at a time when the atmospheric level of this isotope was high. The authors previously tested the validity of their approach in other tissue samples known to be renewable (intestine and skeletal muscle) and showed that the technique detected ^{14}C accurately in these cells, even in specimens collected 50 years after DNA replication had occurred (*11*).

In the present study, the authors found numerous ^{14}C-positive nonneuronal cell types, such as glial and endothelial cells, in all cortical specimens. However, no neurons were labeled in the several structurally and functionally distinct areas of the frontal, parietal, temporal, and occipital lobes of the neocortex of adult specimens. For optimal precision, the authors examined more than 100 million cells in most samples. Because ^{14}C assays theoretically may leave a minute number of labeled cells undetected, the authors also labeled DNA with 5-bromo-2′-deoxyuridine (BrdU, a thymidine analog) in cortical tissue from volunteers afflicted with terminal cancer. These patients were injected with BrdU and died between 4 months and 4 years afterward. Although cortical tissue from these patients exhibited labeling of neuronal and nonneuronal cells (glial and granule cells, respectively) in the hippocampus (*12*), none of the labeled cells in neocortical tissues were neurons.

The lack of addition of new neurons to the adult human neocortex agrees with the progressive decrease in adult neurogenesis that occurred during evolution. Although it was shown that new neurons are added to the mammalian hippocampus and olfactory bulb (*13*) in nonhuman primates, their number is both relatively and absolutely smaller than in rodents (*14*, *15*), and the migratory stream that supplies neurons to the olfactory bulb is absent and/or undetectable in the adult human (*16*). The decrease in neurogenesis in adult primates contrasts with the large addition and/or renewal of neurons in the brains of nonmammalian vertebrates such as fish, frogs, reptiles, and birds, which usually correlates with their capacity for regeneration (*13*). Why is such a useful capacity lost during mammalian evolution? One hypothesis is that preservation of acquired information within a permanent population of cortical neurons may be more valuable for the survival of an organism than the introduction of "naïve" neurons that have not been exposed to experience (*17*). Thus, lack of neurogenesis in the cerebral cortex may be a critical step in the evolution of mental prowess in *Homo sapiens*.

The absence of neurogenesis in the adult human cortex is not a reason to decrease research efforts that seek ways of replacing neurons lost due to trauma, stroke, infection, degeneration, or aging. Various therapies, including cell transplantation, are being explored precisely because neurons are not normally regenerated. However, at present, we lack the knowledge of how to direct endogenous or transplanted neural stem cells to their desired position, and to assume their correct phenotype, functionality, and ability to form cellular connections. Accumulating evidence suggests that the timing of neuron formation, laminar specificity, and phenotypes of cortical neurons are encoded within lineages of individual progenitor cells in the ventricular zone (*18*), making transplantation of stem cells a formidable task. Furthermore, the resistance of mature neurons to multiplication appears to be so powerful that, remarkably, no malignancy of mature neurons has occurred spontaneously or been induced by carcinogens in the adult cortex, although glioma and other types of brain tumors are frequent. Thus, identification of the mechanism that inhibits neuronal proliferation in the adult cortex may provide new insight into how to prevent unwanted cell divisions in various carcinomas.

References and Notes

1. R. D. Bhardwaj *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12564 (2006).
2. J. B. Angevine, R. L. Sidman, *Nature,* **192**, 766 (1961).
3. M. B. Luskin, C. J. Shatz, *J. Comp. Neurol.* **242**, 611 (1985).
4. P. Rakic, *Science* **183**, 425 (1974).
5. E. Gould *et al.*, *Science* **286**, 548 (1999).
6. R. D. Kornack, P. Rakic, *Science*, **294**, 2127 (2001).
7. D. Koketsu *et al.*, *J Neurosci.* **23**, 937 (2003).
8. S. S. Magavi, B.R. Leavitt, J. D. Macklis, *Nature* **405**, 951 (2000).
9. R. S. Nowakowski, N. L. Hayes, *Science* **288**, 771a (2000).
10. P. Rakic, *Nat. Rev. Neurosci.* **3**, 65 (2002).
11. K. Spalding *et al.*, *Cell* **122**, 133 (2005).
12. P. S. Eriksson *et al.*, *Nat. Med.* **4**, 1313 (1998).
13. G. Kempermann, *Adult Neurogenesis* (Oxford University Press, Oxford, 2006).
14. R. D. Kornack, P. Rakic, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5768 (1999).
15. R. D. Kornack, P. Rakic, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4752 (2001).
16. N. Sanai, *Nature* **427**, 740 (2004).
17. P. Rakic, *Science* **227**, 1054 (1985).
18. Q. Shen *et al.*, *Nat. Neurosci.* **9**, 743 (2006).

10.1126/science.1131713

CHEMISTRY

Dendrimers at Work

Brett Helms and E. W. Meijer

Highly branched dendrimer molecules are being used increasingly for such biomedical applications as molecular imaging and drug delivery.

