Chapter

Zeolites as Chameleon Biomaterials: Adsorption of Proteins, Enzymes, Foods, Drugs, Human Cells, and Metals on Zeolite Membranes with Versatile Physics-Chemical Properties

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Abstract

This chapter is dedicated to demonstrating how both the hydrothermal synthesis of crystalline zeolites with precise atomic compositions and the knowledge of their physics-chemical characteristics allow designing selective materials, useful as powerful tools for biomedical applications. The adsorption of proteins and enzymes, dyes, and drugs and the preparation of scaffolds for in vitro testing of new food and cosmetic formulations are discussed according to the configuration, the composition, and the morphology of prepared materials. Finally, the study of the chemical, molecular, and supramolecular interactions between interesting biological species, drugs, cells, and synthetic materials was used to produce advanced materials and active scaffolds.

Keywords: zeolite membranes, adsorption, proteins and enzymes, drug delivery, cell cultures, food analysis

1. Introduction

The problem of creating excellent biomaterials is a scientific challenge with enormous effects on the economy and health field. Today, synthetic zeolitic materials are probably an opportunity to prepare a successful alternative to traditional biomaterials. Study, chemical design, and manufacture of various inorganic structures allow to have active biomaterials which, in all applications, avoid unwanted responses of the body such as thrombosis [1], inflammatory reactions [2], and infections [3, 4]. In fact, the chemical and physical characteristics of the internal and external surfaces of these microporous materials lend themselves to well interact with exchangeable and reactive ions (e.g., toward drugs and microbes), with proteins (preserving their biological reactivity), and with cells (being non-cytotoxic). Certainly, the chemical approach must be modified by replacing the traditional parameters used to characterize biomaterials with novel concepts such as

contact angle with point of zero charge (PZC) and wettability with silicon/aluminum ratio. All the most advanced applications concern materials that occur in a membrane configuration [5], i.e., having chemical and physical selectivity whether they are pure materials, in mixture, or made of overlapping layers (composites). Zeolites, already in the form of crystals, have selectivity (shape selectivity, hydrophobicity/hydrophilicity), which can be modified by means of chemical functionalization, ion exchange, impregnation, etc.

2. Traditional biomaterials

A biomaterial has been defined as any substance (other than a drug) or a combination of substances, synthetic or of natural origin, which can be used for any period of time, in whole or in part of a system that treats, increases, or replaces any tissue, organ, or body function.

In 1992 Black defined biomaterial as "a non-living material used in a medical device, designed to interact with biological systems."

The fundamental requirements of every biomaterial are compatibility with human tissues and possessing all those physical, chemical, and biological characteristics that allow the material to adequately perform the task for which it was designed, such as constituting a resistant support, replacing fabrics lost, and promoting regrowth of damaged tissues.

Today, more and more, research in the field of biomaterials is fueled by the need to find new materials that can last a long time, due to the increase in the average life of the population, the increased need for prostheses even by young people, and the need to reduce the number of revisions that weigh on public health costs. Furthermore, the biological materials deriving from homologous or heterologous transplants have shown important problems: limited availability, need for a further surgical operation, potential transmission of infectious diseases, reduced osteoconductive capacity, and limited ability to be incorporated into the host bone.

Biomaterials can be divided into three main types based on the response that they generate in the host tissue: an inert material does not cause a response in the tissue, a bioactive material is integrated by the surrounding tissues, and a degradable material is reabsorbed and incorporated into the surrounding tissue and can even dissolve completely after a certain time. To date, the materials most frequently used in medical applications are metals, typically inert and used for applications subjected to loads, with sufficient fatigue resistance to withstand daily activity; ceramics, used for their hardness and resistance to stress in applications such as joint surfaces, in teeth and in surfaces in contact with bone; and polymers, used for their stability and flexibility but also for low friction in articular surfaces (**Figure 1**).

2.1 Important physics features

2.1.1 Morphology

Substrate morphology can influence cell adhesion, influencing the substrate's ability to adsorb proteins and/or altering the conformation of adsorbed proteins.

For example, material roughness affects the adhesiveness of platelets. Blood normally coagulates when exposed to surfaces different from the biological endothelial ones; for this reason various attempts have been made to find a synthetic material that is biocompatible with blood. When the surfaces were tested in a laminar flow cell, it is noted that an added surface roughness led to a decrease in platelet adhesion on hydrophilic surfaces, while an increase in platelet adhesion was

