

Non-viral gene delivery

Stretching is the point

CC Conwell and L Huang

Gene Therapy (2006) 13, 377–378. doi:10.1038/sj.gt.3302660;
published online 29 September 2005

Many recent approaches to gene therapy employ non-viral gene delivery with the ultimate aim of improving regenerative medicine and tissue engineering. A recent detailed examination of a matrix-based approach to gene delivery from the group of David J Mooney, published in *Nature Materials*, shows that the 'stretchiness' of cell adhesion substrates has a major influence over the success of such non-viral gene delivery approaches.¹

The uniting of gene delivery and tissue engineering is a monumental advance for those looking to optimize systems for tissue regeneration. Gene delivery systems have been adapted from existing prototypes to function in tissue engineering applications. Recently, the focus has shifted away from optimizing a vector for targeted gene delivery and towards the matrix on which cells are attached. The polymers and hydrogels used to create the matrix are of primary interest in the development of stable systems for gene delivery and tissue growth. The composition of the matrix may be exploited to promote different functions during cell growth.

After an optimal matrix is determined, the nature of the DNA used in the studies may be tailored towards the regulation of specific intracellular functions. Preliminary studies using matrix-based gene delivery for regenerative medicines have been explored, particularly with bone regeneration. A pioneering study published by Bonadio *et al.*² demonstrated that tissue growth can be promoted by polymer matrices (e.g. GAM) embedded with specific genes to promote bone regeneration. Bone injuries treated with engineered gene-activated matrices had nearly 75% more healing than the untreated controls.

Kong *et al.* investigated the mechanical properties of surfaces, specifically defining several charac-

teristics that act to enhance the cellular uptake of vectors in addition to increasing gene expression. They examined the fundamental details of cell adhesion to the matrices and further explored the possibility that altering characteristics of the matrix might enhance gene delivery. DNA was complexed in the presence of the common encapsulating agent, polyethyleneimine (PEI). Fluorescent resonance energy transfer (FRET) experiments were then used to follow the quantity of these complexes taken into the cell as well as the release of DNA from the particles.

By varying the elastic modulus (E) (i.e. the amount of force required to elongate the matrix) of the hydrogel upon which the cells were grown, it was determined that the quantity of DNA complexes taken up into the cell increased with respect to E . Furthermore, the FRET ratio shifted to favor a faster rate of DNA release from the complex with respect to increasing E , allowing for increased gene activity. The observed increase in gene uptake and expression might be attributed to increased cell proliferation, which doubled over the range examined in this study. Although the stiffness of the matrix was clearly linked to both cell proliferation and gene expression, the authors did not define a mechanism to explain the marked improvement.

The ability of the cell to grow and divide efficiently has previously been shown to affect the efficiency of gene uptake and expression. To determine the extent to which proficient cell proliferation accounted for the increased efficiency of delivery in this environment, the authors arrested the cell cycle using several mechanisms, including unfavorable growth conditions and the addition of biochemical agents to halt proliferation. As previously observed, as conditions became unfavorable for cell growth, gene uptake and expression were also diminished.

Other groups are performing exciting tissue regeneration studies that complement the results described by Kong *et al.* For example, the Shea laboratory at Northwestern University has developed systems where DNA complexes are tethered to the matrix prior to the addition of cells.³ They found that growing cells on a matrix containing complexed DNA rather than adding a bulk solution to the adhered cells promotes the longevity and levels of gene expression. Additional studies have focused on the role of substrate-mediated gene delivery and efforts to optimize the matrix conditions.^{4,5} These studies further demonstrate that modifications of specific properties of the DNA-embedded matrices can significantly increase *in vitro* gene activity compared to bolus delivery. A more physical approach, cyclic cell stretching, has also been employed as a means of increasing *in vitro* gene delivery efficiency.⁶

Future studies will likely exploit embedded polymer matrices in combination with undifferentiated stem cells to initiate and promote tissue growth *in vitro* for implantation. Such a system would be widely applicable, as the gene embedded into the matrix could be varied in order to alter the stem cell differentiation pathway. Explorations of such systems might provide interesting insights into the possibilities of using gene delivery methods to improve regenerative medicine.

The challenge remains to mold the existing data into an optimal matrix-based delivery system. These new data show that the elastic modulus of the matrix directly influences both the cell proliferation and the gene uptake, and additionally promotes DNA release from the complexes. So embedding complexed DNA into a matrix of high elastic modulus might further improve cell proliferation and the longevity and levels of gene expression. Moreover, applying the external force to cause cyclic stretching might encourage gene uptake and expression. In combination, these and similar systems could create a far more efficient means of tissue regeneration than those currently defined. The potential application of these systems to stem cells provides vast opportunities for the future of regenerative medicine.

While the matrix-based approaches discussed above may not

offer a universal fix for the limitations currently faced in gene therapy, they certainly provide a solid foundation for the application of gene therapies for tissue regeneration and engineering. As new information becomes available regarding specific mechanisms to improve cell growth and thereby increase gene delivery and activity, it is apparent that matrix-based approaches to gene therapy will provide a concrete method for optimizing cell growth *in vitro* for implantation as well as for insertion *in vivo* for the expression of specific genes at target tissues. ■

Dr CC Conwell and Dr L Huang are in the Division of Drug Delivery and Disposition, University of North Carolina School of Pharmacy, Chapel Hill, NC 27599-7360, USA.
E-mail: cconwell@unc.edu or leafh@unc.edu
Published online 29 September 2005

- 1 Kong H, Liu J, Riddle K, Matsumoto T, Leach K, Mooney D. Non-viral gene delivery regulated by stiffness of cell adhesion substrates. *Nat Mater* 2005; **4**: 640–644.
- 2 Bonadio J, Smiley E, Patil P, Goldstein S. Localized, direct plasmid gene delivery *in vivo*: prolonged therapy results in reproducible tissue regeneration. *Nat Med* 1999; **5**: 753–759.
- 3 Segura T, Shea L. Surface-tethered DNA complexes for enhanced gene delivery. *Bioconjugate Chem* 2002; **13**: 621–629.
- 4 Bengali Z, Pannier A, Segura T, Anderson B, Jang J, Mustoe T *et al.* Gene delivery through cell culture substrate adsorbed DNA complexes. *Biotechnol Bioeng* 2005; **90**: 290–302.
- 5 Huang Y, Riddle K, Rice K, Mooney D. Long-term *in vivo* gene expression via delivery of PEI-DNA condensates from porous polymer scaffolds. *Hum Gene Ther* 2005; **16**: 609–617.
- 6 Taylor W, Gokay K, Capaccio C, Davis E, Glucksberg M, Dean D. The effects of cyclic stretch on gene transfer in alveolar epithelial cells. *Mol Ther* 2003; **7**: 542–549.