Synthesis and Patterning Methods for Nanostructures Useful for Biological Applications

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1   Introduction

The importance and benefits of nanotechnology in biology and medicine are now well-recognized by scientists, technologists, as well as various governmental and private research funding agencies. The basis that enables the application of nano-technology is the availability of nanostructured materials. Therefore, it is essential to provide an insight on the state-of-the-art methods to manufacture various nanostructures.

Nanotechnology has exploded onto the scientific scene in the last 20 years or so after the discovery, followed by a Nobel Prize recognition, of novel forms of carbon as the fullerenes molecules or buckyballs (Kroto et al. 1985), and the rediscovery of carbon nanotubes (CNTs) (Iijima 1991). The subsequent development has impacted nearly every field of scientific research. The infancy of nanotechnology began in materials science laboratories and has now matured at the forefront of several other disciplines, including chemistry, physics, various engineering fields, and, of course, biology and biomedical engineering. Nanotechnology is now rap-idly being expanded for improved biotechnology to the extent that it can now be called as a subfield of "nanobiotechnology." The research and development on syn-thesis of nanowires and nanoparticles, nanopatterning, as well as nanoscale phe-nomena and properties literally exploded in the past several years developing a large variety of nanostructures extending to a myriad of other physical or chemical components. Some of the biological applications of the nanostructures, such as nanopatterns, nanochannels, nanotubes, and nanoparticles, synthesized using the

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methods described in this chapter include nanoparticles for fluorescent biological labeling and tagging, drug and gene delivery, biodetection of pathogens and proteins, nanotubes and nanochannels for probing the structure of DNA, tissue engineering, magnetic nanoparticles for tumor destruction via heating (hyperthermia) and magnetic resonance imaging contrast enhancement, separation and purification of biological molecules and cells, and many others that are still currently under investigation (Salata 2004).

The methods for synthesis and patterning for many of these nanostructures are founded on basic, well-known techniques and their modifications, as is described in this chapter. The nanofabrication processes can be divided into the two well-known approaches: "top-down" and "bottom-up." The "top-down" approach uses traditional methods to guide the synthesis of nanoscale materials. The paradigm proper of its definition generally dictates that in the "top-down" approach it all begins from a bulk piece of material, which is then gradually or step-by-step removed to form objects in the nanometer-size regime (~1 billionth of a meter or 10^{-9} m). Well-known techniques, such as photo lithography and electron beam lithography, anodization, and ion- and plasma etching (PE), that are later described, all belong to this type of approach. The top-down approach for nanofabrication is the one first suggested by Feynman in his famous American Physical Society lecture in 1959, "There is plenty of room at the bottom" (Feynman 1959):

Now comes the interesting question: How do we make such a tiny mechanism? I leave that to you. However, let me suggest one weird possibility. You know, in the atomic energy plants they have materials and machines that they can't handle directly because they have become radioactive. To unscrew nuts and put on bolts and so on, they have a set of master and slave hands, so that by operating a set of levers here, you control the "hands" there, and can turn them this way and that so you can handle things quite nicely.

Most of these devices are actually made rather simply, in that there is a particular cable, like a marionette string, that goes directly from the controls to the "hands." But, of course, things also have been made using servo motors, so that the connection between the one thing and the other is electrical rather than mechanical. When you turn the levers, they turn a servo motor, and it changes the electrical currents in the wires, which repositions a motor at the other end.

Now, I want to build much the same device – a master-slave system which operates electrically. But I want the slaves to be made especially carefully by modern large-scale machinists so that they are one-fourth the scale of the "hands" that you ordinarily maneuver. So you have a scheme by which you can do things at one-quarter scale anyway – the little servo motors with little hands play with little nuts and bolts; they drill little holes; they are four times smaller. Aha! So I manufacture a quarter-size lathe; I manufacture quarter-size tools; and I make, at the one-quarter scale, still another set of hands again relatively one-quarter size! This is one-sixteenth size, from my point of view. And after I finish doing this I wire directly from my large-scale system, through transformers perhaps, to the one-sixteenth-size servo motors. Thus I can now manipulate the one-sixteenth size hands. Well, you get the principle from there on. It is rather a difficult program, but it is a possibility …

The "bottom-up" approach on the other hand takes the idea of "top-down" approach and flips it right over. In this case, instead of starting with large materials and chipping them away to reveal small bits of them, it all begins from atoms and molecules that get rearranged and assembled to larger nanostructures. It is the new paradigm for synthesis in the nanotechnology world as the "bottom-up" approach allows a creation of diverse

types of nanomaterials, and it is likely to revolutionize the way we make materials. It requires a thorough understanding of the short-range forces of attraction, such as Van der Waals forces, electrostatic forces, and a variety of interatomic or intermolecular forces. Since it is not possible to have various minute things come together without some attractive force or active field of force in the region, having the fundamental forces "doing all the work" for us is the key principle underlying this approach.

Some examples of such a synthesis route starting from atoms and molecules are methods like self-assembly of nanoparticles or monomer/polymer molecules, chemical or electrochemical reactions for precipitation of nanostructures, sol–gel processing, laser pyrolysis, chemical vapor deposition (CVD), plasma or flame spraying synthesis, and atomic or molecular condensation. The bio-assisted synthesis of nanomaterials also belongs to this approach (Sarikaya et al. 2003). However, despite being so promising and inviting, our ability to build things from the bottom up is fairly limited in scope. While we can assemble relatively simple structures, we cannot produce complex, integrated devices using the bottom-up approach. Any kind of overall ordered arrangement aside from repeating regular patterns cannot be done without some sort of top-down influence, like lithographic patterning. Until we have fully mastered the bottom-up synthesis approach, we will not be able to fully exploit its speed and accuracy. The important factor is that they are two different approaches to creating nanostructures which can be applied according to the specific needs for each application, often in a complementary way.

