tionation across phase boundaries, even in a homogeneous mantle (*15*). At the 670-km boundary that involves garnet and perovskite and potentially separates stable convection levels, substantial Sm/Nd fractionation therefore may take place.

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Growth of Diamond from Atomic Hydrogen and a Supersonic Free Jet of Methyl Radicals

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The growth of small (~10-micrometer) diamond particles (on 0.1- or 0.25-micrometer seed crystals) using an effusive glow discharge nozzle for $H\cdot$ and a separate supersonic pyrolysis jet for $\cdot CH_3$ is reported. Laser micro-Raman, scanning electron microscopy, and x-ray photoelectron spectroscopy data are presented as evidence that well-crystallized diamond is indeed formed. Resonant multiphoton ionization spectroscopy is used as a diagnostic for the gas-phase chemistry indicating that the radical sources are clean and quantitative and that there is no detectable interconversion of $\cdot CH_3$ to C_2H_2 under the conditions of the experiment. Diamond growth is found at substrate temperatures greater than or equal to 650°C with no marked increase in the rate of growth up to 850°C. Acetylene does not give good quality diamond under similar conditions.

The growth of diamond from mixtures of hydrocarbon gases and H_2 on application of some source of energy, for example, thermal filament–assisted chemical vapor deposition (CVD), plasma CVD, and combustion flame CVD, is now an established technology (*1*, *2*). Considerable progress has been made since the early reports of low-pressure growth of polycrystalline diamond films by Derjaguin and co-workers (*3*), Eversole (*4*), and Angus (*5*), with the emphasis now shifting to the rational optimization of growth rates, reduction of defects and nondiamond impurities, and epitaxial growth (*6*) on diamond or nondiamond substrates. To further these ends, there needs to be a better understanding of the basic chemistry of diamond growth, especially with respect to the key atomic and molecular species involved in the growth process.

Mass balance and kinetic considerations have implicated CH_3 and C_2H_2 as the two most likely candidates for the immediate gas-phase precursors to diamond (*7*, *8*). In most systems, an excess of atomic hydrogen has also been found to be necessary. However, in the typical deposition system, as represented by filament-assisted CVD reactors (*2*), the concentrations of reactive species are coupled by gas-phase reactions that proceed as fast as or faster than transport of the species to the growth surface. The separate contribution of each species is difficult to assess and must be inferred from the composite results by indirect methods. Some of these issues are being addressed in fast-flow experiments, that attempt to decouple the separate contributions of different species to the overall growth process (*9*, *10*). Furthermore, the two distinct events of free radical generation in the gas phase and free radical reaction on the surface are also coupled at the relatively high pressures in the typical reactor in such a way that it is difficult to separately optimize each process.

We report the growth of diamond on small diamond seeds under conditions where the hydrocarbon radicals and atomic hydrogen are produced separately and come to interact only at the surface of the growth substrate. A clean, well-characterized, supersonic pyrolysis jet of $\cdot CH_3$ and an effusive glow-discharge jet of $H\cdot$ were separately, but simultaneously, impinged onto a seeded substrate that could be held thermostatically at temperatures from 450° to 900°C. Mixing of the two flows occurs primarily in the region near the surface where the supersonic free jet of $\cdot CH_3$ in helium is disrupted by interaction with the substrate. In this way, the concentrations of reactive species are decoupled from each other and can be independently varied, and the process of radical production is decoupled from the process of surface reaction of the radicals.

Clean, specific generation of $\cdot CH_3$ was done by a continuous-flow version of the supersonic jet flash pyrolysis technique (*11*, *12*). Azomethane, produced by the HgO oxidation of dimethylhydrazine and purified by trap-to-trap distillation, was seeded into 250-torr He (<0.1% seed ratio) and expanded through a heated Al_2O_3 tube nozzle. The terminal 1.0 cm of the 0.6-mm inner diameter tube was heated to ~1000°C by 40-W dc current passing through a molybdenum coil in an annular space between the tube nozzle and another Al_2O_3 jacket. The jacketing was necessary to suppress contamination of the deposition substrate with Mo, which we found to occur for bare coils. We optimized methyl radical production by monitoring the $\cdot CH_3$ concentration using the $3p^2A_2'' \leftarrow X^2A_2''$ transition (*11*, *13*) at 333.4 nm by 2+1 resonant multiphoton ionization (MPI) spectroscopy. We further characterized the methyl radical source by vacuum-ultraviolet photoionization mass spectrometry of the pyrolysate (*14*) using the Nd^{3+}-YAG ninth-harmonic at 118.2 nm. Production of $\cdot CH_3$ from azomethane was found to be clean and quantitative. Under the conditions of the pyrolysis, the ratio $\cdot CH_3/C_2H_2$ was found to be greater than 100:1, with the limit set by the detectability of acetylene by means of the $^1\Delta_g \leftarrow X^1\Sigma_g^+$ origin band (*15*) at 274.9 nm in 2+1 MPI. We estimate the $\cdot CH_3$ flux to be at least 10^{16} radicals per second delivered to a circular spot (at the nozzle-substrate distance) about 0.5 cm in diameter.

