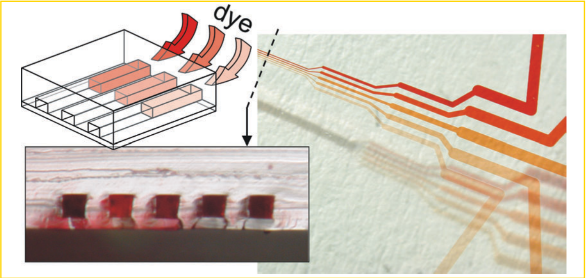
Defining features with fluids

PHOTOLITHOGRAPHY



Schematic diagram of microchannels in PDMS being filled with dye at the desired concentrations. Optical micrographs show the bottom view of a μFPM featuring five channels and its cross-section at the location indicated by the arrow. (Credit: Albert Folch, University of Washington, Seattle. © PNAS 2003.)

The miniaturization of mechanical or fluid devices, optical devices, self-assembling components, and even tissue-engineering scaffolds demands the ability to produce three-dimensional microstructures. Traditional photolithography, which relies on illumination of a photoresist layer through a photomask, is not ideal for this purpose because it produces features of a uniform height. A team of researchers led by Albert Folch at the University of Washington, Seattle, has devised a photomask based on microfluidics that overcomes this limitation [*PNAS* (2003), 10.1073/pnas.0435755100].

The light-absorbing feature of the microfluidic photomask (μFPM) is a fluid containing an UV-absorbing, water-soluble dye, the opacity of which can be tailored to an arbitrary number of gray-scales by altering the concentration. Instead of the all-or-nothing approach of conventional photolithography, features of multiple and/or varying heights can be produced using the μFPMs. Patterns can be reconfigured in a matter of seconds by simply changing the dye concentration in a given microchannel. The μFPMs can be used with standard photolithography equipment (contact mask aligner and collimated UV source) to pattern areas as large as 7.6 cm in diameter to a resolution (not optimized, the researchers stress) of 5 μm. The researchers created a sample pattern using a μFPM that would have required five different masks, five exposures, and four alignment steps using conventional photolithography.

Easy to fabricate because they simply consist of microfluidic channels in transparent sheets of polydimethylsiloxane (PDMS), the μFPMs present a low-cost alternative to current gray-scale photolithography. If handled with care, the photomasks can be reused endlessly, say the researchers. There are disadvantages to the method compared with existing gray-scale photolithography, but applications not bound by these constraints, such as lab-on-a-chip, cell-based microchannel bioreactors, polymer molding, and waveguides, could benefit from such an inexpensive and versatile method. "The designs are constrained by the ability to route fluids to small areas," explains Folch, "but if you are willing to pay that price, our technique offers an infinite number of gray-scale levels for a dollar. And as a bonus, you have a lot of freedom in changing the photomask design using the same microfluidic channels."

The photomasks are particularly well suited to cellular applications, suggests Folch, who believes that the technique could be useful for addressing basic neurobiology and cell biology questions.

Tip of a magnetic iceberg

MAGNETIC MATERIALS

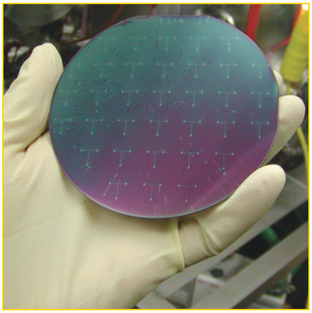
As bits of data get smaller, and more bits are packed onto disk drives, more reliable magnetic sensors are required that are both smaller and more sensitive. Magnetic sensors based on the ballistic magnetoresistance (BMR) effect have been attracting attention in recent years for just this purpose.

Last summer, Susan Hua and Harsh Deep Chopra from the State University of New York at Buffalo reported an unusually large BMR effect in Ni nanocontacts at room temperature in fields of only a few hundred oersteds. "We first saw a large effect of over 3000% resistance change in small magnetic fields," explains Chopra. "That was just the tip of the iceberg. These results point to the beautiful science that remains to be discovered."

In their latest work [*Phys. Rev. B* (2003), **67**, 0604XXR], Hua and Chopra report a BMR effect of 70 000-100 000%, significantly larger than their previous highest result. Compare this with current disk drive technology, which relies on detecting resistance changes as low as 20%. Because the new sensors produce much more distinct and reliable signals, they could allow bit size to be shrunk dramatically.