1.1   Nanofabrication by Top-Down Methods

Top-down methods were originally introduced as a new set of manufacturing tools to solve specific industrial problems in micromachining, such as the microelectro-mechanical systems (MEMS) devices. At the nanoscale though, these methods are generally not suitable for production on a very large scale because they presently encounter technological limitation and require extremely long and costly processes. Nonetheless, they represent some of the most common approaches now used to pattern surfaces and create three-dimensional (3-D) features on substrates. Over the past decades, there has been a wide variety of top-down manufacturing techniques that have been implemented using different media that span from chemical and electrochemical means to photofabrication, laser machining, electron- and ion-beam milling (IBM), plasma etching and powder blasting. Here, we describe only the most common techniques used for nano- or nanobio-related applications.

1.1.1   Nanolithography

Nanolithography is one of the most established techniques for making nanostructured materials and patterns. Nanolithography derives its name from the Greek words *nanos* (dwarf), *lithos* (rock), and *grapho* (to write), which literally means "small writing on rocks." This technique is based on depositing, etching, or writing
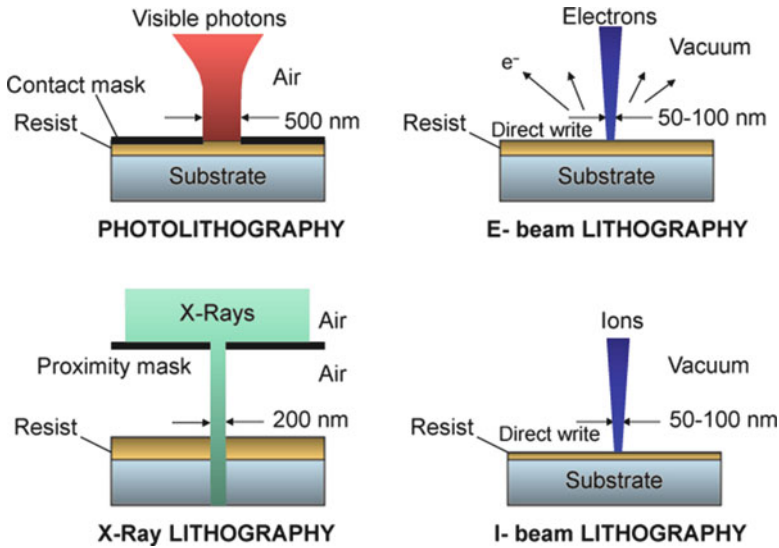
Fig. 1 Comparison of the available lithography techniques (image adapted from Brodie and Muray 1982)

on a surface features with dimensions on the order of nanometers. Lithography (Madou 2002) can be performed using light (optical- or photolithography), electrons (e-beam lithography), ions (i-beam lithography), or X-ray (X-ray lithography, LIGA) depending on the desired minimum feature size of the outputs (Fig. 1).

Photolithography is the most common and widely used technique in applications requiring micron and submicron feature sizes, like electronic, integrated circuits manufacturing, and microfluidic devices. It finds a lot of applications in bio-MEMS devices for its applicability to patterning of organic and inorganic materials, hydrogels, membranes, and ion-selective electrodes (Lambrechts 1992). With this technique, a design or pattern can be transferred at once on the surface of a device by exposure with light. Similarly, e-beam, i-beam, and X-ray lithography use the exposure to beams of electrons, ions, or X-ray for the design transfer.

All the lithographic techniques are multistep processes. As an example, we describe the steps followed in the specific case of photolithography of a Si/SiO$_2$ substrate (Fig. 2), keeping in mind that other techniques follow a very similar path. The first step is to coat the surface of the desired substrate with a thin polymer layer of a positive or negative photoresist (Fig. 2a), which is then covered by a predesigned, light-blocking mask and exposed to UV light (Fig. 2b). After exposure, the cured photoresist remains on the substrate as a protective coating, and the uncured portions are removed by the developer (Fig. 2c). This step is then followed by the etching of the substrate and the final removing of the photoresist (Fig. 2d). The type of resist chosen allows having a direct duplication of the mask used (as in the case of positive resist) or inverse of it (negative resist) exactly like in the case of film photography developing.
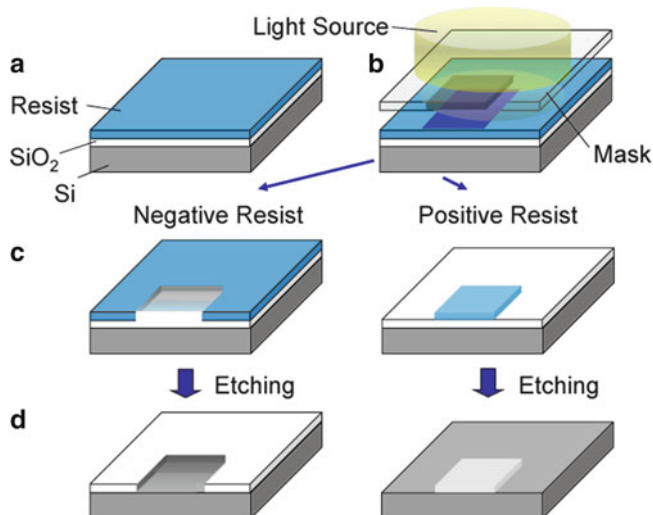
Fig. 2 Photolithography steps of surface patterning

The technical limits of photolithography are related to the wavelength of light used, which also determines the smallest feature sizes achievable. A limitation is dictated by the lens characteristics: if the used wavelength is made too small, the lens simply absorbs the light in its entirety and do not allow curing of the photoresist. This implies that photolithography cannot reach the superfine sizes of some other technologies, such as electron beam lithography, as the wavelength of typical light, even UV lasers, is somewhat limited. Small-wavelength UV light (deep UV techniques) can now be employed to obtain finer nanostructures.