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Atomic hydrogen was produced by a glow discharge (4 mA/400 V) run through an effusive jet (1.0-mm nozzle) expansion of a 0.25% mixture of H_2 in 10-torr He. The production of atomic hydrogen was optimized by monitoring the H· concentration with the $2^2S_{1/2} \leftarrow 1^2S_{1/2}$ transition (*16*) at 243.13 nm in 2+1 MPI. Though the H· could be maximized with respect to total pressure and dilution, an absolute H· flux was not determined.

The two nozzles were oriented with the $\cdot CH_3$ source normal to the substrate surface and the H· source tilted by 45°. The nozzle-to-surface distance was typically 0.5 cm, which places the surface before the extrapolated position of the Mach disk for the supersonic jet expansion (*17*). The substrate was either a thin molybdenum foil or a silicon wafer clamped to the top of the foil. The temperature of the foil was monitored by Cr/Al thermocouple attached to the bottom face of the foil out of the gas flow. Resistive heating of the foil was needed to achieve the 450° to 900°C temperature range, although a temperature rise of up to 200°C occurred when the pyrolysis jet and discharge nozzle were both run. The substrate was prepared by sonication in an aqueous suspension of 0.1-μm or 0.25-μm diamond powder for times up to 1 hour and then examined by scanning electron microscopy (SEM). The seed crystals were observed to be uniformly distributed over the substrate with typical particle-to-particle separations exceeding several tens of micrometers. Use of other solvents, such as acetone, caused aggregation of the diamond particles. Sonication for shorter periods of time (for example, 5 min) gave a lighter loading of seed crystals on the harder substrates like Mo, which would "blow off" when exposed to a supersonic free jet. Typical deposition times are about 20 hours. Deposited samples were imaged by SEM, subjected to x-ray photoelectron spectroscopy (XPS) for elemental analysis, and assayed for diamond by laser micro-Raman at 514.5 nm (30 mW).

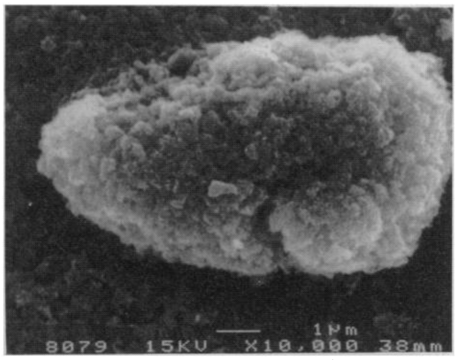

Fig. 1. SEM image at ×10,000 of a 10-μm diamond particle grown on a 0.1-μm diamond seed crystal (on Si) from $\cdot CH_3$ and H· at a substrate temperature of 850°C. Note the 1-μm scale marker below the image.

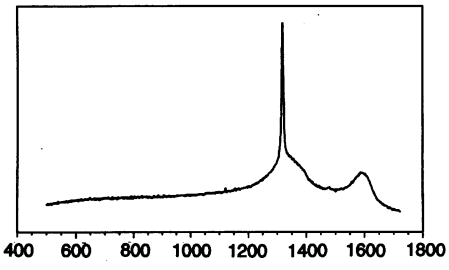

Fig. 2. Laser micro-Raman spectrum (514.5 nm, 30 mW) of a ~10-μm diamond particle from the same sample shown in Fig. 1. The diamond peak at 1320 cm^{-1} is shifted from the usual 1332 cm^{-1} because of local heating effects associated with the tightly focused laser and small particle size. Some graphite is evident from the broad peaks at 1350 and 1600 cm^{-1}.

