ture determines function: the planum temporale, an area in which acoustic information is processed, is larger in persons with absolute pitch[9]. However, function may influence structure as well. In adults who had an arm amputated at very young age, the contralateral primary motor cortex is abnormal, and the typical 'hand-knob' can be missing[10]. Although new techniques may enable us to demonstrate a more refined relation between structure and function than in the days of Franz Gall, we are still far from answering some of the most basic questions. But even if we do not always understand what we see, the more we look, the more we learn.

1. Finger, S. *Origins of Neuroscience* (Oxford University Press, New York, 1994).
2. Watson, J.D.G. *et al*. Area V5 of the human brain: evidence from a combined study using positron emission tomography and magnetic resonance imaging. *Cerebr Cortex* **3**, 79–94 (1993).
3. Yousry, T.A. *et al*. Localization of the motor hand area to a knob on the precentral gyrus. *Brain* **120**, 141–157 (1997).
4. May, A. *et al*. Correlation between structural and functional changes in the brain in an idiopathic headache syndrome. *Nature Med.* **5**, 836–838 (1999).
5. May, A., Bahra, A., Büchel, C., Frackowiak, R.S.J. & Goadsby, P.J. Hypothalamic activation in cluster headache attacks. *Lancet* **351**, 275–278 (1998).
6. Talbot, J.D. *et al*. Multiple representations of pain in the human cerebral cortex. *Science* **251**, 1355–1358 (1991).
7. Weiller, C. *et al*. Brain stem activation in spontaneous human migraine attacks. *Nature Med.* **1**, 658–660 (1995).
8. Raskin, N.H., Hosobuchi, Y.& Lamb, S. Headache may arise from perturbation of brain. *Headache* **27**, 416–420 (1987).
9. Schlaug, G., Jancke, L., Huang, Y. & Steinmetz, H. *In vivo* evidence of structural brain asymmetry in musicians. *Science* **267**, 699–701 (1995).
10. Dettmers, C. *et al*. Abnormal motor cortex organization contralateral to early upper limb amputation in humans. *Neurosci. Lett.* **263**, 41–44 (1999).

Department of Neurology
Friedrich-Schiller-Universität Jena
Philosophenweg 3–5
07743 JENA
Germany
Email: cweiller@neuro.uni-jena.de

The matrix delivers

Gene therapy and tissue engineering team up to speed bone regeneration (pages 753–759).

D.A. LAUFFENBURGER[1] & D.V. SCHAFFER[2]

THREE OF THE most active and exciting areas in biomedical research, especially in bioengineering, are drug delivery, tissue engineering and gene therapy. The paper by Bonadio *et al.* on on page 753 of this issue[1] and a paper recently published by the same group in *Nature Biotechnology*[2] offer salient examples of how the combination of all three fields can yield potentially important advances in tissue regeneration treatment. Effective and rapid restoration of functional tissue as a therapeutic response to injury, disease or aging is an issue of growing importance. Few of the current approaches lead to significant improvements in either the quality or rate of healing of dermal burns, bone fractures, or cartilage degeneration, to name a few examples.

Many current tissue regeneration therapeutic strategies are based on the delivery of a drug or protein that promotes regeneration of the injured tissue. Current techniques, however, suffer from the pharmacokinetic loss of the drug due to a combination of physical and biological degradation mechanisms. Researchers have spent many years pursuing synthetic or natural polymer delivery vehicles that permit the sustained and stable release of protein from an implanted or injected source. Gene therapy techniques may aid in the controlled-release polymer technologies by allowing the delivery of DNA encoding therapeutic proteins via genetically engineered cells, leading to sustained expression and release of the protein to surrounding tissues[3] and the circulation.

Using a tissue engineering approach, polymeric scaffolds may be constructed from either natural or synthetic macromolecules, and cells can either be seeded or migrate into these scaffolds, where they undergo proliferation and differentiation[4]. However, these scaffolds often lack the additional soluble factors needed to properly stimulate or guide the desired cell responses. Thus, the *in vitro* seeding of scaffolds with genetically engineered cells before scaffold implantation is now being explored for several tissue engineering applications[5].

