Cancer nanomedicine has emerged as a revolution in the last decade opening up promising strides for the cancer treatment. The major challenge in these therapeutic approaches resides in the failure of clinical trials owing to the immunological cancer microenvironment. Therefore, the success of next generation nanomedicine depends on tunable physicochemical nanomaterial design and corresponding clinical trials by integrating targeted delivery with mitigated toxicity. The present study deals with the fabrication of nanofibrous scaffold impregnated with molybdenum nanoparticles for targeted skin cancer therapeutics. Molybdenum oxide, a transitional metal oxide is gaining rapid importance due to its vital role in cellular and molecular metabolism. Polycaprolactone nanofibers were chosen as a matrix to localize the nanoparticles topically facilitating selective apoptosis of the tumor cells over the normal cells with mitigated side effects. The scaffold was designed to tailor the physicochemical, mechanical and biological suitability for skin cancer (melanoma and non melanoma). The designed scaffold was found to reduce more than 50% cell viability of the cancer cells selectively through apoptosis as confirmed using AO/PI staining and the probable mechanism could be attributed to the induction of mitochondria dependent apoptosis as observed by JC1 dye staining. *In-vivo* trials in zebra fish were found to reduce cancer progression by more than 30% in 14 days. The fabricated molybdenum trioxide nano constructs not only serve as tunable targeted systems but also open venues capable of ferrying chemotherapeutic drugs sparing normal cells alleviating the trauma due to side effects.

1. Introduction

The rapid globalisation has caused a major environmental degradation that has destroyed the protective ozone covering of the atmosphere. The penetration of the UV rays has increased the incidents of skin cancer. Skin is the largest organ of the human body which serves as a physical barrier against various infections and environment. Skin cancer is a type of cancer that has the least mortality but accounts for almost 40% of the world cancer population. The most common types of skin cancer are the basal cell carcinoma, squamous cell carcinoma and melanoma. The basal cell carcinoma and the squamous cell carcinoma are benign tumors that exhibit a very low rate of metastasis whereas, the melanoma (cancer caused in melanocytes, which are the pigment containing cells) have high tendency to metastasize to other organs of the body. Melanoma causes majorly a high mortality rate than the non-melanoma cancers. The current treatment modalities of skin cancer (melanoma and non-melanoma) include radiation, chemotherapy and surgical removal of the tumour.

Nanotechnology has revolutionized therapeutic strategies through optimization of the biodistribution, bioavailability, stability, pharmacokinetics and targeted delivery to the required site thereby reducing toxicity as well as minimizing side effects. This led to the growing interest in applying this technology to cancer, a deadly disease with a great complexity and heterogeneity. Several therapeutics based on nanoparticles have been attempted for diagnostics, imaging and even treatment either independently or in combination with existing chemotherapy, hyperthermia, gene or interference therapy and immunotherapy. However, the major challenge faced with the nanoparticle therapeutics is to overcome the enhanced permeability and retention effect as well as targeted delivery for maximizing the efficiency. Identifying a suitable carrier system for the nanoparticles would mitigate several challenges. This paper essentially deals with envisaging the role of Molybdenum oxide nanoparticles in cancer therapeutics as well as fabricating a scaffold with suitable physicochemical properties to strategically target the cancer cells.

Molybdenum an essential trace element, has been used in the treatment of oesophageal cancer in early days [1]. The absence of molybdenum in the diet has also been one of the major causes for the

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development of cancer in patients [2]. Molybdenum trioxide nanoparticles are currently used for a variety of applications such as fuel cells [3], antimicrobial paints [4] etc. They are said to be one of the least toxic metal nanoparticle upon application. Since they are less toxic and are one of the essential trace elements that are required for the body metabolism they are used in wide range of therapeutic applications. MoO_3 nanoparticles are powerful anti-microbial agents that have been used to develop antibacterial paints [4]. Their extensive antimicrobial property is through creating membrane stress for microorganisms [5]. Molybdenum serves as a cofactor for enzymes such as xanthine dehydrogenase, aldehyde oxidase, sulphite oxidase, nitrate reductase [6]. Molybdenum sulphite nanoparticles have also been proved to destabilize the amyloid fibers and inhibit Alzheimer's Disease [7]. Surface Plasmon Resonance is an important phenomenon that can be exploited for photo thermal ablation of cancer, Molybdenum oxide nanoparticles are said to posses strong Localized Surface Plasmon Resonance (LSPR) absorption in the near infrared region [8], which would make them as cheap alternative to replace the use of gold nanoparticles in photo thermal therapy. MoO_3 nanoparticles have also shown to have toxicity against MCF-7, breast cancer cell lines [9].

Amongst the nanotechnological carrier systems, electrospun nanofibers are evolving to be a lucrative drug delivery system, as they have an enormous loading capability and targeted delivery of the drug [10,11]. A huge number of anticancer drugs such as paclitaxel [12], doxorubicin [13], cisplatin [14] etc., have been delivered through nanofibers in order to facilitate better loading and delivery of the drug. Paclitaxel loaded nanofiber mats have proved to be greatly efficient in rat models against glioma cells [15]. Polycaprolactone (PCL) is a FDA approved polymer which is widely used in drug delivery applications. They are cheap, easily available, highly biocompatible and biodegradable. They also serve as an excellent dressing material for topical application of drugs to the infected area and have been tested in numerous animal models [16] which authenticate their biocompatibility.