After about 20 hours of deposition at substrate temperatures of either 650° or 850°C, SEM examination (×1500) of the substrate revealed numerous ~10-μm particles which contained no detectable elements other than carbon by XPS. Under higher magnification (×10,000), the particles appeared polycrystalline with some showing cracks and fissures presumably arising from relief of mechanical stress associated with thermal cycling (Fig. 1). The laser micro-Raman spectrum of one of the particles (Fig. 2) exhibited an intense, sharp (~10 cm^{-1} full-width–half-maximum) peak indicative of well-crystallized diamond. The characteristic Raman peak for diamond was shifted slightly (from 1332 to 1320 cm^{-1}) by local heating effects associated with the intense laser irradiation of an ~1-μm^2 area on a small diamond particle which itself was in poor thermal contact with the underlying substrate. The effect has been seen previously and is less pronounced for lower laser power. Evidence for graphitic inclusions also appeared in broad Raman peaks at 1350 and 1600 cm^{-1}. The rest of the substrate surface was covered with a thin layer of graphitic carbon, indicating that saturation of the surface with carbon had been achieved. Other salient results are the following: (i) No growth occurred at substrate temperatures below 500°C. The size of the diamond particles was maximum at 650°C and did not increase further as the substrate temperature was increased to 850°C. (ii) If the pyrolysis was turned off (azomethane instead of $\cdot CH_3$), or if the glow discharge was turned off (H_2 instead of H·), there was no diamond growth. (iii) If there was no seeding of the substrate with small diamond crystals, there was no diamond growth. With seeding of the substrate, Mo and Si gave closely similar results. (iv) Substitution of acetylene for azomethane (with no pyrolysis) gave poorly defined particles whose Raman spectra indicated primarily graphite. The small diamond signal in those spectra could be attributed to the 0.1-μm seed crystals.

The expectation that $\cdot CH_3$ and H· are necessary (and sufficient, it would appear) for the growth of diamond is borne out. This result is important in light of the rapid interconversion of $\cdot CH_3$ and C_2H_2, in the presence of H·, that occurs in the usual filament-assisted CVD process (*2*). Whereas ^{13}C isotopic tracer studies by Chu *et al.* (*18*) under conditions of partial scrambling have suggested that $\cdot CH_3$ is the more important growth species, some fast-flow depositions (*10*) have suggested that C_2H_2 contributes as well. The present results are a direct probe of the relative importance of $\cdot CH_3$ versus C_2H_2 under specialized, but well-characterizable, conditions.

Because radical production is decoupled from radical reactions in the present experiment, the separate effect of substrate temperature can be assessed. In the pyrolysis jet deposition, the gas-phase radical concentrations and temperatures are independent of substrate temperature, at least until the molecules come within a few mean free paths of the surface. The observation that growth occurs at 650°C, the very lower end of the range seen in other studies, and does not improve as the substrate temperature is raised is generally supportive of predictions made by the "generic" kinetic model described by Butler and Woodin (*19*). In that model, the lower limit for diamond growth (with regard to temperature) is set by the kinetics of hydrogen abstraction to create surface radical sites. A steady-state treatment of the kinetics finds that the fraction of open surface radical sites is dependent on temperature (and not [H·]) in the limit of large H· flux and begins to become large between 500° and 600°C, below which there can be no addition of $\cdot CH_3$ to the surface. The absence of a large increase in the overall rate of diamond growth as the surface temperature is increased from 650° to 850°C (as is typically observed in CVD growth) may be attributed to decoupling of the H· (and $\cdot CH_3$) concentration and temperature in the gas phase from the surface temperature in this low-pressure experiment.

The similarity of results for Si and Mo, the absence of growth on unseeded substrates, and the relatively uniform particle sizes seen in SEM all suggest that whereas growth of diamond on existing diamond does occur under our conditions, spontaneous nucleation of new crystals on unseeded parts of the substrate does not. This observation should be of importance to the homoepitaxial growth of diamond on diamond substrates where it is imperative to suppress secondary nucleation events. The observation of growth without new nucleation suggests that deposition on a single-crystal diamond substrate may produce further single-crystal material.