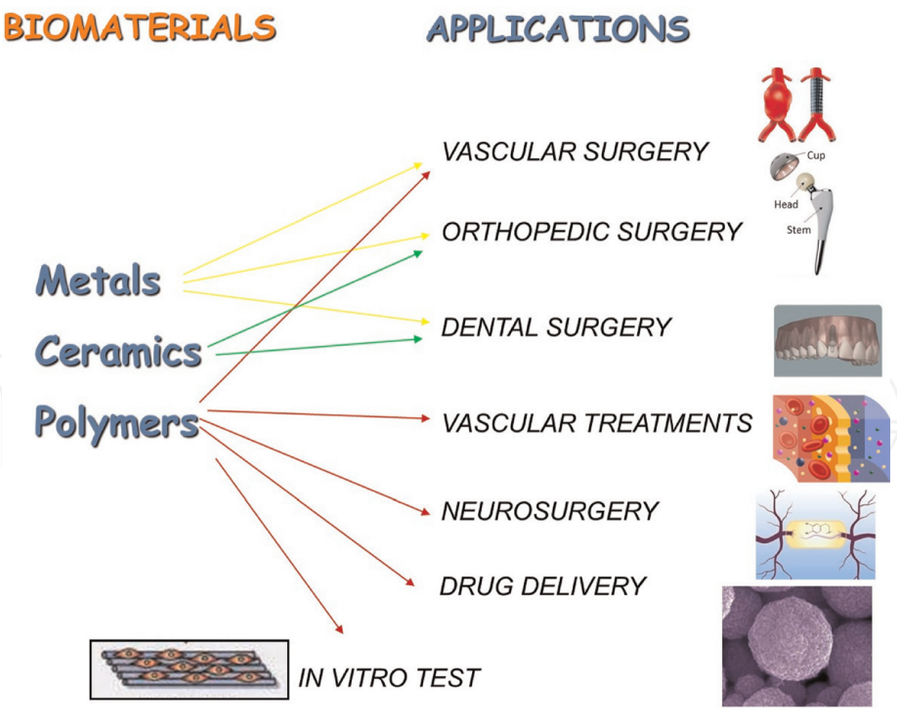

Figure 1.
Type of biomaterials and their biomedical applications.

found on hydrophobic surfaces. This demonstrates that morphology influences the properties of the material and therefore its interaction with the cells.

2.1.2 Topography

Another aspect of the morphology that must be considered to control the cellular response is surface topography. Topography, coupled with biochemical and physical signals, regulates cellular functions such as migration, adhesion, morphogenesis, differentiation, and apoptosis. Topography not only allows the systematic study of cell-substrate interactions but can also control cell orientation and morphology, which in turn controls other cellular responses. Therefore the techniques used to create substrate, precise, high-resolution surfaces acquire great importance. Today, topographies are generated with a resolution up to micrometer level, but with the advancement of modeling techniques and technology, the resolution level will reach the nanometer scale like the most in vivo structures (such as the collagen fibrils of the basement membrane).

As previously stated, topography can induce changes in cell morphology, thus influencing cellular responses such as proliferation, gene expression, and cellular function. These responses also vary depending on the type of cells being used for sowing. For example, the experimentation conducted with surfaces on which channels have been produced revealed that many cell types tend to line up along the main axis of the channels themselves and that often the organization of the cytoskeletal components and the focal contacts is oriented in the same direction. The degree of cellular alignment in the direction identified by the channels depends in a complex way on the characteristics of the topographical surface structure.

Finally, it has been observed that also the symmetry and regularity of the topographical structure are important properties of the substrate that influence cellular behavior. The results showed that regular topography reduces cell adhesion very markedly, while surface discontinuities have improved cell adhesion. This

shows that the substrate topography is important for cell adhesion and therefore for cell-substrate interaction.

2.1.3 Stiffness

Stiffness of a material is measured with the modulus of elasticity or Young's modulus. It is important to have sufficient substrate stiffness for the anchor-dependent cells to adhere to the surface. It is fundamental for the characterization of the interactions that modulate intracellular signaling pathways and cellular events, from gene expression to cellular locomotion. In fact, cell movement can be guided by manipulation of substrate stiffness characteristics. It has been shown how the mechanical properties of the matrix influence the differentiation of stem cells. Moreover, the proliferation and cellular mobility varied as the stiffness of the substrate varied. In particular, different types of substrates with different stiffness seeded with NSPC2 cells showed that the optimal stiffness for proliferation was 3.5 kPa, while for neuronal differentiation, it is less than 1 kPa [6].

2.1.4 Crystallinity

By controlling the amorphous-crystalline microstructure of the surface layer of the substrate, it is possible, for example, to improve the compatibility of blood surfaces. Surfaces with different degrees of crystallinity were tested, and an increase in the adhesiveness of the platelets was noticed on substrates that had less crystallinity. The particular amorphous-crystalline surface microstructure also modified the denaturation of adsorbed proteins. For example, the particular amorphous-crystalline microstructure of apolar surfaces such as propylene (with 55% surface layer crystallinity) has been shown to reduce platelet activity [7].

During the design of scaffolds for in vivo implantation, crystallinity can also influence the biodegradability of the scaffold and consequently the cellular response. The crystalline region is in fact more resistant to water infiltration and therefore delays the degradation of the biomaterial. For example, the adhesion, proliferation, and morphology of human chondrocytes of articular cartilage tested as a function of the crystallinity of various degradable polymers. The results suggested that cell proliferation is slower on crystalline polymers than amorphous polymers. This highlights the interesting dynamics between cell and substrate depending on the crystallinity of the material.

A variation in crystallinity can also affect surface roughness, on a nanometric scale. Osteoblasts seeded on polymeric substrates having different crystallinity and their number were measured using fluorescence microscopy. The results showed that the proliferation rate was greater on the smooth regions of the substrates, while it was smaller on the rough regions; a decreasing monotonic variation of proliferation as a function of roughness was observed. The critical roughness above which there is a significant reduction in the proliferation rate is 1.1 nm. It has therefore been shown that the cells respond directly to the topography of the substrate, as they are sensitive to nanometric variations in the substrate topography.