This paper essentially deals with designing a strategy to localize MoO_3 nanoparticles through a nanofibrous scaffold potentially overcoming the challenges with reference to targeting and bioavailability in addition to mitigate dosages and side effects.

2. Experimental

2.1. Materials and Methods

All solvents used in this study were of analytical grade and were not further purified. Normal cell lines, HaCaT (Keratinocytes), Swiss 3T6 (Fibroblasts) and cancer cells lines A431 (Epidermoid carcinoma), HT1080 (Fibrosarcoma) and G361 (Melanoma) were purchased from National Centre for Cell Science (NCCS), Pune, India. Cell culture medium and fetal bovine serum (FBS) were purchased from Life Technologies, USA. Zebra fishes (*Danio rerio*) were procured from Pentagrit, Chennai, India. All the other chemicals used for the culture studies were procured from Sigma-Aldrich, India and were culture tested.

2.2. Preparation of FITC tagged nanoparticles

The MoO_3 nanoparticles were surface functionalized with the protocol followed by Babitha et al. [17] The surface modification was carried out using a silane coupling agent, (3-aminopropyl)trimethoxysilane (APTMS), The MoO_3 nanoparticles were dispersed in 50 ml dimethyl sulfoxide (DMSO) and ultrasonicated for 1 h. To this sonicated mixture silane coupling agent (APTMS) was added and refluxed for 3 h. The nanoparticles particles were separated by centrifugation at 10,000 rpm for 10 min. The surface modified particles were dried in an oven at 60 °C overnight and cooled in a vacuum chamber for 1 h. The surface modified MoO_3 (dispersed in deionized water) and FITC (Fluorescein Isothiocyanate; Sigma Aldrich) (10 mg/ml in DMSO) was mixed in a ratio of 1:10 and allowed to react by stirring for 4–5 h at room temperature in dark. The solution was then centrifuged at 8000 rpm for 15 min and the pellet was washed several times with deionized water to remove the unbound FITC. The prepared FITC labeled MoO_3 nanoparticles were air dried and stored in amber bottles.

2.3. Preparation of nanoparticles incorporated nanofibers

Polycaprolactone (PCL) nanofibers were fabricated according to Satish et al. [16] 15% (w/v) of PCL was dissolved in Dichloromethane: Ethanol (1:1) and stirred for 4 h to make a homogenous polymer solution. The MoO_3 nanoparticles or FITC conjugated MoO_3 nanoparticles were added at a concentration of 1 mg/ml to the PCL solution and allowed to mix thoroughly by stirring for 4 h in dark. The mixture was then loaded into a 2 ml syringe equipped with a blunt 18 gauge stainless steel needle and was fitted into the electrospinning apparatus (Physics equipment & Co). Molybdenum trioxide containing PCL (Mol-PCL) nanofibers were then obtained by controlling the spinning parameters. The flow rate was maintained at 0.5 ml/hr with an applied voltage of 6–9 kV and the collector distance was set at 12–15 cm. The control PCL nanofibers were also developed following the same procedure, at an voltage of 14–16 kV. The entire process was carried out in a closed Plexiglass chamber at 24 ± 1 °C with a relative humidity of 68 ± 3 °C.

2.4. Characterization of nanoparticles and nanofibers

The surface morphology of the PCL nanofibers and Mol-PCL nanofibers were analyzed by scanning electron microscopy(SEM) (Hitachi, Japan) using an accelerated voltage of 10 kV. The samples were gold sputter coated under argon atmosphere to make them electrically conductive preceding SEM analysis. The average diameter of the fibers was formulated using inbuilt software system coupled with SEM. The Mol-PCL nanofibers were also viewed using transmission electron microscope (TEM) (Technai 10, Philips) accelerated at 100 kV. The samples for TEM observation were prepared by collecting the nanofibers on carbon coated Cu grids. The incorporation of the nanoparticles into the nanofibers was confirmed using Raman Spectroscopic Analysis. Raman measurements were carried out in a black scattering geometry using single monochromator based Raman spectrometer (Horiba Jordan Yvou, Japan) connected to an air-cooled device charge coupled device (CCD) detector. The samples were excited using 514.5 nm line of Ar + ion laser, operated at ~3 mW on the sample. The Raman mappings were recorded over 35 μ × 35 μ area at a step size of 1 μ.The interaction between the nanoparticle and the PCL scaffold was investigated using Fourier Transform Infrared (FTIR) spectroscopy. For performing FTIR spectrometric analysis, the nanofibrous scaffold samples were shredded into tiny particles, ground with KBr powder and pressed into small pellets. These pellet samples were loaded into a FTIR spectrometer (Perkin Elmer, USA) and the spectral measurements were recorded between the IR range 800–4000 cm^{-1} with a resolution of 2 cm^{-1}. The encapsulation of MoO_3 nanoparticles in the fibers was also ascertained using fluorescence microscopic analysis. The PCL nanofibers fabricated with FITC conjugated MoO_3 nanoparticles were collected on glass coverslips and observed under a fluorescence microscope (Leica microsystems, Germany).

2.5. Cell culture

HaCaT, Swiss 3T6 and A431 cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM: High glucose). HT1080 and G361 cell lines were cultured in (DMEM: Low glucose) and McCoy's medium respectively. The media were supplemented with 10% FBS and antibiotics streptomycin (100 μg/ml), penicillin (100units/ml), gentamycin (30 μg/ml) and amphotericin B (2.5 μg/ml). The cells were maintained in 25 cm^2 culture flasks at 37 °C in a humidified incubator (Binder, Germany) supplied with 5% CO_2 and 95% air.