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Zeolite inorganic scaffolds for novel biomedical application: Effect of physicochemical characteristic of zeolite membranes on cell adhesion and viability

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ABSTRACT

The design, preparation and selection of inorganic materials useful as functional scaffolds for cell adhesion is a complex question based both on the understanding of the chemical behavior of the materials and individual cells, and on their interactions. Pure zeolite membranes formed from synthetic crystals offer chemically-capable being modulated silanolic surfaces that are amenable to adhesion and growth of fibroblasts. We report the facile preparation of reusable, very longlasting, biocompatible, easily sterilized synthetic scaffolds in a zeolite membrane configuration, which are very stable in aqueous media (apart from ionic strength and pH values), able to adsorb pollutant species and to confine undesired toxic ions (present in culture media). This may ultimately lead to the development of cell supports for economic antibiotic-free culture media.

1. Introduction

The novelty of the present work lies in the association of the synthesis of zeolite membranes with the adhesion of the cells. The skill to grow isolated human cells outside of the body has led to advances in fields ranging from the materials science to tissue engineering and nanomedicine [1]. Recently, methods to step up human cells from progenitor and stem cells ex vivo have been developed and this approach holds out great therapeutic promise. A huge effort has been directed at the development of synthetic substrates that can provide biomimetic signals to cells, encouraging them to attach and behave as they would in their natural environment [2–4]. Evaluation of novel materials such as components of substrates for cell growth remains an important research goal [5,6].

The adhesion and the subsequent cell activities (spreading, focal adhesion, migration, and proliferation) are highly sensitive to the surface chemistry and its physical environment including the chemical composition (atom ratio, Brönsted's and Lewis's acidity, hydrophobic/hydrophilic properties), structure, porosity, crystallinity of the materials, morphology and topography of the surfaces to which cells adhere [7–10]. For many years polymeric membranes have attracted the interest of researchers for their potentialities [11,12]. However, they are affected the same drawbacks that up to now have limited the wide utilization of these materials [13]. Indeed, the accessibility to using an inorganic material that marries specific performances of membranes and properties of zeolites has an edge over the other scaffolds [14]. Their peculiar physicochemical characteristics and the relatively simple possibility of preparing highly reproducible large-area zeolite membranes has placed these inorganic materials to the forefront of scientific interest correlated to the preparation of designed and synthesized materials for innovative applications in the past decade [15].

For years, zeolite crystals have been used in various technological fields of great industrial interest such as catalysts, ion-exchangers, and adsorbents in chromatographic applications. Today, the preparation of crystalline inter-grown zeolite membranes plays a central role in many new applicative fields such as catalytic zeolite membrane reactors, gas mixture separations, and pervaporation of aqueous solutions and drug delivery [16,17]. The design, synthesis and selection of inorganic materials useful as functional scaffolds for cell adhesion is a complex question

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based both on the understanding of the chemical behavior of the individual cells and materials, and on their interactions. Since it is well known that initial interactions of foreign materials with the living cells begins with adsorption of proteins onto non-living surfaces, to understand how the physicochemical parameters of these innovative zeolite materials can influence biological performances and, then, to rationalize their behaviour with regard to both desired and undesired effects, we have just reported the immobilization of proteins like Bovine Serum Albumin and cytochrome c on zeolite crystals and membranes [18,19]. In this study, pure structure zeolite membranes have been synthesized and utilized, without surface functionalization, for the adhesion and growing of fibroblasts.

2. Experimental part

2.1. Materials

For the synthesis of zeolite membranes the reactants were: sodium aluminate (99%, Sigma–Aldrich), aluminum sulfate octadecahydrate (purum, Fluka), aluminum isopropoxide (98 wt.%, Sigma–Aldrich), fumed silica (aerosol 380, Degussa), colloidal silica (AS-40, Dupont), tetraethylorthosilicate (TEOS, Sigma–Aldrich), ammonium fluoride (RPE, Carlo Erba), sodium fluoride (reagent grade, Sigma–Aldrich), vanadylsulphate pentahydrate ($VOSO_4{\cdot}5H_2O$), tetraethylammonium hydroxide (TEAOH) (35% wt.% sol, Sigma–Aldrich), tetrapropylammonium bromide (TPABr), tetramethylammonium hydroxide (TMAOH, Sigma), tetramethylammonium bromide (TMABr, 98 wt.%, Fisher) tetrabuthylammonium hydroxide (TBAOH, Sigma Aldrich), phosphate buffered saline (PBS) (Life Technologies, Paisley, UK), Dulbecco's Modified Eagle's Medium (DMEM) (Life Technologies, Paisley, UK), penicillin/streptomycin (Eurobio France), Acridine Orange (Sigma–Aldrich, Milan, Italy), glutaraldehyde solution, (Sigma–Aldrich, Milan, Italy), osmium tetroxide (Sigma–Aldrich, Milan, Italy).