The researchers put their success down to a new method, developed by Salah Boussaad and Nongjian Tao at Arizona State University, that enables reliable fabrication of the nanoscale Ni whiskers, which form the nanocontacts between two electrodes. The great advantage of Boussaad and Tao's self-terminating, electrodeposition technique is that it allowed Hua and Chopra to determine the resistance of the Ni nanoscale whiskers in advance. It also eliminates the possibility of introducing any chemical layers during fabrication and is much easier than the previously used method.

The researchers are now working on creating nanoconductors between electrodes made by photolithography. Such patterned devices (as shown) are required for technological applications, but also allow detailed and controlled investigation of the BMR effect, says Chopra.



A wafer containing numerous devices. (Courtesy of Susan Hua and Harsh Deep Chopra, State University of New York.)

Nanostructure cleans up

NANOTECHNOLOGY

It comes as no surprise that nanostructures have unusual properties, but recent work by Danish researchers point to a new, and very useful, application.

Jeppe Lauritsen from the University of Aarhus, together with colleagues from the Technical University of Denmark and Haldor Topsøe A/S, used scanning tunneling microscopy (STM) in combination with density functional theory calculations to study two-dimensional MoS_2 nanoclusters [*Nanotechnology* (2003), **14**, 1]. These nanoclusters demonstrate the ability to hydrogenate and break up thiophene (C_4H_4S) molecules.

"Surprisingly, the nanocluster edges behave just like ordinary catalytically active metals," explains Lauritsen. "This metallic reactive behavior is shown in the STM images as a bright rim extending around the cluster edges."

The researchers' calculations indicate that the behavior can be traced to one-dimensional metallic states located along the edges of the otherwise insulating, triangular nanoclusters. Associated with sulfur-saturated active sites on the MoS_2 nanoclusters, the electronic states could represent a new class of active centers in heterogeneous catalysis, suggests Lauritsen.

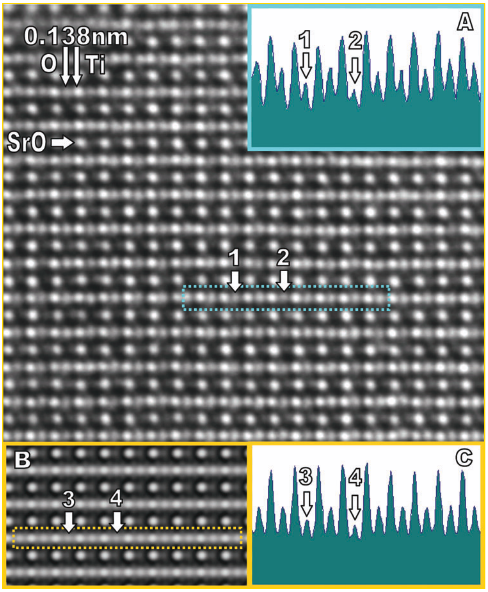
The chemical activity of MoS_2 nanoclusters is particularly significant, add the researchers, because it could have implications for the clean up of sulfur-containing molecules from oil products in the hydrodesulfurization process. "Throughout the last century, most catalysts have been developed by costly time-and-error methods," says Lauritsen. "Nanotechnology is about to change this, since we can now build and view matter directly on the nanoscale."

Solid-state observatory

MICROSCOPY

Researchers from the Institute of Solid State Research in Juelich, Germany have achieved the first atomic-scale resolution images of oxygen atoms, and their vacancies, in crystals [Jia, C. L., *et al.*, *Science* (2003), 299, 870]. Knut Urban and his team used a standard transmission electron microscope with a field emission gun at 200 kV but equipped, crucially, with an electromagnetic hexapole system. This allows the spherical aberration of the objective lens to be corrected. By overcompensating the objective lens aberration to produce negative C_S (spherical aberration coefficient) values, the researchers found that the contrast improved dramatically. The contrast is also inverted, so that the atoms appear bright on a dark background. Images of the perovskite $SrTiO_3$ (shown) reveal atomically resolved oxygen atoms – and, in regions where the contrast appears lower, oxygen vacancies. Similar results were also achieved for the superconductor $YBa_2Cu_3O_7$, including the imaging of a stacking fault. This is a particularly remarkable achievement, says John C. H. Spence in an accompanying article [*Science* (2003), 299, 839]. The results promise, he adds, a true atomic-resolution solid-state chemical observatory for the future.