2.2 Important chemical features

2.2.1 Wettability

Wettability of a solid surface is a measure of its hydrophobicity and hydrophilicity. It concerns to the ease liquid phase spreading on a solid surface, which, for polymeric materials, is generally evaluated by contact angle measurements. Contact angle represents the angle formed by the intersection of liquid-solid and

liquid-vapor interface, obtained by virtual tangent drawing along the vapor-liquid interface. Water molecules are not able to form hydrogen bonds with the hydrophobic support; therefore, they form hydrogen bonds between them generating a more ordered structure with less entropy. Water molecules on a polymeric surface reorganize around proteins, causing the irreversible unfolding and adsorption of native proteins on the substrate surface. The proteins present in the serum can act as surfactants, or they can lower the surface tension of a liquid; the hydrophobic domains interact with the substrate and the hydrophilic domains form hydrogen bonds with the water molecules, thus facilitating the wettability of the surfaces. This involves a release of ordered water molecules, which is energetically favorable due to the increase in entropy, known as hydrophobic effect. In general, proteins are preferentially adsorbed on hydrophobic surfaces, mediated by their hydrophobic domains. Instead of seeing the underlying surface, the cells see the layer of proteins adsorbed on the surface of the substrate, which then modulate cell adhesion.

2.2.2 Surface charge

Polymeric biomaterial surface charge affects the adsorption and the unfolding of proteins on its surface. Unlike wettability, the driving force for protein unfolding on a charged surface is electrostatic interaction, not hydrophobic interactions. Protein unfolding depends on the net charge that proteins and cells encounter on the surface in the cell culture medium. Many proteins have a net negative surface charge, which promotes their adsorption on a positively charged surface.

2.3 Zeolites as biomaterials

We can imagine that the adaptability of zeolitic materials to interact with different biologically active molecular species and with different environments containing cells makes them (within the vast field of biomaterials) entirely comparable to chameleons (in the animal kingdom). In fact, it can be imagined that just as the complex specialized organization of cells can produce a color change in chameleons (by acting on well-defined physical parameters), the changing complex chemical organization in zeolite framework can interact with proteins, enzymes, cells, and foods (by modifying the preparation methods). The chameleon-like characteristics of the zeolite membranes as biomaterials can be inferred from the various applications reported in the literature and are highlighted in **Figure 2**.

Zeolites used as biomaterials can be distinguished according to the origin: natural, artificial, and synthetic. It should be noted that the crystallized structures by means of hydrothermal reactions in the laboratory under controlled conditions have, at the same time, higher crystallinity, purity, and reproducibility of chemical composition, zeolitic structure, dimensions, morphology, and distribution of the pore and channel system. The choices of chemical parameters, in the synthesis and in the pre- or posttreatments, always have repercussions on the macroscopic chemical-physical characteristics of prepared materials, such as the hydrophobicity, the point of zero charge (PZC), and the presence of the various types of ions, present in the form of exchangeable cations or clusters. We can analyze these different characteristics by gradually shifting our analysis from the microscopic atomic field to the macroscopic membrane field.

2.4 Synthesis and characterization of zeolite biomaterials

Zeolites are bi-functional materials having both Lewis and Brönsted acidity. These two types of acidity are not independent of each other but are closely related

to each other, and both participate in the formulation of the total acidity and hydrophilicity of the zeolitic material. Lewis acidity is linked to the presence and relative concentration of trivalent aluminum atoms within the framework (and of other chemically equivalent atoms); therefore, it strongly depends on the so-called SAR ratio. Hydrophobic zeolites are not very acidic (like silicalite-1 or silicalite-2), while hydrophilic zeolites (like zeolite Y or zeolite A) or isomorphically substituted (with cation 3^+) have a high value of Lewis acidity (**Figure 3**).

The second type of acidity is linked to functional silanol groups and more complicated to analyze. We must distinguish between Brönsted acidity of the single bonds (electronically influenced by the neighboring atoms) and the total acidity (depending both on the chemical composition and on the reaction environment used for the synthesis). As a first approximation, we can consider Brönsted total acidity concerning the outer surface of the crystals so it is probably the most important type in interactions with species larger in size than that of zeolite pores such as human cells (**Figure 4**).

