Materials Science and Engineering C 68 (2016) 474–481

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Zeolite scaffolds for cultures of human breast cancer cells. Part II: Effect of pure and hybrid zeolite membranes on neoplastic and metastatic activity control

Palmira Tavolaro [a,*,1], Guglielmo Martino [b], Sebastiano Andò [a], Adalgisa Tavolaro [c,1]

[a] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Cubo 4/c, 87036, Rende, Italy
[b] Department Di.B.E.S.T. (Biologia, Ecologia, Scienze della Terra), Unit of Physiology, University of Calabria, Cubo 4/c, 87036 Rende, Italy
[c] Research Institute on Membrane Technology, Unit of Zeolite Membranes, ITM-CNR, University of Calabria, Cubo 17/c, 87036 Rende, Italy

ARTICLE INFO

Article history:
Received 2 April 2016
Received in revised form 16 May 2016
Accepted 5 June 2016
Available online 08 June 2016

Keywords:
Zeolite scaffolds
MCF-7 breast cancer cells
MDA-MB-231 breast cancer cells
Doxorubicin
Bergapten
Matrix metalloproteinases

ABSTRACT

This work is focused on the response of two invasive phenotypes of human breast cancer cells, MCF-7 and MDA-MB-231, grown on synthesized zeolite scaffolds in order to study the influence of those biomaterials in controlled conditions with and without *anti*-tumoral drug treatments.
Our research was directed to the use of doxorubicin (DOX) and bergapten (5-MOP). The former is broadly considered the most active single agent available for the treatment of breast cancer, the second is a natural psoralen with an apoptotic effect.
The results indicate that both drugs inhibit the cell viability of all cell lines grown on all zeolite scaffolds and that all Pure Zeolite Membranes are more responsive with respect to all Mixed Matrix Membranes. Moreover, the results after treatment with DOX at a concentration of 7.4 μM for 24 h, show that the expression of the matrix metalloproteinases (MMP-2 and MMP-9) is greatly reduced in both cell lines, especially in those adherent on Pure Zeolite Scaffolds.

1. Introduction

Breast cancer is the most common cancer and the second leading cause of cancer mortality in women, with most of the deaths recorded due to metastases, rather than for the localized tumor [1–3]. It is one of the most highly metastatic tumors. Approximately 1 in 8 breast cancer patients will develop invasive breast cancer [4–8].

The distinctive features of tumorigenesis are the most important: the uncontrolled cell division, the alterations of communications between cell and cell, the modifications of communications between cells and extracellular matrix tissue (ECM), a significant increase of cell migration, mutations in several oncogenes and tumor suppressor genes and resistance to apoptosis [9–12].

In addition, breast cancer cell proliferation and invasion involve the breaching of tissue barriers and the subsequent infiltration of cells into the surrounding tissue [13]. Research into the capability for invasion and metastasis has accelerated dramatically over the past decade as powerful new research tools and refined experimental models have become required.

Our research focused on response of cancer cells grown on synthesized zeolite surfaces in order to study the influence of these scaffolds in controlled conditions. The study was directed to the use of zeolites as new biomaterials because they are microporous materials that can be synthesized in different structures [14–18]. We have selected MCF-7 and MDA-MB-231 human breast cancer cell lines as model tumor cell lines because they are adherent cell lines having different characteristics. In particular, MCF-7 are estrogen receptor alpha ($ER\alpha^+$)-positive tumors, HER2 negative, poorly invasive, and low metastasizing human breast carcinoma cells while MDA-MB-231 are triple negative ($ER\alpha^-$, PR^-, $HER2^-$) highly invasive, and metastatic human breast carcinoma cells [19–21]. Estrogen receptors are primarily involved on breast cancer cell invasiveness. The invasive character of MCF-7 and MDA-MB-231 has been studied in vitro using Transwell mobility tests and Matrigel invasion tests [22]. Moreover, MDA-MB-231 cells are a more aggressive and movable cellular phenotype respect to other cells.

We evaluated the suitability of various Pure Zeolite Membranes and Mixed Matrix Membranes, with different percentages of zeolite crystals (5%, 35%, 70%, 80%) and polylactic acid polymer, as scaffolds to be used as support for the adhesion and growth of cancer cells and simultaneously as carrier membranes for selective adsorption and the delivery of two antineoplastic drugs in order to assess the various survival cell responses. These membranes have very different physics-chemical characteristics from each other because while the former are solely

* Corresponding author.
E-mail address: p.tavolaro@unical.it (P. Tavolaro).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.msec.2016.06.013
0928-4931/

made of zeolite crystals and interact with molecules exclusively through the siloxane groups, the latter are formed by a polymeric matrix within which the crystals are dispersed then they have end groups, which depend on the polymeric materials. PLA is becoming to be the most important bio-based polyester due to its favorable properties widely investigated as support material for cell cultures because hydroxyl groups on its surface interact with polar groups on the cell surface.

In particular, the adsorption on Pure Zeolite Membranes and Mixed Matrix Membranes was tested both in buffered solution and in cellular microenvironment. This later experiment was performed both in the presence and in absence of cancer cells. Our research was directed to the use of doxorubicin (DOX) and bergapten (5-MOP). The former is broadly considered the most active single agent available for the treatment of breast cancer, the second is a natural psoralen with an apoptotic effect [23,24].

