analyze and interpret biological data, the current, somewhat broader definition has come to encompass biomathematics (which can be described as the application of mathematical principles to biological processes). Population genetics, cellular biology, comparative genetics, pharmacokinetics, protein folding and cell membrane dynamics are fields of study in which mathematics is important. This also includes data mining (searching for comparative genomic data) and all other comparable techniques being developed to analyze and interpret the mountain of sequence data currently churned out at rates that are estimated to double the amount of available biological data every six months.

Sequence data provides the basic foundation for bioinformatics research. One example where an enormous amount of this 'raw' genetic data has been provided is the Human Genome Project. Biotechnology and pharmaceutical companies face the challenge of developing bioinformatics tools to transform this mass of information into valuable medical and therapeutic products.

Although the end products of genomics and proteomics studies (e.g. isolated compounds based upon sequences, both nucleic and amino acid) provide potentially patentable subject matter, in many cases the tools and techniques themselves will be as valuable – and in some cases more valuable – than the underlying biological data.

Accordingly, bioinformatic companies require the whole slew of various methods of protection afforded, such as intellectual property assets (copyright, trademark, trade secret and patents). Among these, patent protection will provide the main buttress that will support and reinforce a company's position in the marketplace, and in some cases even establishes the position of an organization.

Seeking patent protection for an improved technique or for a special apparatus is valuable both for offensive and defensive purposes. Defensively, research organizations can appreciate that being the first to patent a bioinformatics invention could prevent others from obtaining the same or similar patent that could block work by your organization. From an offensive standpoint, the allure of bioinformatics patents is the potential long-term economic value. Obtaining a patent for novel solutions to industry-specific difficulties provides research organizations with two primary paths to interact with other organizations.

First, if the other organization is a competitor, the patent owner could possibly enforce the patent to prevent the competitor from using the patented process, product or composition. This would give the patent owner an obvious competitive advantage until the competitor either developed its own solution to the problem or until the state of the art changed.

Second, and in many cases more appropriately, the patent owner can license the patented process or apparatus to anyone who might find it useful. The group of potential licensees might include others in the genomics field, as well as organizations in a variety of other fields. If the patent claims are properly drafted and sufficiently broad, they could cover many variations upon the particular process used by the patent owner, all of which could then be licensed.

Given the current and projected amount of capital to be invested in bioinformatics tools and services (US$40 billion over the next five years), companies and organizations that aggressively pursue patent protection for their bioinformatics inventions will be most likely to weather the extremely competitive and stormy milieu that has come to define the biotech marketplace.

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# Blood hot in Boston

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Temperatures of 37°C plus warmed delegates in Boston (MA, USA) for the *5th International Biorelated Polymers Symposium* (at the 224th ACS National meeting, 18–22 August 2002); purportedly the hottest spell since the 1930s. This report is a snap shot of the meeting and will focus on some of the polymeric carrier technologies presented, which are specific to drug delivery and gene therapy.

## Drug delivery

### *Immunoliposomes*

The enhanced permeability and retention (EPR) effect is based on the non-specific penetration of particulate entities into the interstitium through leaky vasculature. Phosphatidylethanolamine-modified polyethylene glycol (PEG–PE) micelles are stable and long circulatory with dimensions that are ideally suited for exploiting the EPR opportunity. To explore this, Vladimir Torchilin (Northeastern University, http://www.northeastern.edu)

 1359-6446/02/$ – see front matter  PII: S1359-6446(02)02495-7

discussed the comparison of the efficiency of polymer–lipid micelles, either naked or modified (immunomicelles) with a nucleosome specific 2C5 monoclonal antibody [1]. This has a broad spectrum of 'anti-cancer' affinity and was used in this study against Lewis lung carcinoma (LLC) mouse model. Micelles were prepared from PEG-$_{750}$-PE and PEG$_{2000}$-PE. *In vivo* administration of $^{111}$In-labelled formulations demonstrated that both micellar constructs accumulate more readily in the tumour than muscle tissue, confirming that the pro-angiogenic state in this model facilitated uptake through the present leaky vasculature. PEG-$_{750}$-PE had a greater targeting index for the tumour (compared with muscle tissue) although the PEG$_{2000}$-PE had reduced elimination rate (≤17 h post-injection).