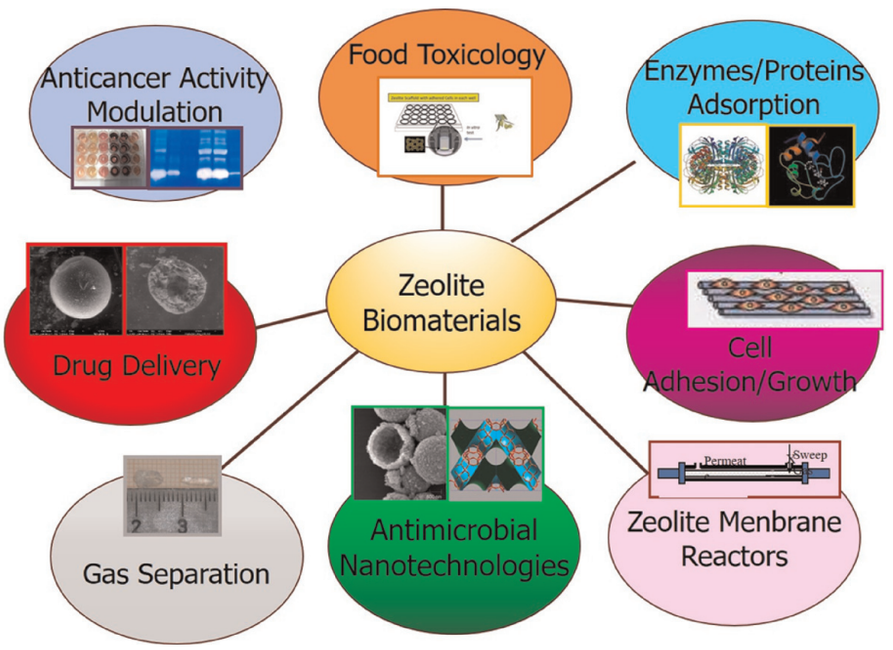

Figure 2.
Application fields of zeolite biomaterials.

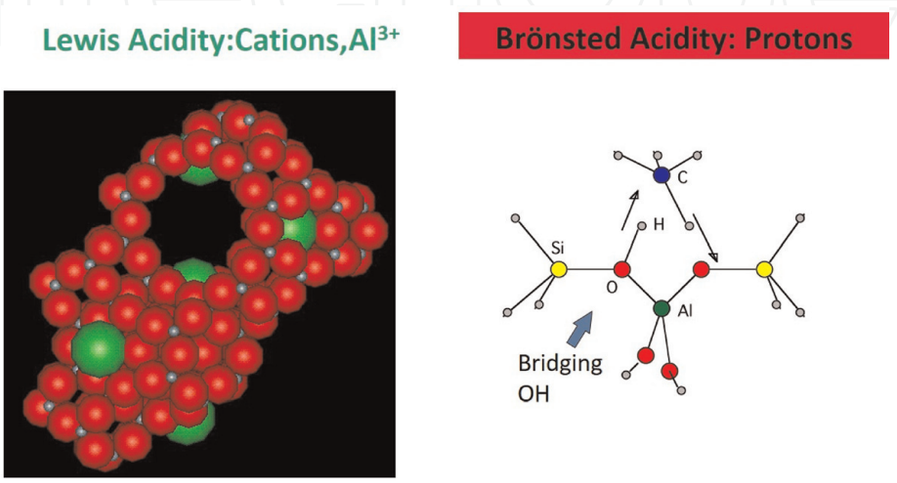

Figure 3.
Scheme of Lewis and Brönsted acidity of single bonds. The substitution of tetravalent Si atoms in the lattice with the trivalent Al atoms generates local negative charges, which are then compensated by extra-framework cations. The charge compensation by protons results in strong Brönsted acid sites.

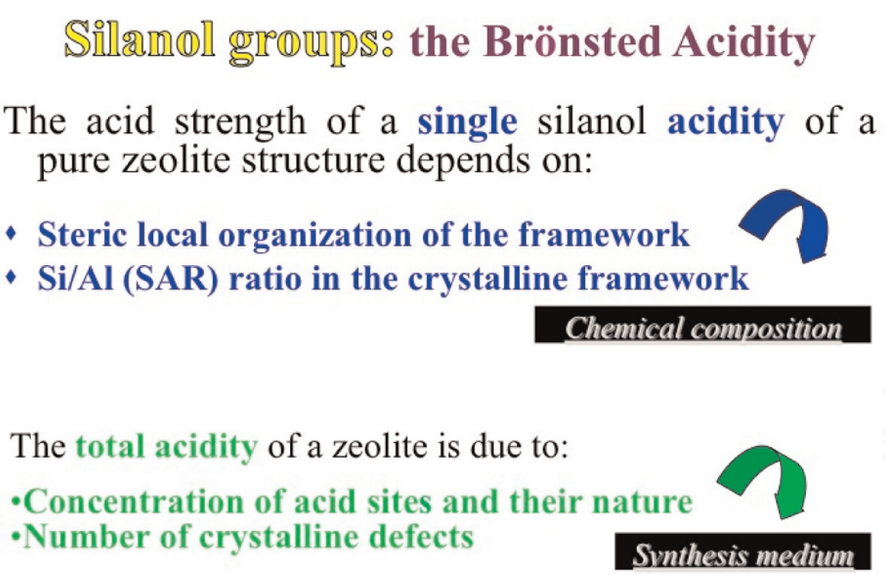

Figure 4.
Schematic summary of the Brönsted acidity for zeolitic biomaterials.

Finally, an extremely important characteristic is point of zero charge (PZC), which is the pH value at which the zeolitic membrane is electrically neutral.

Zeolites have been approved and defined as safe for human consumption by the FDA (Code of Federal Regulations, April 2017). They are also widely used in agriculture as fertilizers because they are declared nontoxic by IARC (IARC, Lyon, Vol. 68, 5061997). Furthermore, they have been approved by the Codex Alimentarius Commission for their use as fertilizer (Codex Alimentarius Commission, 2016) and EFSA experts as flavoring material or food storage adjuvant (EFSA J 11: 3155, 2013) and use as feed additives (EFSA J11: 3039 2013).

