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Fabrication and evaluation of novel zeolite membranes to control the neoplastic activity and anti-tumoral drug treatments in human breast cancer cells. Part 1: Synthesis and characterization of Pure Zeolite Membranes and Mixed Matrix Membranes for adhesion and growth of cancer cells

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ABSTRACT

Novel pure and hybrid zeolite membranes were prepared with appropriate different physicochemical characteristics such as frameworks, hydrophilicity, crystal size, chemical composition, acid-base properties (Point of Zero Charge, PZC) and surface morphology and used in inorganic cell/scaffold constructs. Because the control of cell interactions, as the adhesion, proliferation, remodelling and mobility, is important for differentiation and progression of tumors, this work focused on response of cancer cells adhered and grown on synthesized zeolite surfaces in order to study the influence of these scaffolds in controlled conditions. We have selected the MCF-7 and MDA-MB-231 human breast cancer cell line as model tumor cell lines. This study showed that all the zeolite membranes synthesized are excellent scaffolds because they are very selective materials to support the adhesion and growth of neoplastic cells. All zeolite scaffolds were characterized by FESEM, FTIR ATR, XRD, AFM, PZC and contact angle analyses. Cell adhesion, viability and morphology were measured by count, MTT assay and FESEM microphotography analysis, at various incubation times.

1. Introduction

Biomaterials science involves the design and fabrication of nanomaterials for studying, directing, or mimicking biology. The ability of cells to recognize and interact with the substrate is the first essential step, without which processes such as adhesion, proliferation, migration, cell differentiation and carrying, which presuppose continuous exchanges of ions and molecules among cell and support, would not be possible [1,2]. Therefore, the possibility of modifying and controlling surface properties at the micro/nano level constitutes one of the major breakthroughs, because it opens a whole new range of strategies seeking the desired interaction with the biological environment. In order to prepare a new generation of biomaterials with enhanced properties a different approach needs to be researched, based on a more fundamental understanding of the way in which the structure of a biomaterial controls its biological activity. The chemical properties influence the surface properties of a material and, consequently, cell behavior [3]. When cells are exposed to a suitable scaffold, a layer of proteins is adsorbed on the scaffold surface within a few milliseconds. Thus cells "see" the layer of adsorbed proteins rather than the actual abiotic surface. The chemistry of the surface of a scaffold can be developed in order to control the adsorption of proteins, which in turn controls cell adhesion. According to the hoped for result, the chemical characteristics of the surface of a material can be modified to modulate the interactions of cells adherent to the substrate, with consequent influence on morphology, migration, differentiation, proliferation and cell apoptosis. The effect on cell behavior starts at the point of interaction. Furthermore, the conformation of the surface chemistry also affects the way proteins are immobilized and the adsorption of these on the surface.

For example, polymeric substrates with greater hydrophobicity promote greater osteogenesis in vivo. The hydrophobicity of the biomaterial in fact, most likely, is a design criterion important for polymer scaffolds that should promote the healing of bone defects [4].

However, it is well-known that the driving force for the deployment of proteins on a surface charge is the ionic and not the hydrophobic interaction, because the surface charge of a biomaterial affects adsorption and deployment of proteins on its surface and we have just reported the

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immobilization of proteins like Bovine Serum Albumin and cytochrome *c* on zeolite crystals and membranes [5,6].

Starting from this assumption our research was directed to the identification of an innovative and advanced biomaterial that allows the study in auditable mechanisms of behavior of two different human cancer cell lines (with different growth rates, response to hormones and growth factors, invasiveness) which recognize, adhere and grow on synthetic biocompatible scaffolds.

Biomaterials to use as scaffolds can be divided into three main types based on the response that generated in the host tissue: an inert material, which does not provoke a response in the tissue (namely first generation biomaterial), a bioactive material, which is integrated into the surrounding tissue (second generation biomaterial), a material designed to stimulate specific responses at the molecular level (third generation biomaterial) [7].

To date, the materials most frequently used in medical applications are: metals, typically inert and used for applications subjected to loads, with resistance to hard enough to withstand the daily activities; ceramics, including silicate and phosphate bioactive glasses and titania and calcium phosphate bioceramics, for biomedical applications such as drug delivery systems, in vitro cultures and transplantations [8–10]; polymers, used for their stability and flexibility, but also for the low friction in the joint surfaces. Among them, polylactid acid (PLA) is a biocompatible synthetic polymer approved by the Food Drug Administration for human clinical applications. In the last years, it is largely used as a part of implantable and surgical devices [11,12].

All these biomaterials have an amorphous structure, which is different depending on the preparation method. It is evident that a porous, crystalline material having an inorganic framework with modulable acidity, hydrophilicity and pore size, constitute a stable, homogeneous, ion- and solvent-available support. A zeolite membrane can represent this novel type of scaffold.