A technology that allows for fabrication of much smaller nanostructures than photolithography is that of electron beam (e-beam) lithography. The use of an electron beam to draw a pattern nanometer by nanometer and expose an e-beam resist layer (usually made of a polymer) allows achieving incredibly small sizes (on the order of 20 nm). This technique, though, is much more expensive and time consuming than photolithography, as the e-beam exposure is carried out line-by-line or dot-by-dot, unlike the photolithography by which the whole area of, e.g., 12-in. diameter Si wafer can be exposed all at once. It takes orders of magnitude longer to complete the patterning with e-beam, so it is therefore difficult to sell the e-beam lithography for large-scale industry applications.

New nanolithography technologies are continued to being researched, leading to smaller possible sizes and access to more complex surface geometries and properties. Extreme ultraviolet (EUV) lithography, for example, is capable of using light at wavelengths of 13.5 nm and fabricating ~30-nm regime features. While hurdles still exist in this new field, it promises the possibility of sizes far below those produced by current industry standards. Another lithography technique proposed for the realization of nanoscale features is the direct-write, "dip-pen" nanolithography

(DPN) method. This technique relies on the use of a small tip to deposit molecules on a surface in a positive printing mode (Piner et al. 1999). It can achieve relatively small sizes, but currently it cannot go below 40 nm.

Similarly, scanning probe microscopes (SPMs) and atomic force microscopes (AFMs) are often used in relation to nanolithography. They allow imaging the fine details on surfaces without necessarily modifying it or inducing undesired damage. However, SPM and AFM can also be used to achieve precise etch, write, or print on surfaces obtaining nanometer or even single-atom resolution.

1.1.2 Dry Etching Techniques

In addition to the commonly used wet chemical etching for nanostructure fabrication, dry etching techniques (Lehmann 1991) are also used in the processing of semiconductor wafers in which the material is immersed in plasmas or discharges containing etching species. Dry etching is a process to remove material carried out in the gas phase that uses no aqueous solutions, such as an acid or base, hence the name "dry etching." Dry etching techniques can be either purely chemical (plasma etching), purely physical (ion beam milling, IBM), or a combination of both (reactive ion etching, RIE). The dry etch reactions are generally isotropic, taking place with equal rate in any direction. They can be very selective etching techniques for removal of one material relative to another, generally used in those applications in which directionality (anisotropy) of etching in not required, e.g., in resist stripping.

Plasma etching, like most dry etching techniques, is carried out in chambers in which high-energy electric and magnetic fields rapidly dissociate suitable feeding gasses, forming a plasma. When the surface of a material gets in contact with the plasma, it gets bombarded by molecules, ions, electrons, and photons which stimulate the expulsion of material from the surface, leading to atomically precise removal of materials. Commonly, plasmas can be created in different ways: using direct current (DC) electric fields, applying a radio frequency (RF) voltage source or using a microwave source. One of the most common plasma etching techniques used is RIE because of its relative simplicity and versatility. The RIE method requires process pressures varying between 10^{-3} and 10^{-1} Torr. A schematic diagram summarizing the differences between some of the dry etching methods is shown in Fig. 3 (Madou 2002).

Another common dry etching technique is IBM. In this case, the plasma source is decoupled from the substrate, which is placed on a separate third electrode in a triode setup. IBM can be employed to cover relatively large areas. A commonly used IBM method is focused ion beam (FIB), usually coupled to a high-resolution scanning electron microscope (SEM) to provide visual guidance during the writing process. FIB uses a beam of ions (usually Ga^{2+}) that gets narrowed in a very small area and it is employed as a direct writing tool for patterning holes, thinning samples, and depositing insulating or conductive material almost atom by atom. FIB milling is a powerful technique, now commonly considered a "mechanical" machining technique in which the classical metal drill bit used in all machine shops is
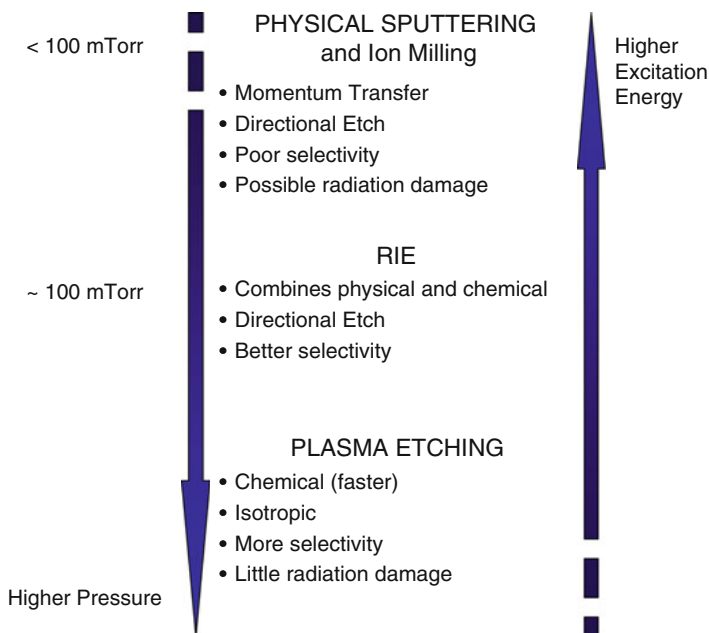
Fig. 3 Schematic diagram of the dry etching techniques spectrum (modified from Madou 2002)

replaced by the focused beam of highly energetic ions. However, the disadvantages of FIB are long processing time, the risk of undesired ion implantation, or damage to the sample.