2.2. Cell cultures

Primary human fetal fibroblasts (cell line GPE 86, ISTGE), were used in this study. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS, 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.

2.3. Synthesis procedure of zeolite crystals

The detailed syntheses of the Mordenite (MOR), zeolite Y (FAU), zeolite β (BEA) and Silicalite-2 (MEL) zeolite crystals were carried out in alkaline media, while the samples Silicalite-1 (MFI) and V-Silicalite-1 (MFI) were prepared in fluoride gel by hydrothermal methods. The organic template ions used for the synthesis of the MOR, FAU, BEA, MEL and MFI crystals were tetraethylammonium (TEA^+), tetramethylammonium (TMA^+), tetraethylammonium (TEA^+), tetrabuthylammonium (TBA^+) and tetrapropylammonium (TPA^+), respectively; the template is generally reported as $(TTA)_2O$. The molar chemical composition used in terms of oxides reported in Table 1.

Depending on the size, crystals were separated by filtration or ultracentrifugation, washed, sonicated and dried then made in the form of disk and placed on top of a Teflon filter in a stainless steel autoclave containing water for steaming.

2.4. Fabrication of zeolite membranes

The zeolite membranes used in this work as inorganic scaffolds were prepared according to the patented Tavolaro et al. method (Tavolaro, PCT 2011) with a diameter equal to 13 mm.

2.5. Powder X-ray diffraction (XRD)

The zeolite structures of the solid products were identified by powder X-ray diffraction (XRD) patterns on a Philips Model PW 1730/10 generator equipped with a PW 1050/70 vertical goniometer (using Cu Ka radiation; $\lambda = 1.54$ Å at 40 kV and 50 mA). The diffractograms were measured from 5 to 45° using a step size of 0.02 and a scanning speed of $2^\circ\,\mathrm{min}^{-1}$. For the measurement of the crystallinity of the prepared samples, the intensity of the main peak (e.g. for MFI structure at $2\theta = 23.10^\circ$) was compared with the intensity of a reference sample, which was obtained by ultrasonic treatment (Soniprep 150) of the most crystalline sample for each reaction investigated.

The elimination of the organic template was performed in a Lindberg/blue STF55346C tube furnace in a static atmosphere. The amounts of aluminum, boron and alkali cations in the crystals were determined by atomic absorption (Shimadzu AA-660).

To investigate the zeolite crystal morphologies and sizes synthesized and the surface and cross-sectional morphology of the scaffolds fabricated, high resolution Field Emission scanning electron microscopy (FESEM, FEI – Philips quanta 200) images were captured. In addition, energy dispersive X-ray spectroscopy (EDX) was used to map the elemental composition and distribution within zeolite scaffolds.

The FTIR spectra were recorded on a spectrophotometer (Perkin-Elmer Spectrum One F.T. 2000) equipped with an attenuated total reflectance accessory (ATR). This is an infrared technique particularly useful for surface analysis of membranes. For each sample, six scans were signal-averaged at a resolution of $4\,\mathrm{cm}^{-1}$. The analysis was performed on the two different surfaces of each prepared membrane.

Table 1
Chemical composition, PZC, zeolite structure, pore size, atom ratios and crystal size of synthesized zeolite.

Zeolite Crystals	*a*	*b*	*c*	*d*	*e*	*f*	*w*	PZC	Structure	Pore Size (Å)	Si/Al ratio	Crystal size L_{max} (μm)
Mordenite	1.5	30.0	1.0	5.7			1500	8.73	MOR	5.7 × 2.6	21.8	1
Zeo Y	2.4	3.4	1.0	0.032			370	8.06	FAU	7.4 × 7.4	3.4	15
Zeo β	5.0	35.0	1.0	3.1	30.0		670	9,01	BEA	5.6 × 5.6;7.7 × 6.6	35	5
Silicalite-2	0.15	1.0					12	6.97	MEL	5.4 × 5.3	∞	Nano
Silicalite-1	0.625	10.0		0.5			330	7.24	MFI	5.6 × 5.3	∞	240
V-Silicalite-1	0.625	10.0			10.0	0.1	330	6.63	MFI	5.6 × 5.3	∞	200

$a(TTA)_2O{:}bSiO_2{:}cAl_2O_3{:}dNa_2O{:}eNH_4F{:}fVOSO_4{:}wH_2O$

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