Zeolites are well-known microporous natural or synthetic crystalline materials, traditionally used as catalysts. The framework of every zeolite is constructed from tetrahedral building blocks, TO_4 (where T represents a silicon or aluminum atom), which are bound to each other with oxygen bridge bonds to form pore-containing complex units. These units can be assembled in many ways to yield a large number of different zeolite structure types, classified according to either their framework symmetry, with an identification code of three letters used by the International Zeolite Association (IZA) [13] or, in technological applications, they are distinguished on the basis of their pore size. In order to modify zeolite adsorptive properties for practical applications, two methods have garnered considerable interest: isomorphous substitution and ion-exchange [14]. The isomorphous substitution of Si^{4+} by Al^{3+}, Fe^{3+} or B^{3+} causes a negative excess charge on the framework that is compensated by the presence of cations located in the crystalline channel system [15].

Their use, just owing to the specific physicochemical characteristics of the crystals, is due to the presence of cavities and channels in their structure and this allows guest chemical species within the zeolite crystalline framework to flow and to interact with molecules and organic structures [16–18] as well as with non-modified human cells [19]. The use of the natural zeolites in animal health and nutrition, and also their long-term chemical and biological stability has been reported in the literature [20]. In particular, several papers on biomedical applications of natural clinoptilolite have been recently published. For example, Pavelic reported a novel use of clinoptilolite as a potential adjuvant in anticancer treatments [21,22]. Clinoptilolite does not produce biological damage to humans and animals [23–29]. However, natural zeolites reported in these studies are generally mixtures of different zeolite structures and/or containing various cations. On the contrary, there is no paper regarding systematic investigations in biomedical applications of synthetic, pure and designed zeolite structures having a controlled chemical composition. Pure and hybrid zeolite membranes are extended materials in membrane morphology characterized by zeolitic selectivity.

In the design of a biomaterial, therefore, must take into account the different parameters that can influence the cell-material interactions.

Based on abovementioned considerations and ours expertise in zeolite hydrothermal synthesis, zeolite membranes preparation and physiological and physiopathological cell cultures, we prepared different scaffolds to analyze the adhesion and the behavior of cancer cells.

Our research was directed to the preparation of various Pure Zeolite Membranes and Mixed Matrix Membranes with different percentages of zeolite crystals (5%, 35%, 70%, 80%) and polylactic acid polymer. These membranes have different zeolite framework, hydrophilicity, crystal size, chemical composition, acid-base characteristics and surface morphology.

We have selected two different human breast cancer cell lines MCF-7 and MDA-MB-231 (with different growth rates, response to hormones and growth factors, invasiveness) as model tumor cell lines. In particular, MCF-7 are poorly invasive, and low metastasizing human breast carcinoma cells while MDA-MB-231 are a more aggressive and movable cellular phenotype respect to other cells as adhesion proteins such as E-cadherin, do not express on their surface [30].

2. Materials and methods

2.1. Reagents

Potassium hydroxide, potassium fluoride (ACS reagent: minimum 99%), tetrabutylammonium hydroxide (TBAOH, 40% water solution), tetrapropylammonium bromide (TPABr, purum), tetrapropylammonium hydroxide (TPAOH, 1 M), fumed silica (99.8%), colloidal silica (Ludox, AS-40, 40% suspension in water), aluminum sulfate octadecahydrate (ACS reagent: minimum 98%), aluminum nitrate nonahydrate (ACS reagent: minimum 98%), hexadecyltrimethylammoniumbromide and boric acid (ACS reagent: minimum 99.5) were purchased from Sigma Aldrich (USA). Sodium aluminate (reagent grade: minimum, 98%), and tetraethyl orthosilicate (TEOS, 98%) were obtained from Janssen Chimica (Belgium). Sodium hydroxide and sodium fluoride were purchased from Carlo Erba Reagents (Italy). Polylactic polymer granules (PLA) were obtained from Cargill-Dow Inc. (USA) with the trade name of Nature Green® 2100D. This highly crystalline type is mostly consisted of the L co-monomer containing less $1.47 \pm 0.2\%$ of the D co-monomer.

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), trypsin, sodium orthovanadate, dimethyl sulfoxide (DMSO), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltatrazolium bromide), glutaraldehyde solution and osmium tetroxide were obtained from Sigma Aldrich (USA).

2.2. Breast cancer cell lines

The estrogen receptor (ER)-positive, poorly invasive, and low metastasizing human breast carcinoma cell line MCF-7 and the triple negative, highly invasive, and metastatic human breast carcinoma cell line MDA-MB-231 were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). MCF-7 and MDA-MB-231 cells were 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, France). Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2–95% air.