1.1.3   Anodization

Anodization is a term that stands for anodic oxidation and it is "the process of growth of an oxide on the surface of a biased conductive solid (e.g., semiconductor wafer) immersed in a liquid electrolyte or gas. With this method, under specific process conditions, a thin, dense, barrier oxide of uniform thickness can be grown on several different metals" (Alwitt 2002). The properties of the grown oxide layer vary as a function of the materials selected and the process conditions. The commercial application of anodic oxidation extends to different surface coatings, mostly based on aluminum, tantalum, niobium, and zirconium. Recently, titanium has been also receiving increasing attention in bio-related applications (Oh et al. 2005). Aluminum in particular is commonly used because of its ease and versatility of processing. In addition to the creation of a thin barrier oxide, the anodization of aluminum, under specific conditions, can produce a thick oxide coating with a high density of vertically oriented nanopores (see Fig. 4) (Asoh et al. 2001). This porous substrate can then function as template for other assisted
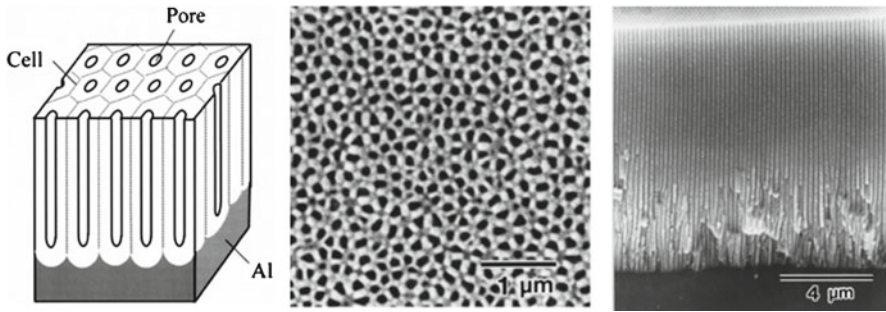
Fig. 4 Porous-anodized aluminum with neighboring cells of aluminum oxide merges together to form the matrix supporting the aligned nanopores. *Left*, scheme; *center*, top view transmission EM; *right*, side view SEM (Reproduced from Asoh et al. 2001 with permission from The Electrochemical Society, Inc.)

growth of CNTs or other nanomaterials or as substrate for several different applications, like corrosion prevention for automobile and aerospace structures or electrical insulation.

The anodization process takes place in a processing cell containing a cathode and an anode. Usually, the metallic sample of choice functions as the anode, and it is connected to the positive terminal of a DC power supply. A different conductive material, inert in the anodizing bath, functions as the cathode (some example materials used as cathodes are carbon, lead, nickel, or stainless steel), and it is connected to the negative terminal of the supply. When this circuit is closed, electrons are allowed to move, being attracted by the positive terminal, and permit the reaction between the ions present on the surface of the metal with the water molecules that forms the oxide layer.

1.2 Nanofabrication by Bottom-Up Methods

With the first decades of the new millennium, standard top-down techniques for nanomanufacturing have approached a critical limit in the ability to miniaturize components. Further size reduction has required innovative chemical routes. Eric Drexler, a pioneer in nanotechnology, in 1986 said, "our ability to arrange atoms lies at the foundation of technology." From this point on, several bottom-up approaches have blossomed, trying to build nanostructures one atom at a time, in the most efficient and precise way possible. On this aspect, nature is well-ahead of human engineering: proteins build structures quickly and effectively mastering the self-assembly of molecules as the basic building blocks of life. Nanoengineering is today putting a lot of effort in combining old, well-known chemical methods with new systems, even looking for inspiration from nature in bio-inspired structures and bio-assisted synthesis of nanodevices (Madou 2002).

One of the basic bottom-up techniques used to fabricate nanomaterials is chemical precipitation. This method allows producing nanoparticles of metals, alloys, oxides, etc. from aqueous or organic solutions following relatively simple and cost-effective steps. The drawback of this synthesis method, however, is the difficulty in the ability to control the distribution of particle size and shape, which limits the use of the produced nanomaterials in applications where a random particle distribution is undesired. Nanoscale precipitates can be obtained with various techniques, for example by a controlled phase transformation guided by the free energy diagrams or by controlling the solid-state diffusion, following a composite route approach, for example mixing two different materials and stirring them mechanically. Other approaches can be found in exploiting internal oxidation of materials, in thin film deposition of coatings, or sputtering.

The phase transformation approaches rely mostly on the rapid cooling of a solution that, not given enough time to reestablish equilibrium transformations, becomes supersaturated and precipitates into more stable and fine solid states. Common methods for rapidly cooling a solution (achieving cooling rates between 10^5 and 10^8 degrees per second) are splat cooling, quenching in salt baths, or melt spinning. These techniques are often used to obtain thin films and bulk materials with an extremely fine microstructure that influences positively its mechanical and electrical properties. Another common example of nanofabrication of nanoparticles or nanostructured films based on phase transformation of materials is called "spinodal decomposition." It is a spontaneous reaction driven by the free energy minimization of the material that guarantees the formation of a periodic structure of nanoparticles of equal sizes.

