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Potential Application of Nanoporous Materials in Biomedical Field

Saraswati Prasad Mishra, Shweta Dutta, Anil Kumar Sahu, Koushlesh Mishra and Pankaj Kashyap

Abstract

Nanoporous materials are the substances having pores of size 100 nanometers in a frame work organic or inorganic substance. These substances are used in medical devices such as bioartificial organ and biosensing. Nanoporous material has also importance in the field of diagnostics. This chapter basically explains about the nanoporous material in detail along with its types. The methods of fabrication of these nanoporous material area also explained. The chapter also deals with the characterization of the materials. Moreover present application of nanoporous material such as in the field of biomedical along with the future prospects is explained in the present chapter.

Keywords: nanoporous material, biosensing, organic, inorganic, medical device

1. Introduction

Nanoporous material is a structure containing framework of organic or inorganic substances having pores of size 100 nanometers. The pores found in nanoporous material contain either gas or liquid filled in it. A Nanoporous material is used recently in novel medical devices, implants or making bioartificial organs and biosensing. Advancement in the field of nanofabrication made it possible to produce nanoporous material with desired size of pores, distribution of pores in the nanoporous material as well as their porosity and chemical nature. Eventually it made the nanoporous material more attractive to carry out process of regulation and transportation at the molecular level. Basically nanoporous material is used for size sorting; antibiofouling behavior along with it is used in medical devices as mentioned above. In near future it is possible that nanoporous material can be functionalized with smart polymers that can initiate or modulate transportation at bio-molecular level in response to different kind of stimuli such as ion, change in pH or temperature [1]. This can eventually help in development of such medical device that can act in accordance to the changing physiological needs. The body cells naturally have proteins of nano-size that helps in regulating movement of biomolecules across the membranes. In similar way nanoporous material functionalized with smart polymer will differentiate between the biomolecules that has to be transported from the biomolecules that are not to be transported. As nanoporous material have small pore size but contains a larger surface porosity it becomes ideal

to be used in activities like ion exchange, catalysis, sensing [2–4]. Nanoporous material has an important role to play diagnostic field as it is used in combinatorial biochemistry on-a-chip, in analysis of DNA, in activity like cell manipulation and chromatography as well [5, 6]. Moreover it can also be used in boosting devices that are used to store energy as nanoporous material shows a greater conductivity to electrolytes. The present chapter explains about the nanoporous materials, their relevance in present day as well as their future prospects and their classification. The chapter also elaborates about the fabrication methods, nanopores techniques along with the characterization of nanoporous material and their applications [7–10].

2. Types of nanoporous materials

Nanoporous material are generally grouped into two class i.e. bulk material and membranes. Under bulk material activated carbon and zeolites are the examples whereas when membranes are concerned then cell membrane is an example of nanoporous membrane. Nanoporous materials are made using a chemical reagent that is basically inorganic and a structure is provided by using the organic templates. It can be said that nanoporous material is made by polymerization of inorganic monomers that are associated by the templates of organic molecules. Many nanoporous material are also made by using minerals instead of chemicals reagent as inorganic source. In case of mineral nanoporous material templating is based on the initial structure of the mineral itself [11, 12].

2.1 Classifications

Nanoporous materials can be of different types as discussed above. Below are classification of nanoporous material based on pore size and the network material used.

2.1.1 Classification by pore size

The pores of nanoporous material vary from 1 nm to 1000 nm. In accordance to IUPAC there are following class of nanoporous material [11–13].

- **Microporous material:** The pore size in this kind of material is between 0 to 2 nm
- **Mesoporous material:** In mesoporous material the pore size ranges from 2 to 50 nm
- **Macropores material:** Macroporous materials are those materials where the pores size is greater than 50 nm.

Comparison between these three pore systems is given in **Figure 1**. There is no order found between above mentioned pore materials, mostly they are random in nature (**Table 1**) [14–17].

2.1.2 Classification based on network material

In the field of nanoporous material one of the most important thing is to have network material of desired chemical composition. These network materials can be classed into two categories

- i. Organic material
- ii. Inorganic material

One of the most important goals in the field of nanoporous materials is to achieve any possible chemical composition in the network materials “hosting” the pores. It makes sense to divide the materials into two categories:

- i. Organic materials
 - ii. Inorganic materials.
- i. Organic material:

As organic material act as template for the inorganic material to form the structure so it is the smaller group used nanoporous material. Different kinds of polymers are used under this category [18, 19].

ii. Inorganic Material: Inorganic material are the main group in nanoporous material. Following nanoporous materials are used [20].