Only a natural type of fibrous zeolite is considered dangerous and erionite [8, 9]. It can induce, if breathed, tumors (pleural and peritoneal mesothelioma) and pathologies of the respiratory system, caused by its microfibrous morphological characteristics.

Another use of zeolites, with good results, is to add them as additives for animal feed. The integration of zeolites in animal feed has been studied on different animals (sheep, calves, pigs, etc.), and the results obtained have shown that these substances allow preventing diseases. Moreover, the use of zeolite in animal nutrition improves the assimilation of nutrients and therefore, consequently, promotes an increase in weight of the animal itself [10].

In recent years, zeolitic membranes have been studied as new biomaterials used for biomedical applications. These membranes, in fact, are considered as an ideal support for the immobilization of biological molecules not having the limitations associated with traditional polymeric membranes [11].

Numerous biological molecules were adsorbed and immobilized on zeolite crystals and membranes. These species include cytochrome c [11], bovine serum albumin (BSA) [12], glucose [13], uremic toxins [14, 15], nitrosamines [16], and catalase [17].

An important characteristic of zeolitic membranes is that the basic/acid nature of the material can be modified by varying the Si/Al ratio or by introducing different metals (Me) into the crystalline structure and changing the Si/Me ratio [12].

Cytochrome c is a water-soluble electron carrier that is efficiently immobilized onto zeolitic membranes, but the composition of the membrane is an important factor that influences the immobilization performance. We also studied adsorption of BSA protein [12] on FAU, BEA, and MFI zeolite crystals synthesized under hydrothermal conditions and membranes showing that the chemical composition

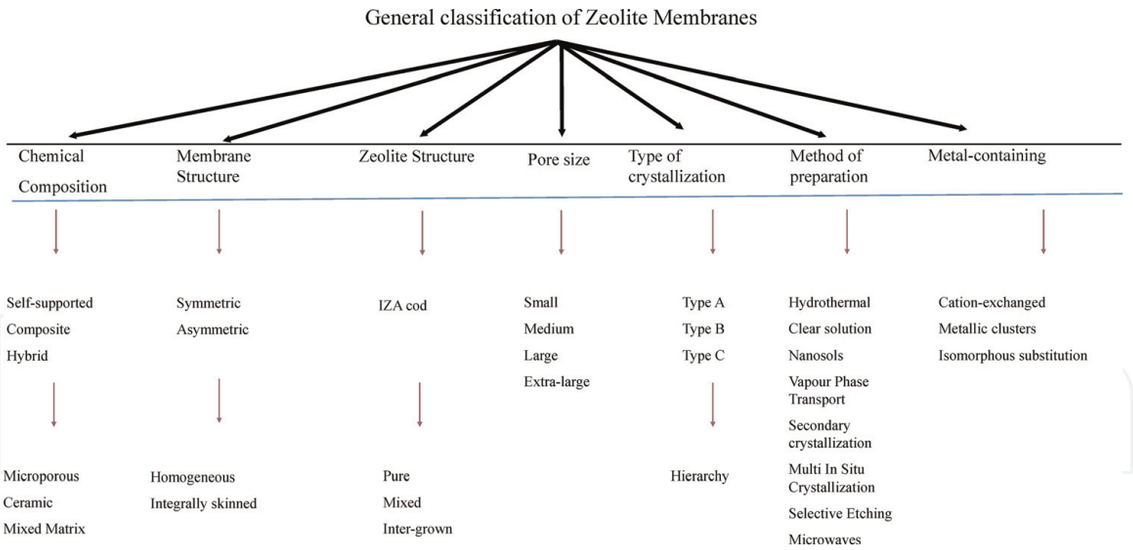

Figure 5.
Classification of zeolite membranes.

and structure of zeolitic membranes influences protein adsorption kinetics. In our work the acidity of zeolite structures was modulated considering several frameworks and MFI structures (having isomorphous vanadium atoms incorporated into the crystalline structure), and Si/Al, Si/V, and Al/V ratios were varied changing the chemical composition of the gels' reaction precursors.

Obtained results shows that zeolite Y surface adsorbs largest amounts of BSA and its percentage of adsorption increases with temperature and depends on the pH of the solution used, being absolute maximum in correspondence of protein pI value. The adsorption difference between the various types of zeolite also depends on the type of hydrothermal crystallization within the inorganic support [12].

2.5 Classification of zeolite membranes

Zeolitic membranes are membranes in which the selectivity is due to the zeolitic structure regardless of the membrane constitution and morphology (**Figure 5**). Composite, self-supported, mesoporous, and mixed-matrix membranes can certainly be considered zeolitic if the chemical-physical zeolite characteristics influence the process to which they are applied.

Zeolitic membranes consist of intergrown crystals of sizes in the range of a few nanometers up to several hundred microns. They can be formed in self-supported zeolite membranes, which are very fragile, and therefore, for applications that require the use of pressure gradients, microporous film is grown using permanent inorganic (e.g., ceramic and metal) and organic (e.g., plastic and wood) supports.