In order to control or limit the growth of nanomaterials produced with bottom-up methods, two techniques are conventionally used: arrested precipitation and physical restriction. Arrested precipitation relies on the exhaustion of one of the reactants or on the introduction of chemicals that would block the reaction. The physical restriction method relies on the confinement of the volume available during growth of the individual nanoparticles. Example of physical restriction includes using the micelle-reverse-micelle reactions or using templates (like, for example, the porous alumina described in the Sect. 1.1.3 above).

There are numerous bottom-up methods used to produce nanomaterials; we limit the rest of the chapter to the description of a few: sol–gel processing, CVD, self-assembly and bio-assisted synthesis, laser pyrolysis, electroplating, plasma or flame spraying synthesis, atomic or molecular condensation, and supercritical fluid synthesis.

1.2.1   Sol–Gel Processing

The sol–gel process in general is based on the transition of a system from a liquid "sol" (mostly a colloidal suspension of particles) into a gelatinous network "gel" phase. With this, it is possible to create at low temperature ceramic or glass materials in a wide variety of forms. It is a long-established industrial process that is

Fig. 5 System model for nanocomposites produced by sol–gel (modified from Luther 2004)
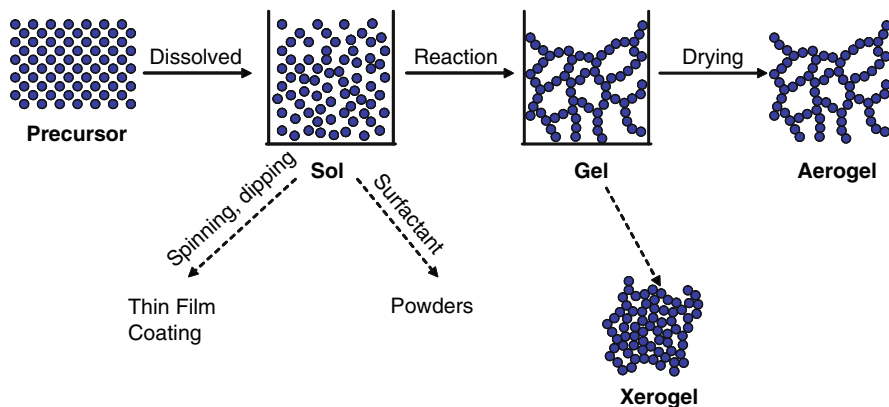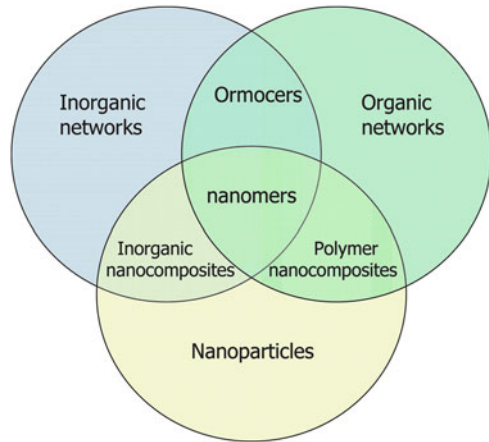

Fig. 6 Basic flow of a sol–gel process (modified from Madou 2002)

very cost-effective and versatile. It has been further developed in last year for the production of advanced nanomaterials and coatings but also in bio-MEMS application for the production of piezoelectrics, such as lead–zirconium–titanate (PZT) (Madou 2002) or membranes (Guizard et al. 1992). Sol–gel processes are well-adapted for oxide nanoparticles (Lakshmi et al. 1997) and composite nanopowder synthesis as well as for access to organic–inorganic materials. A summary of all the possible interlinked combination of organic and inorganic nanocomposites that are produced by the sol–gel method is provided in Fig. 5.

The basic flow of the sol–gel process is described in Fig. 6. The "sol" is prepared by mechanically mixing a liquid alkoxide precursor (such as tetramethoxysilane, TMOS, or tetraethoxysilane, TEOS), water, a cosolvent, and an acid or base catalyst at room temperature. During this step, the alkoxide groups are removed by the acid- or

base-catalyzed hydrolysis reactions, and networks of O–Si–O linkages are formed in subsequent condensation reactions. After this step, the treatment of the sol is varied depending on the final products desired. For example, spinning or dipping techniques can create thin film coating, and the exposure of the sol to a surfactant can lead to powders. Depending on the water–alkoxide molar ratio R, the pH, the temperature, and the type of solvent chosen, additional condensation steps can lead to different polymeric structures, such as linear, entangled chains, clusters, and colloidal particles. In some cases, the resulting sol is cast into a mold and dried to remove the solvent. This leads to the formation of a solid structure in the shape of the mold (e.g., aerogels and xerogels) with large surface-to-volume ratios, high pore connectivity, and narrow pore size distribution. They can be doped with a variety of organic/inorganic materials during the mixing stage to target specific applications.