The technological importance of these materials makes these results particularly significant, say the researchers. Perovskites have great potential as high-permittivity dielectrics in microelectronics and nonvolatile memory devices, but the properties of such devices are highly sensitive to the local oxygen content. The same is also true for cuprate high-temperature superconductors.



Experimental TEM image of $SrTiO_3$ [011]. Atoms appear bright on a dark background, and the oxygen is atomically resolved. At positions 1 and 2, the oxygen contrast is lower, as shown quantitatively in the inset intensity trace (A). Inset (B) shows a calculated image and a corresponding intensity trace (C), which agree well with the experimental data. (© 2003 AAAS.)

Pyramids get smaller

NANOTECHNOLOGY

A team of researchers from Pacific Northwest National Laboratory and Washington State University led by Lai-Sheng Wang have created nanoclusters of Au atoms [*Science* (2003), **299**, 864] with unusual properties.

"As a metal particle's size approaches the nanometer dimension, all of its properties change," explains Wang. "The properties not only differ from those of the bulk material, but also show strong dependence on the particle's size and shape." The researchers amply demonstrate this with a tetrahedral, 20-atom Au cluster that they created using laser vaporization. The technique creates a range of clusters, but Au_{20} were selected using time-of-flight mass spectrometry and analyzed with photo-electron spectroscopy.

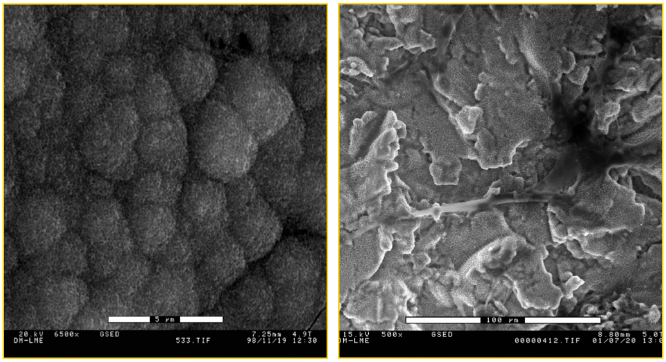
Au_{20} is a unique molecule, say the researchers, with atomic packing similar to bulk gold, but very different properties. "We observed experimentally that the 20-atom Au clusters exhibit large energy gaps," says Wang, of 1.8 eV. The implications are that, unlike metallic bulk Au, Au_{20} would be chemically inert, highly stable, and exhibit insulating or semiconducting behavior. For comparison, Au_{20} has a larger energy gap than C_{60} and a comparable electron affinity.

"We hope that the chemical inertness of the Au pyramids will allow chemists to use them as potential building blocks to assemble new materials," says Wang. For example, they suggest that the four (111) faces of the pyramid could provide ideal sites to bind molecules for catalysis.

With the growth in the aged population and the need to extend average individual healthspan, biomaterials have an increasingly important role in the development of new generation medical devices, drug delivery systems, and medical diagnostic technologies. This column seeks to provide an insight into the latest developments in biomedical materials and related technologies through brief synopses and expert commentaries of recent presentations, publications, and patents. Andrew Lloyd, University of Brighton.

Treating surfaces for implants

SURFACE MODIFICATION



SEM micrograph (left) showing the apatitic surface formed on the NaOH-treated Ti in simulated body fluids. SEM micrograph (right) showing human osteoblasts adherent to the NaOH-treated Ti. (©2002 Elsevier Science Ltd.)

The longevity of dental implants and orthopedic prostheses is dependent on the establishment of an effective integration of the metal implants with the surrounding bone. Previously, various groups have focused on the need to modify the surface of Ti implants to improve osteointegration. One approach has been to use plasma-sprayed hydroxyapatite coatings, which provide a bioactive surface for prosthetic materials. A key limitation of the use of plasma-spray techniques is the lack of control over chemical composition and crystallinity of the surface coated material. In 1996, Kokubo and coworkers [*J. Am. Ceram. Soc.* (1996) 79, 1127] demonstrated that the deposition of bone-like apatite on Ti surfaces could be achieved by treating the surface with NaOH and incubation of the surface treated material in simulated body fluids.

More recently, Gil *et al.* [*Mat. Sci. Eng. C* (2002) 22, 53] have repeated these initial experiments using environmental scanning electron microscopy (ESEM) to monitor the growth of a dense bone-like apatite layer on a Ti surface in aqueous solution. The researchers demonstrate the initial formation of a microporous alkaline titanate hydrogel during the alkaline/heat treatment. On incubation in simulated body fluids, the alkali titanate layer hydrates to give a TiO_2 hydrogel and releases an OH ion into the surrounding solution. The localized rise in pH appears to cause a change in the local solubility of apatite, leading to deposition on the substrate surface (see left-hand image).

