NET TIP
INTERNET BROWSERS
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Turning the Big Five-O

Spend a few minutes looking at World Wide Web pages and commonly used patterns emerge. You see similar colors used in Web pages, similar fonts, and similar layouts. Ten people viewing the same site will see identical images, because sites do not adapt to the user or retain preferences set during a previous visit. The current Web style seems to have reached a steady state over the past year. However, this is about to change.

Why? Because the core technology behind the building of Web pages will change dramatically with the introduction later this year of the next generation of browser software. In December 1994, Netscape's first browser was offered to the public. As of September 1998, the two dominant companies in the browser field, Microsoft and Netscape, had released fourth generation products. Now, by significantly enhancing the languages and the browser itself, the 5.0 versions of browsers will transform the Web into a sophisticated development platform. The sky is truly the limit with these new tools.

What features can we expect to see in the 5.0 browsers? For Netscape Communicator 5.0, there is little information to report. The company has decided to build this next version in an open way, by assembling contributions from individual developers around the world who will design components of the new browser. Netscape hints that the product will be available by late 1998.

Microsoft, however, has just released a working preview version of Internet Explorer 5.0 (IE5) to developers, and a final version is slated for late 1998. IE5 was constructed at Microsoft and has not been a public effort. IE5 is a powerful piece of software that will radically alter a Web page in three general areas: document structure, user preferences, and speed.

The document structure of today's Web pages is derived from Hypertext Markup Language (HTML has evolved to version 4.0). HTML will not be replaced, instead it will be augmented. IE5 will allow developers to position all items and figures on a page precisely, to construct one page from the content of multiple URLs, and to build a page quickly from data packets coded in Extensible Markup Language (XML). These changes describe a fraction of the possibilities that will become available.

The user experience will change with the increased use of Dynamic HTML (DHTML), first released in Internet Explorer 4.0. DHTML in IE5 will make all elements on a page (the graphics, text, buttons, forms, and so forth) dynamic. With this ability, Web developers can make pages that change to fit the preferences of the user. For example, an electronic journal could be designed in which the sections of articles could be rearranged based on viewer preferences. Would you want to see only abstracts? Click on a button to show only the abstract component of the articles. Would you want to see the results sections first? Click on a button to switch the order of the pieces of the article. Also, with the new technology of IE5, the browser will retain your layout preference for future visits.

The IE5 browser will also be faster. Each Web page must be processed for viewing, or rendered, by code within the heart of the browser. This code has been overhauled in IE5 and optimized for speed. For instance, for table structures used to position text and graphics, the time it takes to visualize a page after transfer to your computer could become 1/100 of what it is now.

Scientists will soon have desktop tools to distribute and format the most complex data and information. For more information on the next generation of browsers, see the links collected at www.mednav.com/zone/science.

—ROBERT SIKORSKI AND RICHARD PETERS

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TECHSIGHTING
IMAGING
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The Art of Bubble Making

Magnetic resonance imaging (MRI) reflects detection of the relaxation of ^{1}H nuclei after a biological specimen has been exposed to a magnetic field. Because such specimens are mainly water, MRI has had enormous utility. When the proton signal is weak, as it is in blood, contrast agents that magnify the relaxation of ^{1}H (such as gadolinium–diethylenetriamine pentaacetic acid) are usually added. For visualizing the lungs, which lack a significant proton signal source altogether, laser-polarized gases (^{3}He and ^{129}Xe) are used. Then the gas itself becomes the source of the signal. These particular gases offer at least a 20-fold increase in signal-to-noise ratio compared with ^{1}H MRI images. In addition, these gases do not occur naturally in the body, and thus there is no background signal. Such contrast agents have been used with great success for visualizing anatomical air space. Furthermore, ^{129}Xe dissolves reasonably easily, so researchers have been able to view tissues such as blood, muscle, and brain, either by letting the gas dissolve in the pulmonary blood flow or by dissolving it in lipid vesicles. Helium-3 has a larger magnetic moment and currently a higher degree of polarization than ^{129}Xe and thus would offer a 10-fold improvement in signal over ^{129}Xe. Unfortunately, ^{3}He has very poor solubility (one-tenth to one-hundredth that of ^{129}Xe), so it has been exclusively used for air space imaging of biological samples.

A report published last month in the *Proceedings of the National Academy of Sciences U.S.A.* may promote the use of ^{3}He for visualizing fluid and solid tissues (*1*). In a preliminary feasibility study, researchers at the Duke University Medical Center were able to generate microbubbles of ^{3}He and to view the vasculature of live rats. To generate these microbubbles, they used two 10-cm^3 syringes connected via a three-way stopcock. The gas generated by the laser polarizer was collected in a holding cell. Then, 2 cm^3 of it was quickly withdrawn from the cell into the first syringe, which had been evacuated to prevent paramagnetic oxygen in the syringe from depolarizing ^{3}He. Next, 8 cm^3 of fluid in the second syringe was manually flushed back and forth between the syringes, producing a suspension of ^{3}He microbubbles. This was done rapidly because the microbubbles quickly rise out of the suspension. For this study, the time between agitation and image acquisition was reduced to about 10 s. The authors measured the suspended microbubbles with a Coulter counter. Mean bubble diameter was 31.8 μm with a mean standard deviation of 10.4 μm. The large distribution of bubble size was due to the hand agitation technique, and more homogeneous distribution can be expected with other mechanical methods (such as sonication). The size of the bubbles was also significantly larger than the ideal size of 8 μm (the size of a red blood cell), but the authors decided this size was acceptable for this in vivo feasibility study. They also noted that the ^{3}He suspension differed significantly in different fluids: in all, they tried six fluids and found that Hexabrix (Mallinckrodt) offered by far the most homogeneous distribution of bubbles and the best signal-to-noise ratio.