- Inorganic oxide type materials such as porous silica or porous titania or porous material of zirconia is used.
- Nanoporous carbon materials are also used where active carbons are used. Mesoporous carbon materials are example under these groups.
- Sulphide and nitrites are also used under inorganic material. An $AlPO_4$ material also comes under this.

3. Fabrication methods

The area of fabrication in materials of nanostructure is ever improving area with involvement of innovative techniques that helpful to different field of research and

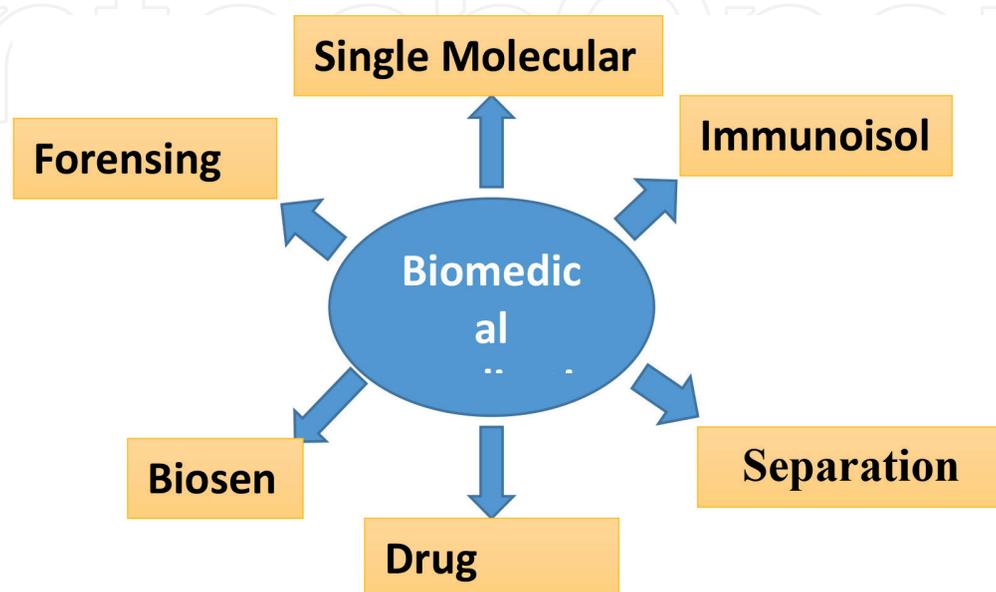


Figure 1.
Biomedical applications of nanoporous materials.

CONTENT	POLYMERIC	CARBON	GLASS	ALUMINOSILICATE	OXIDASE	METAL
PORE SIZE	Meso-macro	Micro-meso	Meso-macro	Micro-meso	Micro-meso	Meso-macro
SURFACE AREA / POROSITY	Low 0.6	High-0.3–0.6	Low-0.3–0.6	High0.3–0.6	Medium 0.3–0.6	Low 0.1–0.7
PERMEABILITY	Low- medium	Low- medium	High	Low	Low- medium	High
STRENGTH	Medium	Low	Strong	Weak	Weak- medium	Strong
THERMAL STABILITY	Low	High	Good	Medium- high	Medium- high	High
CHEMICAL STABILITY	Low –medium	High	High	High	Very high	High
COST	Low	High	High	Low medium	Medium	Medium
LIFE	Short	Long	Long	Medium long	Long	Long

Table 1.
That table contains various properties of nanoporous.

development [21]. Improvement seen in the field of nanofabrication and the growing interest in the domain of nano-manufacturing can help in the enhancement in methods of ultrafiltration [22]. Ideal properties of a protein sieve or a molecular membrane is that it contains uniformly distributed pores on an ultrathin membrane and that is fabricated in such a way that can be used in scalable and robust manner. It should be cleanable and reusable after sterilization. In this fabrication method, nanoporous membrane are made with ratio of pore size to thickness is around one. The said ratio between pore size to thickness helps in effective mass transportation due to enhanced selectivity and permeability. Fabrication of membrane is done at very low cost so that it is scalable enough to have manufacturing at large scale. The defects that are seen during fabrication of membrane are pore size variation and absence of pores in membrane. As far as ultrafiltration is concerned absence of pore size is not that important and optimization of variation in pore size can be performed to have better functioning of membrane and optimum efficiency.