Dendrimers are synthetic polymers with a highly branched architecture and nearly perfect molecular structure (see the figure). They are identical, monodisperse macromolecules that expose many end groups at their globular periphery. In academic laboratories, dendrimers have been used as light- and energy-harvesting materials, for drug delivery, as catalysts, and in optoelectronic applications. Yet they have not been widely introduced commercially. This situation is about to change, with several dendrimers now entering the market.

Paul Flory first envisaged dendrimers in 1941 (*1*), but four decades passed before chemists could produce these materials with high precision (*2*–*4*). These early dendrimers were prepared in an iterative sequence of steps to achieve growth and branching, starting from a central core unit; this approach would later be termed "divergent." A paradigm shift occurred in the early 1990s, when Hawker *et al.* constructed dendritic materials from the periphery inward (*5*). This "convergent" approach provided access to dendrimers of unprecedented purity and offered greater control over the placement of functional groups in the macromolecule than could be achieved with divergent methods.

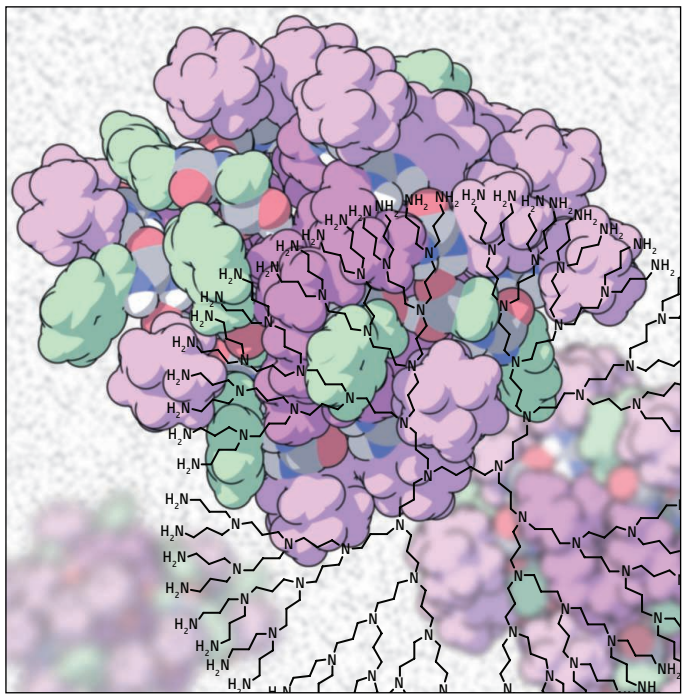
Since then, many new dendrimers have been reported, using both divergent and convergent synthesis methods (*6*), but commercialization has been slow to follow. It was not until 1993 that de Brabander-van den Berg *et al.* described a divergent industrial-scale synthesis that allowed the preparation of kilogram quantities of the poly(propylene imine) dendrimer (*7*).

Today, a wide range of dendrimers are commercially available and tuned for specific applications. However, for large-scale applications, the focus has switched to hyperbranched polymers, which are easy to manufacture. They share some of the key properties of dendrimers, including a large number of end

The authors are in the Laboratory of Macromolecular and Organic Chemistry and Department of Biomedical Engineering, Eindhoven University of Technology, 5600 MB Eindhoven, Netherlands. E-mail: e.w.meijer@tue.nl

groups and a globular conformation, but have an ill-defined microstructure with irregular branches. They are increasingly used in coatings, as viscosity mediators, and to prevent gas hydrate–ice crystal formation in oil pipelines (*8*). For such bulk applications, hyperbranched polymers are more cost-effective than dendrimers and have become the industry standard.

For smaller scale applications, especially in the biomedical sector, many companies have continued to develop products based on dendrimers. With their unique and perfect molecular composition, dendrimers are more likely than other polymers to meet the strict regulatory requirements for polymer-based materials intended for use in humans. Moreover, the biocompatibility and toxicity of dendrimers can be regulated by synthesis, especially through the judicious choice of functional groups at the periphery (*9*).



Dendrimer structure. The molecular structure of a dendrimer resembles a tree. The globular molecules expose many end groups at their periphery.

For example, Starpharma (Australia) has used a dendrimer as the active pharmaceutical ingredient in VivaGel, a topical vaginal microbicide for HIV prevention (*10*). Their polylysine dendrimer displays 32 naphthalene disulfonate units at its periphery. The dendrimers prevent infection by binding to receptors on the viral coat of HIV-1, which in turn prevents the virus from entering target cells. VivaGel has entered phase II clinical trials in humans. The company expects to introduce the product to the market by 2008.

Dendrimers have also been used to improve existing molecular imaging technologies. Schering AG (Germany) introduced Gadomer-17 as a magnetic resonance imaging (MRI) probe. The polylysine dendrimer is functionalized at its periphery with gadolinium chelates, which act as intravascular contrast agents. Gadomer-17 has been particularly effective in magnetic resonance angiography and tumor differentiation, because its moderate size limits the material to the vascular space and slows renal clearance.

