Biomaterials 120 (2017) 115–125

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Toxicity evaluation of magnetic hyperthermia induced by remote actuation of magnetic nanoparticles in 3D micrometastasic tumor tissue analogs for triple negative breast cancer

Nathanael A. Stocke [a], Pallavi Sethi [b], Amar Jyoti [b], Ryan Chan [b], Susanne M. Arnold [c], J. Zach. Hilt [a], Meenakshi Upreti [b, *]

[a] Department of Chemical and Materials Engineering, University of Kentucky, Lexington, KY 40506, USA
[b] Department of Pharmaceutical Science, University of Kentucky, Lexington, KY 40506, USA
[c] College of Medicine, Department of Internal Medicine, University of Kentucky, Lexington, KY 40506, USA

ARTICLE INFO

Article history:
Received 28 June 2016
Received in revised form
10 November 2016
Accepted 16 December 2016
Available online 23 December 2016

Keywords:
Magnetic nanoparticles (MNP)
Tumor tissue analogs (TTA)
Alternating magnetic field (AMF)
Triple negative breast cancer (TNBC)
Metastasis and Transmission electron microscopy (TEM)

ABSTRACT

Magnetic hyperthermia as a treatment modality is acquiring increased recognition for loco-regional therapy of primary and metastatic lung malignancies by pulmonary delivery of magnetic nanoparticles **(MNP)**. The unique characteristic of magnetic nanoparticles to induce localized hyperthermia in the presence of an alternating magnetic field **(AMF)** allows for preferential killing of cells at the tumor site. In this study we demonstrate the effect of hyperthermia induced by low and high dose of MNP under the influence of an AMF using 3D tumor tissue analogs **(TTA)** representing the micrometastatic, perfusion independent stage of triple negative breast cancer **(TNBC)** that infiltrates the lungs. While application of inhalable magnetic nanocomposite microparticles or magnetic nanocomposites **(MnMs)** to the micrometastatic TNBC model comprised of TTA generated from cancer and stromal cells, showed no measureable adverse effects in the absence of AMF-exposure, magnetic hyperthermia generated under the influence of an AMF in TTA incubated in a high concentration of MNP (1 mg/mL) caused significant increase in cellular death/damage with mechanical disintegration and release of cell debris indicating the potential of these inhalable composites as a promising approach for thermal treatment of diseased lungs. The novelty and significance of this study lies in the development of methods to evaluate *in vitro* the application of inhalable composites containing MNPs in thermal therapy using a physiologically relevant metastatic TNBC model representative of the microenvironmental characteristics in secondary lung malignancies.

1. Introduction

Breast cancer is the second leading cause of death in women, surpassed only by lung cancer, where one in every eight women that is diagnosed develops the invasive form of this dreadful malignancy [1]. Triple negative breast cancer **(TNBC)** is an aggressive subtype of breast cancer wherein the tumor cells do not express the routinely targeted receptors for estrogen **(ER)**, progesterone **(PR)**, and human epidermal growth factor 2 **(HER2)** [2] and therefore has limited treatment options. Accounting for approximately 20% of all breast cancers, TNBC has a disproportionately high rate of metastasis [3]. Additionally, patients with distant metastatic disease have a significantly shorter survival time relative to the other breast cancer subtypes [4]. The most common ectopic sites for TNBC to metastasize are the lungs, the liver, bones and the brain [5] with a higher propensity of metastasis to the lungs [3,6]. Currently there is an unmet and compelling need for novel approaches to treat patients with metastatic TNBC.

Pulmonary delivery presents a promising approach for local treatment of both primary and secondary lung cancers by facilitating increased drug concentration at the malignant site and reduced systemic side effects [7–9]. Clinical trials with stage IV lung cancer patients have shown a statistically significant increase in median survival time when carboplatin was administered via inhalation combined with injection as opposed to injection only

* Corresponding author. Department of Pharmaceutical Sciences, University of Kentucky 789 S. Limestone St., Lexington, KY 40536, USA.
E-mail address: m.upreti@uky.edu (M. Upreti).

http://dx.doi.org/10.1016/j.biomaterials.2016.12.019
0142-9612/

[10]. These results suggest that pulmonary delivery has the potential to complement traditional approaches for patients with metastatic disease in the lungs. Current initiatives employing the delivery of magnetic nanoparticles to the lung is gathering pace for developing novel therapeutic approaches that involve thermal sensitization and/or ablation at the malignant/metastatic site [11,12]. Treatment of recurrent TNBC that metastasizes primarily to the lungs aims to improve the quality of life and overall survival. Conventional treatment options are however, not defined and systemically administered, therefore result in increased overall toxicity. Remote actuation of inhalable magnetic nanoparticles to induce magnetic hyperthermia at the metastatic lesions of TNBC in the lungs is an attractive concept for management of the malignancy in advanced stages with reduced systemic toxicity.

The biocompatibility and magnetic properties of iron oxide magnetic nanoparticles (MNP) categorizes them.

in a unique class of materials that have found increasing use in a variety of commercialized biomedical applications [13,14]. The ability of magnetic nanoparticles **(MNP)** and nanocomposites **(MnM)** to be actuated remotely under the influence of an alternating magnetic field **(AMF)** has sparked interest in their use for thermal sensitization of cancer cells/tumors to maximize therapeutic outcomes [15–17]. In the presence of an AMF, iron oxide magnetic nanoparticles generate heat through Brownian and Néel relaxation [18]. The regional hyperthermia so generated is utilized to actuate the onset of therapy or as a form of therapy itself [19–23]. For cancer patients, researchers have been interested in the potential advantages of thermal therapy both in isolation and in combination with anti-cancer agents but the translation to clinical settings of such therapies has been limited [24–28], owing in part due to the inability of existing tumor models to accurately simulate the biological processes and therapeutic response that occur in patients with primary and secondary lung cancer.