3.1 Nanopore techniques

Nanopores are nothing but pores having size in nanometer. They can be made either by using proteins that can form pores or by creating pores of nanosize in molecules. When nanopores are coated with iron and are present in a membrane which is electrically insulating act as single molecule identifier. Additionally it also acts as network of biological protein in bilayer of phospholipid. Nanopore technology is used as a detector for detecting the biological and chemical agent in nanoscale at molecular level. By the use of principle of electrophoresis a device based on nanopores pulls the molecules through nanopores into the solution and detect the molecule and ascertain their competence at analytically. Characterization of nucleic acid polymer is done in narrow and confined space in the nanopores. Nanopore sequencing technique has made DNA sequencing inexpensive and fast as characterization of single stranded DNA and RNA without labelling and amplification of it [23]. As nanopores are highly sensitive that lead to many research that helps in analyzing nucleic acid [24, 25].

3.2 Biological nanopores

Proteins are also capable of forming nanopores [26]. This kind of protein are typically have a structure like mushroom and the core of the mushroom shaped structure has hollow in it. Examples of some proteins capable of pores are α hemolysin, Phi 29 connector and MspA porin. The most initial biological nanopore is α hemolysin (α -HL) which is used in the area DNA sequencing. α -HL is produced from bacterium *Staphylococcus aureus* as an exotoxin. The specification of mushroom shaped protein is 232.4 kDa of transmembrane channel with a cap of diameter around 3.6 nm and barrel of 2.6 diameter barrel [27]. Then it is inserted in lipid bilayer and then manipulation is done [28].

3.3 Solid-state nanopores

These kinds of nanopores are made from silicon film, mostly silicon nitride. Various techniques are employed for solid state nanopores manufacturing which involves “fabrication by electron beam” and “Deploying and sculpting with ion beam” [29]. Solid nanopores have diameter ranging from sub nanometers to nanometers in hundreds and the change in diameter is based on the requirement of experimental parameter. SiN used in manufacturing of solid state nanopores shows better chemical and thermal stability as compared to lipid membrane [30].

Nanopores made of graphene expressed chemical properties that are unique and shows better gains over the biological complements [31]. Solid state nanopores created many paths for research especially in DNA sequencing. Identifications of protein interaction nanofluidic device assembly. Solid state nanopores are suitable substitute for biological nanopores due to the unique chemical properties. Various measurement technique such as electronic and optical measurement are compatible with solid state nanopores. Recent nanopores fabrication techniques are membrane technology for ion tracking [32, 33]. Production of metallic surfaced oxidative film ionic beam sculpting.

3.4 Anodic oxidation method on the metal aluminum

When electrochemistry and electrophysiology of anodic oxidation of metals was observed it resulted in fabrication of nanoporous oxides of metals that are self-ordering. Metals included are anodized form of aluminum oxide, nanotubular titania oxide and silicon [34]. The reasons due to which the anodic alumina oxide stands out are its hardness, high surface area and stability it shows chemically and thermally [35]. Selective metals such as Al, Nb, Ti, are studied for ordering behavior during the process of anodic oxidation. These metals are known as valve element [36]. Factors responsible for enhancement of the process are electrolyte type, its pH as well as concentration, temperature, surface and the voltage and current applied [37, 38].

3.5 Ion track-etching technology

This technology is used for generation of pore in materials that are insulating in nature. Several polymers are used to produce filtration films. The underlying principle is that when a material comes in the path of straight ion, due to penetration by high energy heavy ion a pore is seen in the material. By the help of appropriate reagent etching is done to enlarge the pores. Pore size can be made of dimension of nanometers to micrometers and cylindrical pores as well [39, 40].

To have a uniform etching surfactant are added during the process of ion track etching [41].

While using surfactants following few things are to be taken into consideration.

- When surfactant used gets adsorbed on the surface it tends to change susceptibility to chemical attack.
- Size of surfactant molecule is quite small in nanometer range [42].

3.6 Ion-beam sculpting

Ion beam sculpting has been matter of interest for the researchers for the meeting the challenges of nanopores. As it has low rate of shattering of ions, it gives better firmness and patterning of substrate which makes it crucial in meeting nanopore challenges High resolution of focused ion beam offers nanometer based sculpting [43].

3.7 Ion current rectification

Specific kind of transportation effect has been seen in nanocapillaries or nanopores having uneven shape and the reason being the nanosize of the opening. It is seen that there is rectification of ion current in this kind of nanopores whereas pH

of electrolyte and the concentration remains the same. For the purpose of observation of rectification current voltage curves are used [44, 45]. Ion current rectification is behavior seen in many nanoporous system. A biological nanopore as well as artificial nanopores shows rectifying behavior [46, 47].