1.2.2 Chemical Vapor Deposition

The CVD process is now probably the most common of all bottom-up approaches. It is used today to grow structures, like nanotubes, nanowires, and nanoparticles aided by several different types of chambers and growth-enhancing methods. The process consists of decomposing a gaseous precursor that adheres and accumulates onto a substrate (i.e., a silicon wafer or a quartz slide) (Jensen 1989). The presence of a catalyst, either predeposited on the substrate or provided in the gas feedstock, activates the chemical reaction between the substrate surface and the gaseous precursor. The CVD reaction can be achieved either with temperature (thermal CVD) or with plasma (PECVD). Plasmas can be obtained with DC electric fields, RF fields, or microwave fields, and their presence allows decreasing significantly the process temperature compared to the thermal CVD process. The presence of plasma also enables a more aligned or directional growth of the desired nanomaterials. A number of forms of CVD systems are in wide use and are frequently referenced in the literature. Some examples include atomic-layer CVD (ALCVD) in which two complementary precursors [e.g., $Al(CH_3)_3$ and H_2O] are alternatively introduced into the reaction chamber; metal-organic CVD (MOCVD) in which metal-organic precursors are used to obtain specific crystalline structures [e.g., tantalum ethoxide, $Ta(OC_2H_5)_5$, to create TaO nanostructures, and tetra dimethyl amino titanium (TDMAT) to create TiN]; laser-assisted CVD (LCVD); rapid thermal CVD (RTCVD) that uses heating lamps or other methods to rapidly heat the wafer substrate; ultrahigh vacuum CVD (UHVCVD); and more (Adams 1988; Jensen 1989).

The CVD process is widely used to produce CNTs and semiconductor nanowires, such as Si, GaN, and ZnO. CNTs have received much attention in the recent years for their potential application in several fields of bioengineering, from enhanced cell growth to biosensing, biomanipulation, and drug delivery. The most common synthesis routes for CNTs are CVD processes because they allow large-scale production of CNTs with high purity and good yield. To clarify how the CVD process works, we show a schematic diagram of a thermal CVD growth system used for the synthesis of CNTs (Fig. 7). In this example, a carbon-containing precursor is
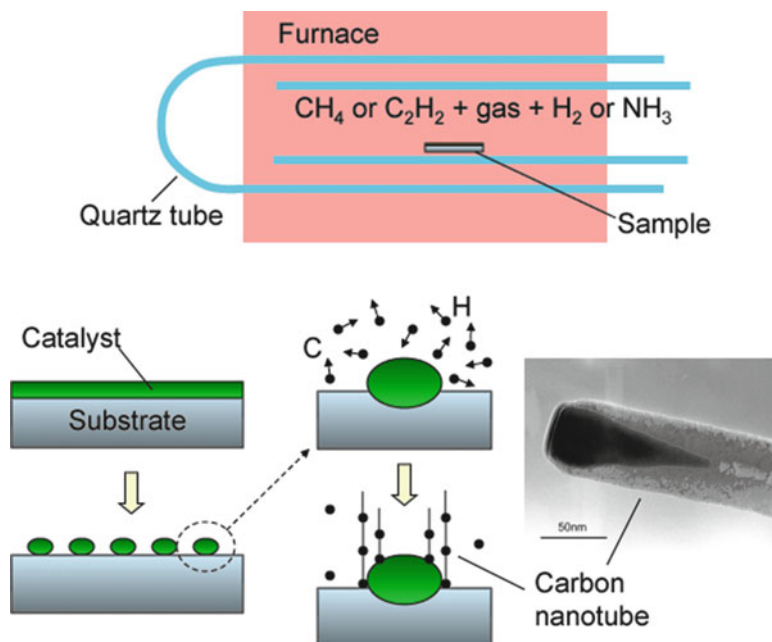
Fig. 7 Schematics of a thermal CVD furnace (*top*) and basic flow of a carbon nanotube growth process (*bottom*)

decomposed in the furnace at high temperature. The growth reaction is activated by the presence of a catalyst that can be deposited on the sample's surface (as in the figure) or in the gas mixture fed to the reactor (e.g., injecting ferrocene $C_{10}H_{10}Fe$). The most common carbon sources used in thermal CVD growth of CNTs are mixtures of ammonia (NH_3) and acetylene (C_2H_2) while the catalyst metal can be cobalt, iron, or nickel. The growth proceeds through several steps: first, the catalyst metal breaks up into islands at high temperatures and forms metal seeds for the reaction; then, the hydrocarbon gas supplied in the quartz tube decomposes creating floating C and H atoms; the floating atoms are then attracted by the catalyst seeds that become supersaturated and condensate forming ordered tube-shaped graphene sheets. The catalyst particle can then either stay attached to the substrate (base growth) or get pushed to the tip of the formed nanotube (tip growth) conferring different properties to the final structure. This type of growth process, although being relatively inexpensive, forms a randomly distributed array of CNTs with a complex hierarchical microstructure. Bundles of tubes grow vertically, but the tubes are intertwined and partially curled at small scales. This lack of complete alignment is normally overcome by the use of PECVD systems, which are a bit more complex and expensive, since they need additional constituents like electrodes, pumps, voltage supplies, etc.

1.2.3   Self-Assembly and Bio-Assisted Synthesis

As mentioned in the earlier section for the case of CNT growth, the assembly of nanostructures or nanoparticles in a periodically aligned fashion or in a functionally engineered geometry is often desired over random or disordered structures. Approaches for ordering or assembling nanostructures in a desired geometry have been widely investigated in the past and are still pursued in many different laboratories. Self-assembly provides the most convenient way for alignment, especially considering that at such small scales, single-structure manipulation and postgrowth rearrangement appear to be very expensive if not at all impractical. Chemistry and molecular biomimetics are the dominant fields leading the design of hybrid technologies combining the tools of molecular biology and nanotechnology. Molecular biomimetics combines the physical and biological fields to assemble nanomaterials using the recognition properties of proteins.