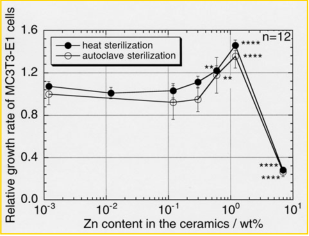
The researchers were also able to demonstrate that this simple chemical method of modifying the surface of Ti improves osteoblast adhesion and differentiation *in vitro* (see right-hand image). This low temperature approach clearly offers a useful alternative to plasma-spray techniques for the deposition of bioactive apatite-like coatings onto orthopedic and dental implants.

Zinc stimulates bone formation

TISSUE ENGINEERING

In recent years, there has been increasing interest in the use of pharmacological agents to promote tissue regeneration around implanted biomaterials. Although a wide range of ceramics and metals employed as hard tissue replacements integrate favorably with bone tissue, major benefits would accrue from the development of materials that enhance bone integration or inhibit bone resorption.

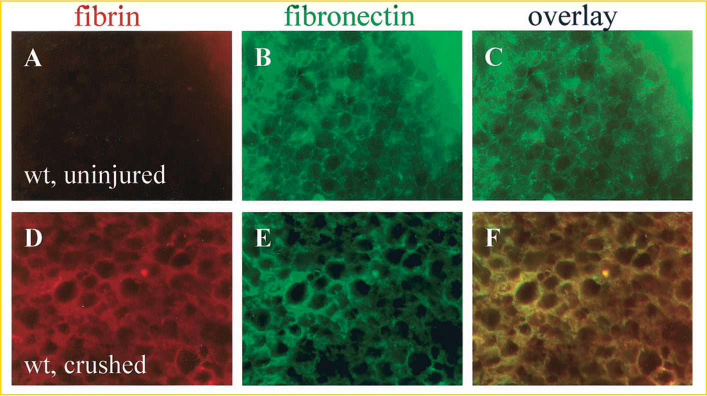
Zn is well established as an essential nutrient known to have stimulatory effects on bone formation at low concentrations. It is also known to be cytotoxic at higher concentrations. The use of Zn as a stimulant for bone formation in ceramic biomaterials will, therefore, require the controlled release of low levels of Zn over a prolonged period. Ito and coworkers have recently investigated the potential incorporation of Zn into calcium phosphate bone cements as a means of achieving promotion of bone formation at the cement interface [*Mat. Sci. Eng. C* (2002) **22**, 21]. Tricalcium phosphate is an ideal slow release carrier as the Zn ions can be incorporated directly into the crystal structure of this resorbant biomaterial. The group prepared Zn calcium phosphate ceramics and composite ceramics of Zn calcium phosphate and hydroxyapatite by sintering at 1100°C, and demonstrated that Zn release could be sustained for over 50 days in NaCl solution. The composite materials containing 1.2% Zn were shown to increase the proliferation of osteoblastic MC3T3-E1 cells (as shown) and alkaline phosphatase activity of rat stromal cells in culture. Examination of the materials in a rabbit model demonstrates that the incorporation of 0.316% Zn into the composite materials increases bone formation by 51% relative to Zn-free control samples after four weeks. These studies suggest that the incorporation of low levels of Zn into tricalcium phosphate bone cements may offer an advantage in respect of enhancing bone formation.



Relative growth rate of osteoblastic cells. (© 2002 Elsevier Science Ltd.)

Regulating nerve regeneration

TISSUE ENGINEERING



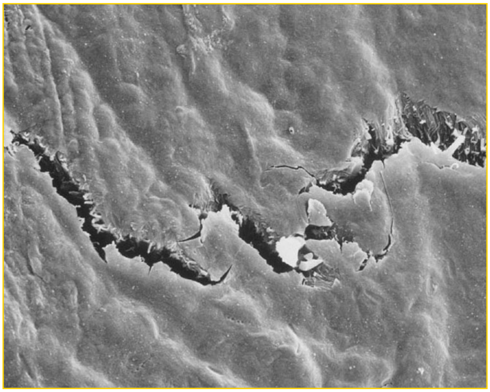
Cross-sections of uninjured and crushed sciatic nerve showing the relative amounts of fibrin and fibronectin present. (© 2003 Elsevier Science Ltd.)