3.8 Electron-beam fabrication

Fabrication of solid state nanopore with small diameter is difficult. It is almost impossible to fabricate the nanopores which are less than 30 nm in terms of shape and size. By use of FIB nanopores can be etched but due to low etch rate limitation on film thickness can be seen [48–50]. Nanopores can be significantly condensed to almost 10 nanometer from 50 to 100 nanometers by use of ion beam or electron beam having high energy. Solid state nanopores are very effective in detection of single molecule when pore diameter is as equal as molecule diameter [51, 52].

4. Characterization

4.1 FTIR spectroscopy

Fourier transform infrared spectroscopy (FTIR) is a type of spectroscopy that concerned with the infrared portion of the electromagnetic spectrum that helps in identifying a compound by investigating the composition of a sample. Specific frequencies of Infra-red (IR) radiation is absorbed by molecule based the functional group present in it [53].

4.2 Raman spectroscopy

It is a type of vibrational spectroscopy at molecular level which originated as inelastic light scattering process. In this spectroscopy sample molecules scatters a laser photon and there will be gain or loss of energy. Energy lost is indicator of change in energy or wavelength of the laser photon. Energy lost is characteristic to a specific bond in molecule. With Raman spectroscopy an exact spectral fingerprint can be obtained specific to molecule or any molecular structure [54].

4.3 UV-Vis spectroscopy

UV-Vis spectroscopy is different from earlier two as it is concerned with electronic transition occurring within a molecule. When a continuous striking of radiation is done on a molecule then some portion of the radiation get absorbed and the remaining radiation is passed across a prism it gives spectrum that has gap in between. This spectrum is called as absorption spectrum and due to absorption of energy there is transition of molecule from low energy to higher energy state [55].

4.4 Energy-dispersive X-ray spectroscopy (EDX)

These spectroscopies are used for analysis of element and determine the characteristics of chemical aspect of sample. X-ray is a form of energy released when sample is being bombarded with high energy beam that leads to ejection of excited electron from inner shell creating a hole and the hole formed is filled by electron from a high energy outer cell and during this energy. To measure the X-ray in terms of number and energy the instrument used is energy-dispersive spectrometer. X-ray helps in determining composition of element in a specimen [56].

4.5 X-ray diffraction (XRD)

X-ray Diffraction (XRD) is a technique which studies the diffraction produced by X-ray through the lattice and determines the characteristics of lattice. It helps in determine structure of zeolite. The sample preparation for this technique is easy and the pace of the test is quick [57].

4.6 Scanning electron microscope (SEM)

Scanning electron microscope (SEM) is an instrument that is different from normal microscope as it makes image by using electrons rather than light. In SEM when scanning of sample is done by the beam of primary electron, the surface electrons get excited and that leads to release or emission secondary electron from the surface that results in formation of image. SEM is capable of producing images having high resolution that enables the observer to examine the close features with higher magnification. The images formed from SEM gives details about particle size and surface of sample [58].

4.7 Transmission electron microscope

In TEM utilizes the electron beam that has transmitted partially across a very thin specimen. This beam helps in getting the image. TEM helps in determining or acquiring information about structure and particle size of the sample under study. TEM is slightly better in magnitude than SEM [59].

4.8 Nitrogen adsorption/desorption isotherms

This technique is used for determination of characteristics of surface zeolite. It provides information related to the entire surface such as internal, external along with the diameters of mesopores [60].

5. Biomedical applications of nanoporous materials

Applications of nanoporous materials in biomedical field has been explored and discovered and there are many more under exploration still to be discovered. Nanoporous membranes act as semipermeable membrane or compartment in many implantable devices that keep the drug or the implant and allow the passage of desired molecule. Moreover nanoporous material has application in variety of biomolecular application. It is also used in field of diagnosis and separation of protein [61].

5.1 Separation and sorting of biomolecules

Sorting or separation is essential to purify and isolate the molecules from the stream of biological feed. This application has a huge importance in the industry like pharmaceutical manufacturing, biotechnology and food industry. Currently techniques like gel electrophoresis or size exclusion chromatography are relevant and used in separation science [62, 63]. Examination of biomolecular separation in pores which are more ordered has been done recently. Synthetic nanoporous membrane has been used as support system for the cells of kidney as they filter blood and retain proteins present in serum and filter out the waste

materials [64]. The material that flow through the nanoporous material can be regulated externally [65].