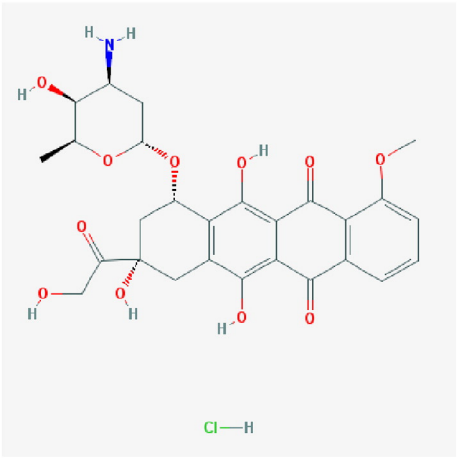
DOX is among the most effective antitumor drugs used for the management of breast cancer (Scheme 1). It is an anthracycline drug first extracted from *Streptomyces peucetius* var. *caesius* in the 1970's and routinely used in the treatment of several cancers including breast, lung, gastric, ovarian, thyroid, multiple myeloma, non-Hodgkin's and Hodgkin's lymphoma and sarcoma [23,25–29].

5-MOP is a linear furanocoumarins, belonging to psoralens (Scheme 2). It is a substance that is found mainly in the essential oil of bergamot (*Citrus bergamia* L.) but also in many other citrus essential oils and as well as in the extract of fig leafs (*Ficus carica* L.), one of the most popular plants in China.

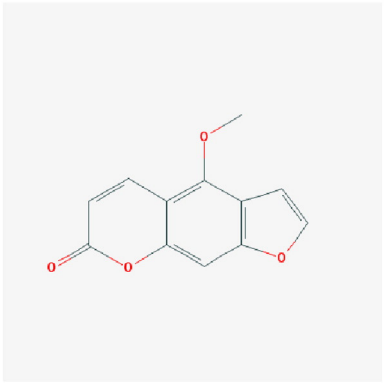
Psoralenes are very interesting due to their photobiological and phototherapeutic activities. The feature activity of photo-induction of the psoralenic molecules is widely used for the treatment of pathologies of the epidermis in combination with UVA light [30,31]. In fact, because of their planar structure, they easily intercalate into DNA and this allows a cycloaddition reaction promoted by the UV radiation between the pyrimidine bases from the DNA, and their furan ring [32,33]. The reaction with psoralens inhibits DNA duplication and reduces the rate of cell division. Furthermore, the bergapten by itself is able to stop cell growth, especially of breast cancer cells, and trigger apoptotic responses. In fact, psoralen induces an “up-regulation” of some proteins involved in the proliferative arrest, effectively counteracting the signals of cell survival [24,34].

Another aspect examined in this study concerns the enzymes, which “mark” the power metastatic and invasive own tumor cells and the influence of the novel supports on the proteinase activities on drug adsorption in adhered cells.

It is known, in fact, that the process of metastasis formation is a complex multistep process involving detachment of tumor cells from the primary tumor, invasion through basal membrane into the surrounding



Scheme 1. Chemical structure of DOX.



Scheme 2. Chemical structure of 5-MOP.

stroma, intravasation into the circulatory system, extravasation at a distant site and outgrowth of a secondary tumor [35]. Many studies showed that metastasis formation is partly associated with the expression and activity of a specialized group of extracellular-matrix (ECM)-degrading proteinases, the matrix metalloproteinases (MMPs). Among the members of this MMP family shown to play a role in tumor-cell invasion and metastasis are MMP-2 (gelatinase A/72-kDa type-IV collagenase) and MMP-9 (gelatinase B/92-kDa type-IV collagenase) [36,37]. In various tumor systems, increased expression of MMP-2 has been associated with metastatic potential [38]. In human breast cancer, elevated MMP-2 protein have been detected and compared to benign breast tissue [39]. Indeed, a correlation between a potential for surface binding and activation of MMP-2 and the metastatic capacity of breast-cancer cells have been observed both in vitro and in nude mice [40,41].

Those scientific discoveries and the SEM microphotography observation have led us to evaluate the behavior of gelatinase with and without antineoplastic drug treatment.

2. Materials and methods

2.1. Reagents

The reagents used to prepare Pure Zeolite Membranes and Mixed Matrix Membranes were described in detail in the paper Part I.

Phosphate buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12), L-glutamine, penicillin/streptomycin was purchased from Eurobio (France), fetal bovine serum (FBS) was purchased from Life Technologies, (Life Technologies, Paisley, UK), tripsine, sodium orthovanadate, phenylmethylsulfonyl fluoride (PMSF), hydrocortisone, dimethyl sulfoxide (DMSO), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltatrazolium bromide, ECL system, acrilamide bis 29:1, Coomassie Brilliant Blue G, doxorubicin hydrochloride (DOX), bergapten (5-MOP), acetic acid, glutaraldehyde solution and osmium tetroxide were obtained from Sigma Aldrich (USA).

DOX solutions were prepared both in water and in DMSO while 5-MOP solutions were prepared in ethanol absolute.

2.2. Breast cancer cell lines

MCF-7 and MDA-MB-231 human breast carcinoma cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and authenticated, stored according to the supplier's instructions, and used within a month after frozen aliquots resuscitations. They were maintained in Dulbecco's Modified Eagle's Medium (DMEM and DMEM/F12, Eurobio, France) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Paisley, UK), antibiotics (100 IU/mL penicillin, 100 mg/mL streptomycin) and 2 mM glutamine (Eurobio,