Streamlining the Process From Gene to Pure Protein

The proteomic era has increased the demand for simple and reliable methods to clone and express new target genes and purify their encoded proteins. Research in this area has recently delivered two new methods that independently simplify these goals. On the cloning end, the Invitrogen corporation has developed Gateway technology, allowing rapid recombination of genes between various expression vectors. Independently, the purification of recombinant proteins has been simplified through the development of a variety of self-cleaving purification tags. In this issue, Gillies and coworkers have combined Gateway cloning technology with self-cleaving purification tags to generate a significant advance in high-throughput cloning, expression and purification of arbitrary protein targets. These methods are simple, general and reliable, and case studies are presented for two target proteins using four different purification tags. These studies demonstrate the utility of the system for high-throughput screening of proteins and optimization of purification processes. Page 229

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Stem Cells: How Do They Behave When Cultured Under Physiological Conditions?

Embryonic stem (ES) cells hold promise for providing unlimited quantities of specialized cells to treat a wide range of diseases. Little is known about their proliferation and differentiation at low partial pressures of oxygen (pO₂), even though the early embryo is normally exposed to such conditions. Powers and co-workers investigate the effects of oxygen on undifferentiated mouse ES cell growth, phenotype retention, and cellular energetics. Growth rate is maximal at intermediate pO₂ and declines modestly at the extremes over the range of 285–0 mmHg. When cultured at low pO₂, stem cell state, expression of self-renewal genes decreases, but pluripotency is maintained. Following a decrease to low pO₂, aerobic metabolism decreases and anaerobic metabolism increases so that the total ATP generation rate remains constant. This work helps us understand the behavior of ES cells at physiological oxygen levels. Page 241

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Is it Possible to Completely Camouflage the Surface of Red Blood Cells?

The ever increasing shortage of donated human blood has prompted the development of hemoglobin-based oxygen carriers (HBOCs) for use in transfusion medicine. HBOCs in development range from polymerized hemoglobins to particle encapsulated hemoglobins. However, by taking hemoglobin outside of its native environment—the red blood cell (RBC)—HBOCs exhibit increased NO scavenging compared to RBCs. Previous work addressed this problem by PEGylating bovine RBCs (bRBCs) in order to camouflage potential antigens while maintaining hemoglobin in its native environment. In this issue, Gundersen and Palmer demonstrate that we unfortunately cannot fool the immune system by camouflaging xenogenic cells with methoxypolyethylene glycol. In this B&B issue, Gundersen, Kennedy and Palmer demonstrate that we unfortunately cannot fool nature yet. The primary xenoantigen, Galα(1,3)Gal, still remains exposed on the surface of the RBC and is still immunoreactive at all tested levels of PEGylation. The authors recommend a completely fresh approach using siRNA to knockout Galα(1,3)Gal synthesis, creating a immunologically silent, natural, hemoglobin carrier. Page 337

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Modeling Nanoparticle Penetration in Tumor Tissue

Nanoparticle vehicles that mediate intracellular delivery can be a double-edged sword: the vehicles can help delivery by stabilizing the therapeutic and facilitating cellular uptake, but can also impede efficient tissue penetration due to their size. Previous studies have demonstrated that nanoparticles suffer in vivo from significantly hindered diffusion within tissues due to the extracellular matrix and tightly packed cells. In this work, Goodman and co-workers develop a mathematical model to describe nanoparticle penetration in multicellular spheroids. This model extends existing models by accounting for radial changes in tumor architecture that affect nanoparticle diffusion. Model parameters were determined experimentally and the model was then used to accurately predict nanoparticle concentration profiles in spheroids for several test cases. This mathematical model can therefore be used to facilitate the design and development of nanoparticle delivery vehicles with improved tissue delivery profiles. Page 388

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