3D co-culture of different cell types that exist in the tumor and its microenvironment provide a more physiologically relevant representation of *in vivo* tissue morphology and function. Inclusion of such *in vitro* tumor models embracing characteristics of the tumor microenvironment for understanding and advancing the parameters for thermoablative therapy will provide more effective treatment options. To this end, our laboratory has developed a 3D co-culture system that incorporates tumor cells, endothelial cells and fibroblasts as color-coded murine tumor tissue analogs (TTA) to better recapitulate the micrometastatic perfusion independent TNBC tumor biology *in vitro* [29]. The 3D spheroidal co-culture of neoplastic tumor cells with the non-neoplastic stromal components facilitates the formation of a natural extracellular matrix **(ECM)** allowing for a more accurate recreation of the tumor microenvironment that is often overlooked in *in vitro* assays.

In the current study, we have devised cost-effective and reproducible *in vitro* methods using color coded TNBC micrometastatic TTA developed in the laboratory to investigate the potential of inhalable formulations of MNP for secondary lung cancer patients with metastatic TNBC. The murine TNBC micrometastatic TTA are comprised of mCherry fluorescent protein expressing 4T1 tumor cells, the GFP expressing C166 endothelial cells and murine embryonic fibroblasts (MEF) that are grown in in hanging drops of medium without the use of any artificial matrices to a size of ~600 μm [30]. The murine 4T1 breast carcinoma cell line does not express the ER, PR and HER2 receptors and is therefore representative of the TNBC phenotype [31]. The spray dried MNP are formulated into inhalable magnetic nanocomposites or magnetic composites (MnM) [32] before incubating with the TTA. Remote controlled thermal therapy on TTA is accomplished using a custom AMF and the treated tissues are analyzed both quantitatively and qualitatively.

2. Material and methods

2.1. Cell lines and culture

4T1-mCherry is a red fluorescent protein-expressing murine metastatic mammary carcinoma cell line that closely mimics the triple negative subtype of human breast cancer [33]; was a kind gift from Dr. D. D. Schlaepfer (University of California, San Diego, CA). 2H11, murine tumor endothelial cell line [34], and C166-GFP, a murine green fluorescent protein-expressing endothelial cell line, were purchased from ATCC (Manassas, VA). Murine embryonic fibroblasts (MEF) were a kind gift from Dr. V. Rangnekar (University of Kentucky, Lexington, KY). The cell lines were routinely cultured in high glucose DMEM containing 10% (v/v) fetal bovine serum and 100 IU/mL penicillin, 100 IU/mL streptomycin at 37 °C, 5% CO_2, and 95%.

2.2. Chemicals and reagents

Iron(II) chloride tetrahydrate ($FeCl2{\cdot}4H2O$), iron(III) chloride hexahydrate ($FeCl3{\cdot}6H2O$), and d-mannitol were purchased from Sigma–Aldrich (St. Louis, MO). Ammonium hydroxide (NH4OH) was obtained from EMD chemicals (Gibbstown, NJ). Chloroform, anhydrous methanol, and high performance liquid chromatography-grade methanol were purchased from Fisher Scientific (Pittsburgh, PA). Ultrahigh-purity nitrogen gas was purchased from Scott-Gross (Lexington, KY).

2.3. Formulation and characterization of MnM and MNP

Detailed discussion on formulation and characterization of the MnM and MNP is as described in our previous publication [12]. Briefly, magnetic nanocomposite microparticles or magnetic composites (MnMs) were formulated by spray drying of iron oxide MNPs and D-mannitol. The physicochemical properties of these MnMs were evaluated and the *in vitro* aerosol dispersion performance of the dry powders was measured by the Next Generation Impactor®. For all powders, the mass median aerosol diameter (MMAD) was <5 μm and deposition patterns revealed that MnMs could deposit throughout the lungs. Heating studies with a custom AMF showed that MNPs retain excellent thermal properties after spray drying into composite dry powders, with specific absorption ratios (SAR) > 200 W/g.

2.4. Formation of TNBC micrometastasic TTA

4T1-mcherry tumor cells, C166-GFP endothelial cells and murine embryonic fibroblasts (MEF) were used to generated 3D BCM tumor tissue analogs in “hanging drops” of media (Dulbecco modified Eagle medium with 10% fetal bovine serum and antibiotic mix) as previously described [29,30]. Briefly, the single a cell suspension of 4T1-mcherry cells, C166-GFP cells and MEF cells in equal proportion (3000 cells/20 μL) was dispensed on the inside of the lid of each well of a 48-well cell culture plate (Greiner Cellstar, Kaysville, UT). The growth of tumor tissue analogs was monitored over time until day 10 in a hanging drop of medium, following which they were subject to MNP (Magnetic nanoparticle) or AMF (Alternating magnetic field) treatment. The tumor tissue analogs were transferred to optically clear repellent plates (Greiner Cellstar, Kaysville, UT) or placed on Millicell culture inserts (Millipore, Billerica, MA) for MNP and AMF treatment respectively. Subsequent imaging of TTAand analysis of the treatment response was performed in the repellent plates (Griener, Cellstar, Kaysville, UT).