Target-specific immunomicelles architecture was achieved using the versatile [2] protein and peptide coupling reagent pNP–PEG–PE. This is added as a minor component to the micelles to introduce the reactive pNP groups onto the micelle surface through which the tumour-specific antibody was attached. These modified PEG–PE micelles were 5.5-times more efficient at binding to a monolayer of nucleosomes *in vitro* compared with the antibody free. *In vivo* the antibody functionalized micelles achieved a substantial threefold higher accumulation in the tumour compared with the non-targeted micelles, clearly demonstrating that PEG–PE micelles with tumour-specific antibodies substantially increase tumour accumulation.

*Ionizing radiation sensitive liposomes*

A novel ionizing radiation sensitive liposomal vehicle has been designed for site-specific release of anticancer agents (D.F. O'Brien *et al.* [3]; University of Arizona, http://www.arizona.edu). This technology is based around the inclusion of a polymerizable lipid within the formulation, in this case bis-SorbPC, which can form either intramolecular crosslinks or poly(lipid) following exposure to an ionizing radiation source (cobalt-60), which acts as a free radical generator. Cross-linkage results in disruption in the continuity of the liposomal bilayer, which results in leakage and release of the drug. *In vitro*, following exposure to 50–100 rads (maximum clinical daily dose is 200 rads) the most sensitive liposomal formulations released up to 80% of their contents within an hour after exposure. Radiation induced leakage was found to be independent of PEG–lipid content.

## Mechanism of *in vivo* nanoparticulate polymer coating

The immediate fate of polystyrene particles following intravenous administration is rapid clearance by the macrophages present in the liver and spleen. This can be circumvented using a coating of polymer poloxamine 908 that results in the formation of a steric barrier, which inhibits macrophage sequestration generating long circulating particles [4]. Interestingly, it has been demonstrated that the administration of poloxamine 908 followed by non-coated polystyrene particles gives identical long circulatory *in vivo* results to the precoated particles [5]. S. Moein Moghimi (University of Brighton, http://www.brighton.ac.uk) examined how variable concentrations of poloxamine 908 in the presence of serum can modify the surface of polystyrene nanoparticles using electrophoretic mobility measurements and surface plasmon resonance (SPR) to monitor the interaction between poloxamine and poloxamine pre-treated serum with polystyrene surfaces.

Pre-incubation of the nanoparticles in a solution of poloxamine at ≥1mg ml$^{-1}$ prior to intravenous injection dramatically suppressed their capture by the macrophages of the reticuloendothelial system (RES). Concentrations of ≤0.5mg ml$^{-1}$ were significantly less effective in suppressing macrophage recognition. Particles with prolonged circulation times were found to have an electrophoretic mobility of $-0.82 \pm 0.24 \times 10^{-8}$ m$^2$ Vs$^{-1}$ or lower in buffer. Shadowing the previous *in vivo* methodology by incubation of the particles in a range of poloxamine-treated serum resulted in electrophoretic mobility values supporting their *in vitro* modification. Thus, even in the presence of serum proteins poloxamine can still modify the surface of nanoparticles and generate long circulating entities. SPR was used to determine how poloxamine 908 can modify a polystyrene surface. Adsorption profiles obtained for the poloxamine 908 [~75 milli-degree-angle change (mda)] and poloxamine treated serum (96 mda) were similar, compared with that of pure serum. This suggests that following the passage of poloxamine-treated serum, the polystyrene surface is coated either with poloxamine and/or some poloxamine–protein complexes. These results could be indicative of the underlying *in vivo* coating mechanism and stability under shear flow

## Gene delivery

Clearly, the hot area of research in biodirected synthetic polymer chemistry is the use of living radical polymerization, especially atom transfer radical polymerization (ATRP). The advantages of this technique include highly controlled architecture, predictable molecular weight, low reaction temperatures and narrow polydispersity. For example, poly(dimethylaminoethyl methacrylate) is cationic, forms complexes with DNA and has been incorporated into a triblock polymer for the vectorization of DNA for gene therapy using transition metal (CuI)-mediated living radical polymerization by investigators at the University of Warwick (S. Monge and D.M. Haddleton; http://www.warwick.ac.uk) [6]. Poly(butyl methacrylate) was used as the initiator and reacted in the presence of poly(DMAEMA) and PEG to form the triblock PEG-*b*-PBMA-*b*-PDMAEMA. Inclusion of the PEG block was essential to afford solubility in solvents used for DNA transfection. Initial

transfection results for this vehicle have been reported as promising. It will be interesting to see if the PEG could act as a steric barrier and reduce recognition by opsonic proteins of the blood and sequestering Kupffer cells (liver macrophages) of the reticuloendothelial system, thus enhancing the probability of reaching its target for nucleic acid transfection.