Zeolitic membranes can be classified into various categories based on their shared physics-chemical and morphological characteristics, using various classification parameters. Currently, we can identify eight possible classification systems that can coexist and are preferred by scientists depending, for example, on the material application or the feature studied. These systems can thus be identified on the inclusion criterion used:

- Chemical composition
- Membrane morphology
- Type of crystalline zeolite framework

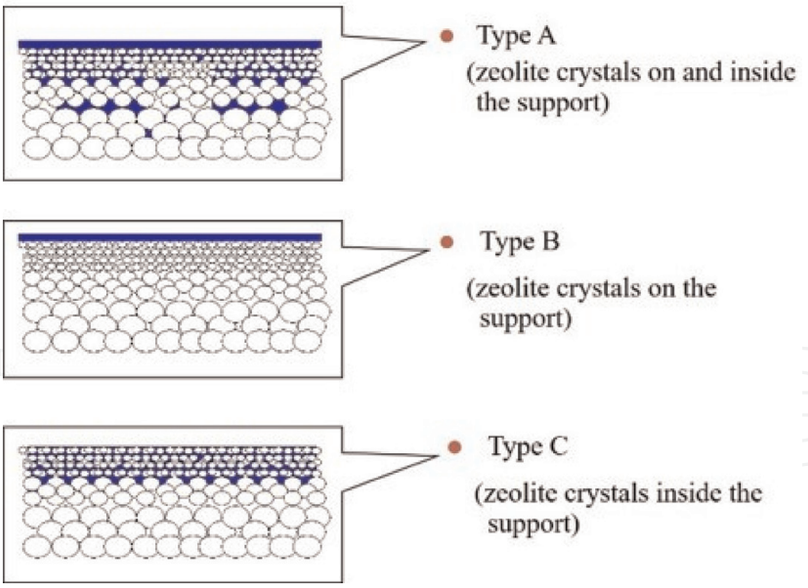

Figure 6.
Schematic representation of different types of zeolite membrane crystallizations.

- Pore size
- Type of crystallization (**Figure 6**)
- Synthesis methodology
- Metal-containing frameworks
- Origin (natural, artificial, synthetic)

Classification based on the membrane chemical composition includes inorganic, composite, and hybrid membranes. Inorganic zeolitic membranes are solid membranes made of zeolitic crystals and/or inorganic materials such as metals, oxides, amorphous silica, and ceramic particles. These membranes are very stable and resistant to mechanical stress at high temperatures and pressure gradients.

Composite membranes are constituted by a superposition of different materials layers, which can be evidenced by an orthogonal section to the surface like zeolite/alumina membranes.

Hybrid membranes (mixed-matrix membranes) include membranes consisting of zeolitic crystals dispersed in a polymeric film. These membranes have great tensile strength and great elasticity but low thermal and mechanical resistance as well as poor aging stability. They are prepared easily and quickly by means of inclusions or crystal depositions.

3. Adsorption

The possibility of modulating the specific characteristics of zeolites in a membrane configuration using inorganic zeolitic membranes in biotechnological applications such as molecular separations, enzymatic membrane reactors, protein chips, drug delivery, etc. is an attractive perspective that would offer remarkable potential applications. Naturally, the selection of materials suitable for specific applications cannot ignore the study of the interaction between the biological species and the crystalline inorganic support and therefore the understanding of adsorption.

Although the zeolitic materials have been well characterized and widely used in chromatographic applications, the analysis of protein adsorption on zeolitic crystals is poorly reported in the literature, and even less numerous are the research activities concerning the zeolitic membranes.

Adsorption is a surface phenomenon characterized by the interaction of a molecular species present in a solution (adsorbate) with the external or internal porous surface of a solid (absorbent). In the thermodynamic sense, most studies have considered adsorption as a reaction, which, of course, is more extensive if the solid material has a high surface area Eq. (1):

$$A + B_{solid} \leftrightarrow AB \tag{1}$$

The species A dissolved in the solution reacts with the adsorbent B to form AB.

In a thermodynamic equilibrium situation, the Gibbs free energy change tends to zero, whereas the two chemical potentials are equal according to Eq. (2):

$$\Delta G = \mu_{s-l} - \mu_l = \Delta G^0 + RTln(K_e) = 0 \tag{2}$$

where ΔG is the Gibbs free energy change, $\mu_{s\text{-}l}$ is the chemical potential in the solid–liquid interface, μ_l is the chemical potential in the liquid phase, R is the universal gas constant, and K_e is the equilibrium thermodynamic constant (Cheng and Zhang, 2014 da Bonilla):

$$\ln(K_e) = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} = 0 \tag{3}$$

Eq. (3) permits to calculate the adsorption thermodynamic values of $\Delta H°$ and $\Delta S°$ plotting $ln(K_e)$ versus I/T values in the van't Hoff plot. A reasonable physic meaning of K_e can be given considering:

$$K_e = \frac{activity\ of\ occupied\ sites}{(\text{activity of empty sites})\ (\text{activity of adsorbate in solution})} \tag{4}$$

$$K_e = \frac{\frac{q_e}{q_m}}{\left(1 - \frac{q_e}{q_m}\right)\frac{C_e}{C°}} \tag{5}$$

and

$$q_e = \frac{q_m K_e \left(\frac{C_e}{C°}\right)}{1 + K_e \left(\frac{C_e}{C°}\right)} \tag{6}$$

This last equation allows to obtain the dimensionless value of K_e by plotting the experimental data obtained for q_e (expressed in moles/grams) versus C_e (expressed in moles per liter) and considering the value of $C°$ equal to 1 mole per liter.