Researchers have found ways to process nanoparticles of a wide range of materials – including organic and biological compounds, inorganic oxides, metals, and semiconductors – using chemical self-assembly techniques. In these cases, molecules are attached, patterned, or clustered on specific substrates or to themselves using chemical and biomolecular recognition (e.g., the preferential docking of DNA strands with complementary base pairs). Other techniques, like micelle, reverse micelle, and photochemical and sonochemical synthesis, are also employed to realize one-, two-, and three-dimensional self-assembled nanostructures. Lately, viruses have also been used to assemble specific nanostructures (Mao et al. 2004). A review of the path to nanotechnology through biology is provided in (Sarikaya et al. 2003). It was shown that proteins, through their specific interactions with inorganic and other macromolecules, could be used in nanotechnology to control structures and functions just like they do biological tissues in organisms. Taking lessons from nature, polypeptides can be genetically engineered to specifically bind to selected inorganic compounds for specific applications, as outlined in Fig. 8 (Sarikaya et al. 2003).

The use of organic molecules is also a powerful technique in the synthesis and arrangement of semiconducting nanoparticles, for example quantum dots, used in biological labeling and tagging. One of the major issues in the synthesis of these isolated islands of materials is to prevent particles' agglomeration and coarsening. Micelle processes and organic ligands capping (TOPO) are synthesis methods based on the simple mixing of surfactant with desired materials' sources. With these methods, it is possible to synthesize and/or align a wide range of nanomaterials spanning from magnetic nanoparticles (i.e., Fe_3O_4 particles found in magnetotactic bacteria) to the bright CdS quantum dots used for bioimaging. Micelles are employed as small chemical-reacting chambers in which nanomaterials are synthesized in fixed-sized cells (Fig. 9). One of the first examples of nanoparticle synthesis using a reverse-micelle process was for fabrication of FePt nanoparticles (Sun et al. 2000) using a combination of oleic acids and oleyl amine to stabilize the monodisperse FePt colloids and to prevent oxidation. With this method, the authors showed that it is also possible to control the size of the synthesized nanoparticles and their composition,
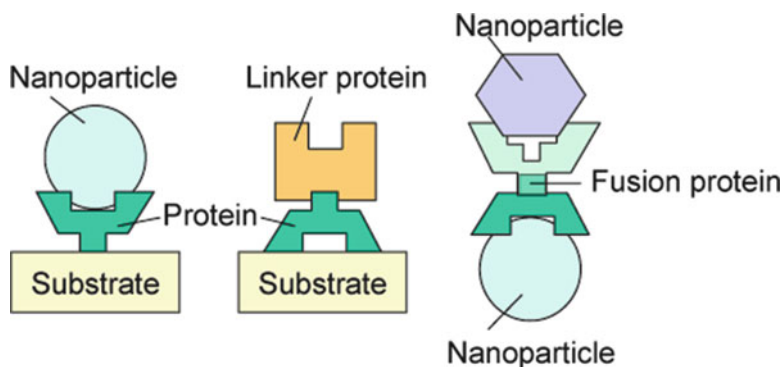
Fig. 8 Examples of molecular biomimetics (adapted from Sarikaya et al. 2003). Proteins can be used, for example, to bind nanoparticles to a functionalized substrate, to create linkers onto a specific substrate, or to connect different nanoparticles with each other
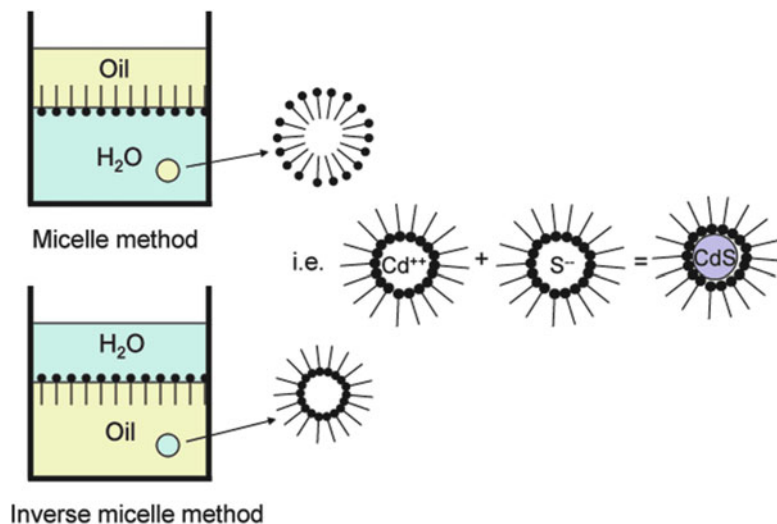

Fig. 9 Micelle and reverse micelle processing for synthesis of nanoparticles. On the *right*, it is shown the schematic for the synthesis of CdS quantum dots via reverse (inverse) micelle method

simply tuning the molar ratio of the solvents. Upon drying, the particles could self-assemble into three-dimensional superlattices that are chemically and mechanically robust and can, for example, support high-density magnetization reversal transitions.

The micelle process is also commonly used in the synthesis of semiconducting quantum dots. For example, CdSe nanoparticles capped with ZnS coating offer significant advantages as bioimaging agents as compared to the commonly used organic

fluorescent dye materials. Quantum dots are ~20 times brighter than the typical organic dye molecules, and their emission is ~100 times more stable to photobleaching than conventional fluorescent dyes (e.g., rhodamine). They can be made water soluble by chemical functionalization and can also be made specific cell- or organ-targetable via chemical or biological conjugation (Chan and Nie 1998; Akerman 2002). They can be imaged with a conventional fluorescence microscope. Besides the visible-emitting quantum dots, both near-IR and UV-emitting dots can also be synthesized.