As the delivery of DNA encoding therapeutic proteins may improve tissue engineering techniques, so may tissue engineering improve gene therapy. One setback of current gene therapy techniques is the need for vehicles that can provide selective, efficient and targeted gene delivery in a nontoxic, noninflammatory and nonimmunogenic manner. Synthetic gene delivery vectors represent an especially promising avenue to reach these goals, especially for safety manufacturing reasons, and many advances in this direction have evolved from early drug delivery research[6]. Synthetic support matrixes would also be useful in the frequently occurring situation where, after transplantation of genetically altered cells expressing a therapeutic protein, transgene expression decreases too rapidly to achieve any therapeutic benefit. Recent reports have suggested that the level and duration of transgene expression by implanted cells may be enhanced by including a supporting matrix[7].

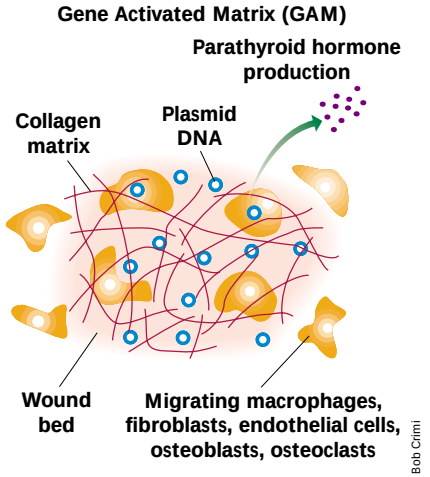


Fig. 1 Expression of parathyroid hormone mediated by a gene activated matrix

Bonadio *et al.* have intertwined these diverse approaches in an attempt to enhance regeneration of bone tissue in dogs after a defect injury. By physically entrapping a plasmid carrying the gene for an active fragment of human parathyroid hormone (hPTH1-34) within a bovine collagen matrix, they formed a moldable three-dimensional porous sponge called a 'gene activated matrix', or GAM (Fig. 1). They implanted different amounts of the collagen carrier and varying concentrations of plasmid into tibia or femur defects of different sizes, including a resected segment of variable length in mid-diaphysis. Bone tissue growth was assessed by X-ray determination of mineral density and by immunostaining for alkaline phosphatase and type I procolla-

gen. A dose-dependent increase in new bone formation was observed over a period of 2–6 weeks in the largest defects receiving the GAM, but not in controls. Results for the smaller defects were qualitatively consistent with this general trend although different in rough quantitative terms. Histological analysis of the regenerated tissue morphology and immunohistochemical determination of osteoclast presence likewise showed substantial healing at the higher DNA doses, in contrast to its absence at the lower doses. Expression of plasmid-derived mRNA and protein was reported for 2–3 weeks after implant, even for the lowest levels of plasmid, although not in sufficient amounts to indicate correlation with DNA dose.

These important findings need to be followed by continuing and broader studies, including a more quantitative assessment of the factors that govern the success of such implants. For example, more information is needed about methods to improve plasmid availability, uptake and expression by various cell types. Further analysis of the protein concentrations and distribution range required to achieve a therapeutic effect should help us to better interpret the current tissue regeneration outcomes and to improve fundamental design principles.

One interesting observation reported by Bonadio, *et al.* is that low doses of administered plasmid did not lead to bone regeneration, whereas higher doses did, even though both high and low plasmid concentrations result in transgene expression by 30–50% of the cells in the wound bed granulation tissue. Another interesting and related question is why plasmid DNA entrapped within a matrix works more effectively than plasmid directly injected into a wound site simply as naked DNA (ref. 8). Finally, there is the issue of selectivity of cell uptake and expression. The wound bed attracts macrophages, fibroblasts and endothelial cells along with tissue-specific cell types such as osteoclasts and osteoblasts or precursors, so the effect of the transgene-encoded therapeutic protein could be strongly influenced by the cell type that takes it up and expresses it—important not only for the degree of benefit but also for toxicity. Developing strategies to target specific cell types through receptor–ligand interactions could enhance GAM performance by providing better control of protein expression and function. Such techniques, however, will involve further, more complex design issues[9].