They injected this suspension of ^{3}He into both the arterial and venous systems

of live, anesthetized Harlan–Sprague-Dawley rats. Images were collected in less than 30 s and revealed excellent delineation of all major vessels [see figures 3 and 4 of (*1*)]. Although the report is encouraging, it raises questions for future studies. First, can smaller ^{3}He bubbles be generated while significant polarization is maintained? Also, concerns about embolism lead to safety questions about microbubbles of such a poorly soluble gas in the body. Finally, the researchers injected large amounts of the fluid into the rat vasculature (about 25% of the animal's body weight), making the current approach impractical in the clinical setting. The authors believe that the bubble concentration and the degree of ^{3}He polarization can be increased, so that in the future less material can be used.

Despite these significant limitations, the approach offers hope that very high-resolution angiographic images with little background signal may be safely made in the future. In addition to revealing blood vessels, ^{3}He microbubbles could also be used to study myocardial function without the risks of nuclear medicine approaches. Also, the ^{3}He microbubbles are short-lived so they would not generate a significant signal from a second-pass effect as the dye recirculates.

—RICHARD PETERS AND ROBERT SIKORSKI

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TECHSIGHTING
ELECTROSTIMULATION
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Muscular Electronics

A number of techniques are under investigation for the effective transfer of DNA into tissue. In the field of gene therapy, one interesting technique that does not involve the use of viral vectors is injection of plasmid DNA directly into skeletal muscles. This approach is theoretically attractive for several reasons. It is simple; the intramuscular injection is like a vaccination. It does not involve viruses, it seems to have no adverse effects, and many delivery sites are available in the muscular system. Last, the transferred plasmid produces a protein that is secreted by the muscle systemically. This approach has been used successfully in animal models for the production of growth factors and cytokines such as erythropoietin and interleukin-5 (IL-5). Typically, the muscle is treated several days before the injection with a toxic compound (cardiotoxin or bupivacaine), because after an injection of plasmid, regenerating muscle tends to produce more protein than healthy muscle. The secreted proteins are produced for several months after one injection; however, the overall low level of expression of the transferred gene has precluded the use of this approach in human gene therapy.

A group of researchers in Japan have just published a modification of the intramuscular approach, attempting to improve the expression level by applying an electrical current around the intramuscular injection site (*1*). In this way, the technique of electroporation was applied to living cells with the expectation that it would increase uptake of plasmid DNA and result in greater expression of the transferred gene. During electroporation, cells are stimulated in vitro with electric discharges as the cells are bathed in a solution of plasmid DNA.

The authors used a plasmid that expresses IL-5 and injected it in the tibialis anterior muscles of 2-month-old mice. A pair of electrode needles with a 5-mm gap were inserted into the muscle to encompass the DNA injection sites. Three 50-V electric pulses of 50 ms each were delivered at a rate of one pulse per second, followed by three pulses of the opposite polarity. When the scientists measured IL-5 levels in the blood of the mice 5 days later, they found that mice injected with control plasmid lacking IL-5 had IL-5 levels undetectable by enzyme-linked immunosorbent assay (ELISA). Mice injected with IL-5 plasmid had serum concentrations of 0.1 ng/ml, whereas mice that received electric pulses showed concentrations >20 ng/ml.

Additional studies revealed that neither the orientation of the electrode needles (longitudinal versus transverse to the myofibers) nor pretreatment with muscular toxin affected the efficiency of electroporation. Instead, 3 weeks after the injection, concentrations of IL-5 were lower in mice that had been pretreated with bupivacaine, suggesting that the muscle necrosis induced by the toxin may reduce the long-term expression of transferred genes. A dose-response curve (voltage delivered versus expression of IL-5) indicated that the optimal voltage was 100 V. Higher voltage decreased expression of IL-5, possibly because of damage to the muscle cells. In addition, the 100-fold rise in IL-5 production resulted from increases in both the number of plasmids per myofiber and the number of muscle cells taking up the plasmid.

Although these results were encouraging, the procedure did not increase the overall duration of IL-5 production: time-course experiments revealed peak levels of IL-5 at days 5 to 7 and then a decay to 0.9 ng/ml 10 weeks later (*2*). Additional work will have to focus on optimization of other parameters such as pulse length, volume of injection, and solution type, to further improve the efficiency of gene transfer. It will be interesting to see this procedure used in larger animals in follow-up experiments.

—RICHARD PETERS AND ROBERT SIKORSKI

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TECHSIGHTING
DNA ANALYSIS
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Pocket Laboratory

The use of simple laboratory kits for everything from running the polymerase chain reaction to plasmid cloning has revolutionized the analysis of DNA. Now, a group from the University of Michigan, led by David Burke, has developed an apparatus that might put an entire molecular biology laboratory into one device small enough to fit into a pocket (*1*).

How does it work? The tiny device (47 mm by 5 mm by 1 mm) was built by photolithography and microfabrication from a variety of materials, including silicon, platinum, and glass. The investigators designed a microchannel architecture in which reagents could be processed completely without human intervention. One key to the operation is a clever means of creating and moving the droplets that carry reagents, buffers, and DNA samples. The liquid flow pauses at various places because of hydrophobic patches in the channel. A small puff of air in perpendicular channels serves to separate nanoliter-sized droplets, which are then fed into the reaction chamber. From there, the tiny drops are passed to a matrix of polyacrylamide with embedded electrodes. Electrophoresis is performed in situ, and fluorescence detectors are used to measure the flow of DNA fragments.

Although hurdles exist, such as preventing evaporation and fine-tuning the electronics and fabrication techniques, the proof of concept for a pocket molecular biology laboratory has been demonstrated convincingly.

—ROBERT SIKORSKI AND RICHARD PETERS

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