Fibrin-based matrices have been suggested for a number of tissue engineering applications. In nerve regeneration, Schwann cells are responsible for both axonal elongation and remyelination of the damaged nerve. Akassoglou and coworkers from The Rockefeller University and New York University Medical Center have investigated the effects of fibrin deposition on Schwann cell migration after sciatic nerve damage in mice [*Neuroscience Letts.* (2003) 338, 185].

***In vivo* studies demonstrate that both fibrin and fibronectin deposition occurs at the site of nerve injury (as shown above). Examination of the migration of Schwann cells on fibrin containing increasing concentrations of fibronectin demonstrates that fibrin inhibits the migration of Schwann cells in a dose-dependent fashion. This has important implications for the potential use of fibrin-based biomaterials in certain tissue engineering applications.**

Mechanistic understanding of bone

MECHANICAL PROPERTIES



High-magnification scanning electron micrograph showing evidence of uncracked-ligament bridging, which contributes to toughening mechanisms in bone. (© 2003 Nature Materials.)

Understanding how bone fractures is essential to predicting – and possibly preventing – damage associated with age or disease. One of the difficulties is that bone has a complex hierarchical microstructure that affects the initiation and propagation of cracks, which lead, ultimately, to fracture. It is assumed that fracture in bone is strain-controlled, i.e. ductile fracture akin to the process seen in structural steels at high temperatures, but there is little or no experimental evidence for this. Researchers from the University of California at Berkeley and San Francisco believe they have provided this evidence [Nalla, R. K., *et al.*, *Nature Materials* (2003), nmat832].

Using a double-notched, four-point bend test, Robert O. Ritchie and his coworkers were able to examine the nature of a local fracture event in human cortical bone at the onset of failure. Scanning electron micrographs reveal that not only is the fracture strain-based, but also confirm the marked anisotropy in the toughness of bone. One such extrinsic toughening mechanism that is commonly seen in metal-matrix composites and intermetallics, uncracked-ligament bridging, is demonstrated and quantified.

Compatibility of blood-contacting biomaterials

COATINGS

A wide range of biomaterials are used in blood-contacting applications. A key challenge for such materials is the ability to resist thrombus formation as a result of activation of the fibrinolytic blood clotting cascade.

In recent years, various groups have demonstrated that both poly(ethylene oxide) (PEO) and biomimetic phosphorylcholine-based phosphobetaine coatings offer a means of improving the hemocompatibility of blood-contacting biomaterials. Various groups have shown that sulfobetaine polymers may be used as alternatives to the phosphobetaine coatings for reducing inflammatory cell adhesion.

Recent work by Zhang Jun and coworkers from Nanjing University in China [*Colloids and Surfaces B: Biointerfaces* (2003) **28,** 1] has extended this to the evaluation of sulfobetaine polymer coatings to improve hemocompatibility of segmented poly(ether urethane), a widely used biomaterial.

The researchers hydroxylated segmented poly(ether urethane) using potassium peroxosulfate and grafted N,N-dimethyl-N-methacryloxyethyl-N-(3-sulfopropyl) ammonium onto the surface using ceric ammonium nitrate as an initiator.

In vitro biological assessment demonstrates that the sulfobetaine-functionalized materials are resistant to both platelet adhesion and thrombus formation following incubation in whole blood. These sulfobetaine-based coatings, therefore, offer a potential alternative to PEO and phosphorylcholine-based material as thromboresistant coatings for blood-contacting applications.

Golden future for DNA scaffold

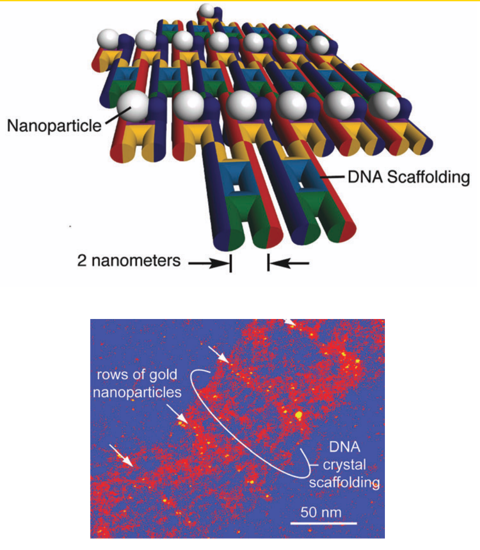
NANOBIOTECHNOLOGY

The ability to design DNA structures by specifying base sequence and the 0.34 nm base pair separation in a DNA helix allows the controlled, bottom-up assembly of components on an ultra-small scale.