4. Adsorption of proteins and enzymes

It is known that protein molecules selectively bind to non-biological surfaces such as those of the metals, of carbonate oxides, and semiconductors. Naturally, in order to use these inorganic supports as biomaterials, it is necessary that the protein

biological activity is preserved with immobilization. In fact, it is possible that the interaction of the inorganic matrix with the protein causes its inactivity or functional slowdown as a consequence of structural or conformational changes or steric unavailability of the active site. Therefore, it is evident that a suitable selection of the matrices is essential to obtain immobilized and, at the same time, active biological species. Zeolites have a large surface area and thermal, mechanical, and chemical resistance; therefore, they are well suited to the role of adsorbent supports for biological molecules. The acid/base nature of the material can be modified changing the silicon/aluminum ratio (called SAR) of the framework or introducing different metal atoms into it (creating isomorphic substitution) and varying the silicon/metal ratio by synthesis. Furthermore, it is possible to modify the acidity of zeolites by ionic exchange of the extra-framework cations present in the microporous channels, for example, with protons.

5. Drug delivering zeolite biomaterials

Most biomaterials used for implants are inert, non-immunogenic, and nontoxic, but devices made with such materials often contain parts that trigger the so-called foreign body reaction, a material rejection complex process still not completely understood and probably related to the presence of histamine and the fibrinogen adsorption onto the implant surfaces [18]. These reactions can produce thrombosis, infections, inflammations [19], formation of fibrotic tissues around implantation, and prostheses. To realize novel active drug-releasing biomaterials, we prepared low-cost, specific drug carrier membranes for innovative biomedical drug delivering materials for implants [20]. In order to achieve this purpose, we synthesized MOR and MFI zeolite nanocrystals and composite membranes using porous stainless steel permanent supports; then we prepared ion-exchanged structures Cu(II) and Zn(II)-containing hydrophilic frameworks.

Our work on the adsorption, and the subsequent release, of a model drug revealed that these zeolite materials are useful to immobilize famotidine (3-[[[2-diathiazolyl]methyl]thio]sulfamoylpropionamidine), a histamine H_2 receptor. Furthermore, we evidenced that the synthesized materials, having different types of zeolitic structure and bivalent counter-cations, show different performances suitable to biomedical applications. In fact, the adsorption percentage of on transition metal-containing nanocrystalline zeolites was greater with respect to the as-made materials suggesting that these cations chemically interact with the drug and that cupric ions form stable organometallic complexes.

6. Interaction of zeolite materials and cells

The composition of traditional scaffolds has changed considerably since the end of the 1980s, when the field of tissue engineering was started in a systematic way. This improvement reflects the greater scientific understanding of the needs of the cells in the adhesion and management of their behavior, which are fundamental in tissue engineering applications. The success of a new scaffold is not only based on its mechanical characteristics or on the surrounding chemical environment but also on its detailed chemical surface and topography (in a nanometer scale). These last two characteristics are not so easy to achieve by chemical synthesis for a large number of inorganic or polymeric materials.

The analysis of cell-substrate interaction is of fundamental importance in order to design biomimetic scaffolds capable of replacing damaged vital organs, or tissues, or

to assist the body's natural healing processes. The ability of a cell to recognize and interact with the substrate represents the first indispensable step, without which processes such as cell adhesion proliferation, migration, and differentiation would not be possible. Therefore the understanding of the mechanisms that determine the early phases of cell-material adhesion, as well as their control, is indispensable for the design of biomaterials. Both the mechanical and biochemical properties of the material determine the efficacy and agreed with which the cells recognize the material.

The possibility of modifying and controlling surface properties at the micro-/nanolevel 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 reached, 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. 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. Starting from this assumption, we designed and prepared various crystalline zeolite scaffolds, which are 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 constitutes a stable, homogeneous, ion- and solvent-available support. Zeolite membranes symbolize this novel type of chameleonic scaffold.

7. Zeolite scaffolds

Zeolite scaffolds provide a framework above and within which cells seeded in the culture medium can adhere and over time populate them. These processes require that the scaffold structure must also be able to support a growing number of cells allowing the transport of sufficient amounts of nutrients and the removal of waste products having an extended surface where the cells can adhere and migrate freely so as to form a mass of cells with subsequent deposition of an active extracellular matrix (ECM) [21].

A scaffold is a critical component of tissue engineering, as it is intended to release, contain, and form new tissue in vitro or to promote tissue repair in vivo. Porosity, architecture, and rate of degradation are important aspects of the material that allow the growth of cells that guide the formation of tissues such as bone.