The essence of bottom-up processing of nanotechnologies is mastered by nature in biological systems. For example, cells function on similar length scales to those of synthetic nanosystems. The molecules that operate and react in living cells are similar to the devices imagined for building nanosystems. Rather than using biological elements for the assembling of inorganic nanostructures, another way proposed for the synthesis of nanomaterials relies on incorporating the biological components themselves into artificial nanoscale structures and devices. Biological molecular motors, for example, have been incorporated into artificial structures, and the light harvesting complexes of plants or photosynthetic bacteria have been incorporated into synthetic membranes. From this point on, a vast array of applications that combine artificial structures with biological components can be envisioned and investigated, defining the future of nanobiotechnology.

1.2.4   Laser Pyrolysis

Laser pyrolysis is a technique that uses IR laser energy to decompose materials by heating gas-phase reactants in an oxygen-deprived environment. This method is used to produce extremely pure spherical nanoparticles with small-diameter, small-size distribution and with a low agglomeration level (Herlin-Boime et al. 2004). Such particles are used, for example, as precursors for surface treatments of ceramics. A similar technique, called laser ablation, is also used for the production of CNTs and nanowires.

1.2.5   Electrochemical Deposition/Electroplating

Electrochemical deposition is a technique used for manufacturing ordered arrays of nanomaterials, like quantum dots on a flat surface, for coating different surfaces or for patterning thin films (Allred et al. 2005). It represents one of the most powerful techniques that allow obtaining high-density and high-aspect ratio designs, with excellent reproducibility of the process and with great precision of the final products. Similarly to lithographic techniques, electrochemical deposition requires a polymer mask through which metal is deposited. Differently from other mask-based techniques like isotropic etching, ion milling, and RIE, electrochemical deposition avoids the problem of shadowing near edges or interfaces and builds structures atom by atom. The techniques rely on the presence of an electric field in a solution, which allows metal ions to discharge and conform to the smallest features of a mold.

Metal deposition can be carried on under two major techniques (Madou 2002): with current (pulsed or DC) or electroless (via catalytic, exchange, or electrophoretic reactions). The first method takes place in an electrolytic cell and involves a reaction under an imposed bias and current flow. In this case, important process parameters to control are pH, current density, temperature, agitation, and solution composition. The second technique, the electroless deposition, is based on a substantial oxidation reaction that replaces the dissolution of a sacrificial substrate.

1.2.6 Spraying Synthesis

Spraying processes are a viable technology for coating large area devices, and represent a simple and inexpensive alternative to produce sensing devices and nano-sized coating of surfaces (Mooney and Radding 1982). Spraying processes take place in a chamber containing a high-energy flame produced, for example, using plasma spraying equipment or using a carbon dioxide laser. A flow of reactants (gas or liquid in form of aerosols or a mixture of both) is forced into the flame and decomposes, forming particles by homogeneous nucleation and growth. The subsequent rapid cooling of the material completes the formation of nanoscale particles. In its simplest form, the reagent is simply dissolved in a carrier liquid and sprayed on a hot surface in the form of tiny droplet of ~100-nm diameter. In this case, the spray is formed from a liquid pressurized by compressed air or mechanically compressed through a small nozzle.

1.2.7 Atomic or Molecular Condensation

Condensation is a well-known technique used to produce primarily metal-based nanoparticles. The process takes place in a vacuum chamber: first, a bulk piece of metallic material is heated, melted, and vaporized to produce a stream of atomized matter. A gas flow (either inert or reactive) is then introduced into the reaction chamber to rapidly cool the metallic vapor. The cooled metallic atoms form condensed liquid nanoparticles, which then coalesce in a controlled environment, preserving a spherical shape with smooth surfaces. As the liquid particles are further cooled, they solidify and stop growing. The so-formed nanoparticles are very reactive and sensitive to agglomeration, which is prevented by adding coating or surfactants to keep them separated. If oxygen is present in the gaseous stream of the second chamber, the reaction results in the formation of metal oxide nanoparticles.

1.2.8 Supercritical Fluid Synthesis

Supercritical fluids are materials forced in a state above their critical point by regulating their pressure and temperature. They are obtained by vaporizing a liquid in a closed chamber, until the vapor phase becomes as dense as the liquid phase.

This supercritical state is very sensitive to the process' parameters and tunable. Methods using fluids in such a unique state are now used for the synthesis of nanoparticles, exploiting the rapid expansion of the supercritical solution, and they are also used for coating and filling nanostructures (Ye et al. 2004). The most common reaction media used in this process are supercritical carbon dioxide ($scCO_2$) and water (scH_2O) because they are nontoxic, nonflammable, inexpensive, and environmentally benign. In addition, they ensure much higher reaction rates and an increased selectivity. Although it has many advantages, supercritical fluid synthesis remains technically demanding, and it is still being adapted for large-scale production.

2   Conclusions

Top-down and bottom-up approaches are complementary in nanofabrication and comprise a numerous set of techniques. Their use and development have been at the center of material research for the past few decades, promising a wide range of applications in (non)biologically related nanotechnology fields. The use of a particular approach is often dictated by the specific need for a particular characteristic, shape, or composition of the nanomaterials for the different applications. Often, methods are used in combination to complement each other.

This chapter presented a brief overview of the most common techniques used for the fabrication of nanostructured bulk materials and nanomaterials with potential applications in biology and bioengineering fields. Most of the techniques described are continuously evolving even as we write and they represent a wide and active area of research in physics, chemistry, and engineering fields. A deep understanding of the mechanisms behind all the nanofabrication methods requires a broad and profound knowledge of the interdisciplinary aspects that regulate the properties of the materials and of the processes used. The details of the chemistry, physics, and mechanics of these methods go beyond the scope of this chapter and can be found in the related technical literature.

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