Building on the success in MRI contrast agents, self-assembly may play an increasingly important role in emerging technologies for molecular imaging. Encapsulation of guest molecules in dendrimers via self-assembly was first demonstrated more than 10 years ago (*11*). Since then, researchers in biomedical imaging have used the void spaces within dendrimers to localize contrast agents for MRI. Guest molecules have included paramagnetic gadolinium chelates, such as Magnevist (*12*), and molecular cages for localizing polarized xenon, a relatively new contrast agent (*13*). In these applications, the contrast agent is bound to a generic, nontoxic dendrimer through relatively weak noncovalent interactions. The assembly must be stable only for the duration of the MRI experiment, after which the assembly falls apart into nontoxic species that can be easily filtered through the kidneys.

Encapsulation of guest molecules in dendrimers can also be used to deliver therapeutic agents throughout the body. The dendrimer can improve the solubility, efficacy, toxicity, and targeting ability of many drugs. For example, Dendritic Nanotechnologies uses the voids in a polyamidoamine dendrimer to host cisplatin or carboplatin anticancer drugs (*14*). The dendrimer-drug conjugates are active against aggressive tumor models that are resistant to cisplatin at its maximum tolerated dose via intravenous administration. They are less toxic than the unbound drug, water soluble, and stable on storage.

In 1993, Szoka and co-workers showed that dendrimers can be used as vehicles to introduce a gene into a cell (*15*). In this case, positively charged dendrimers cause negatively charged DNA to condense into highly compact structures, resulting in supramolecular assemblies that can be delivered to cells (*16*). Commercial kits using similar cationic dendrimer-based materials are available from Qiagen (Germany). Their SuperFect reagent should provide the means for various high-throughput transfection schemes that use recombinant DNA technology for drug discovery and development (*17*–*19*).

Dendrimer formulations used in humans must conform to current Good Manufacturing Practice (cGMP) to ensure the correct identity, strength, quality, and purity. These regulatory hurdles are often difficult to overcome. In contrast, some ex vivo applications can enter the market quite rapidly. For example, Dade Behring has used dendrimer technology in a biosensor for cardiac markers. Their Stratus CS provides quantitative determination of chemical indicators of impending heart damage from whole blood or plasma within 15 min with excellent sensitivity and reproducibility (*20*). The U.S. Army Research Labs are exploring similar techniques for anthrax detection (*21*). Dendrimer technology has also moved beyond the life sciences. Cambridge Display Technology has developed an energy-efficient dendrimer-based diode that emits red light (*22*).

As specialty polymers, dendrimers can be prepared with the precision of small organic molecules, yet they behave like macromolecules. Early successes in dendrimer technology have relied on covalent modification of dendrimers to generate active pharmaceutical ingredients. In the future, more products may use the encapsulation of biologically active agents in dendrimers. Formulations based on simple universal dendrimers may conform more easily with regulations and may be the key to the further success of these materials.

References

1. P. J. Flory, *J. Am. Chem. Soc.* **63**, 3083 (1941).
2. R. G. Denkewalter *et al.*, U.S. patent 4,289,872 (1981).
3. D. A. Tomalia *et al.*, *Polym. J.* **17**, 117 (1985).
4. G. R. Newkome *et al.*, *J. Org. Chem.* **50**, 2003 (1985).
5. C. J. Hawker *et al.*, *J. Am. Chem. Soc.* **112**, 7638 (1990).
6. D. K. Smith, *Chem. Commun.* **2006**, 34 (2006).
7. E. M. M. de Brabander-van den Berg *et al.*, *Angew. Chem.* **105**, 1370 (1993).
8. P. Froehling, *J. Polym. Sci. A* **42**, 3110 (2004).
9. N. Malik *et al.*, *J. Control. Release* **65**, 133 (2000).
10. Y.-H Jiang *et al.*, *AIDS Res. Hum. Retroviruses* **21**, 207 (2005).
11. J. F. G. A. Jansen *et al.*, *Science* **266**, 1226 (1994).
12. H. Gries *et al.*, U.S. patent 4,957,939 (1990).
13. J. L. Mynar *et al.*, *J. Am. Chem. Soc.* **128**, 6334 (2006).
14. N. Malik *et al.*, U.S. patent 7,005,124 (2006).
15. J. Haensler *et al.*, *Bioconjugate Chem.* **4**, 372 (1993).
16. M. Tang *et al.*, *Bioconjugate Chem.* **7**, 703 (1996).
17. T. Hudde *et al.*, *Gene Ther.* **6**, 939 (1999).
18. J. Zhou *et al.*, *Chem. Commun.* **2006**, 2362 (2006).
19. F. Tack *et al.*, *J. Drug Target.* **14**, 69 (2006).
20. S. Altinier *et al.*, *Clin. Chem.* **46**, 991 (2000).
21. R. Yin *et al.*, *Polym. Mater. Sci. Eng.* **84**, 856 (2001).
22. D. Ma *et al.*, *Appl. Phys. Lett.* **81**, 2285 (2002).

10.1126/science.1130639