Martin Woodle (Intradigm Corporation, http://www.intradigm.com) presented the use of a novel self-assembled delivery system based on a trifunctional polymer PEI–PEG–RGD for the complexation and condensation of DNA (PEI), enhanced *in vivo* circulation (PEG) and a specific cellular binding function (RGD) [7]. In the absence of the targeting ligand, the PEI–PEG conjugate readily formed stable particles when mixed with DNA, even at a charge ratio of 1. Electrophoretic mobility measurements demonstrated that these constructs had low surface charge indicating the imparted steric shielding effect of the outer PEG moiety. *In vivo* this was confirmed with enhanced circulation times for PEG–PEI and reduced lung accumulation compared to the PEI–DNA complex. It would be interesting if the presence of the coupled PEG forces more efficient complexation of the DNA to the cationic PEI, resulting in a smaller construct and hence reduced sequestration in pulmonary capillaries. Comparative cell binding and transfection experiments using the PEI–PEG and PEI–PEG–RGD conjugates were performed. The results demonstrated gene expression only when the targeting ligand was present, which suggests a ligand-mediated delivery of the nucleic acid into the cell. This technology has now been taken further by the inclusion of a cleavable linker in a prototype construct to unmask the nucleic acid payload once in the cell. During the question session, Moghimi raised two fundamental issues, which have been highlighted recently [8]: First, why is gene expression transient and, second, what is the fate of the PEI in the cytoplasm of the cell. Woodle suggested that, although the answer to both questions is at present unknown, it is possible that both events are related.

A.J. Domb (The Hebrew University of Jerusalem, http://www.huji.ac.il) discussed the synthesis of cationic polysaccharides, which are efficient and versatile vectors for gene delivery [9]. These are based on dextran, which is a fully degradable natural product and has several advantages; these include simple and efficient synthesis, straightforward addition of ligands, including markers, water solubility, hydrophobic and hydrophilic sites, and a branched nature facilitatating both 2D and 3D interaction with an ionic surface. This work has led to a new versatile polycation system based on spermine grafted onto dextran, which is capable of complexing various plasmids and delivering them into various cell lines in high yield to achieve transcription. A range of natural fatty acids (saturated and unsaturated C6–18) were covalently linked to the representative polycations to assess the effect of hydrophobicity on transfection.

Grafting of the oligoamines on oxidized ($KIO_4$) dextran was readily achieved by the slow addition of this polyaldehyde to a concentrated basic solution of the desired oligamine. A slow rate of addition reduced the degree of cross-linking facilitating the grafting of the diamine moieties onto the polymer backbone. Most of the tested polycations achieved negligible transfection rates relative to the commercial references Tranfast™ and DOTAP/Chol 1:1. However, when spermine was used as the grafting oligoamine, high protein expression was obtained in line with the commercial references. Similar protein expression was achieved with the hydrophobized dextran–spermine construct compared to the unmodified form. The oleate modified polycation achieved a threefold increase in expression, which was better than that achieved with the DOTAP/Chol 1:1 in serum free medium.

## Concluding remarks

Carrier technology requires the synthesis and use of increasingly exotic polymeric materials, which is being supported by relatively new synthetic techniques, such as ATRP. It is important to know what is the fate of the polymeric materials during drug and gene delivery, especially if this is the underlying reason why, for example, only transient gene expression is achieved following transfection. As demonstrated, polymers can self-assemble on nanoparticles *in vivo* and are then liable to be driven to a potentially consequential unique biological interaction. Therefore, to fully realize the potential of the elegant synthetic techniques being applied to increasingly ingenious carrier constructs, there is a need to gain a better mechanistic understanding of macromolecular and biological interactions *in vivo* as well as *in vitro.* Understanding of these events is essential for the future design of efficient and safe [8] synthetic drug-delivery vehicles.

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