5.2 Biosensing

Proteins pores that are membrane bound are used by sensory system as a detector of stimuli and facilitate the cells to respond accordingly. Biosensing has its application in fields like pharmaceutical industry, in the sector of medical diagnosis and it is also used for detecting of hazardous biomolecules. In these applications there is combination of physiochemical detection component with biological component for detection of analytes in stream of biological feed. Sensory systems use a variety of membrane-bound protein pores to detect molecules and facilitate cells to respond to stimuli. Such biosensing is also important in many technological areas including pharmaceutical industry, medical diagnosis, and detection of hazardous biomolecules. In a majority of these applications the biosensing device combines a biological component with a physiochemical detection component to detect analytes in biological feed streams [66].

5.3 Single molecular analysis

Nanoporous materials are also used probing of biomacromolecules such as DNA, RNA, and proteins one by one for single-molecule analysis. Information of biomacromolecules such as concentration, sequence, size or structure can be accessed by measurement of magnitude, frequency and blockage duration of ion current when the biomolecules are passed through the nanopore which is embedded in insulating membrane [67]. Earlier research in the field of single molecule analysis had utilized lipid membrane that had been incorporated in polymeric films like Teflon having aperture of microsize. The only drawback with micro-sized pores having polymeric support is rupture of lipid membrane after a small period of use and this technique has to be improved to have better durability. But nanoporous membrane shows better result in supporting protein pores in the process of single molecule analysis [68].

5.4 Immunoisolation

Immunoisolation means to protect implanted cells or the drug release systems from any kind of an immune reaction. It is done by encapsulating the implanted cell or drug in a nanoporous semipermeable membrane. This nanoporous material isolate the encapsulated drug or cell from the immune system of body. The pores allows entry of glucose, insulin and oxygen to pass through but it is impervious to immunoglobulins. Only requirement for nanoporous material to use in immunoisolation is that it should be compatible foul resistant for *in vivo* functions [69].

5.5 Drug delivery

In vivo delivery systems are developed to supply of drugs in a controlled manner where it is needed. Controlled delivery system is used to deliver drugs in effective way so as to eliminate any kind of improper dosing. Nanoporous membranes having controlled pore size, desired membrane thickness and porosity can deliver controlled release drugs in capsule form [70]. By coupling it with biosensors a smart drug delivery systems can be developed that will respond according to the physiological conditions.

5.6 Forensing analysis

Nanoporous gold (NPG) being a good conductor and having suitable pore-size distribution with large surface area, and can enhance the electrochemical response to the enzymatic substrates namely NADH and H₂O₂ depending on their low coordinated Au atoms. All said advantages make it perfect for construction of dehydrogenase- and oxidase-based biosensors which will show improved sensitivity and anti-interference ability. DNA sensor which is based on an NPG electrode and is prepared by the process of dealloying Ag from Au/Ag alloy and multifunctional encoded AuNP. The active surface area of the NPG electrode is 9.2 times larger as compared to bare flat as characterized by CVs. Fabrication of DNA biosensor was done by immobilizing capture-probe DNA on the NPG electrode and hybridization with target DNA, which further hybridized with the reporter DNA loaded on the AuNP. The AuNP contained two kinds of bio bar-code DNA, one complementary to the target DNA, while the other was not, reducing the cross reaction between the targets and reporter DNA on the same AuNP. Besides DNA detection, NPG is also used in making an amperometric immunosensor [71–73].

6. Application

Nanoporous materials can enhance the performance devices used in biomedical field such as immunoisolation devices, devices used for dialysis, targeted drug delivery systems, bioanalytical devices, and biosensors. The main properties that nanoporous membranes should have so that it can be used in biomedical applications are having a pore size of a few tens of nanometers or below it and the pore size distribution should be in order that help us to achieve high biomolecule selectivity; high porosity as well as low thickness in order to enable high analyte flux; mechanical stability; and chemical stability [74]. The central issue of membrane is Pore geometry, biofouling resistance, and biocompatibility so that it can be used like interfaces in implantable devices. Porous material has become a potential drug delivery system for lots of biomedical application. They can be modified internally as well as externally to load the required molecule efficiently. Moreover outer layer can acts as a barrier and help in delaying the release of drug. Porous material has many advantages over the prominently used organic material for the drug delivery. They show better stability, better loading capacity, and provide better protection to the loaded material from degradation. Although porous material has potential to used but the obstacle is how it can be transferred to the clinic successfully [75].

7. Future prospects

Porous materials are the materials of future as they show many advantages over the prominent materials used in recent times. They provide versatile porosity and the pore size can be tailored according to the need. It also has better drug loading capacity. With all the said advantages nanopores material can be in demand in future in many fields.

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