We have demonstrated that diamond can be grown from $\cdot CH_3$ and $H\cdot$ with an arrangement by which the reactive species are segregated until they impact the growth substrate. Growth occurs at the lower end of temperatures seen in filament-assisted diamond CVD and requires the presence of diamond seed crystals.

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An in Vitro System for Adhesion and Fusion of Maize Gametes

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The development of in vitro fertilization systems in flowering plants is important for understanding and controlling the mechanisms of fertilization. Here a method is described in which isolated maize gametes fuse. In a medium containing 5 mM calcium chloride, sperm and egg cells adhere for several minutes and then fuse within 10 seconds. The method is specific to male-female gamete pairs and results in 80 percent fusion, whereas fusions with other combinations of gametic and mesophyllic cells are less frequent. Eggs fertilized in vitro do not fuse with additional male gametes, which suggests that a block to polyspermy exists.

Studies using in vitro fertilization systems in animals and lower plants have led to a better understanding of the critical first steps of fertilization, including species-specific recognition (*1*), binding and fusion of gametes (*2*), intracellular signaling (*3*), and blocking of polyspermy (*4, 5*). In contrast, little is known about the process of double fertilization that is characteristic of flowering plants (*6*). During double fertilization, two sperm cells, each without a cell wall, are released from the pollen tube into a cell of the female gametophyte, the synergid. The two sperm cells move through the degenerated synergid, enter the intercellular space between the two adjacent female gametes—the egg cell and the central cell—and fuse with them to form the embryo and a nutritive tissue, the endosperm (*7*). All of these steps occur within the embryo sac embedded in the ovule, which is itself enclosed in the flower ovary. Thus the main barrier to studying fertilization has been the inaccessibility of the female gametes and the zygotes. This has restricted our knowledge of double fertilization largely to microscopic observations. For better understanding of the molecular and cellular events of fertilization, methods that allow the in vitro fusion of gametes are important. Over the past few years, techniques to isolate gametes from the paternal and maternal structures have been described (*8*), and more recently, in vitro fusion of gametes has been achieved in maize by use of electrofusion (*9*). This approach leads to karyogamy (*10*) and fertile plant regeneration (*11*). However, electrofusion does not allow studies of gametic recognition and fusion (*12*), because mesophyll protoplasts as well as sperm cells can be induced to fuse with the eggs (*13*). We have therefore developed a gametic fusion system that more closely resembles the in vivo situation.

We isolated gametes from the inbred maize line A188 (*14*). Male gametes were released from freshly collected pollen grains (*9, 15*) after a pH shock in 0.5 M mannitol. Egg cell and central cell protoplasts were isolated from the ovules by enzymatic treatment followed by manual microdissection (*9, 16, 17*). Pairs of male and female gametes isolated under sterile conditions were then combined in a simple fusion medium composed of 0.5 M mannitol containing 1 mM, 5 mM, or 10 mM calcium chloride ($CaCl_2$) (*18, 19*). The fusion medium was delivered in droplets of 3 to 4 μl under mineral oil on a glass cover slip with siliconized edges (*9, 20*). The isolated gamete protoplasts were transferred into the droplets with a manual tube clamp (Micropipex, Assistent 558/E) connected to glass microcapillaries with Teflon tubing (*21*). The male and female gametes were then moved into contact by use of glass microneedles (*21*).

At each of the three $CaCl_2$ concentrations studied, we observed adhesion (*22*) of the sperm cell with the egg cell protoplast, followed by fusion (Fig. 1) (*23*). In 1 mM $CaCl_2$, 15.4% of sperm-egg pairs fused; in 5 mM $CaCl_2$, 79.7% fused; and in 10 mM $CaCl_2$, 66.7% fused. When fusion did not occur, a new sperm cell was introduced in each droplet and placed in contact with the egg. After two new attempts at fusion, 15.4% of the egg cell protoplasts fused with sperm cells in 1 mM $CaCl_2$, 96.9% in 5 mM $CaCl_2$, and 79.2% in 10 mM $CaCl_2$. The lower percentage of fusions in 1 mM $CaCl_2$ suggests that there is a calcium requirement for gamete fusion, as is the case in mammals (*2, 24*).

The fusion of gametes occurred rapidly. In 5 mM $CaCl_2$, adhesion (*22*) generally lasted 3 min 50 s (±1 min 50 s), after which time most of the fusions occurred in less than 10 s. Within the first 15 min after

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