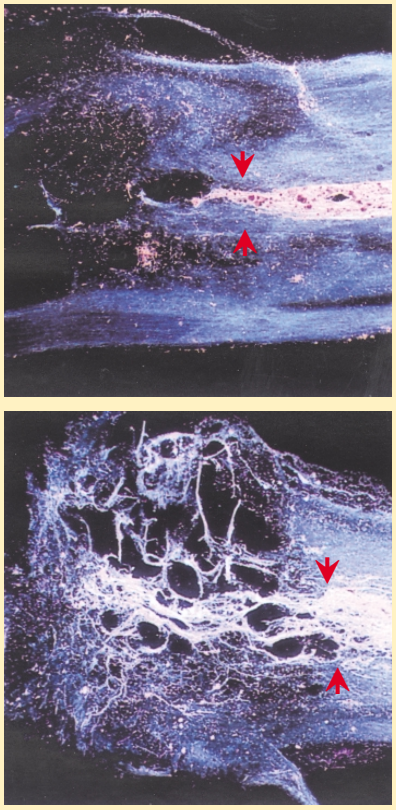
Overall, the findings by Bonadio *et al.* auger considerable promise for combining gene therapy and tissue engineering methodologies to enhance tissue regeneration. Similar multi-disciplinary efforts combining molecular biology, cell biology, biochemistry and bioengineering will lead to substantial advances in this technology.

1. Bonadio, J., E. Smiley, P. Patil, & Goldstein, S. Localized, direct plasmid gene delivery in vivo: prolonged therapy results in reproducible tissue regeneration. *Nature Med.* **5**, 753–759 (1999).
2. Shea, L.D., Smiley, E., Bonadio, J. & Mooney, D.J. DNA delivery from polymer matrices for tissue engineering. *Nature Biotechnol.* **17**, 551–554 (1999).
3. Barr, E. & Leiden, J. Systemic delivery of recombinant proteins by genetically modified myoblasts. *Science* **254**, 1507–1512 (1991).
4. Langer, R. & Vacanti, J.P. Tissue engineering. *Science* **260**, 920–926 (1993).
5. Eming, S.A., M.L. Yarmush, & Morgan, J.R. Enhanced function of cultured epithelium by genetic modification: cell-based synthesis and delivery of growth factors. *Biotechnol. Bioeng.* **52**, 15–23 (1996).
6. Legendre, J.-Y. & Szoka, F.C.Jr. Delivery of plasmid DNA into mammalian cell lines using pH-sensitive liposomes: comparison with cationic liposomes. *Pharmacol. Res.* **9**, 1235–1242 (1992).
7. Petersen, M.J. *et al.* Sustained production of human transferrin by transduced fibroblasts implanted into athymic mice: a model for somatic gene therapy. *J. Invest. Dermatol.* **104**, 171–176 (1995).
8. Truong-Le, V.L., August, J.T. & Leong, K.W. Controlled gene delivery by DNA-gelatin nanospheres. *Hum. Gene Ther.* **9**, 1709–1717 (1998).
9. Schaffer, D.V. & Lauffenburger, D.A. (1998). Optimization of cell surface binding enhances efficiency and specificity of molecular conjugate gene delivery. *J. Biol. Chem.* **273**, 28004–28009 (1998).

[1]*Biotechnology Process Engineering Center and Division of Bioengineering & Environmental Health, Massachusetts Institute of Technology Cambridge, Massachusetts 02139, USA*

[2]*Department of Chemical Engineering, University of California at Berkeley Berkeley, California, 94720, USA*

Two lesions are better than one for spinal cord regeneration



Whereas it once seemed a matter of biological fact that neurons of the central nervous system (CNS) do not regrow after being severed, advances in neuroscience research have made considerable progress in turning this dogma on its head. Another piece of evidence to contradict the doctrine that CNS lesions are irreversible was provided recently by Simona Neuman and Clifford Woolf (*Neuron* **23**, 83–91; 1999), who found that sectioning a peripheral nerve before its central transection can actually stimulate regrowth of the neuronal fibers in the CNS.

Axonal growth into the dorsal column of the CNS stops typically at the site of injury (arrows) when a spinal lesion occurs (upper panel). However, Neuman and Woolf made preconditioning lesions by peripheral sciatic nerve transection 1 or 2 weeks before or 2 weeks after bilateral dorsal column lesions of rat spinal cords (T6–T7).

Anterograde neuronal tracing by injection of conjugated horseradish peroxidase showed that preconditioning results in growth of the ipsilateral sciatic central axons into and across the severed spinal cord (pink fibers, lower panel). The most extensive fiber regrowth was seen with preconditioning at 1 week before central transection. No regrowth was found when peripheral lesions were made after central transection.

The findings reinforce the idea that inhibition of CNS regrowth depends at least partly on the internal state of the growing axons, rather than solely on the neuronal environment. If the way in which preconditioning lesions alter the instrinsic growth state of CNS neurons can be understood and therapeutically harnessed, this basic research could offer clinical hope to paralyzed individuals.

Karen Birmingham