An ideal scaffold should have the following characteristics:

- Biocompatibility suitable not to induce any adverse reaction
- Degradation rate appropriate to match the tissue regeneration process
- Narrow pore size distribution to allow cells to have a sufficient space to grow and access to nutrients and metabolites

- High surface area to boost the cell adhesion
- Chemical composition appropriate to promote cell differentiation and growth
- Structural parameters appropriate to modulate cellular biosynthesis

When the adhered cells increase in number, they begin to enter the internal pores of the scaffold. If the porosity and interconnection between the pores are good, the cells grow and colonize the entire scaffold releasing their extracellular matrix. The upper layer of cells consumes more oxygen and nutrients, thus limiting the amount available for the cells that are migrating into the scaffold; the maximum depth at which cells can survive corresponds to the depth of cellular penetration. We studied many types of both self-supported and hybrid PLA-containing zeolitic membranes (MMMs) to study interactions with different types of normal [22] or carcinogenic (MDA-MB-231 [23] and MCF-7 [24]) cells. Initial cell tethering and filopodia exploration are followed by lamellipodia ruffling, membrane activity, and cell spreading. With time endogenous matrix is secreted by the cells, and matrix assembly sites form on the ventral plasma biological membrane. Later, with increased integrin recruitment, these early cell-matrix contacts form anchoring focal complexes at the lamellipodium leading edge that are reinforced intracellularly to form larger focal adhesion plaques upon increased intracellular and/or extracellular tension. The regulation of focal adhesion formation in adherent cells is highly complex and involves both the turnover of single integrins and the reinforcement of the adhesion plaque by protein recruitment. It follows that focal adhesions emerge as diverse protein networks that provide structural integrity and dynamically link the ECM to intracellular actin filaments, directly facilitating cell migration and spreading through continuous regulation and turnover. Furthermore, in combination with growth factor receptors, these adhesive clusters initiate signaling pathways and regulate the activity of nuclear transcription factors and processes crucial to cell growth and differentiation. The adhesion sites act as mechanosensors that form additional contact points with the underlying substratum in response. Preceding focal adhesion reinforcement, a tightly regulated series of temporospatial events occurs, mediating integrin clustering in an anisotropic manner in the direction of force. Our works underlined that the cells of both lines assume a specific morphology under the influence on the major peculiarities of scaffolds.

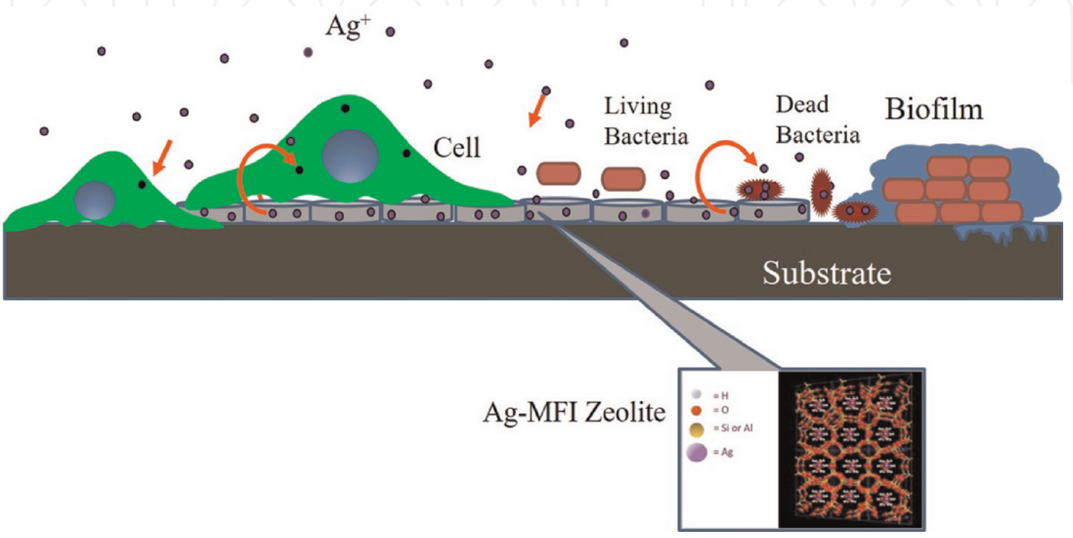

Figure 7.
Schematic representation of the antimicrobial activity of zeolite scaffolds.

Synthetic zeolite scaffolds have been successfully applied to in vitro studies regarding both adhesion and cell growth kinetics [25] as well as to the analysis of new formulation cosmetics and foods [26] (**Figure** 7).

8. Conclusions

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 zeolite membranes plays a central role in many new applicative fields such as catalytic zeolite membrane reactors, gas separations, drug delivery, anticancer activity modulation, food toxicology, enzyme/protein adsorption, and antimicrobial nanotechnologies. Zeolite crystals and membranes are key materials for selective adsorption of biological molecules, drugs, and chemotherapy drugs. Moreover, the zeolite membranes represent a synthetic scaffold suitable, ideal, and able to guarantee the survival, growth, and correct differentiation of human normal and cancer cells. The development of zeolite membranes with versatile physics-chemical properties may constitute the goal for new components in biomedical and biotechnological applications.

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Conflict of interest

The authors declare no conflict of interest.

Nomenclature

In this work, the authors use framework type codes of the IZA Commission.

Abbreviations

PZC	point of zero charge
$NSPC_2$	neural stem/progenitor cells
SAR	silicon/aluminum ratio
FDA	Food and Drug Administration
IARC	International Agency for Research on Cancer
EFSA	Education and Skills Funding Agency
BSA	bovine serum albumin
FAU	faujasite structure
BEA	beta-structure
MFI	MFI structure
IZA	International Zeolite Association
ECM	extracellular matrix

PLA	polylactic acid polymer
MOR	mordenite structure
MMM	mixed-matrix membrane
MDA-MB-231	human breast adenocarcinoma cells
MCF-7	human breast ductal carcinoma cells

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