Researchers at the University of Minnesota, New York University, and Brookhaven National Laboratory have assembled ordered arrays of Au nanoparticles using DNA crystals as a scaffold [*J. Nanoparticle Res.*, (2002), 4, 313]. The authors hope that the use of DNA crystals in this way will provide a new basis for nanoscale assembly in many fields, including nanoelectronics, nanorobotics, and nanomaterials. "The technique is well suited to laying out locally interconnected circuitry," says Richard A. Kiehl, who led the work.

The researchers followed the approach of previous work that used double-crossover (DX) molecules as building blocks to form two-dimensional DNA crystals (Liu, *et al.*, *J. Am. Chem. Soc.*, (1999), 121, 917; see also *Materials Today*, (2003), 6 (1), 24). By including one DX molecule with a covalently linked Au nanoparticle, the researchers were able to construct two-dimensional structures with an ordered pattern of nanoparticles.

Other groups have used DNA strands to program assembly or glue nanoparticles together, but Kiehl believes that these are prone to structural flaws in comparison with the virtually perfect arrangement of molecules within a DNA crystal.



Schematic diagram (top) and scanning transmission electron microscope (STEM) image (bottom) of arrays of Au nanoparticles on a DNA crystal scaffold. (Top image: courtesy of Richard A. Kiehl. Bottom image: © 2002 Kluwer Academic Publishers.)

The next challenge is to demonstrate the potential of the technique for assembling nanoelectronic structures. "We're working on instrumentation to do electrical characterization of Au nanoparticles and other nanocomponents on DNA," says Kiehl. These nanocomponents could include nanotubes or molecules. "We hope to show, for example, that DNA doesn't interfere with the electrical functioning of the nanocomponents."

H_2 storage promise is dealt a blow

CARBON NANOTUBES

After initial excitement surrounding suggestions that carbon nanotubes (CNTs) could be used to store considerable amounts of H_2, recent research has produced results that are less encouraging.

A number of recent reports have found very different values for the H_2 storage capacity of CNTs. The data have been obtained using various experimental techniques, with both single-walled and multi-walled nanotubes (SWNTs and MWNTs) prepared by different methods.

The importance of CNT storage capacity to a future H_2 economy and the disparity of recent results has led C. N. R. Rao and coworkers at the Jawaharlal Nehru Centre for Advanced Scientific Research and the Centre for Energy Research in India to carry out systematic H_2 absorption measurements with various CNT samples [*J. Mater. Chem.*, (2003), **13**, 209].

The researchers used SWNTs, MWNTs, and bundles of aligned MWNTs prepared by different methods. Samples treated with acid to remove the catalyst particles were also investigated. Aligned MWNTs showed the best results, where a H_2 storage capacity of 3.7 wt% was measured in absorption experiments performed at 300 K. This maximum capacity is well below the storage capacity reported by others.

The group used different sample weights and pressures to confirm the validity of the results. Electrochemical measurements gave similar values to the gas storage experiments, adding further credence to the data.

Rapid synthesis

DIAMOND

Polycrystalline diamonds are harder and tougher than single crystal diamonds and are in demand for cutting and polishing applications. However, naturally occurring polycrystalline diamond is rare, while synthetic versions are slow to produce using current methods.

Researchers at Ehime University and Sumitomo Electric Industries in Japan report a new method for the production of ultrahard diamond [Irifune, T., *et al.*, *Nature* (2003) **421**, 599]. They produced pure, sintered polycrystalline diamond by direct conversion of graphite under static high pressure and temperature.

The researchers observed the transformation of a pure, polycrystalline graphite rod into a transparent, colorless phase in only a few minutes at 2300-2500°C and a pressure of 12-25 GPa. X-ray diffraction and Raman spectroscopy showed that sample was cubic diamond. Transmission electron microscopy of a thin section revealed granular crystals 10-20 nm in size, and electron diffraction indicated that these were randomly oriented.

The Knoop hardness of the samples varied between 110-140 GPa, compared to values of 60-120 GPa for single crystal diamond. The polycrystalline sample is as hard, if not harder, than single-crystal diamond and, furthermore, this hardness is constant throughout the sample.

This new synthesis method can be compared favorably to chemical vapor deposition of diamond thin films and sintered diamond produced commercially using a binder of metal or inorganic material. The researchers believe improving their synthesis method could give rise to better quality products and new industrial applications.